**Supplemental Data S3.** The National Center for Biotechnology Information’s nucleotide database accession numbers for each mtCOI haplotype, the haplotype code (Species\_haplotype number) and the number of individuals from each stream with each haplotype. The total number of different haplotypes detected for each species is represented in parentheses. FV= *Faxonius validus*; FE = *Faxonius erichsonianus*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Haplotype | Bear Creek Drainage | | | Cahaba River Drainage | |  |
| Accession Number | code | Little Bear | Cedar | Rock | Little Cahaba | Shades | Total |
| *Faxonius validus* (28) |  |  |  |  |  |  |  |
| MN053979 | FV\_Hap1 | 8 | 14 | 0 | 0 | 0 | 22 |
| MN053980 | FV\_Hap2 | 31 | 7 | 0 | 0 | 0 | 38 |
| MN053981 | FV\_Hap3 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053982 | FV\_Hap4 | 0 | 2 | 0 | 0 | 0 | 2 |
| MN053983 | FV\_Hap5 | 0 | 2 | 0 | 0 | 0 | 2 |
| MN053984 | FV\_Hap6 | 12 | 11 | 0 | 0 | 0 | 23 |
| MN053985 | FV\_Hap7 | 0 | 3 | 0 | 0 | 0 | 3 |
| MN053986 | FV\_Hap8 | 1 | 4 | 0 | 0 | 0 | 5 |
| MN053987 | FV\_Hap9 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053988 | FV\_Hap10 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053989 | FV\_Hap11 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053990 | FV\_Hap12 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053991 | FV\_Hap13 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053992 | FV\_Hap14 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053993 | FV\_Hap15 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN053994 | FV\_Hap16 | 1 | 1 | 0 | 0 | 0 | 2 |
| MN053995 | FV\_Hap17 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN053996 | FV\_Hap18 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN053997 | FV\_Hap19 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN053998 | FV\_Hap20 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN053999 | FV\_Hap21 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN054000 | FV\_Hap22 | 0 | 0 | 27 | 0 | 0 | 27 |
| MN054001 | FV\_Hap23 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054002 | FV\_Hap24 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054003 | FV\_Hap25 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054004 | FV\_Hap26 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054005 | FV\_Hap27 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054006 | FV\_Hap28 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Faxonius erichsonianus* (42) |  |  |  |  |  |  |  |
| MN054007 | FE\_Hap1 | 0 | 17 | 0 | 0 | 0 | 17 |
| MN054008 | FE\_Hap2 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054009 | FE\_Hap3 | 0 | 2 | 0 | 0 | 0 | 2 |
| MN054010 | FE\_Hap4 | 0 | 11 | 0 | 0 | 0 | 11 |
| MN054011 | FE\_Hap5 | 0 | 3 | 0 | 0 | 0 | 3 |
| MN054012 | FE\_Hap6 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054013 | FE\_Hap7 | 0 | 2 | 0 | 0 | 0 | 2 |
| MN054014 | FE\_Hap8 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054015 | FE\_Hap9 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054016 | FE\_Hap10 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054017 | FE\_Hap11 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054018 | FE\_Hap12 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054019 | FE\_Hap13 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054020 | FE\_Hap14 | 0 | 1 | 0 | 0 | 0 | 1 |
| MN054021 | FE\_Hap15 | 11 | 0 | 0 | 0 | 0 | 11 |
| MN054022 | FE\_Hap16 | 30 | 0 | 0 | 0 | 1 | 31 |
| MN054023 | FE\_Hap17 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN054024 | FE\_Hap18 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN054025 | FE\_Hap19 | 1 | 0 | 0 | 0 | 0 | 1 |
| MN054026 | FE\_Hap20 | 0 | 0 | 0 | 3 | 0 | 3 |
| MN054027 | FE\_Hap21 | 0 | 0 | 0 | 4 | 0 | 4 |
| MN054028 | FE\_Hap22 | 0 | 0 | 0 | 1 | 0 | 1 |
| MN054029 | FE\_Hap23 | 0 | 0 | 0 | 2 | 0 | 2 |
| MN054030 | FE\_Hap24 | 0 | 0 | 0 | 1 | 0 | 1 |
| MN054031 | FE\_Hap25 | 0 | 0 | 0 | 2 | 0 | 2 |
| MN054032 | FE\_Hap26 | 0 | 0 | 0 | 13 | 13 | 25 |
| MN054033 | FE\_Hap27 | 0 | 0 | 0 | 2 | 1 | 3 |
| MN054034 | FE\_Hap28 | 0 | 0 | 0 | 2 | 0 | 2 |
| MN054035 | FE\_Hap29 | 0 | 0 | 0 | 2 | 0 | 2 |
| MN054036 | FE\_Hap30 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054037 | FE\_Hap31 | 0 | 0 | 21 | 0 | 0 | 21 |
| MN054038 | FE\_Hap32 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054039 | FE\_Hap33 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054040 | FE\_Hap34 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054041 | FE\_Hap35 | 0 | 0 | 2 | 0 | 0 | 2 |
| MN054042 | FE\_Hap36 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054043 | FE\_Hap37 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054044 | FE\_Hap38 | 0 | 0 | 1 | 0 | 0 | 1 |
| MN054045 | FE\_Hap39 | 0 | 0 | 0 | 0 | 7 | 7 |
| MN054046 | FE\_Hap40 | 0 | 0 | 0 | 0 | 1 | 1 |
| MN054047 | FE\_Hap41 | 0 | 0 | 0 | 0 | 1 | 1 |
| MN054048 | FE\_Hap42 | 0 | 0 | 0 | 0 | 5 | 5 |