**Table** **S1**. Characteristics of the 24 polymorphic microsatellites in *Habrobracon hebetor*. Allelic diversity and heterozygosity were estimated in 614 specimens from Iran and Niger.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |
| Locus | Size range (bp) | *N*A | *H*0 | *H*E  | *F*IS | *f* null-allele(%GIND) |
| **PCR multiplex** | **set 1** |  |  |  |  |  |
| Pet-Heb2-02 | 106-130 | 13 | 0.7145 | 0.8565 | 0.166 | 0.021 (99.8) |
| Vic-Heb2-13 | 183-193 | 6 | 0.4870 | 0.5945 | 0.181 | 0.025 (100) |
| Fam-Heb2-17 | 235-266 | 21 | 0.7883 | 0.8891 | 0.114 | 0.016 (100) |
| Fam-Heb2-25 | 388-406 | 10 | 0.4631 | 0.6113 | 0.243 | 0.046 (97.2) |
| Vic-Heb3-01 | 119-139 | 8 | 0.5537 | 0.6937 | 0.202 | 0.021 (100) |
| Ned-Heb3-04 | 170-180 | 2 | 0.0185 | 0.1211 **\*** | 0.847 | 0.001 (100) |
| Ned-Heb3-07 | 246-261 | 5 | 0.5024 | 0.6636 | 0.243 | 0.016 (99.8) |
| Pet-Heb3-08 | 292-298 | 3 | 0.2855 | 0.4790 **\*** | 0.404 | 0.068 (93.3) |
| Vic-Heb3-09 | 295-319 | 6 | 0.4581 | 0.5587 | 0.180 | 0.034 (99.2) |
| **PCR multiplex** | **set 2** |  |  |  |  |  |
| Fam-Heb2-04 | 097-115 | 10 | 0.3098 | 0.8753 **\*** | 0.541 | 0.106 (96) |
| Ned-Heb2-06 | 108-170 | 24 | 0.7386 | 0.8410 | 0.122 | 0.012 (99.7) |
| Pet-Heb2-09 | 164-192 | 12 | 0.5945 | 0.6902 | 0.136 | 0.008 (100) |
| Vic-Heb2-16 | 216-234 | 9 | 0.7463 | 0.7965 | 0.063 | 0.014 (99.5) |
| Fam-Heb2-21 | 284-325 | 10 | 0.6248 | 0.6785 | 0.079 | 0.019 (98.6) |
| Fam-Heb3-03 | 175-204 | 7 | 0.4519 | 0.5416 | 0.166 | 0.029 (99.8) |
| Pet-Heb3-06 | 237-252 | 7 | 0.1080 | 0.1819 | 0.406 | 0.002 (99.5) |
| Ned-Heb4-02 | 301-325 | 7 | 0.6007 | 0.7563 | 0.215 | 0.016 (98) |
| **PCR multiplex** | **Set 3** |  |  |  |  |  |
| Fam-Heb2-03 | 103-115 | 7 | 0.6287 | 0.7379 | 0.148 | 0.013 (100) |
| Vic-Heb2-07 | 133-155 | 12 | 0.7259 | 0.8405 | 0.136 | 0.009 (99.8) |
| Ned-Heb2-11 | 183-193 | 6 | 0.6069 | 0.7522 | 0.193 | 0.031 (99.8) |
| Vic-Heb2-14 | 187-209 | 9 | 0.5745 | 0.6804 | 0.156 | 0.039 (99.5) |
| Pet-Heb2-15 | 199-215 | 8 | 0.6360 | 0.6422 | 0.010 | 0.004 (94.3) |
| Fam-Heb3-05 | 178-199 | 8 | 0.5945 | 0.6776 | 0.123 | 0.018 (100) |
| Ned-Heb4-01 | 113-129 | 4 | 0.1743 | 0.2694 | 0.353 | 0.005 (100) |

Loci with significant deviations (*P* < 0.05) from Hardy-Weinberg equilibrium after Bonferroni correction

*N*A, number of observed alleles per locus

*H*0, observed heterozygosity per locus \_ *H*E, expected heterozygosity per locus

*F*IS*,* inbreeding coefficient (Weir & Cockerham, 1984)

*f* null-allele, estimated null allele frequency per locus \_ %GIND, percentages of successfully genotyped individuals

**Tables S2**. Characteristics of the *mtCOI* (**A**) and *16S rDNA* (**B**) haplotypes identified across the *Habrobracon hebetor* sequenced in our study, along with the correspondence with the sub-populations defined using Structure and nuclear markers. Sites with alignment gaps were considered.

A)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Haplotype****641bp-COI** | **Polymorphic sites****(*N* = 44)** | **Sequences****(*N* = 82)** | **Sequence names** | ***K*** |
|  | 000000000011111222222333333333333445555555660112667889045890114452345556688890300124780332910921798416876907579801504124750477860294 |  |  |  |
| **Hap-1****Hap-2****Hap-3****Hap-4****Hap-5****Hap-6****Hap-7****Hap-8****Hap-9****Hap-10****Hap-11****Hap-12** | **AAGTACAGTTTAACTCCACTCTACGTATGCCTGAACCCTTTTAT****.G..........................................****...........T................................****................T...T.......................****...................C........................****............................A...............****....G...........T...........................****....G.......................................****................................A...........****..A.....C...................................****G...............................A...........****T.ACTATACCA.GTCT.GT..CTTACGA.TTAATTTTTCGCCGC** | **49****1****1****1****2****1****5****1****13****4****1****3** | **\*****F-Ng8-7****F-Ng30-13****F-Ng30-8****F-Ng20-1, F-Ng20b-6****F-Ng29-3****%****F-Ng3-10****#****F-Ng4-7, F-Ng13-6, F-Ng15-4, F-Ng15-5****L-Ng9-9****F-Ir1-2,F-Ir1-20,F-Ir1-12** | **\******K*1C*****K*1C*****K*1C*****K*1E*****K*1C*****%******K*1C****#*****K*1C,1B*****K*1A*****K*2** |

The sample code for each sample is as listed in Table 1, (i.e. L for Laboratory, F for field and C for crossing tests).

 **\*** [**Hap-1**]: F-Ng1-24, F-Ng1-4, F-Ng7-5, L-Ng12-10, F-Ng3-23, F-Ng6-3, F-Ng5-18, F-Ng7-13, F-Ng8-15, F-Ng5-2, F-Ng8-16, F-Ng4-13, F-Ng8-3, F-Ng6-11, F-Ng5-5, F-Ng3-2, F-Ng13-1, L-Ng14-12, L-Ng14-24, C-Ng17-1, C-Ng17-13, L-Ng19-8, F-Ng24-6, F-Ng24-9, F-Ng36b-22, F-Ng36-2, F-Ng37-1, F-Ng37b-1, F-Ng34-12, F-Ng34b-1, F-Ng34b-10, F-Ng21-5, F-Ng21-8, F-Ng23-11, F-Ng23-3, F-Ng25-6, F-Ng25-21, F-Ng28-1, F-Ng28-2, F-Ng28-4, F-Ng31-5, F-Ng31-13, F-Ng32-1, F-Ng33-16, F-Ng33-11, F-Ng35-13, F-Ng26-2, F-Ng26-9, F-Ng29-1. **[*K*1A, *K*1B, *K*1C, *K*1D, *K*1E]**

 **%** [**Hap-7**]: F-Ng11-3, F-Ng11-18, F-Ng11-21, F-Ng34-5, F-Ng32-2 **[*K*1A, *K*1G]**

 **#** [**Hap-9**]: L-Ng-HhF1, L-Ng-HhF2, L-Ng-HhM3, L-Ng-HhM4, L-Ng-HhM5, F-Ng1-23, F-Ng1-10, L-Ng9-21, L-Ng9-8, L-Ng9-24, F-Ng4-10, L-Ng12-15, F-Ng35-19. **[*K*1A, *K*1C]**

****

The sample code for each sample is as listed in Table 1, (i.e. L for Laboratory, F for field and C for crossing tests).

\* [**Hap-5**]: F-Ng8-16, F-Ng8-15, L-Ng9-8, F-Ng8-7, F-Ng6-11, F-Ng1-10, F-Ng8-3, L-Ng12-10, F-Ng3-23, L-Ng9-24, F-Ng7-13, L-Ng12-15, L-Ng14-12. **[*K*1A, *K*1B, *K*1C, *K*1E]**

**#** [**Hap-12**]: F-Ng6-3, F-Ng1-4, L-Ng14-24, L-Ng19-8, F-Ng20-1, F-Ng24-6, F-Ng24-9, F-Ng36-2, F-Ng37b-1, F-Ng34-12, F-Ng21-5, F-Ng21-8, F-Ng23-1, F-Ng25-6, F-Ng25-21, F-Ng28-4, F-Ng30-8, F-Ng30-13, F-Ng31-13, F-Ng32-1, F-Ng33-16, F-Ng33-11, F-Ng35-13, F-Ng26-2, F-Ng26-9, F-Ng29-1 **[*K*1B, *K*1C, *K*1D, *K*1E].**

**Table S3**. Net divergence (percentage nucleotide differences) among the putative *Habrobracon hebetor* *mtCOI* and *16S-rDNA* haplotypes and between those from related braconids used as outgroups in the phylogenetic analysis, respectively.

|  |  |  |
| --- | --- | --- |
|  | ***Habrobracon hebetor*** | **Related braconids** |
| **641bp-COI****haplotype** | **Niger** **Hap1-Hap11** | **Iran****Hap12** | **KY484509****KX371823** | **GBMIN73841** | **NZHYM803** | **KJ627789** | ***B. brevicornis*** | ***B. lissogaster*** | ***B. nigricans*** | ***B.*** ***cephi*** | ***Bracon******sp1*** |
| **Niger****Hap1-Hap11** | 0.2% 🡪0.7% | 5.7 % 🡪6.3% | 1.1%🡪0.5% | 0.4%🡪0.7% | 6%🡪6.6% | 2.4%🡪2.7% | 1% 🡪 1.3% | 13.3% 🡪 14.9% | 10.3% 🡪10.8% | 13.2% 🡪 14.4% | 11.6% 🡪 11.9% |
|  |  |  |  |  |  |  |  |  |  |  |  |

|  |  |  |
| --- | --- | --- |
| ***Habrobracon hebetor*** |  | **Related braconids** |
| **506bp-16S haplotype** | **Niger****Hap4-Hap32** | **Iran****H1-H3** | **AF003483** |  | ***B.******brevicornis*** | ***B. lissogaster*** | ***B.******nigricans*** | ***B.******cephi*** | ***Bracon sp1*** | ***Bracon******sp2*** | ***Callibracon******limbatus*****AF003482** |
| **Niger****Hap4-Hap32** | 0.3% 🡪2.7% | 1.9%🡪4% | 8.6%🡪10.3% |  | 0.3%🡪2.3% | 12%🡪16.2% | 9.7%🡪11.1% | 12%🡪14.4% | 16.1% 🡪14.6% | 14.4%🡪15.6% | 22.4%🡪23.8% |

**Table S4**. The table (**A**) of the parameters estimated according to the Evanno ‘s method (Evanno *et al.,* 2005) and the figure (**B**) representing the relation between Delta *K* and *K* as implemented in the Structure Harvester programme (Earl & vonHoldt, 2012)

(**A**)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *K* | Reps | Mean LnP(*K*) | Stdev LnP(*K*) | Ln'(*K*) | |Ln''(*K*)| | Delta *K* |
| 1 | 15 | -32812.2000 | 0.1897 | NA | NA | NA |
| 2 | 15 | -31688.6833 | 121.6340 | 1123.516667 | 147.150000 | 1.209777 |
| 3 | 15 | -30712.3167 | 70.8993 | 976.366667 | 279.633333 | 3.944094 |
| 4 | 15 | -30015.5833 | 74.8232 | 696.733333 | 46.100000 | 0.616119 |
| 5 | 15 | -29364.9500 | 99.0876 | 650.633333 | 148.783333 | 1.501533 |
| 6 | 15 | -28863.1000 | 77.2399 | 501.850000 | 71.183333 | 0.921587 |
| 7 | 15 | -28290.0667 | 38.5150 | 573.033333 | 187.200000 | 4.860447 |
| 8 | 15 | -27904.2333 | 101.9845 | 385.833333 | 19.100000 | 0.187283 |
| 9 | 15 | -27499.3000 | 68.8708 | 404.933333 | 12.466667 | 0.181015 |
| 10 | 15 | -27081.9000 | 116.0739 | 417.400000 | 152.733333 | 1.315828 |
| 11 | 15 | -26817.2333 | 65.2712 | 264.666667 | 27.400000 | 0.419787 |
| 12 | 15 | -26525.1667 | 96.8394 | 292.066667 | NA | NA |

**(B)**



**Table S5.** Pairwise estimates of *F*ST-ENA over the 21 polymorphic lociamong the 14 field samples **(F-Ng20 to F-Ng37)** from the Louga area in Niger.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *F*ST-ENA |  |  |  |  |  |  | Samples codes |  |  |  |  |  |  |  |
| **F-** | **Ng20** | **Ng21** | **Ng23** | **Ng24** | **Ng25** | **Ng26** | **Ng28** | **Ng29** | **Ng30** | **Ng31** | **Ng32** | **Ng33** | **Ng34** | **Ng35** | **\*Ng36** | **\*Ng37** |
| **Ng20** | 0 | 0.304 | **0.144** | 0.191 | **0.140** | 0196 | 0.165 | 0.166 | 0.178 | 0.157 | 0.179 | 0.157 | 0.168 | 0.183 | - | - |
| **Ng21** |  | 0 | 0.158 | 0.185 | 0.159 | 0.187 | 0.206 | 0.163 | 0.186 | 0.155 | 0.217 | 0.250 | 0.226 | 0.178 | - | - |
| **Ng23** |  |  | 0 | **0.057** | **0.025** | **0.084** | **0.039** | **0.031** | **0.061** | **0.048** | **0.074** | **0.107** | **0.063** | **0.056** | - | **-** |
| **Ng24** |  |  |  | 0 | **0.048** | **0.088** | **0.051** | **0.033** | **0.056** | **0.063** | **0.086** | **0.101** | **0.061** | **0.076** | - | **-** |
| **Ng25** |  |  |  |  | 0 | **0.064** | **0.029** | **0.020** | **0.049** | **0.056** | **0.080** | **0.102** | **0.039** | **0.055** | - | **-** |
| **Ng26** |  |  |  |  |  | 0 | **0.071** | **0.081** | **0.102** | **0.100** | **0.136** | **0.153** | **0.075** | **0.065** | - | **-** |
| **Ng28** |  |  |  |  |  |  | 0 | **0.032** | **0.080** | **0.071** | **0.080** | **0.095** | **0.029** | **0.088** | - | **-** |
| **Ng29** |  |  |  |  |  |  |  | 0 | **0.055** | **0.042** | **0.088** | **0.114** | **0.043** | **0.053** | - | **-** |
| **Ng30** |  |  |  |  |  |  |  |  | 0 | **0.074** | **0.112** | **0.135** | **0.094** | **0.094** | - | **-** |
| **Ng31** |  |  |  |  |  |  |  |  |  | 0 | **0.072** | **0.118** | **0.082** | **0.070** | - | **-** |
| **Ng32** |  |  |  |  |  |  |  |  |  |  | 0 | **0.013** | **0.104** | **0.111** | - | **-** |
| **Ng33** |  |  |  |  |  |  |  |  |  |  |  | 0 | **0.131** | **0.134** | - | - |
| **Ng34** |  |  |  |  |  |  |  |  |  |  |  |  | 0 | **0.080** | - | **-** |
| **Ng35** |  |  |  |  |  |  |  |  |  |  |  |  |  | 0 | - | **-** |
| **\*Ng36** |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0 | - |
| **\*Ng37** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0 |

\* samples with *N* < 5

**In bold**, pairwise *F*ST-ENA values indicating low level of genetic differentiation (*F*ST-ENA < 0.15)

The sample code for each sample is as listed in Table 1.

**Table S6.** Pairwise estimates of *F*ST-ENA among the 7 subpopulations (*K*1A to *K*1G) and the non-assigned specimens (*K*1H)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *K*1A | *K*1B | *K*1C | *K*1D | *K*1E | *K*1F | *K*1G | *K*1H |
|  |  |  |  |  |  |  |  |  |
| *K*1A | 0 | 0.163 | **0.136** | 0.270 | 0.215 | 0.433 | 0.299 | 0.167 |
| *K*1B |  | 0 | **0.056** | **0.150** | **0.129** | 0.260 | 0.229 | **0.064** |
| *K*1C |  |  | 0 | **0.108** | **0.052** | 0.196 | 0.167 | **0.036** |
| *K*1D |  |  |  | 0 | 0.204 | 0.402 | 0.307 | 0.201 |
| *K*1E |  |  |  |  | 0 | 0.247 | 0.254 | 0.098 |
| *K*1F |  |  |  |  |  | 0 | 0.431 | 0.313 |
| *K*1G |  |  |  |  |  |  | 0 | 0.228 |
| *K*1H |  |  |  |  |  |  |  | 0 |

**In bold**, pairwise *F*ST-ENA values indicating low level of genetic differentiation (*F*ST-ENA < 0.15)

**Fig. S1.** Topology of the *H. hebetor* samples either originating in the laboratory rearing or crossing-tests, or pearl millet field in Niger obtained by Principal Component Analysis (PCoA) using the 21 microsatellite loci.

\* samples with N < 5

The sample code for each sample is as listed in **Table 1** with the addition of the collection date.

