Development and characterization of 20 polymorphic microsatellite markers for *Epinephelus marginatus* (Lowe, 1834) (Perciformes: Epinephelidae) using 454 pyrosequencing

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Abstract

The dusky grouper, *Epinephelus marginatus*, is a well-known and widespread marine fish assessed as endangered by the International Union for the Conservation of Nature. Analyzing the genetic diversity of this species is, therefore, of utmost importance and necessary for conservation purposes. Microsatellites are molecular tools with advantages that are ideal for population analyses. This study provides the first set of species-specific microsatellite loci for *E. marginatus* that can be applied when assessing both intra- and interpopulation genetic variation. Twenty microsatellite loci were isolated and characterized for the dusky grouper by genotyping 20 individuals obtained from the North Eastern Atlantic Ocean (n = 4) and from the South Western Atlantic Ocean (n = 16). The number of alleles per locus varied from 2 to 11, while the observed and expected heterozygosities ranged from 0.25 to 0.94 and 0.34 to 0.89, respectively. The polymorphic information content varied from moderately to highly informative. This suite of markers provides the first specific nuclear tools for *E. marginatus* and, thus, allows to assess with more specificity different populations’ structures.

Keywords: Short tandem repeat, population genetics, conservation, fishery, marine resources.

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Epinephelidae, known as groupers, are considered commercially important marine resources by commercial and recreational coastal fisheries (Begossi and Silvano, 2008; Schunter et al., 2011). In 2009, approximately 275,000 metric tons of the global catch were groupers (Epinephelidae), which represented approximately 90 million fish (Sadovy de Mitcheson et al., 2013). Groupers play an important role in trophic foodwebs in coral and rocky reef ecosystems (Condini et al., 2015). They are protogynous hermaphrodites characterized by high site fidelity, slow growth, delayed sexual maturation in males, and large body size (Marino et al., 2001; Koeck et al., 2014). This group of Perciformes is especially susceptible to overfishing (Morris et al., 2000).

The dusky grouper, *Epinephelus marginatus* (Lowe, 1834), is broadly distributed in the Atlantic Ocean from the Mediterranean Sea to southern Africa, in the Indian Ocean northwards to Madagascar, and from the British Isles to South Africa, including the Macaronesian Archipelagos of Azores, Madeira, Canaries, and Cape Verde (Heemstra, 1991; Heemstra and Randall, 1993). On the Atlantic coast of South America, this species occurs from Rio de Janeiro (Brazil) to Patagonia (Argentina) (Rico and Acha, 2003; Irigoien et al., 2005). Fishing data have shown a 50% decline in the overall dusky grouper catches from European countries between 1994 (7699 metric tons) and 2011 (869 metric tons) (Harmelin-Vivien and Craig, 2015). This population reduction combined with their life-history has led to an assessment of Endangered A2d status by the International Union for the Conservation of Nature (Cornish and Harmelin-Vivien, 2004; Harmelin-Vivien and Craig, 2015).

Knowledge of intra- and intergenetic diversity is important for planning the long-term conservation and recovery of marine fish resources through legal environmental protection and ecosystem-based fisheries management (Zhou et al., 2010). Therefore, molecular markers are needed to acquire knowledge on the genetic diversity of a given species, and microsatellite markers are the most frequently used tool in marine fish (Cuéllar-Pinzón et al.,...
Although, species-specific microsatellite markers have been developed for different species of the genus Epinephelus: E. querrus – 9 loci (Rivera et al., 2003); E. septemfasciatus – 12 loci (Zhao et al., 2009a) and 22 loci (An et al., 2014); E. awara – 12 loci (Zhao et al., 2009b); E. fuscoguttatus – 10 loci (Mokhtar et al., 2011); E. merra – 13 loci (Muths and Bourjea, 2011); E. aakaara – 12 loci (Watanabe et al., 2011) and 10 loci (Xie et al., 2015); E. lanceolatus – 32 loci (Yang et al., 2011) and 24 loci (Kim et al., 2016); E. striatus – 15 loci (Bernard et al., 2012); E. bruneus – 28 loci (Kang et al., 2013); E. polyphakadion – 12 loci (Ma et al., 2013); E. itajara and E. quinquefasciatus, 29 and 25 loci, respectively (Seyoum et al., 2013); and E. ongus – 18 loci (Nanami et al., 2017). As species-specific microsatellite marker have not yet been developed for E. marginatus, previous studies of population genetic diversity used microsatellites by cross-amplification (Sola et al., 1999; De Innocentiis et al., 2001; De Innocentiis et al., 2008; Schunter et al., 2011; Elgild et al., 2015; Buchholz-Sørensen and Vella, 2016; Reid et al., 2016).

Now that next generation sequencing (NGS) has become more accessible, the development of species-specific microsatellite loci is much faster and less labor intensive (Kumar and Kocour, 2017). In addition, microsatellites are generally found in non-coding regions, where the substitution rate is higher than in the coding regions, and hence, the flanking regions of the microsatellites, where the primers are designed, are prone to mutations (Primmer et al., 2005). Mutations in these regions may result in null alleles that affect the cross-amplification success rate (Maduna et al., 2014). Another issue regarding microsatellite cross-amplification is that the ascertainment bias phenomenon may be operating, that is, the chance of the median allele length of microsatellites being longer in one species in which it was first developed than in other cognate species (Crawford et al., 1998; Barbarà et al., 2007; Li and Kimmel, 2013). With this in mind we have now used NGS to develop a novel set of 20 specie-specific microsatellite markers to provide support to conservation management programs for the dusky grouper.

Genomic DNA was extracted from the caudal fins of five Epinephelus marginatus specimens using QIAGEN DNeasy blood and tissue extraction kits (QIAGEN Inc., Valencia, CA) following the manufacturer protocols, and these samples were forwarded to GenoScreen (Lille, France) for commercial microsatellite library production. The extracted DNA was fragmented (~1500 bp) by sonication (S220 Focused-ultrasonicator; Covaris, Newtown, CT, USA) and used to construct a shotgun library (GS-FLX Titanium kit; Roche Diagnostics Corporation, Branford, CT, USA), which was then sequenced in a 454 GS-FLX Titanium pyrosequencer (Roche Diagnostics Corporation). The following DNA probes were used to perform the enrichment: TG, CT, AAC, AAG, AGG, ACG, ACAT, and ACTC. The software QDD (Meglécz et al., 2010) was used to identify the microsatellites from the raw sequences. A total of 5526 sequences were recovered with microsatellite motifs. Of these, 165 sequences presented simple and perfect repetitions with a minimum of five repeat motifs, where 125 resulted in dinucleotides, 26 in tri-, 13 in tetranucleotides and one pentanucleotide. Forty-eight microsatellite loci were initially selected for polymorphism assessment, and 20 of these loci amplified reliably and showed evidence of polymorphism (Table 1). Primer sets were designed from the microsatellite flanking regions using the QDD software. Twenty individuals of E. marginatus were analyzed to validate the panel of species-specific microsatellite markers, with 16 individuals being from the Southwestern Atlantic Ocean near Brazil (São Paulo, n = 4; Rio de Janeiro, n = 4; Parana, n = 4 and Santa Catarina coast, n = 4) and 4 individuals from the Northeastern Atlantic Ocean (Spain / Mallorca (n = 2), Greece / Cyclades Islands (n = 1) and Azores Archipelago (n = 1)).

For each primer set, the forward primer was 5’-end-tailed with the M13 universal sequence (5’-TGTTAAAACGACGGCCAGT-3; Schuelke, 2000). The polymerase chain reactions (PCR) were performed in 20 μL reaction volumes (~100 ng genomic DNA, 1X PCR buffer, 0.25 mM dNTPs, 1.5-3.0 mM MgCl₂, 0.5 U Taq DNA polymerase, 0.2 μM of the IRDye700 fluorescently labeled universal M13 primer, 10 μM of the forward primer, and 10 μM of the reverse primer) in a Veriti thermal cycler (Applied Biosystems). The amplification thermal profile for all markers had an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 40 s at 94 °C, 1 min at the primer set annealing temperature (Table 1), and 40 s at 72 °C, with a final extension of 10 min at 72 °C. Amplicons were separated on 6.0% polyacrylamide gels using an automated DNA sequencer (DNA Analyzer 4300; LI-COR Biosciences, Lincoln, NE, USA). Alleles were sized using a 50-350 bp standard (LI-COR Biosciences), and genotypes were scored using SAGA v.3.3 software (LI-COR Biosciences).

The number of alleles per locus, estimates of observed (Ho) and expected heterozygosity (He), and deviations from Hardy-Weinberg expectations were determined with an exact test using the Markov chain-randomization approach (Guo and Thompson 1992) with HW-Quickcheck (Kalinowski, 2006). Linkage disequilibrium was assessed using GENEPOP v.4.2 (Rousset, 2008), and MicroChecker v.2.2.3 (Van Oosterhout et al., 2004) was used to test for null alleles and allele dropout. The polymorphic information content (PIC) was analyzed using Cervus v.3.0.7 (Kalinowski et al., 2007).

Table 1 summarizes the information for each primer sequence, providing GenBank accession number, repeat motif, number of alleles, diversity estimates (Ho and He),...
| Locus | GenBank accession | Primer sequence (5'-3') | Repeat motif | Ta (°C) | N | A | Size range (bp) | Ho | He |
|-------|-------------------|-------------------------|--------------|---------|---|---|-----------------|----|----|
| Ema01 | KU762341          | F: ACAGCAACCATGTGAGCAG   | (AC)14       | 60      | 20 | 09 | 158-180        | 0.70 | 0.82 |
|       |                   | R: TGGAGTGATAGTCCTTGTGG  |              |         |    |    |                 |     |     |
| Ema02 | KU762342          | F: CAGACGTATGCATCCTGGCCT | (TG)9        | 62      | 20 | 07 | 123-165        | 0.85 | 0.78 |
|       |                   | R: ATATGTCAGCCTCACCCTCC  |              |         |    |    |                 |     |     |
| Ema03 | KU762343          | F: CCAACATGCCCTCCAAATA  | (ATCT)5      | 58      | 19 | 11 | 125-165        | 0.89 | 0.89 |
|       |                   | R: GACCCAGTGTAATGACACT  |              |         |    |    |                 |     |     |
| Ema04 | KU762344          | F: CAGAGGGAACTCCAAATTTAATC | (GATG)8      | 52      | 20 | 09 | 183-223        | 0.80 | 0.87 |
|       |                   | R: TCTGACTGAGACATGAAACAAGG   |              |         |    |    |                 |     |     |
| Ema05 | KU762345          | F: GCTCAAGGAGACTGACAGA  | (GA)7        | 56      | 18 | 09 | 177-195        | 0.83 | 0.81 |
|       |                   | R: GTGACCAAAGAGGCGACAG    |              |         |    |    |                 |     |     |
| Ema06 | KU762346          | F: TGTAGCTTGTCTGAATGTTGTG | (CAA)5       | 55      | 20 | 05 | 204-216        | 0.75 | 0.70 |
|       |                   | R: CTGAACTGCTACTGACATCGTC |              |         |    |    |                 |     |     |
| Ema07 | KU762347          | F: CCTCTACTGCTACATGACTTCCTCC | (TAC)5       | 52      | 12 | 02 | 205-208        | 0.25 | 0.34 |
|       |                   | R: ACAGTTGAAATATGAGCTGAGA |              |         |    |    |                 |     |     |
| Ema12 | KU762348          | F: AAGATGCAGCTGTGACGCAG  | (TCC)7       | 58      | 17 | 03 | 221-230        | 0.94 | 0.56 |
|       |                   | R: TGATGTTGCAAGCAAGAAGA   |              |         |    |    |                 |     |     |
| Ema17 | KU762350          | F: GGTCAGTGACGGTAGACATTT | (CTAT)3      | 56      | 20 | 09 | 142-182        | 0.80 | 0.82 |
|       |                   | R: CAAAGGCGAATTACAACTC   |              |         |    |    |                 |     |     |
| Ema18 | KU762351          | F: GGCAAAAGGTGACATTTGCG  | (CTAT)6      | 60      | 20 | 09 | 175-207        | 0.80 | 0.89 |
|       |                   | R: AACCAGGAGCTTATGCGTCT  |              |         |    |    |                 |     |     |
| Ema20 | KU762352          | F: TGATTATGAGTCAAAGGAGTGAT | (CATC)5      | 60      | 18 | 04 | 150-162        | 0.78 | 0.63 |
|       |                   | R: AGGCGCATGTGACATTGTA   |              |         |    |    |                 |     |     |
| Ema22 | KU762353          | F: GTTGGCAGGTGTGCTGAGTCT | (TATG)7      | 58      | 20 | 07 | 111-135        | 0.85 | 0.81 |
|       |                   | R: TAGGGTGGGTAGTGGACTG    |              |         |    |    |                 |     |     |
| Ema23 | KU762354          | F: AACATGATCCGATAGGCTGA  | (ACAG)7      | 60      | 19 | 07 | 214-234        | 0.84 | 0.74 |
|       |                   | R: CAGAAGGCTCCAGTCAAGTAT |              |         |    |    |                 |     |     |
| Ema26 | KU762355          | F: CAGGTGGAGGTATTGGTGGC  | (TTC)6       | 52      | 20 | 06 | 128-143        | 0.65 | 0.76 |
|       |                   | R: TTACCTRATGGGAATGTGA   |              |         |    |    |                 |     |     |
| Ema35 | MG640563          | F: ACCTCCACCTGCCTGCCAG  | (AC)14       | 56      | 20 | 11 | 169-197        | 0.55 | 0.84 |
|       |                   | R: ACCTGCAAAATTTTGGGACA  |              |         |    |    |                 |     |     |
| Ema38 | MG640564          | F: TGTCGTTGAGCAAGCTCGCC | (TG)9        | 60      | 20 | 07 | 160-172        | 0.35 | 0.82 |
|       |                   | R: CCACTCTACTCTGCTCCTC   |              |         |    |    |                 |     |     |
| Ema42 | MG640565          | F: AAATGATCGTAATTTGACGCA | (CTG)7       | 54      | 20 | 06 | 147-162        | 0.55 | 0.66 |
|       |                   | R: CACCCCTAGACAGCACAAT   |              |         |    |    |                 |     |     |
| Ema43 | MG640566          | F: TGGGAGAACAGGCTTCTCAG  | (GT)9        | 58      | 15 | 04 | 189-195        | 0.33 | 0.71 |
|       |                   | R: CGTGCCTGTTGCTGACCTCA  |              |         |    |    |                 |     |     |
| Ema45 | MG640567          | F: GGAGCTTGTCTAGAAACAGCC | (TGT)7       | 56      | 17 | 04 | 158-167        | 0.47 | 0.62 |
|       |                   | R: CAGACGTGCAAGAAACACGC  |              |         |    |    |                 |     |     |
| Ema48 | MG640568          | F: TCAAAGTGATTCCACACTGCC | (ATCC)7      | 50      | 19 | 04 | 113-125        | 0.68 | 0.57 |
|       |                   | R: ATGGATAGATGATGAGGCTG   |              |         |    |    |                 |     |     |

Ta: Annealing temperature; N: number of individuals; A: number of alleles; Allele size in base pairs; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content; HWE: Hardy-Weinberg Equilibrium p-values.

*p < 0.05 significant departure from Hardy-Weinberg Equilibrium.

Primer 5’ end labeled with M13 tail (5’TGTAAAACGACGGCCAGT 3’) (Schuelke, 2000).
PIC, and probability of Hardy-Weinberg equilibrium (HWE).

Among the 20 individuals, the number of alleles per locus ranged from 2 (Ema07) to 11 (Ema03 and Ema35), with an average of 6.65, while the observed and expected heterozygosities ranged from 0.25 (Ema07) to 0.94 (Ema12) and 0.34 (Ema07) to 0.89 (Ema03 and Ema18), respectively (Table 1). Sixteen loci (Ema01, Ema02, Ema03, Ema04, Ema05, Ema06, Ema07, Ema17, Ema18, Ema20, Ema22, Ema23, Ema26, Ema42, Ema45, and Ema48) were in HWE ($p > 0.05$), while four loci significantly deviated ($p < 0.05$; Ema12, Ema35, Ema38, and Ema43). Three loci (Ema35, Ema38, and Ema43) showed evidence of null alleles, but no allele dropout was detected. No statistical evidence for linkage disequilibrium was found between any of the 20 loci pairwise comparisons. The number of alleles was high and exhibited moderate to high levels of polymorphism (PIC), ranging from 0.27 (Ema07) to 0.85 (Ema02 and Ema18), with an average of 0.67. When separated in di- (0.74), tri- (0.54) and tetranucleotides (0.72), the loci that presented the highest levels of polymorphism (PIC) were the dinucleotides.

These novel polymorphic microsatellite loci developed using NGS technology will aid in achieving better resolution when assessing stock structure and population connectivity for the dusky grouper’s long-term conservation and the sustainable use of this valuable marine resource.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this study.

Author contributions

J.O.V., K.G.M. and A.P.O. conducted the experiments; J.O.V. and A.W.S.H. analyzed the data and wrote the manuscript; J.P.B. and R.G.M. contributed to data analysis and revising the manuscript; A.W.S.H. supervised the whole project.

References

An HS, Cho JK, Kim KM, Son MH, Myeong JI and Na CM (2014) Characterization of 22 polymorphic microsatellite markers for seven-band grouper Epinephelus septemfasciatus developed using a 454 pyrosequencing approach. Conserv Genet Resour 6:665-667.

Barbára T, Palma-Silva C, Paggi GM, Bered F, Fay MF and Lexer C (2007) Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol Ecol 16:3759-3767.

Begossi A and Silvano RAM (2008) Ecology and ethnoecology of dusky grouper [garoupa, Epinephelus marginatus (Lowe, 1834)] along the coast of Brazil. J Ethnobiol Ethnomed 4:20.

Bernard AM, Feldheim KA, Richards VP, Nemeth RS and Shivji MS (2012) Development and characterization of fifteen novel microsatellite loci for the Nassau grouper (Epinephelus striatus) and their utility for cross-amplification on a suite of closely related species. Conserv Genet Resour 4:983-986.

Buchholz-Sørensen M and Vella A (2016) Population structure, genetic diversity, effective population size, demographic history and regional connectivity patterns of the endangered dusky grouper, Epinephelus marginatus (Teleostei: Serranidae), within Malta’s fisheries management zone. PLoS One 11:e0159864.

Condini MV, Hoeninghaus DJ and Garcia AM (2015) Trophic ecology of dusky grouper Epinephelus marginatus (Actinopterygii, Epinephelidae) in littoral and neritic habitats of southern Brazil as elucidated by stomach contents and stable isotope analyses. Hydrobiologia 743:109-125.

Cornish A and Harmelin-Vivien M (Grouper & Wrasse Specialist Group) (2004) Epinephelus marginatus. The IUCN Red List of Threatened Species 2004:c.T7859A12857009.

Crawford AM, Kappes SM, Paterson KA, de Gotari MJ, Dodds KG, Freking BA, Stone RT and Beattie CW (1998) Microsatellite evolution: Testing the ascertainment bias hypothesis. J Mol Evol 46:256-260.

Cuéllar-Pinzón J, Presa P, Hawkins SJ and Pita A (2016) Genetic markers in marine fisheries: Types, tasks and trends. Fish Res 173:194-205.

De Innocentiis S, Sola L, Cataudella S and Bentzen P (2001) Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (Epinephelus marginatus) within the Mediterranean Sea. Mol Ecol 10:2163-2175.

De Innocentiis S, Longobardi A and Marino G (2008) Molecular tools in a marine restocking program for the endangered Dusky Grouper, Epinephelus marginatus. Rev Fish Sci 16:269-277.

Elglid A, Crouau-Roy B, Bradai MN and Fadhlouai-Zid K (2015) Population genetic structure of Epinephelus marginatus in the Central Mediterranean Sea (Gulf of Gabès and the coast of Libya). J Adv Biol 8:1571-1580.

Guo SW and Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

Harmelin-Vivien M and Craig MT (2015) Epinephelus marginatus. The IUCN Red List of Threatened Species 2015:c.T7859A44904558.

Heemstra PC (1991) A taxonomic revision of the eastern Atlantic groupers (Pisces: Serranidae). Bol Mus Mun Funchal 43:45-71.

Heemstra PC and Randall JE (1993) FAO Species Catalogue. Vol. 16. Groupers of the world (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of
the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO, Rome, 382 p.

Irigoyen AJ, Galvaan DE and Venerus LA (2005) Occurrence of dusky grouper Epinephelus marginatus (Lowe, 1834) in gulfs of northern Patagonia, Argentina. J Fish Biol 67:1741-1745.

Kalinowski ST (2006) HW-QUICKCHECK: An easy-to-use computer program for checking genotypes for agreement with Hardy-Weinberg expectations. Mol Ecol Notes 6:974-979.

Kalinowski ST, Taper ML and Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099-1106.

Kang JH, Yang SG, Moon TS, Park JY and Choi TJ (2013) Development of microsatellite markers for the kelp grouper Epinephelus bruneus by 454 pyrosequencing and transfer to related species. Genet Mol Res 12:5485-5493.

Kim KS, Noh CH, Moon SJ, Han SH and Bang IC (2016) Development of novel tetra- and trinucleotide microsatellite markers for giant grouper Epinephelus lanceolatus using 454 pyrosequencing. Mol Biol Rep 43:541-548.

Koeck B, Pastor J, Saragoni G, Dalias N, Payrot J and Lefant P (2011) Characterization of thirteen new microsatellite markers from the common brown marbled grouper, Epinephelus merra. Conserv Genet Resour 3:629-632.

Koizumi Y, Kajihara K, Kageyama Y, Oshida T, Atsumi T, Yano K and Itoh M (2012) Development and characterization of 29 polymorphic microsatellite markers for the endangered Atlantic goliath grouper (Epinephelus itajara), and the Pacific goliath grouper (E. quinquefasciatus). Conserv Genet Resour 5:1226-1229.

Kumar G and Kocour M (2017) Applications of next-generation sequencing in fisheries research: A review. Fish Res 186:11-22.

Li B and Kimmel M (2013) Factors influencing ascertainment bias of microsatellite allele sizes: Impact on estimates of mutation rates. Genetics 195:563-572.

Ma KY, Sadovy de Mitcheson Y and Chu KH (2013) Isolation and characterization of microsatellite markers from the camouflage grouper, Epinephelus polyphekadion (Epinephelidae). Conserv Genet Resour 5:1129-1132.

Maduna SN, Rossouw C, Roodt-Willing R and Merwe AEB (2014) Microsatellite cross-species amplification and utility in southern African elasmobranchs: A valuable resource for fisheries management and conservation. BMC Res Notes 7:352.

Marino G, Azzurro E, Massari A, Finoia MG and Mandich A (2001) Reproduction is the dusky grouper from the southern Mediterranean. J Fish Biol 58:909-927.

Meglécz E, Costedoat C and Dubut V (2010) QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403-404.

Mokhtar MAA, Normah MN, Kumar SV and Baharum SN (2011) Characterization of 10 novel microsatellite loci for the brown marbled grouper, Epinephelus fuscoguttatus (Serranidae). Genet Mol Res 10:885-888.

Morris AV, Roberts CM and Hawkins JP (2000) The threatened status of groupers (Epinephelinae). Biodivers Conserv 9:919-942.

Muths D and Bourjea J (2011) Characterization of thirteen new polymorphic microsatellite markers from the honeycomb grouper Epinephelus merra. Conserv Genet Resour 3:629-631.

Namani A, Saihok K and Sekino M (2017) Development of 18 microsatellite markers for the white-streaked grouper, Epinephelus ongus (Bloch, 1790). J Appl Ichthyol 33:121-123.

Prammer CR, Painter JN, Koskinen MT, Palo JU and Merilä J (2005) Factors affecting avian cross-species microsatellite amplification. J Avian Biol 36:348-360.

Reid K, Crochelet E, Bloomer P and Hoareau TB (2016) Investigating the origin of vagrant dusky groupers, Epinephelus marginatus (Lowe, 1834), in coastal waters of Réunion Island. Mol Phylogenet Evol 103:98-103.

Rico MR and Acha EM (2003) Southernmost occurrence of Epinephelus marginatus in the south-west Atlantic. J Fish Biol 63:1621-1624.

Rivera MAJ, Graham GC and Roderick GK (2003) Isolation and characterization of nine microsatellite loci from the Hawaiian grouper Epinephelus quernus (Serranidae) for population genetic analyses. Mar Biotechnol 5:126-129.

Rousset F (2008) Genepop’007: A complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103-106.

Sadovy de Mitcheson Y, Craig MT, Bertocinii AA, Carpenter KE, Cheung WWL, Chaoit JH, Cornish AS, Fennessey ST, Ferreira BP, Heemstra PC et al. (2013) Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. Fish Fish 14:119-136.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233-234.

Schunter C, Carreras-Carbonell J, Planes S, Sala E, Ballesteros E, Zabala M, Harmelin JG, Harmelin-Vivien M, Macpherson E and Pascual M (2011) Genetic connectivity patterns in an endangered species: the dusky grouper (Epinephelus marginatus). J Exp Mar Biol Ecol 401:126-133.

Seyoum S, Tringali MD, Barthel BL, Puchulutegui C, Davis MC, Collins AB and Craig MT (2013) Isolation and characterization of 29 polymorphic microsatellite markers for the endangered Atlantic goliath grouper (Epinephelus itajara), and the Pacific goliath grouper (E. quinquefasciatus). Conserv Genet Resour 5:729-732.

Sola L, Papalia S, Rossi AR, Gornung E, De Innocentiis S, Marino G, Di Marco P and Cataudella S (1999) Genetic characterization of Epinephelus marginatus through cyto genetic, allozyme and microsatellite analyses: Preliminary results. Mar Life 9:67-68.

Van Oosterhout C, Hutchinson WF, Willis DPM and Shipley P (2004) MICROCHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Watanabe M, Shimizu T, Kamarudin ASB, Kuniyoshi H, Ohara K, Takagi M and Umino T (2011) Ten novel polymorphic microsatellite loci of Red-spotted grouper (Epinephelus akaara) revealed from full-sib progeny and unrelated individuals. Conserv Genet Resour 3:613-616.

Xie ZZ, Zheng LY, Tang L, Tang ZJ, Li SS, Zhang Y and Lin HR (2015) Isolation and characterization of novel polymorphic microsatellite markers for Epinephelus akaara. Genet Mol Res 14:13663-13666.

Yang S, Wang L, Zhang Y, Liu XC, Lin HR and Meng ZN (2011) Development and characterization of 32 microsatellite loci in the giant grouper Epinephelus lanceolatus (Serranidae). Genet Mol Res 10:4006-4011.

Zhao L, Shao C, Liao X and Chen S (2009a) Isolation and characterization of polymorphic microsatellite loci from a dinu-
cleotide-enriched genomic library of seven-band grouper (Epinephelus septemfasciatus) and cross-species amplification. Conserv Genet 10:627-629.

Zhao L, Shao C, Liao X, Ma H, Zhu X and Chen S (2009b) Twelve novel polymorphic microsatellite loci for the yellow grouper (Epinephelus awoara) and cross-species amplifications. Conserv Genet 10:743-745.

Zhou S, Smith ADM, Punt AE, Richardson AJ, Gibbs M, Fulton EA, Pascoe S, Bulman C, Bayliss P and Sainsbury K (2010) Ecosystem-based fisheries management requires a change to the selective fishing philosophy. Proc Natl Acad Sci USA 107:9485-9489.

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