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| **Table S6.** Observed genetic diversity at each microsatellite loci across all sampled subpopulations of each darter species in the Pearl River basin. |
| **Species** | **Locus** | ***N*** | ***NA*** | ***AR*** | ***HE*** | ***HO*** |
| *Ammocrypta beanii* | *EosC6* | 20 | 11.22 | 10.39 | 0.838 | 0.779 |
| ***NP*** = 9 | *EosD11* | 28 | 16.78 | 15.19 | 0.918 | 0.916 |
|  | *Esc26b* | 46 | 27.00 | 23.34 | 0.963 | 0.811 |
|  | *Esc132b* | 43 | 26.56 | 22.99 | 0.956 | 0.906 |
|  | *Esd17* | 44 | 22.56 | 19.43 | 0.936 | 0.858 |
|  | MEAN | 36.2 | 20.82 | 18.27 | 0.922 | 0.854 |
|  |  |  |  |  |  |  |
| *Etheostoma swaini* | *Ebl4* | 25 | 14.00 | 12.48 | 0.902 | 0.819 |
| ***NP*** = 8 | *Ebl7* | 43 | 23.63 | 20.12 | 0.949 | 0.861 |
|  | *Eca10* | 18 | 8.13 | 7.32 | 0.754 | 0.668 |
|  | *Eca11* | 48 | 24.13 | 20.36 | 0.949 | 0.920 |
|  | *Eca49* | 47 | 25.13 | 21.14 | 0.953 | 0.870 |
|  | *Eca70* | 41 | 18.63 | 16.06 | 0.916 | 0.869 |
|  | *Eche001* | 5 | 2.50 | 2.39 | 0.362 | 0.351 |
|  | *Esc26b* | 22 | 15.25 | 13.46 | 0.892 | 0.872 |
|  | *Esc132b* | 10 | 2.88 | 2.40 | 0.097 | 0.087 |
|  | *Prex41* | 9 | 2.50 | 2.29 | 0.107 | 0.096 |
|  | MEAN | 26.8 | 13.68 | 11.80 | 0.688 | 0.641 |
|  |  |  |  |  |  |  |
| *Percina nigrofasciata* | *Ebl4* | 33 | 21.00 | 18.57 | 0.946 | 0.721 |
| ***NP*** = 9 | *Eca10* | 28 | 16.11 | 14.33 | 0.914 | 0.890 |
|  | *Eca46* | 27 | 18.89 | 16.67 | 0.935 | 0.935 |
|  | *Eca48* | 19 | 14.56 | 13.30 | 0.915 | 0.909 |
|  | *EosD107* | 28 | 17.78 | 15.79 | 0.928 | 0.916 |
|  | *Esc26b* | 38 | 23.44 | 20.09 | 0.950 | 0.930 |
|  | *Prex41* | 42 | 20.22 | 17.89 | 0.944 | 0.878 |
|  | MEAN | 30.7 | 18.86 | 16.66 | 0.933 | 0.883 |
| *N* is total number of alleles; *NA* is mean number of alleles; *AR* is mean allelic richness; *HE* is expected heterozygosity; *HO* is observed heterozygosity; *NP* is total number of populations sampled |