

**Table S1.** Invasive red lionfish sample sizes and genetic variation by location. Lionfish samples are assembled by the 22 locations, ordered geographically (north to south) with the sighted study noted in superscript. For reference, sample sizes are shown for the nuclear microsatellite (nDNA) dataset (see Supplemental Table 1 for details). The mitochondrial (mtDNA) d-loop diversity parameters and test of neutrality are N, the number of sequences; S, number of polymorphic sites;  $\pi$ , nucleotide diversity; k, average number of nucleotide differences; *D*, Tajima’s *D*; *H*, number of haplotypes; and *h*, haplotype diversity. Haplotypes identified in this study are also presented with the number of lionfish samples for each haplotype displayed. The mitochondrial data include both the current study and previously published haplotypes (N = 1,795). Locations in alphabetical order by region: The Bahamas (BAH); Bermuda (BER); North Carolina (NC); Barbados (BAR); Belize (BEL); Bonaire (BON); Grand Cayman (CAY); Cuba (CUB); Honduras (HON); Jamaica (JAM); Jacksonville, Florida (JAX); Florida Keys (KEY); northwest Florida (NWF); Panamá (PAN); Puerto Rico (PR); San Andres Island (SAI); Santa Marta (SAM); southeast Florida (SEF); Saint Petersburg, Florida (STP); Texas (TEX); Trinidad and Tobago (TO); Virgin Islands (VI).

| Local        | <sup>a</sup> Present study |            | <sup>c</sup> Previous studies        |                                  |                                      |   |                         |                                     |                                     | Compiled mtDNA dataset |
|--------------|----------------------------|------------|--------------------------------------|----------------------------------|--------------------------------------|---|-------------------------|-------------------------------------|-------------------------------------|------------------------|
|              | nDNA                       | mtDNA      | <sup>b</sup> Whitaker & Janosik 2020 | <sup>d</sup> Johnson et al. 2016 | <sup>e</sup> Butterfield et al. 2015 | <sup>f</sup> Toledo-Hernández et al. 2014 | <sup>g</sup> Sealy 2013 | <sup>h</sup> Betancur-R et al. 2011 | <sup>i</sup> Freshwater et al. 2009 |                        |
| BAH          | 40 <sup>b,c</sup>          | 6          | 21                                   | -                                | 23                                   | -   | -                       | -                                   | 127                                 | 177                    |
| BAR          | -                          | -          | -                                    | -                                | -                                    | -   | 178                     | -                                   | -                                   | 178                    |
| BEL          | 57 <sup>c</sup>            | -          | -                                    | -                                | 59                                   | -   | -                       | -                                   | -                                   | 59                     |
| BER          | -                          | -          | -                                    | -                                | -                                    | -   | -                       | 45                                  | -                                   | 45                     |
| BON          | 21 <sup>e</sup>            | -          | -                                    | -                                | 21                                   | -   | -                       | -                                   | -                                   | 21                     |
| CAY          | -                          | -          | -                                    | -                                | -                                    | -   | -                       | 79                                  | -                                   | 79                     |
| CUB          | 37 <sup>e</sup>            | -          | -                                    | -                                | 24                                   | -   | -                       | -                                   | -                                   | 24                     |
| HON          | -                          | -          | -                                    | -                                | 15                                   | -   | -                       | -                                   | -                                   | 15                     |
| JAM          | 36 <sup>e</sup>            | -          | -                                    | -                                | 37                                   | -   | -                       | -                                   | -                                   | 37                     |
| JAX          | 24 <sup>b</sup>            | -          | 24                                   | -                                | -                                    | -   | -                       | -                                   | -                                   | 24                     |
| KEY          | 43 <sup>a</sup>            | 119        | -                                    | -                                | -                                    | -   | -                       | -                                   | -                                   | 119                    |
| NC           | 17 <sup>c</sup>            | 2*         | -                                    | -                                | -                                    | -   | -                       | -                                   | 269                                 | 271                    |
| NWF          | 6 <sup>a</sup>             | 29         | -                                    | 82                               | -                                    | -   | -                       | -                                   | -                                   | 111                    |
| PAN          | 16 <sup>a</sup>            | 22         | -                                    | 47                               | 5                                    | -   | -                       | -                                   | -                                   | 74                     |
| PR           | 24 <sup>e</sup>            | -          | -                                    | -                                | 20                                   | 118                                       | -                       | -                                   | -                                   | 138                    |
| SAI          | -                          | -          | -                                    | -                                | -                                    | -   | -                       | 47                                  | -                                   | 47                     |
| SAM          | -                          | -          | -                                    | -                                | -                                    | -   | -                       | 166                                 | -                                   | 166                    |
| SEF          | 49 <sup>a</sup>            | 59         | -                                    | -                                | -                                    | -   | -                       | -                                   | -                                   | 59                     |
| STP          | 18 <sup>b</sup>            | -          | 20                                   | 61                               | -                                    | -   | -                       | -                                   | -                                   | 81                     |
| TEX          | -                          | -          | -                                    | 45                               | -                                    | -   | -                       | -                                   | -                                   | 45                     |
| TO           | 1 <sup>b</sup>             | -          | 15                                   | -                                | -                                    | -   | -                       | -                                   | -                                   | 15                     |
| VI           | 5 <sup>e</sup>             | -          | -                                    | -                                | 10                                   | -   | -                       | -                                   | -                                   | 10                     |
| <b>Total</b> | <b>394</b>                 | <b>237</b> | <b>80</b>                            | <b>235</b>                       | <b>214</b>                           | <b>118</b>                                | <b>178</b>              | <b>337</b>                          | <b>396</b>                          | <b>1795</b>            |

<sup>a</sup>current study; <sup>b</sup>Freshwater et al. 2009; <sup>c</sup>Betancur-R et al. 2011; <sup>d</sup>Sealy et al. 2013; <sup>e</sup>Toledo-Hernández 2014; <sup>f</sup>Butterfield et al. 2015; <sup>g</sup>Johnson et al. 2016; <sup>h</sup>Bors et al. 2019; <sup>i</sup>Whitaker and Janosik 2020; \*Unpublished sequences from Freshwater.

**Table S2.** Invasive red lionfish mitochondrial genetic variation by colonization location and collection year. The invasive red lionfish D-loop sequence at 22 locations, ordered geographically (north to south). Haplotypes identified in this study are also presented with the number of lionfish samples for each haplotype displayed. The mitochondrial data include both the current study and previously published haplotypes (N = 1,795). Locations in general alphabetical order by region: The Bahamas (BAH); Bermuda (BER); North Carolina (NC); Barbados (BAR); Belize (BEL); Bonaire (BON); Grand Cayman (CAY); Cuba (CUB); Honduras (HON); Jamaica (JAM); Jacksonville, Florida (JAX); Florida Keys (KEY); northwest Florida (NWF); Panamá (PAN); Puerto Rico (PR); San Andres Island (SAI); Santa Marta (SAM); southeast Florida (SEF); Saint Petersburg, Florida (STP); Texas (TEX); Trinidad and Tobago (TO); Virgin Islands (VI)

| Location        | Collection Year | Haplotype number |      |    |     |    |    |    |   |   |    |    |    | Grand Total |
|-----------------|-----------------|------------------|------|----|-----|----|----|----|---|---|----|----|----|-------------|
|                 |                 | 1                | 2    | 3  | 4   | 5  | 6  | 7  | 8 | 9 | 15 | 22 | 46 |             |
| Northern Region |                 |                  |      |    |     |    |    |    |   |   |    |    |    |             |
| BAH             | 2012            | 13               | 9    | 1  |     |    |    |    |   |   |    |    |    | 23          |
|                 | 2013            | 4                | 2    |    |     |    |    |    |   |   |    |    |    | 6           |
|                 | 2016            | 6                | 13   |    | 2   |    |    |    |   |   |    |    |    | 21          |
|                 | No year data    | 61               | 44   | 7  | 4   | 5  | 1  | 4  | 1 |   |    |    |    | 127         |
| BER             | 2008            | 18               | 21   | 1  |     |    | 4  | 1  |   |   |    |    |    | 45          |
| NC              | 2007            |                  | 1    |    |     |    |    |    |   |   |    |    | 1  | 2           |
|                 | 2008            |                  | 1    |    |     |    |    |    |   |   | 1  |    |    | 2           |
|                 | No year data    | 102              | 98   | 15 | 16  | 9  | 14 | 10 |   | 1 | 1  | 1  |    | 267         |
| Southern Region |                 |                  |      |    |     |    |    |    |   |   |    |    |    |             |
| BAR             | No year data    | 54               | 117  | 1  | 4   | 1  |    | 1  |   |   |    |    |    | 178         |
| BEL             | 2009            | 17               | 39   |    | 3   |    |    |    |   |   |    |    |    | 59          |
| BON             | 2009            | 4                | 17   |    |     |    |    |    |   |   |    |    |    | 21          |
| CAY             | 2008            | 21               | 56   | 1  | 1   |    |    |    |   |   |    |    |    | 79          |
| CUB             | 2013            | 5                | 18   |    | 1   |    |    |    |   |   |    |    |    | 24          |
| HON             | 2013            | 5                | 8    |    | 2   |    |    |    |   |   |    |    |    | 15          |
| JAM             | 2011            | 7                | 28   |    | 2   |    |    |    |   |   |    |    |    | 37          |
| JAX             | 2016            | 9                | 15   |    |     |    |    |    |   |   |    |    |    | 24          |
| KEY             | 2010            | 5                | 29   |    | 3   |    |    |    |   |   |    |    |    | 37          |
|                 | 2011            | 23               | 52   |    | 7   |    |    |    |   |   |    |    |    | 82          |
| NWF             | 2014            | 1                | 12   |    | 1   |    |    |    |   |   |    |    |    | 14          |
|                 | 2015            | 5                | 9    |    | 1   |    |    |    |   |   |    |    |    | 15          |
|                 | No year data    | 27               | 44   |    | 11  |    |    |    |   |   |    |    |    | 82          |
| PAN             | 2013            |                  | 4    | 1  |     |    |    |    |   |   |    |    |    | 5           |
|                 | 2016            | 10               | 11   |    | 1   |    |    |    |   |   |    |    |    | 22          |
|                 | No year data    | 16               | 26   |    | 5   |    |    |    |   |   |    |    |    | 47          |
| PR              | 2011            | 34               | 81   | 1  | 2   |    |    |    |   |   |    |    |    | 118         |
|                 | 2012            | 5                | 14   |    | 1   |    |    |    |   |   |    |    |    | 20          |
| SA              | 2009            | 18               | 26   |    | 3   |    |    |    |   |   |    |    |    | 47          |
| SEF             | 2013            | 2                | 3    |    |     |    |    |    |   |   |    |    |    | 5           |
|                 | 2014            | 10               | 39   | 2  | 3   |    |    |    |   |   |    |    |    | 54          |
| SM              | 2009            | 42               | 106  |    | 18  |    |    |    |   |   |    |    |    | 166         |
| STP             | 2016            | 4                | 13   |    | 3   |    |    |    |   |   |    |    |    | 20          |
|                 | No year data    | 10               | 44   |    | 7   |    |    |    |   |   |    |    |    | 61          |
| TEX             | No year data    | 15               | 25   |    | 5   |    |    |    |   |   |    |    |    | 45          |
| TO              | 2014            | 6                | 8    | 1  |     |    |    |    |   |   |    |    |    | 15          |
| VI              | 2009            | 1                | 8    |    | 1   |    |    |    |   |   |    |    |    | 10          |
| Grand Total     |                 | 560              | 1041 | 31 | 107 | 15 | 19 | 16 | 1 | 1 | 2  | 1  | 1  | 1795        |

**Table S3.** Geographic distance and break matrices for invasive red lionfish collection locations. Geographic distances in kilometers measured in Google Earth for the different locations studied. The bottom diagonal is a geographic matrix illustrating breaks in connectivity while the upper diagonal is a geographic matrix illustrating high connectivity among all locations. Locations are organized alphabetically by region and abbreviations are as follows: The Bahamas (BAH); Bermuda (BER); North Carolina (NC); Barbados (BAR); Belize (BEL); Bonaire (BON); Grand Cayman (CAY); Cuba (CUB); Honduras (HON); Jamaica (JAM); Jacksonville, Florida (JAX); Florida Keys (KEY); Northwest Florida (NWF); Panamá (PAN); Puerto Rico (PR); San Andres Island (SAI); Santa Marta (SAM); Southeast Florida (SEF); Saint Petersburg, Florida (STP); Texas (TEX); Trinidad and Tobago (TO); Virgin Islands (VI). The bold borderlines separate the Northern Region locations from the Southern Region.

|     | BAH  | BER  | NC   | BAR  | BEL  | BON  | CAY  | CUB  | HON  | JAM  | JAX  | KEY  | NWF  | PAN  | PR   | SAM  | SAI  | SEF  | STP  | TEX  | TO   | VI   |
|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| BAH |      | 1542 | 2762 | 2247 | 1450 | 2925 | 1525 | 178  | 1453 | 1024 | 3266 | 480  | 1040 | 2184 | 1531 | 1898 | 1598 | 3670 | 692  | 1957 | 2725 | 1832 |
| BER | 1527 |      | 1220 | 2407 | 3233 | 2225 | 2529 | 1747 | 3087 | 2049 | 1724 | 1947 | 2528 | 3216 | 1470 | 3004 | 2801 | 2118 | 2173 | 3490 | 2525 | 1776 |
| NC  | 1054 | 1212 |      | 3658 | 2246 | 4006 | 2775 | 1229 | 2471 | 2186 | 504  | 1251 | 1859 | 3342 | 2602 | 3111 | 2811 | 892  | 1492 | 2890 | 3814 | 2931 |
| BAR | 2272 | 3814 | 5034 |      | 3128 | 1032 | 2419 | 2264 | 2937 | 1939 | 5538 | 2754 | 3453 | 2918 | 866  | 2688 | 2572 | 6179 | 3100 | 4237 | 350  | 602  |
| BEL | 2339 | 3881 | 5101 | 3440 |      | 4481 | 809  | 2235 | 204  | 1288 | 5605 | 1020 | 1725 | 2424 | 2589 | 2207 | 2004 | 6008 | 1366 | 1779 | 3760 | 2838 |
| BON | 3334 | 4876 | 6096 | 1041 | 2226 |      | 1692 | 3311 | 2062 | 1203 | 6600 | 3053 | 3761 | 1569 | 1905 | 1325 | 1025 | 6287 | 3399 | 3486 | 721  | 1643 |
| CAY | 1481 | 3023 | 4243 | 2587 | 680  | 3628 |      | 1417 | 596  | 453  | 4747 | 900  | 1386 | 1576 | 1703 | 1373 | 1184 | 5172 | 1181 | 1826 | 2907 | 1985 |
| CUB | 188  | 1730 | 2950 | 2290 | 1363 | 2714 | 1500 |      | 977  | 933  | 3454 | 495  | 1098 | 2091 | 1355 | 1195 | 895  | 3884 | 748  | 1889 | 2566 | 1641 |
| HON | 2115 | 3657 | 4877 | 3207 | 207  | 4248 | 581  | 2000 |      | 1055 | 5381 | 1055 | 1547 | 2199 | 2352 | 2000 | 1801 | 5777 | 1363 | 1956 | 3527 | 2605 |
| JAM | 1024 | 2566 | 3786 | 2151 | 1140 | 3192 | 475  | 940  | 998  |      | 4290 | 1376 | 2077 | 1127 | 1226 | 931  | 631  | 4684 | 1722 | 2233 | 2471 | 1549 |
| JAX | 710  | 2009 | 518  | 3167 | 1186 | 3725 | 2277 | 907  | 1978 | 1874 |      | 910  | 1476 | 3016 | 2269 | 2629 | 2329 | 348  | 1107 | 2391 | 3436 | 2572 |
| KEY | 3323 | 4865 | 6085 | 4426 | 967  | 5467 | 1794 | 3230 | 1178 | 2277 | 6589 |      | 693  | 3413 | 3574 | 3194 | 2982 | 6978 | 339  | 1432 | 4746 | 3824 |
| NWF | 4030 | 5572 | 6792 | 5135 | 1658 | 6176 | 2500 | 3924 | 1886 | 2983 | 7296 | 706  |      | 4103 | 4283 | 3890 | 3688 | 7689 | 348  | 1121 | 5455 | 4533 |
| PAN | 2160 | 3702 | 4922 | 3307 | 1287 | 4348 | 1148 | 2114 | 1143 | 1142 | 5426 | 2278 | 2983 |      | 2392 | 194  | 506  | 5840 | 2636 | 2788 | 3627 | 2705 |
| PR  | 1334 | 2876 | 4096 | 951  | 2388 | 1418 | 1760 | 1335 | 2052 | 1237 | 4600 | 1854 | 2496 | 2388 |      | 1653 | 1353 | 5252 | 2139 | 3489 | 1214 | 301  |
| SAM | 1962 | 3504 | 4724 | 3082 | 1153 | 4123 | 930  | 1888 | 946  | 952  | 5228 | 1753 | 2468 | 199  | 2151 |      | 300  | 5625 | 2135 | 2625 | 3402 | 2480 |
| SAI | 1772 | 3314 | 4534 | 2884 | 882  | 3925 | 678  | 1699 | 697  | 735  | 5038 | 1897 | 2402 | 1869 | 1989 | 1656 |      | 5437 | 2196 | 2299 | 3204 | 2282 |
| SEF | 331  | 1877 | 775  | 2788 | 1535 | 3290 | 1876 | 519  | 1585 | 1458 | 383  | 563  | 1130 | 2608 | 1804 | 2215 | 1915 |      | 780  | 1987 | 2924 | 2113 |
| STP | 3686 | 5228 | 6448 | 4794 | 1317 | 5835 | 2152 | 3582 | 1544 | 2635 | 6952 | 359  | 349  | 3756 | 3933 | 3547 | 3342 | 7347 |      | 1360 | 5114 | 4192 |
| TEX | 5190 | 6732 | 7952 | 6264 | 2808 | 7305 | 3637 | 5071 | 3029 | 4127 | 8456 | 1851 | 1129 | 5227 | 5429 | 5021 | 4834 | 8853 | 1489 |      | 6584 | 5662 |
| TO  | 2617 | 4159 | 5379 | 320  | 2943 | 717  | 2453 | 2607 | 2729 | 1920 | 5883 | 3770 | 4478 | 2285 | 1193 | 2034 | 1734 | 6518 | 4116 | 4163 |      | 922  |
| VI  | 1651 | 3193 | 4413 | 571  | 2734 | 1053 | 2044 | 1643 | 2444 | 1528 | 4917 | 2137 | 2742 | 2695 | 264  | 1909 | 1609 | 5547 | 2384 | 3695 | 923  |      |

**Table S4.** Invasive red lionfish microsatellite multiplexes. The 17 invasive lionfish microsatellites (Schultz et al. 2013) were incorporated into six multiplexes to increase laboratory and cost efficiency. For each locus, annealing temperatures are reported with respective dyes and repeat motifs. The size range of alleles found for each locus are also reported. Due to poor amplification or chromatograms, loci PVM12, PVM38, and PVM41 were dropped resulting in 14 successfully genotyped loci.

| Multiplex | Primer | T <sub>m</sub><br>(°C) | Dye | Repeat Motif | Range (bp) |
|-----------|--------|------------------------|-----|--------------|------------|
| MP1       | PVM11  | 58                     | NED | tetra        | 192-204    |
| MP1       | PVM14  | 58                     | FAM | tetra        | 245-281    |
| MP1       | PVM37  | 58                     | HEX | tri          | 222-231    |
| MP2       | PVM07  | 58                     | FAM | tetra        | 260-288    |
| MP2       | PVM15  | 58                     | NED | tetra        | 201-213    |
| MP3       | PVM12  | 60                     | NED | tetra        | 191-251    |
| MP3       | PVM32  | 60                     | FAM | tri          | 182-185    |
| MP3       | PVM46  | 60                     | HEX | penta        | 220-240    |
| MP4a      | PVM10  | 60                     | HEX | tetra        | 219-235    |
| MP4a      | PVM27  | 60                     | FAM | tri          | 238-253    |
| MP4b      | PVM42  | 62                     | NED | tri          | 214-217    |
| MP5       | PVM18  | 60                     | HEX | tetra        | 209-225    |
| MP5       | PVM31  | 60                     | FAM | tri          | 156-186    |
| MP5       | PVM41  | 60                     | FAM | tri          | 228-240    |
| MP6a      | PVM17  | 62                     | HEX | tetra        | 216-244    |
| MP6a      | PVM21  | 62                     | NED | tetra        | 175-243    |
| MP6b      | PVM38  | 63                     | FAM | tri          | 278-287    |

**Table S5.** Invasive lionfish microsatellite genetic variation by locus. Values are reported by the average number of individual genotypes,  $N$ ; average number of alleles,  $N_A$ ; average effective number of alleles,  $E_A$ ; observed,  $H_O$  and expected heterozygosity,  $H_E$ ; and the inbreeding coefficient,  $F_{IS}$ . Parameters were averaged over all loci and all locations (global) and the standard errors were reported (SE). Microsatellite multiplex information can be found on Supplemental Table 3.

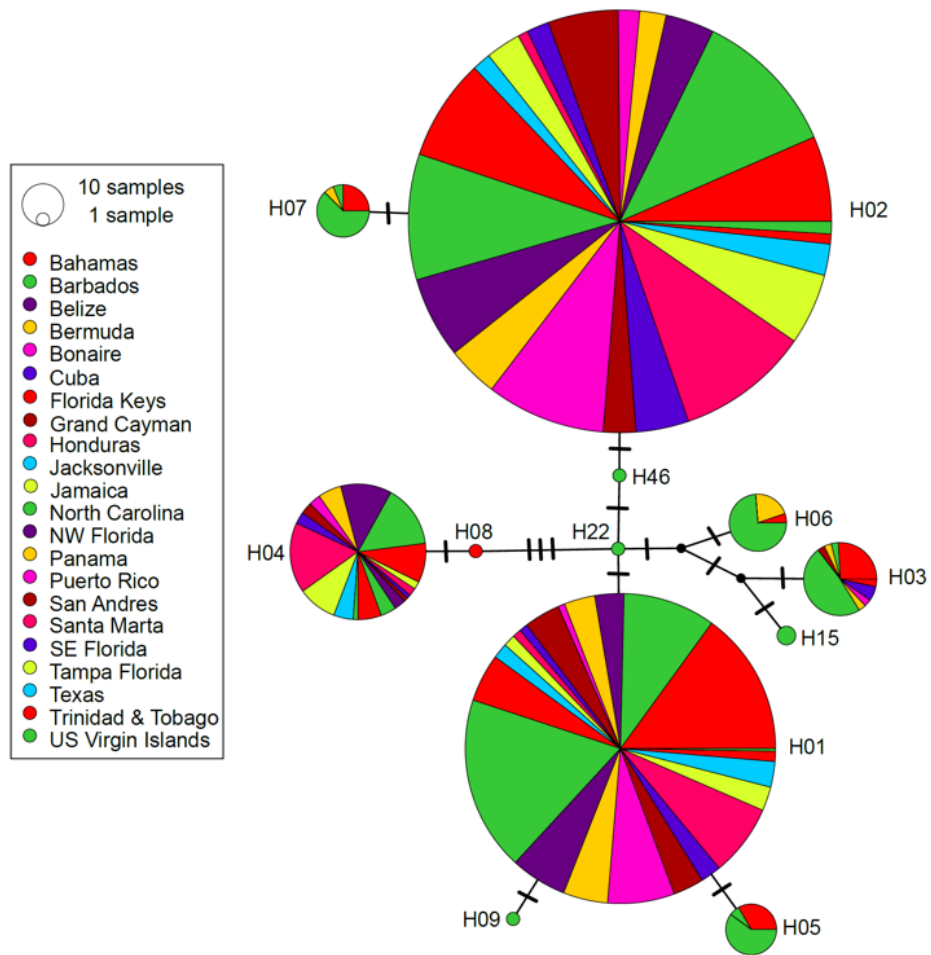
| Locus  | $N$   | $N_A$ | $E_A$ | $H_O$ | $H_E$ | $F_{IS}$ | Multiplex |
|--------|-------|-------|-------|-------|-------|----------|-----------|
| PVM7   | 27.21 | 5.71  | 4.43  | 0.78  | 0.76  | -0.02    | MP2       |
| PVM10  | 28    | 4.43  | 2.7   | 0.64  | 0.62  | -0.04    | MP4a      |
| PVM11  | 28    | 3     | 2.23  | 0.57  | 0.55  | -0.03    | MP1       |
| PVM14  | 25.86 | 6.43  | 5.03  | 0.8   | 0.8   | -0.01    | MP1       |
| PVM15  | 27.14 | 3.14  | 2.66  | 0.65  | 0.61  | -0.07    | MP2       |
| PVM17  | 26.43 | 3.79  | 1.91  | 0.44  | 0.44  | 0.01     | MP6a      |
| PVM18  | 21.93 | 1.93  | 1.83  | 0.46  | 0.43  | -0.06    | MP5       |
| PVM21  | 24.86 | 4.57  | 2.24  | 0.53  | 0.52  | -0.02    | MP6a      |
| PVM27  | 26.57 | 3.79  | 2.38  | 0.62  | 0.57  | -0.08    | MP4a      |
| PVM31  | 27.57 | 4.43  | 3.31  | 0.68  | 0.69  | 0.01     | MP5       |
| PVM32  | 28.07 | 2     | 1.26  | 0.22  | 0.2   | -0.09    | MP3       |
| PVM37  | 27.5  | 3     | 2.15  | 0.49  | 0.53  | 0.07     | MP1       |
| PVM42  | 27.71 | 2.64  | 1.9   | 0.49  | 0.45  | -0.08    | MP4b      |
| PVM46  | 27.93 | 3.86  | 2.24  | 0.55  | 0.54  | -0.01    | MP3       |
| Global | 26.77 | 3.77  | 2.59  | 0.57  | 0.55  | -0.03    |           |
| SE     | 1.06  | 0.1   | 0.08  | 0.01  | 0.01  | 0.01     |           |

**Table S6.** Invasive red lionfish microsatellite genetic diversity values. The averaged genetic diversity for each location was reported for the number of individuals genotypes (N), average number of alleles ( $N_A$ ), average effective number of alleles ( $E_A$ ), observed ( $H_O$ ) and expected heterozygosity ( $H_E$ ), and the inbreeding coefficient ( $F_{IS}$ ). Parameters were averaged over all loci and all locations (Global) and the standard errors were reported (SE). Locations are in alphabetical order and abbreviated as follows: The Bahamas (BAH); Belize (BEL); Bonaire (BON); Cuba (CUB); Jamaica (JAM); Jacksonville, Florida (JAX); Florida Keys (KEY); North Carolina (NC); northwest Florida (NWF); Panamá (PAN); Puerto Rico (PR); southeast Florida (SEF); Saint Petersburg, Florida (STP); Virgin Islands (VI). \*The single lionfish from Trinidad and Tobago was not included in these calculations.

| Location | N    | $N_A$ | $E_A$ | $H_O$ | $H_E$ | $F_{IS}$ |
|----------|------|-------|-------|-------|-------|----------|
| BAH      | 40   | 4.29  | 2.74  | 0.57  | 0.57  | 0.00     |
| BEL      | 57   | 4.07  | 2.70  | 0.60  | 0.58  | -0.05    |
| BON      | 21   | 3.71  | 2.56  | 0.58  | 0.56  | -0.03    |
| CUB      | 37   | 4.00  | 2.65  | 0.58  | 0.57  | -0.01    |
| JAM      | 36   | 3.86  | 2.61  | 0.58  | 0.57  | -0.02    |
| JAX      | 24   | 3.86  | 2.59  | 0.61  | 0.56  | -0.10    |
| KEY      | 43   | 3.93  | 2.73  | 0.59  | 0.57  | -0.03    |
| NC       | 17   | 3.93  | 2.64  | 0.54  | 0.57  | 0.06     |
| NWF      | 6    | 3.14  | 2.37  | 0.53  | 0.50  | -0.04    |
| PAN      | 16   | 3.79  | 2.60  | 0.56  | 0.56  | 0.00     |
| PR       | 24   | 3.93  | 2.71  | 0.58  | 0.57  | -0.02    |
| SEF      | 49   | 3.86  | 2.71  | 0.57  | 0.57  | 0.01     |
| STP      | 18   | 4.00  | 2.78  | 0.60  | 0.59  | -0.01    |
| VI       | 5    | 2.36  | 1.91  | 0.44  | 0.39  | -0.15    |
| Global   | 393* | 3.77  | 2.59  | 0.57  | 0.55  | -0.03    |
| SE       |      | 0.10  | 0.08  | 0.01  | 0.01  | 0.01     |

**Table S7.** Invasive red lionfish effective population size estimation. The estimated effective population sizes for each location and the year(s) of collection. The number of lionfish sampled (N), estimated effective population size ( $N_E$ ), and 95% CI reported as the lower and upper limits. The Bahamas was the only location with non-consecutive sampling years and therefore is reported as two different estimations for  $N_E$ . Locations are organized alphabetically and abbreviated as follows: The Bahamas (BAH); Belize (BEL); Cuba (CUB); Jamaica (JAM); Jacksonville, Florida (JAX); Florida Keys (KEY); North Carolina (NC); Puerto Rico (PR); southeast Florida (SEF); Saint Petersburg, Florida (STP); Virgin Islands (VI). Note that this analysis was conducted with a subset of samples that were published with the inclusion of specific collection dates (as opposed to date ranges).

| Location | Collection<br>Year(s) | N  | $N_E$ | Lower | Upper    |
|----------|-----------------------|----|-------|-------|----------|
| BAH      | 2015-2016             | 11 | 5.3   | 4.4   | 6.7      |
| BAH      | 2012-2013             | 26 | 77.2  | 42.9  | 273.7    |
| BEL      | 2009                  | 57 | 43.6  | 34.1  | 58.3     |
| CUB      | 2012-2014             | 37 | 76.8  | 47.6  | 170.3    |
| JAM      | 2011                  | 36 | 46.8  | 31.7  | 81.2     |
| JAX      | 2016                  | 24 | 12.4  | 9.9   | 15.8     |
| KEY      | 2010-2011             | 43 | 87.7  | 55.9  | 178.8    |
| NC       | 2008                  | 17 | 12.2  | 9.6   | 16.0     |
| PR       | 2012                  | 24 | 36.7  | 25.0  | 63.3     |
| SEF      | 2013-2014             | 49 | 154.8 | 81.8  | 762.5    |
| STP      | 2016                  | 18 | 9.8   | 8.0   | 12.4     |
| VI       | 2009                  | 5  | 13.1  | 5.7   | $\infty$ |



**Figure S1.** Invasive red lionfish haplotype network created using the TCS software. Black hatch marks refer to the number of nucleotide differences between the respective haplotypes. Refer to Figure 1 for the haplotype frequencies for each location.