**Supplementary information**

**Genomics overrules mitochondrial DNA, siding with morphology on a controversial case of species delimitation**

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**Extended Materials and Methods**

**Supplemental Figures and Tables**

**Supplementary References**

**Data repository:** datasets are available from Dryad digital repository: https://datadryad.org/handle/10255/3/submit?workspaceID=361068

**Extended Materials and Methods: molecular protocols, mitochondrial data, SNP genotyping, and Bayes factor delimitation analyses.**

DNA extractions were performed from fin-clip tissues using Qiagen DNeasy Blood and Tissue kit (Qiagen, Inc.), following the manufacturer’s protocol. Library preparation was carried out at the Sequencing and Genotyping Facility (SGF) at the University of Puerto Rico – Río Piedras (UPR-RP). We applied the double-digest RADseq (ddRADseq) protocol using MsI and PsI restriction enzymes as initially described by Peterson *et al.* (2012). This protocol provides the advantage of reducing genome-wide sequences to size-selected digested fragments (300-600bp in this study) [1]. Each library contained up to 20 individuals indexed by a set of six base-pairs barcodes in combinatorial schemes, and further sequenced in two Illumina HiSeq 4000 lanes using 100 base pair-ended sequencing at the Knapp Center of Biomedical Discovery (KCBD) Genomics Facility at the University of Chicago. For *COI* barcoding, PCR products were checked by electrophoresis on 1.8% agarose gels and sequenced in both directions at the SGF at the UPR-RP. Accession numbers and additional information are available from Table S2.

Sequenced libraries were demultiplexed the using the process\_radtags.pl script as implemented in Stacks v1.49 [2]. Raw reads were trimmed to 86 bp after removing restriction sites. The quality of raw reads was further verified using FastQC v0.11.5 (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and selected a Phred score threshold of 33 for filtering sequencing reads. The total number of raw reads was 1.68 X 109, of which 1.184 X 109 (~70%) passed quality filters. *De novo* assembling of putative loci and calling of single nucleotide polymorphisms (SNP) was carried out in Stacks using the denovo\_map.pl routine. Selection of assembly parameters that adjusted best to our data was performed based on alternative strategies [3] (see below). RAD loci were assembled by applying default settings on all samples for a pilot run, which resulted in 4,326,243 putative loci. Different combinations of assembly parameters were tested on a subset of 30 samples that were selected on the basis of sequence quality (>40% of all loci represented after a pilot test using the default settings). Combinations of parameters were tested as in Mastretta-Yanes [3], including minimum number of raw reads required to form a stack (*m* = 2-15), number of mismatches allowed between stacks (*M* = 2-10), number of mismatches allowed between loci upon catalogue building (*n* = 0-5), and maximum number of stacks allowed per single locus (--*max\_locus\_stacks* = 2-6). In all tests, only one parameter was changed at a time, while keeping others at their default value (*m*=3, *M*=2, *n*=1, and *max\_locus\_stacks* = 0; Fig. S2). Results of *de novo* assembly tests varied from 1 to ~8 million putative loci for 30 individuals (Fig. S2). The number of putative RAD loci stop dropping strongly after *m* = 5, suggesting that many low coverage loci are discarded (Fig. S2a). The same pattern is observed at *M* = 2 and *n* = 3 where values also stop, roughly showing large changes. When only polymorphic loci are present in a minimum of 6 populations (80%) and in at least 75% of individuals within populations included, values start to stabilize at *M* = 2 and *max\_loc\_stacks* = 3 (Fig. S2b). Exponential increases are observed from 12,000 to 60,000 SNPs for *n* = 0. In this case, higher values of *n* reflect an increase in RAD loci as this parameter allows more stacks and loci to be collapsed. Final selected parameters were *m*=5, *M*=2, *n*=3.

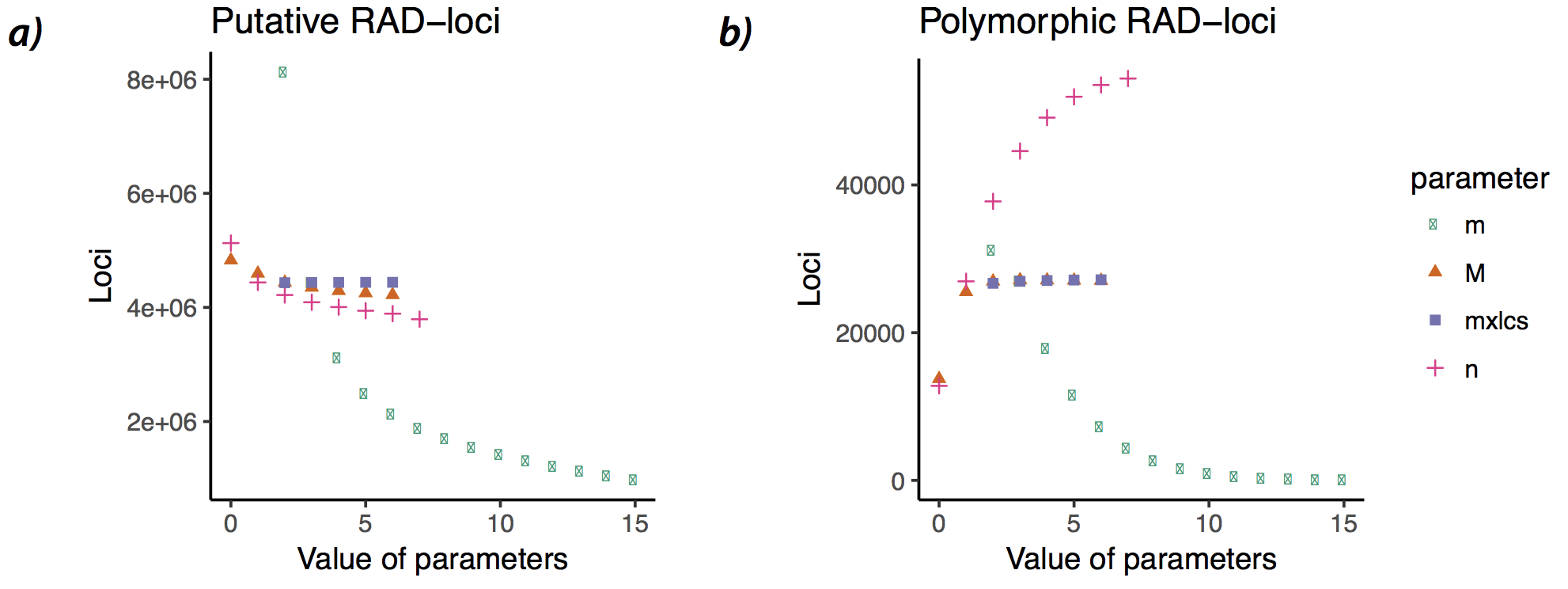
Biallelic loci were filtered in the populations pipeline component of Stacks, varying population coverage constraints (*p* = 15-8 and *r* = 1.0-0.50) and selecting only one SNP per tag to avoid linkage between loci. To remove low frequency and paralogous loci, SNPs were further filtered using a minimum allele frequency of 0.05 and a maximum observed heterozygosity of 0.70. Finally, we were interested in evaluating the sensitivity of results to number of individuals and missing data. Therefore, four of the previous datasets that contained between 21,431 and 55,795 loci were selected (p11r50, p12r50, p9r60 and p8r60), and applied a second filter removing individuals based on the amount of missing sites (minimum proportion of sites present of 0.75, 0.50, 0.25 and 0.05) using the program Tassel v5.2.43 [4]. We refer to this threshold as “min. sites.” Missing data was measured in the output datasets using VCFtools v0.1.15 [5]. These tests resulted in 20 datasets (Table S4), of which six were selected based on the amount of missing data (9–46%), number of individuals present (44–155), number of SNPs (15,112–42,406), and number of populations (8–15).

For the Bayes factor delimitation (BFD\*) analyses, additional filters were applied to the p12r50 dataset (with 15,112 SNPs from 12 populations) by retaining both loci and individuals from each population with the lowest proportions of missing data (e.g., using Tassel v5.2.43 [4]). The subsets were assembled: subset 1: 58 individuals and 149 loci (each locus is present in at least 55 individuals); subset 2: 58 individuals and 938 loci (each locus is present in at least 51 individuals); subset 3: 108 individuals and 957 loci (each locus is present in at least 85 individuals). The XML files for the BFD analyses performed are available from Dryad.

**Supplementary Figures and Tables**

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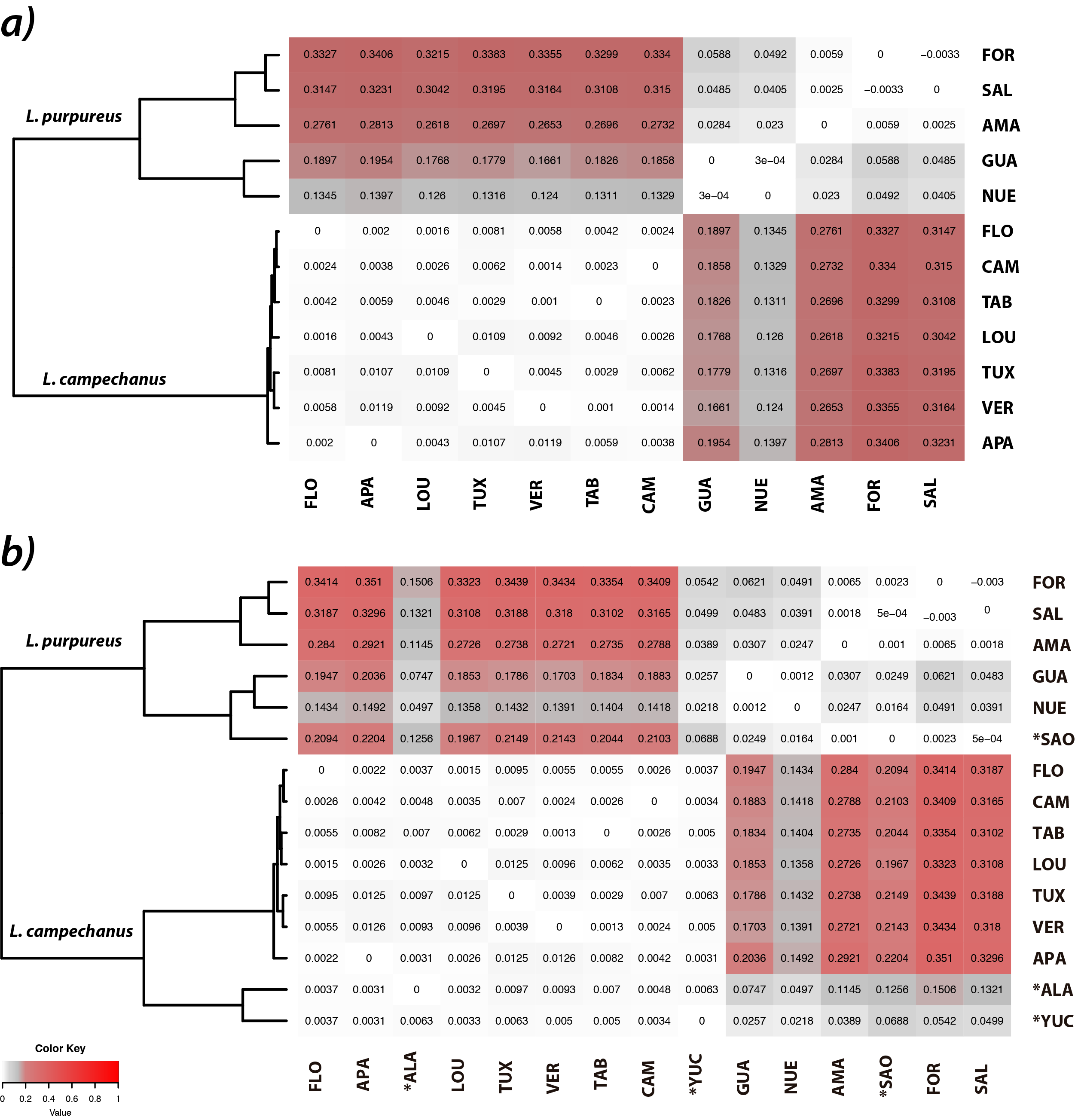
**Figure S1. a) map illustrating reports of *Lutjanus campechanus* and *Lutjanus purpureus* throughout their range and over time; b) heatmap of records of both species.** Red stars in (a) indicate localities that were exhaustively but unsuccessfully surveyed for samples: 1) San Andrés, Colombia; 2) Puerto Rico.



**Figure S2. Tests of parameter combinations (following Mastretta-Yanes et al.** [3]**).**

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**Figure S3. Principal component analyses of allele frequency data from a matrix based on (a-c) 15,112 SNPs and (d-f) 42,406 SNPs.**

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**Figure S4. Weir and Cockerham estimates of genetic differentiation among populations of *L. campechanus* and *L. purpureus*,including a) 12 populations (with minimal amount of missing data), and b) 15 populations.** Populations removed form final analyses in a) are denoted with an asterisk in b). Some calculations in b) appear underestimated despite their great geographic isolation, possibly as result of missing data: e.g., Yucatán vs. Salvador, *FST*=0.0499 (1646 loci); Salvador-Campeche *FST*=0.3165 (4282 loci); Salvador-Florida *FST*=0.3187 (14102 loci).

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**Figure S5. Mantel test simulations (a,b) and correlograms (c,d) for *L. campechanus* (red) and *L. purpureus* (green), respectively.** Geographic distances reflect Least Cost Paths (LCPs); similar results were obtained using Euclidean distances (not shown).

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**Figure S6. Bayesian estimates of the Hybrid index (HI) estimated with gghybrid for each individual in all studied populations of *L. campechanus*, and *L. purpureus***. Red and blue points represent the parental reference sets of *L. campechanus* (Florida-Apalachicola) and *L. purpureus* (Fortaleza-Salvador), respectively. Individuals are color-coded according to their corresponding population. Population codes on the upper left corner correspond to abbreviations given in Table S1. Points indicate HI estimated values, where 0.0 denotes pure *L. campechanus* individuals and 1.0 denotes pure *L. purpureus* individuals; lines represent 95% credibility intervals.

**Table S1. Sampling localities**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| **Population abbreviation** | **Locality** | **Country** | **Geographic coordinates** | **Number of individuals** |
| ***Lutjanus campechanus*** | | | | |
| FLO | West of Florida, Florida | USA | 27.1666°N -83.3833°W | 18 |
| APA | Apalachicola, Florida | USA | 28.7900°N -85.1635°W | 18 |
| ALA | Dauphin Island, Alabama | USA | 29.7836°N -88.0732°W | 18 |
| LOU | Louisiana | USA | 28.2443°N -91.4573°W | 17 |
| TUX | Puerto de Tuxpan, Veracruz | Mexico | 21.0544°N -97.1926°W | 3 |
| VER | Puerto de Veracruz, Veracruz | Mexico | 19.2802°N -96.0216°W | 3 |
| TAB | San Pedro, Tabasco | Mexico | 18.7480°N -93.1829°W | 7 |
| CAM | Champotón, Campeche | Mexico | 19.4576°N -91.1248°W | 9 |
| YUC | Puerto Progreso, Yucatán | Mexico | 21.6932°N -89.6345°W | 12 |
| ***Lutjanus purpureus*** | | | | |
| GUA | Camarones, Guajira | Colombia | 11.8624°N -72.8027°W | 16 |
| NUE | Nueva Esparta | Venezuela | 11.3417°N -63.8558°W | 7 |
| AMA | Amapá | Brazil | 4.33564°N -50.5537°W | 13 |
| SAO | Maranhão, São Luís | Brazil | 0.2172°N -46.246°W | 13 |
| FOR | Ceará, Fortaleza | Brazil | 2.9071°S -39.1452°W | 13 |
| SAL | São Salvador da Bahia, Salvador | Brazil | 13.226°S -38.6245°W | 12 |

**Table S2. GenBank (NCBI) accession numbers for raw data (Bioproject PRJNA524905) and mitochondrial sequences generated.** See Table S1 for population abbreviations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| **Species** | **Sample code** | **Population** | **COI** | **Raw data (ddRAD)** |
| **Accession no.** | **Accession no.** |
| *Lutjanus campechanus* | 30915-1 | FLO | - | SAMN11038299 |
| *Lutjanus campechanus* | 30915-10 | FLO | - | SAMN11038300 |
| *Lutjanus campechanus* | 30915-3 | FLO | - | SAMN11038301 |
| *Lutjanus campechanus* | 30915-4 | FLO | - | SAMN11038302 |
| *Lutjanus campechanus* | 30915-5 | FLO | - | SAMN11038303 |
| *Lutjanus campechanus* | 30915-6 | FLO | - | SAMN11038304 |
| *Lutjanus campechanus* | 30915-7 | FLO | - | SAMN11038305 |
| *Lutjanus campechanus* | 30915-8 | FLO | - | SAMN11038306 |
| *Lutjanus campechanus* | 30915-9 | FLO | - | SAMN11038307 |
| *Lutjanus campechanus* | 31215-10 | FLO | - | SAMN11038308 |
| *Lutjanus campechanus* | 31215-11 | FLO | - | SAMN11038309 |
| *Lutjanus campechanus* | 31215-12 | FLO | - | SAMN11038310 |
| *Lutjanus campechanus* | 31215-13 | FLO | - | SAMN11038311 |
| *Lutjanus campechanus* | 31215-14 | FLO | - | SAMN11038312 |
| *Lutjanus campechanus* | 31215-15 | FLO | - | SAMN11038313 |
| *Lutjanus campechanus* | 31215-16 | FLO | - | SAMN11038314 |
| *Lutjanus campechanus* | 31215-17 | FLO | - | SAMN11038315 |
| *Lutjanus campechanus* | 31215-9 | FLO | - | SAMN11038316 |
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| *Lutjanus campechanus* | ABR60 | ALA | - | SAMN11038323 |
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| *Lutjanus purpureus* | RB1446 | AMA | - | SAMN11038393 |
| *Lutjanus purpureus* | RB1458 | AMA | - | SAMN11038394 |
| *Lutjanus purpureus* | RB1459 | AMA | - | SAMN11038395 |
| *Lutjanus purpureus* | RB1460 | AMA | - | SAMN11038396 |
| *Lutjanus purpureus* | RB1470 | FOR | - | SAMN11038397 |
| *Lutjanus purpureus* | RB1471 | FOR | - | SAMN11038398 |
| *Lutjanus purpureus* | RB1473 | FOR | - | SAMN11038399 |
| *Lutjanus purpureus* | RB1474 | FOR | - | SAMN11038400 |
| *Lutjanus purpureus* | RB1475 | FOR | - | SAMN11038401 |
| *Lutjanus purpureus* | RB1476 | FOR | - | SAMN11038402 |
| *Lutjanus purpureus* | RB1477 | FOR | - | SAMN11038403 |
| *Lutjanus purpureus* | RB1478 | FOR | - | SAMN11038404 |
| *Lutjanus purpureus* | RB1479 | FOR | - | SAMN11038405 |
| *Lutjanus purpureus* | RB1482 | FOR | - | SAMN11038406 |
| *Lutjanus purpureus* | RB1484 | FOR | - | SAMN11038407 |
| *Lutjanus purpureus* | RB1490 | FOR | - | SAMN11038408 |
| *Lutjanus purpureus* | RB1496 | SAO | - | SAMN11038409 |
| *Lutjanus purpureus* | RB1497 | SAO | - | SAMN11038410 |
| *Lutjanus purpureus* | RB1498 | SAO | - | SAMN11038411 |
| *Lutjanus purpureus* | RB1499 | SAO | - | SAMN11038412 |
| *Lutjanus purpureus* | RB1502 | SAO | - | SAMN11038413 |
| *Lutjanus purpureus* | RB1503 | SAO | - | SAMN11038414 |
| *Lutjanus purpureus* | RB1504 | SAO | - | SAMN11038415 |
| *Lutjanus purpureus* | RB1505 | SAO | - | SAMN11038416 |
| *Lutjanus purpureus* | RB1507 | SAO | - | SAMN11038417 |
| *Lutjanus purpureus* | RB1510 | SAO | - | SAMN11038418 |
| *Lutjanus purpureus* | RB1514 | SAO | - | SAMN11038419 |
| *Lutjanus purpureus* | RB1515 | SAO | - | SAMN11038420 |
| *Lutjanus purpureus* | RB1516 | SAO | - | SAMN11038421 |
| *Lutjanus purpureus* | RB1520 | SAL | - | SAMN11038422 |
| *Lutjanus purpureus* | RB1522 | SAL | - | SAMN11038423 |
| *Lutjanus purpureus* | RB1523 | SAL | - | SAMN11038424 |
| *Lutjanus purpureus* | RB1524 | SAL | - | SAMN11038425 |
| *Lutjanus purpureus* | RB1525 | SAL | - | SAMN11038426 |
| *Lutjanus purpureus* | RB1526 | SAL | - | SAMN11038427 |
| *Lutjanus purpureus* | RB1527 | SAL | - | SAMN11038428 |
| *Lutjanus purpureus* | RB1528 | SAL | - | SAMN11038429 |
| *Lutjanus purpureus* | RB1531 | SAL | - | SAMN11038430 |
| *Lutjanus purpureus* | RB1532 | SAL | - | SAMN11038431 |
| *Lutjanus purpureus* | RB1533 | SAL | - | SAMN11038432 |
| *Lutjanus purpureus* | RB1534 | SAL | - | SAMN11038433 |
| *Lutjanus purpureus* | UMSNH16756 | NUE | MK534325 | SAMN11038466 |
| *Lutjanus purpureus* | UMSNH16996 | NUE | MK534324 | SAMN11038467 |
| *Lutjanus purpureus* | UMSNH16997 | NUE | MK534326 | SAMN11038468 |
| *Lutjanus purpureus* | VEN001 | VEN | - | SAMN11038471 |
| *Lutjanus purpureus* | VEN002 | VEN | - | SAMN11038472 |
| *Lutjanus purpureus* | VEN003 | VEN | - | SAMN11038473 |
| *Lutjanus purpureus* | VEN004 | VEN | - | SAMN11038474 |
| *Lutjanus purpureus* | VEN005 | VEN | - | SAMN11038475 |
| *Lutjanus purpureus* | VEN006 | VEN | - | SAMN11038476 |

**Table S3. Accession numbers for sequences mined from GenBank (NCBI).**

|  |  |  |  |
| --- | --- | --- | --- |
| Organism | Region/Location information | Genetic marker | GenBank accession no. |
| *Lutjanus campechanus* | Gulf of Mexico and Atlantic coast of Florida | D-loop | AF356750-AF356776 |
| *Lutjanus campechanus* | US coast | COI | EU752115 |
| *Lutjanus campechanus* | Florida | COI | FJ998466 |
| *Lutjanus campechanus* | Texas | COI | HQ162371-HQ162373 |
| *Lutjanus campechanus* | NA | COI | JN021303 |
| *Lutjanus campechanus* | Alabama | COI | KF461194-KF461195 |
| *Lutjanus campechanus* | NA | COI | KX119461-KX119464 |
| *Lutjanus campechanus* | NA | COI | KX119465 |
| *Lutjanus campechanus* | Gulf of Mexico | COI | MF041054 |
| *Lutjanus campechanus* | Gulf of Mexico | COI | MF041257 |
| *Lutjanus campechanus* | Gulf of Mexico | COI | MF041562 |
| *Lutjanus campechanus* | Gulf of Mexico | COI | MG856504 |
| *Lutjanus campechanus* | Gulf of Mexico | COI | MF041450 |
| *Lutjanus purpureus* | Brazilian coast | D-loop | KC122929-KC123167 |
| *Lutjanus purpureus* | NA | COI | EU752118 |
| *Lutjanus purpureus* | Brazilian coast | COI | KJ907265 |
| *Lutjanus purpureus* | Brazilian coast | COI | KU313736-KU313755 |

**Table S4. Selected output datasets based on alternative population parameters.** Missing percentage of SNPs ranged between 9 and 46 %, where the inclusion of more individuals was the driving factor affecting this value.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Dataset | Number of Individuals | Min. sites | Number of Populations | Number of loci | SNPs | Missing percentage of SNPs |
| **p12r50** | 178 | 0 | 15 | 21431 | 15112 | 0.43 |
| **\*155** | **0.05** | **15** | **21431** | **15112** | **0.35** |
| **122** | **0.25** | **14** | **21431** | **15112** | **0.2** |
| 108 | 0.5 | 13 | 21431 | 15112 | 0.15 |
| **89** | **0.75** | **12** | **21431** | **15112** | **0.1** |
| p11r50 | 178 | 0 | 15 | 40210 | 29798 | 0.48 |
| 149 | 0.05 | 15 | 40210 | 29798 | 0.38 |
| 115 | 0.25 | 13 | 40210 | 29798 | 0.23 |
| 97 | 0.5 | 12 | 40210 | 29798 | 0.16 |
| 73 | 0.75 | 10 | 40210 | 29798 | 0.1 |
| p9r60 | 178 | 0 | 15 | 30138 | 21850 | 0.56 |
| 136 | 0.05 | 14 | 30138 | 21850 | 0.43 |
| 97 | 0.25 | 11 | 30138 | 21850 | 0.25 |
| 76 | 0.5 | 9 | 30138 | 21850 | 0.13 |
| 67 | 0.75 | 9 | 30138 | 21850 | 0.1 |
| **p8r60** | 178 | 0 | 15 | 55795 | 42406 | 0.61 |
| **125** | **0.05** | **13** | **55795** | **42406** | **0.46** |
| **81** | **0.25** | **9** | **55795** | **42406** | **0.23** |
| 73 | 0.5 | 9 | 55795 | 42406 | 0.1 |
| **44** | **0.75** | **8** | **55795** | **42406** | **0.09** |
| *p* denotes the minimum number of populations where each locus must be present in order to be selected; *r* denotes the minimum number of individuals within a population where a locus must be present to be selected. \* final selected matrixes used in downstream analyses. | | | | | | |

**Table S5. Results of Bayes factor delimitation (BFD\*) analyses for WA red snappers using three SNP datasets.** BF values were estimated with three subsets assembled from 12 populations (filtered from the p12r50 dataset). Positive BF values (calculated as 2 x (MLE of base model - MLE of alternative model)) indicate in all cases overwhelming support in favor of the traditional, two-species delimitation (base) model (BF scale: 0 < BF < 2, non-significant; 2 < BF < 6, positive evidence; 6 < BF < 10, strong support; BF > 10, decisive support; [6]. MLE: marginal likelihood estimates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | Individuals | SNPs | MLE – 2 species (base – traditional taxonomy) | MLE – 1 species (alternative – mtDNA) | BF |
| Subset 1 | 58 | 149 | -6,537.45 | -7,692.59 | 2,310.28 |
| Subset 2 | 58 | 938 | -40,948.80 | -47,287.26 | 12,676.92 |
| Subset 3 | 108 | 957 | -67285.99 | -78,464.10 | 22,356.22 |

**Table S6. List of character transformations (SNPs) that differentiate populations of *L. campechanus* from *L. purpureus***. Alignment site indicates the position of the diagnostic SNP in the concatenated nexus file (available from Dryad). Although there are no unambiguous SNPs differentiating all individuals of *L. campechanus* from *L. purpureus*, a combination of SNP sites can be used for diagnostic purposes; for instance, by designing PCR primers from the flanking sites in the nexus file provided.

|  |  |
| --- | --- |
|  |  |
| **Alignment site** | **Character state transformation** |
| 1059 | C --> T |
| 41919 | G --> A |
| 45777 | C --> T |
| 53139 | C --> A |
| 68641 | C --> T |
| 88992 | G --> A |
| 207540 | A --> G |
| 214748 | T --> C |
| 239812 | C --> T |
| 248229 | G --> A |
| 257027 | G --> A |
| 270080 | C --> T |
| 270830 | G --> A |
| 288972 | G --> A |
| 328042 | G --> A |
| 459746 | C --> T |
| 510028 | G --> T |
| 521086 | C --> T |
| 532482 | C --> T |
| 629127 | G --> A |
| 644504 | A --> G |
| 648340 | T --> G |
| 668691 | T --> C |
| 681224 | T --> C |
| 690051 | G --> A |
| 769005 | A --> C |
| 833330 | C --> T |
| 836965 | A --> G |
| 850906 | A --> C |
| 886936 | G --> C |
| 975574 | A --> C |
| 987321 | T --> C |
| 1041916 | A --> G |
| 1144840 | T --> C |
| 1192851 | C --> T |
| 1198134 | T --> G |
| 1202462 | C --> T |
| 1228440 | G --> A |
| 1248434 | G --> A |
| 1274734 | C --> T |
| 1278851 | A --> G |
| 1283521 | C --> G |
| 1317037 | G --> A |
| 1358528 | G --> C |
| 1379595 | G --> T |
| 1387126 | C --> A |
| 1510047 | G --> T |
| 1534354 | T --> C |
| 1600837 | A --> C |
| 1638177 | T --> G |
| 1697315 | A --> G |
| 1772989 | C --> T |
| 1805758 | T --> C |

**Supplementary References**

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