# Reproductive interference explains persistence of aggression between species

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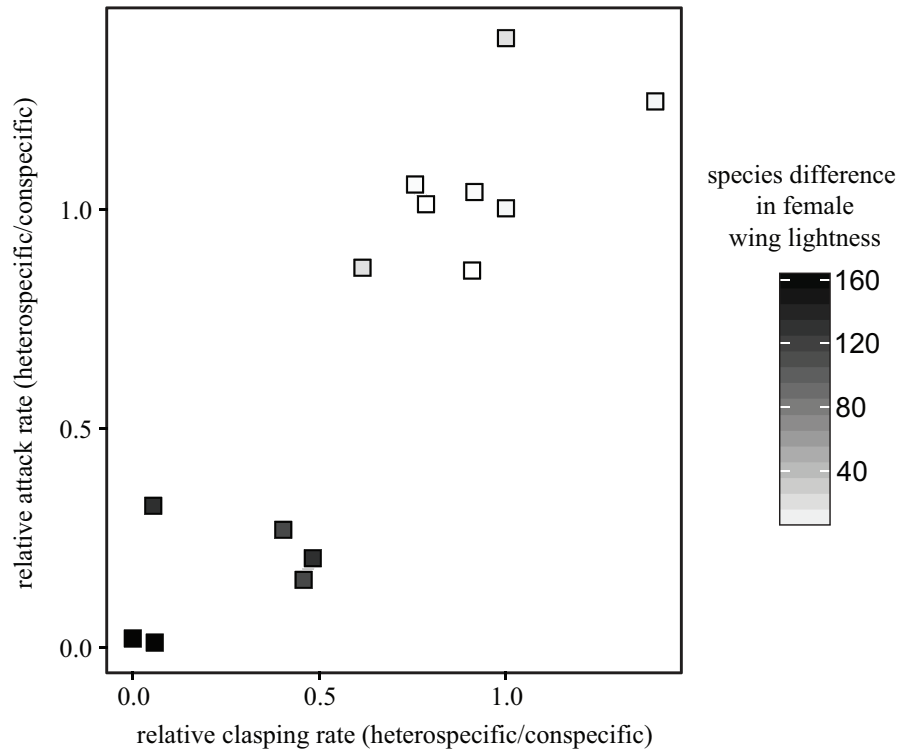
**Figure S1.** Evidence for a link between reproductive interference and interspecific aggression in *Hetaerina* damselflies. This alternative version of figure 4 shows that the results remain qualitatively unchanged if data from the early (PA1) and late (PA2) season at the La Palma site are pooled (relative clasping rate vs. relative attack rate, mean ± s.d. Spearman $\rho = 0.87 \pm 0.07$, $P < 0.01$; relative clasping rate vs. lightness difference, mean ± s.d. $\rho = -0.75 \pm 0.10$, $P < 0.01$; relative attack rate vs. lightness difference, mean ± s.d. $\rho = -0.73 \pm 0.10$, $P < 0.01$).
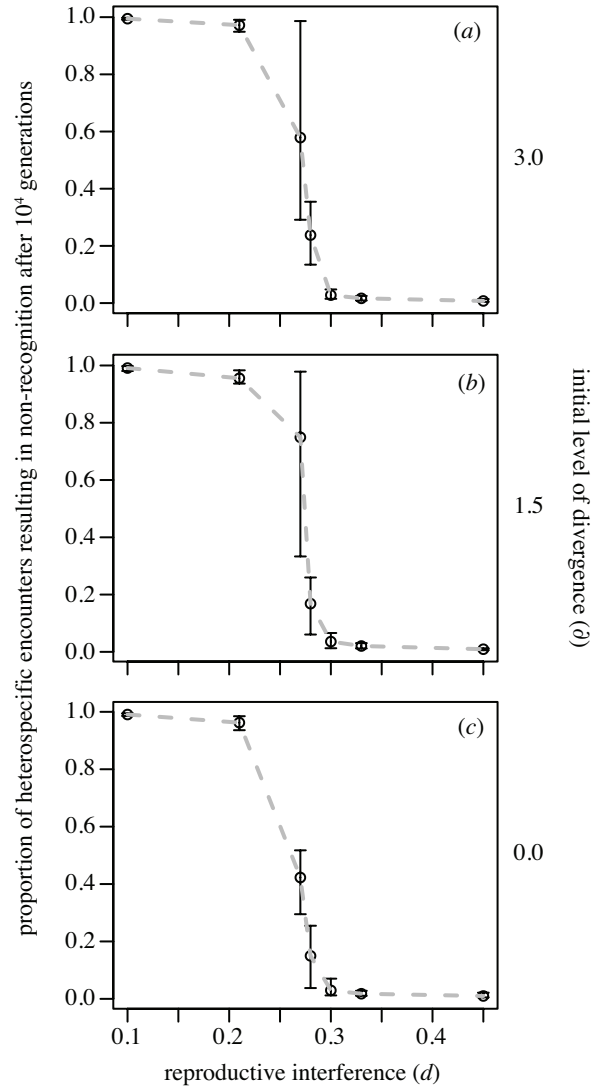
**Figure S2**. Summary of simulation results. (*a-c*) illustrate the proportion of heterospecific encounters resulting in mutual non-recognition 10000 generations after secondary contact begins as a function of the intensity *d* of reproductive interference. Except when *d* = 0.1 and $\partial$ = 0, open circles represent the average of 15 simulation runs, and the ends of the error bars represent the 5th and 95th percentiles for each set of runs. When *d* = 0.1 and $\partial$ = 0, simulations resulting in the loss of territoriality were excluded.
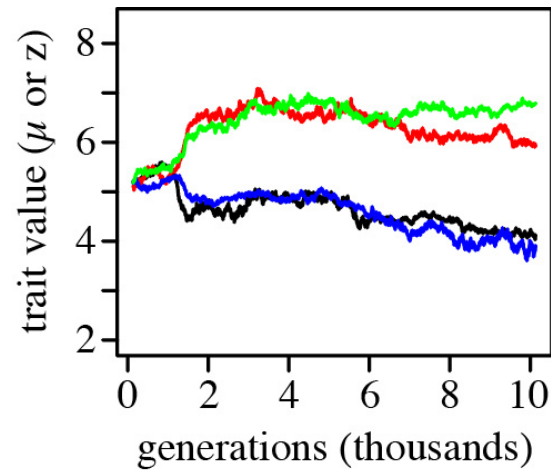
**Figure S3.** Example of a simulation in which intraspecific territoriality was lost as the species diverged from each other. In this and all other cases in which territoriality was lost, the species had the same mean values of $z$ and $\mu$ at the time of secondary contact ($\partial = 0$) and reproductive interference was minimal ($d = 0.1$). Instead of tracking each other within species, the male trait $z$ and central location of the recognition parameter $\mu$ diverged from each other within (as well as between) species. Territoriality was lost in 5 of 15 simulations with $\partial = 0$ and $d = 0.1$, but 0 of 255 simulations with $\partial > 0$ or $d > 0.1$. The color scheme matches figure 1.

**Table S1.** Examples of interspecifically territorial species pairs in which reproductive interference occurs.

| Taxon | Species | Location | References |
|---|---|---|---|
| *Insects* | Speckled wood butterflies (*Pararge xiphia* & *P. aegeria*) | Madeira | [1] |
| | Seed-eating bugs (*Neacoryphus bicrucis* & *Margus obscurator*) | United States | [2] |
| | Whiteface dragonflies (*Leucorrhinia* spp.) | United States | [3,4] |
| | *Mnais* damselflies (*Mnais nawai* & *M. pruinosa*) | Japan | [5] |
| | Beautiful & western demoiselles (*Calopteryx virgo* & *C. xanthostoma*) | Spain | [6] |
| | Beautiful & banded demoiselles (*Calopteryx virgo* & *C. splendens*) | Finland | [7–10] |
| | River & ebony jewelwings (*Calopteryx aequabilis* & *C. maculata*) | United States | [11] |
| *Crustaceans* | European & signal crayfish (*Astacus astacus* & *Pacifastacus leniusculus*) | Sweden | [12] |
| | Fiddler crabs (*Uca* spp.) | United States | [13–15] |
| *Amphibians* | Red-cheeked & Northern slimy salamanders (*Plethodon jordani* & *P. glutinosus*) | United States | [16] |
| | Cope's gray tree & gray tree frogs (*Hyla chrysoscelis* & *H. versicolor*) | United States | [17,18] |
| | Daruma pond & dark-spotted frogs (*Pelophylax porosa brevipoda* & *P. nigromaculata*) | Japan | [19] |

**Table S1 (cont.)**

| | | | |
|---|---|---|---|
| *Birds* | Barred & spotted owls (*Strix varia* & *S. occidentalis*) | United States | [20] |
| | Anna's and Allen's hummingbirds (*Calypte anna* & *Selasphorus sasin*) | United States | [21] |
| | Indigo & lazuli buntings (*Passerina cyanea* & *P. amoena*) | United States | [22] |
| | Chiffchaffs & willow warblers (*Phylloscopus collybita* & *P. trochilus*) | Norway | [23] |
| | Redstarts & black redstarts (*Phoenicurus phoenicurus* & *P. ochruros*) | Czech Republic | [24] |
| | Eastern & western meadowlarks (*Sturnella magna* & *S. neglecta*) | United States | [25] |
| | Seaside sparrow & short-tailed sparrow (*Ammodramus maritimus* & *A.* sp.) | United States | [26] |
| | Dusky indigobirds & paradise whydahs (*Vidua purpurascens* & *V. paradisaea*) | Zambia | [27] |
| | Vinaceous & ring-necked doves (*Streptopelia vinacea* & *S. capicola*) | Uganda | [28] |
| | Hermit & Townsend's warblers (*Setophaga occidentalis* & *S. townsendii*) | United States | [29] |
| | Melodious & Icterine warblers (*Hippolais polyglotta* & *H. icterina*) | Northern Europe | [30] |
| | Pied & collared flycatchers (*Ficedula hypoleuca* & *F. albicollis*) | Sweden | [31] |
| | Reed warblers (*Acrocephalus* spp.) | Europe | [32–34] |
| | Common & thrush nightingales (*Luscinia megarhynchos* & *L. luscinia*) | Europe | [35] |
| *Mammals* | Wolves & coyotes (*Canis lupus* & *C. latrans*) | United States | [36] |
| | Lar & pileated gibbons (*Hylobates lar* & *H. pileatus*) | Thailand | [37] |

**Table S2.** Study site locations (in decimal degrees) and *Hetaerina* species present.

| Site name | Species 1 | Species 2 | Latitude | Longitude |
|---|---|---|---|---|
| Armeria (AR) | *H. americana* | *H. titia* | 18.95001 | -103.93351 |
| Bonita Creek (BC) | *H. americana* | *H. vulnerata* | 32.91627 | -109.49282 |
| Castroville (CV) | *H. americana* | *H. titia* | 29.34079 | -98.88156 |
| Cuetzalapan (CT) | *H. cruentata* | *H. occisa* | 18.37100 | -95.00148 |
| El Limon (EL) | *H. americana* | *H. cruentata* | 21.36673 | -104.61673 |
| Laguna Escondida (ES) | *H. sempronia* | *H. occisa* | 18.59245 | -95.08390 |
| Lampasas (LM) | *H. americana* | — | 31.08271 | -98.01973 |
| La Palma (PA) | *H. occisa* | *H. titia* | 18.56187 | -95.06134 |
| Otapa (OT) | *H. occisa* | *H. titia* | 18.68339 | -96.38350 |
| Pixquiac (PX) | *H. vulnerata* | *H. cruentata* | 19.46679 | -96.95018 |
| Upper Cuetzalapan (UC) | *H. sempronia* | *H. capitalis* | 18.36733 | -94.96500 |

**Table S3.** Comparisons of territorial males' responses to tethered conspecifics and heterospecifics of both sexes.

| Focal species | Sympatric congener | Site | Female tethering $n$ | $P*$ | Male tethering $n$ | Statistic† | $P$ |
|---|---|---|---|---|---|---|---|
| *H. americana* | *H. titia* | AR | — | — | 16 | $t = 3.611$‡ | 0.002 |
| *H. americana* | *H. vulnerata* | BC | 18 | 0.69 | 16 | $t = -0.051$ | 0.96 |
| *H. americana* | *H. titia* | CV | 24 | 0.0065 | 33 | $t = 7.78$‡ | <0.0001 |
| *H. americana* | *H. cruentata* | EL | — | — | 17 | $t = 0.02$‡ | 0.98 |
| | | | | | | | |
| *H. cruentata* | *H. occisa* | CT | 17 | 0.28 | 15 | $t = 0.71$‡ | 0.49 |
| *H. cruentata* | *H. americana* | EL | — | — | 10 | $t = 0.85$‡ | 0.42 |
| *H. cruentata* | *H. vulnerata* | PX | 14 | 0.22 | 17 | $t = 0.68$ | 0.50 |
| | | | | | | | |
| *H. occisa* | *H. cruentata* | CT | 20 | 1 | 16 | $t = 0.14$‡ | 0.89 |
| *H. occisa* | *H. sempronia* | ES | 20 | 0.80 | 19 | $t = 1.32$ | 0.20 |
| *H. occisa* | *H. titia* | OT | 7 | 0.0006 | 39 | $t = 7.33$‡ | <0.0001 |
| *H. occisa* | *H. titia* | PA1 | 64 | 0.09 | 54 | $V = 1144.5$ | <0.0001 |
| *H. occisa* | *H. titia* | PA2 | 42 | <0.0001 | 68 | $V = 1653$ | <0.0001 |
| | | | | | | | |
| *H. sempronia* | *H. occisa* | ES | 10 | 0.37 | 14 | $t = 3.98$ | 0.002§ |
| *H. sempronia* | *H. capitalis* | UC | — | — | 16 | $V = 59$ | 0.6685 |
| | | | | | | | |
| *H. titia* | *H. americana* | AR | — | — | 14 | $t = 5.91$‡ | <0.0001 |
| *H. titia* | *H. americana* | CV | 22 | <0.0001 | 30 | $t = 8.26$‡ | <0.0001 |
| *H. titia* | *H. occisa* | OT | 17 | <0.0001 | 23 | $t = 6.56$‡ | <0.0001 |
| *H. titia* | *H. occisa* | PA1 | 38 | 0.037 | 19 | $V = 4$ | 0.0004 |
| *H. titia* | *H. occisa* | PA2 | 24 | <0.0001 | 22 | $V = 8$ | 0.006 |
| | | | | | | | |
| *H. vulnerata* | *H. americana* | BC | 18 | 0.15 | 16 | $t = 2.42$ | 0.03§ |
| *H. vulnerata* | *H. cruentata* | PX | 11 | 1 | 10 | $t = 0.04$ | 0.97 |

Sample sizes are the number of males tested with tethered individuals of both species. Dashes indicate where, for logistical reasons, responses to females were not measured.
*Fisher's exact tests; in all cases where $P < 0.05$, males responded more strongly to conspecific females than to heterospecific females.
†Paired t-tests ($t$) or Wilcoxon paired sign rank tests ($V$); if $P < 0.05$, males responded more strongly to conspecific males than to heterospecific males, except where noted otherwise.
‡Previously published data [38].
§Cases in which males responded more strongly to heterospecific males than to conspecific males.

**Table S4.** Variation in the level of interspecific territoriality relative to intraspecific territoriality, inferred from behavioral observations, including all observed fights.

| Site | Year | Number of fights, observed (expected)* | | | $\chi^2$ | $P$† |
| | | Species 1 | Interspecific | Species 2 | | |
|---|---|---|---|---|---|---|
| AR | 2005‡ | 10(8) | 20(36) | 58(44) | 12.74 | 0.002 |
| AR | 2008‡ | 6(2) | 8(18) | 42(35) | 12.63 | 0.002 |
| BC | 2012 | 31(28) | 12(16) | 4(2) | 2.57 | 0.28 |
| CT | 2006‡ | 9(7) | 2(9) | 8(3) | 15.78 | <0.001 |
| CV | 2008 April‡ | 6(2) | 8(18) | 42(35) | 9.4 | 0.009 |
| CV | 2008 August‡ | 24(19) | 18(30) | 18(12) | 23.9 | <0.001 |
| CV | 2012 | 15(5) | 7(25) | 39(31) | 34.27 | <0.001 |
| EL | 2008‡ | 37(30) | 7(19) | 8(3) | 17.9 | <0.001 |
| ES | 2013 | 26(17) | 12(26) | 16(10) | 15.61 | <0.001 |
| OT | 2006‡ | 13(11) | 14(59) | 120(77) | 58.55 | <0.001 |
| OT | 2007‡ | 8(8) | 4(25) | 41(19) | 42.00 | <0.001 |
| OT | 2010 | 13(4) | 10(60) | 244(203) | 66.67 | <0.001 |
| PA2 | 2007‡ | 13(19) | 6(24) | 32(8) | 88.18 | <0.001 |
| PA1 | 2011 | 87(133) | 120(136) | 97(35) | 126.44 | <0.001 |
| PA2 | 2011 | 20(22) | 21(35) | 29(14) | 23.06 | <0.001 |
| PA1 | 2012 | 16(16) | 11(21) | 17(7) | 17.46 | <0.001 |
| PA2 | 2012 | 30(19) | 14(41) | 38(22) | 35.55 | <0.001 |
| PX | 2010 | 38(40) | 23(24) | 7(3) | 4.46 | 0.10 |
| PX | 2011 | 25(22) | 13(15) | 2(3) | 0.64 | 0.76 |

Species numbers follow table S2.
*Expected number of fights generated through binomial expansion of the relative proportiosn of each species at the site. When expected values were < 5, we calculated $P$ values using Monte Carlo simulations.
†In all cases with $P < 0.05$, the rate of interspecific fighting was reduced relative to intraspecific fighting.
‡Previously published data [39].

**Table S5.** Variation in the level of interspecific territoriality relative to intraspecific territoriality, inferred from behavioral observations, including only escalated fights.

| Site | Year | Number of fights, observed (expected)* | | | $\chi^2$ | $P$† |
|------|------|------------|--------------|-----------|--------|---------|
| | | Species 1 | Interspecific | Species 2 | | |
| BC | 2012 | 19(18) | 8(10) | 2(1) | 0.74 | 0.69 |
| CV | 2012 | 8(2) | 2(11) | 17(14) | 22.78 | < 0.001 |
| ES | 2013 | 23(14) | 9(21) | 11(8) | 13.93 | <0.001 |
| OT | 2010 | 6(2) | 0(33) | 142(113) | 44.83 | <0.001 |
| PA1 | 2011 | 69(101) | 90(102) | 70(26) | 82.64 | <0.001 |
| PA2 | 2011 | 15(17) | 15(28) | 26(11) | 27.32 | <0.001 |
| PA1 | 2012 | 11(9) | 4(13) | 11(4) | 16.33 | <0.001 |
| PA2 | 2012 | 26(14) | 9(29) | 23(15) | 29.11 | <0.001 |
| PX | 2010 | 15(16) | 7(9) | 4(1) | 6.43 | 0.04 |
| PX | 2011 | 13(11) | 6(8) | 1(1) | 0.62 | 0.78 |

Species numbers follow table S2.
*Expected number of fights generated through binomial expansion of the relative proportion of each species at the site. When expected values were < 5, we calculated $P$ values using Monte Carlo simulations.
†In all cases with $P < 0.05$, the rate of interspecific fighting was reduced relative to intraspecific fighting.

**Appendix S1.** Phylogenetic correction

*(i) Statistical approach*

We employed a simulation approach to calculate phylogenetically corrected test statistics (after [40–42]. In the R program we wrote, species' female wing lightness values are simulated 1,000 times under Brownian motion [BM] and Ornstein-Uhlenbeck [OU] models of evolution across a phylogeny (see *Phylogeny reconstruction*, below) using the fastBM() function in the phytools package for R [43]. We used the empirical data and the fitContinuous() function in geiger [44] to scale the simulation data. After each simulation, differences between sympatric species are calculated. In this way, only the relevant species comparisons are included, and a full species interaction matrix is not necessary, as it is in phylogenetically permuted partial Mantel tests (for other issues with partial Mantel tests, see [42,45,46]). Using these simulated differences, the Spearman correlation simulations described in the main text were carried out between the raw (observed) response variables (i.e., heterospecific clasping ratio or heterospecific aggression ratio) and the simulated species differences in female wing lightness. For each simulated dataset, we created a distribution of 1,000 $\rho$ values from simulated female wing lightness and empirical clasping and aggression ratios and stored the mean value of this distribution. We then compared a distribution of 1,000 simulated mean $\rho$ values (each the mean of 1,000 simulations) to the empirically calculated mean $\rho$ value using a one-sample t-test.

*Hetaerina titia* female wings exhibit a seasonal polyphenism in their wing lightness (Drury et al. MS). In the overall analysis, one site's (PA1) measurements were taken on light-morph females. To determine if the phylogenetically corrected statistic is robust to changes in how we modeled the evolution of wing color, we ran analyses including a mean value of *H. titia* female wings calculated across all sites and another analysis excluding data from PA1 where most females were light-phase morphs.

*(ii) Phylogeny reconstruction*

To construct the phylogeny, we included 32 specimens from 9 *Hetaerina* species, sampling several individuals from different populations where possible (table S6). We included individuals of *Calopteryx maculata* and *Calopteryx aqueabilis* as outgroups.

We obtained a matrix of adult female and male morphological characters used in the creation of the key to the genus *Hetaerina* [47] (R. Garrison, pers. comm.). We also included morphological character data from a published account of *Hetaerina* larvae (see table 3 in [48]).

We extracted DNA from wing muscle tissue of ethanol-preserved specimens using Qiagen DNEasy kits (Qiagen, Valencia, CA, USA). Several target mitochondrial and nuclear sequences (table S7) were amplified using the polymerase chain reaction (PCR) and sequenced at a sequencing core (UCLA GenoSeq Core, Los Angeles, CA, Cornell Genomics Facility, Ithaca, NY, or Beckman Coulter Genomics, Danvers, MA). Resulting forward and reverse chromatograms were aligned in Geneious 4.8.3 (Biomatters, Inc.), checked, and assembled into contigs. Consensus sequences for each locus were aligned using Muscle v.3.8.31 alignment software [49], inspected visually, and altered manually if necessary. We excluded three sequences referenced with BLAST (Altschul et al. 1990) that returned taxonomically distant matches. Concatenated sequence files were created using SequenceMatrix [50]. Our final concatenated matrix totaled 3853 nucleotides (table S7) and 89 morphological characters, which were added to one individual for each species.

When it was possible to verify sequence alignments to ensure the accuracy of codon partitioning using BLAST [51], we partitioned loci at the codon level. We then used PartitionFinder v. 1.1.1 [52] to identify the best-fit partitioning scheme and suitable models of evolution for each partition using BIC model selection and the "greedy" search algorithm. Final phylogenetic inferences were conducted on alignments with the partitions identified in PartitionFinder and three additional partitions for morphological characteristics (females, males, and larvae).

We used MrBayes 3.2.2 for Bayesian reconstruction of the phylogeny [53]. The analysis was run for 20 million generations, sampling every 5000 generations, with four chains (one cold, three heated). Chain convergence was assessed using Tracer 1.6 [54], 25% of trees were discarded as burnin, and the maximum clade credibility tree was calculated from post-burnin trees using TreeAnnotator v.1.7.4 [55] (figure S4).

Analyses were run using the Cipres Web Portal [56]. To obtain an ultrametric tree for modeling trait evolution, we rendered the maximum clade credibility tree obtained from mrBayes ultrametric using the chronos() function in the ape package in R [57] and dropped tips so that the topology had a single tip for each species in our analyses.

*(iii) Phylogenetic results excluding data from PA1*

As with the results based on data from all sites, species differences in female wing lightness (excluding PA1) were negatively correlated with the level of reproductive interference after the phylogenetic correction (excluding early season La Palma: mean observed $\rho$ = -0.730; BM model of evolution, $t$ = 52.16, *d.f.* = 999, $p < 0.001$; OU model of evolution, $t$ = 50.74, *d.f.* = 999, $p < 0.001$).

Likewise, species differences in female wing lightness (excluding PA1) were negatively correlated with the magnitude of interspecific aggression after the phylogenetic correction (mean observed $\rho$ = -0.804; BM model of evolution, $t$ = 55.31, *d.f.* = 999, $p < 0.001$; OU model of evolution, $t$ = 53.55, *d.f.* = 999, $p < 0.001$; excluding early season La Palma: mean observed $\rho$ = -0.722; BM model of evolution, $t$ = 51.34, *d.f.* = 999, $p < 0.001$; OU model of evolution, $t$ = 50.65, *d.f.* = 999, $p < 0.001$)

**Table S6.** Specimens used in the reconstruction of the phylogeny and their GenBank accession numbers. Locus numbers follow codes in table S7 (shaded cells correspond to missing sequences).

| new tree name | collection location | decimal degrees (lat, long) | Locus 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Calopteryx aequabilis* | Horse Creek, CA | 41.824, -123.000 | | KM383956 | KM383925 | KM383900 | KM383991 | KM383865 | | KM383796 |
| *C. maculata* | Burr Ferry, LA | 31.076, -93.489 | KM383849 | KM383957 | KM383926 | | KM383992 | KM383864 | | |
| *Hetaerina americana* 1 | Arroyo de Piedra | 19.456, -96.479 | KM383858 | KM383984 | KM383951 | | KM384000 | KM383870 | KM383832 | KM383824 |
| *H. americana* 2 | Bonita Creek, AZ | 32.916, -109.493 | KM383853 | KM383982 | KM383950 | KM383899 | | KM383872 | KM383833 | KM383825 |
| *H. americana* 3 | Bonita Creek, AZ | 32.916, -109.493 | KM383854 | KM383983 | KM383949 | KM383898 | KM383999 | KM383871 | KM383834 | KM383826 |
| *H. capitalis* 1 | Los Organos | 18.657, -95.151 | | KM383978 | KM383928 | KM383914 | | KM383889 | | KM383813 |
| *H. capitalis* 2 | Upper Cuetzalapan | 18.367, -94.965 | | KM383979 | KM383929 | KM383915 | | KM383890 | | KM383811 |
| *H. capitalis* 3 | Upper Cuetzalapan | 18.367, -94.965 | | KM383980 | KM383930 | KM383916 | | KM383891 | | KM383812 |
| *H. capitalis* 4 | Río Limón | 21.367, -104.617 | KM383861 | KM383981 | KM383927 | KM383913 | KM384009 | KM383888 | | KM383810 |
| *H. cruentata* 1 | Cuetzalapan | 18.371, -95.001 | | KM383986 | KM383954 | KM383918 | KM383998 | KM383893 | KM383842 | KM383818 |
| *H. cruentata* 2 | Los Organos | 18.657, -95.151 | KM383850 | KM383987 | KM383955 | KM383919 | KM383996 | KM383894 | KM383841 | KM383819 |
| *H. cruentata* 3 | Pixquiac | 19.467, -96.95 | | KM383985 | KM383953 | KM383917 | KM383997 | KM383892 | KM383840 | KM383820 |
| *H. miniata* 1 | Bartola | 10.989, -84.334 | | KM383966 | KM383939 | KM383922 | KM384014 | KM383884 | KM383831 | KM383801 |
| *H. miniata* 2 | Bartola | 10.989, -84.334 | | KM383967 | KM383942 | | KM384015 | KM383885 | KM383828 | KM383802 |
| *H. miniata* 3 | Bartola | 10.989, -84.334 | | KM383968 | KM383940 | KM383905 | KM384016 | KM383886 | KM383829 | |
| *H. miniata* 4 | Bartola | 10.989, -84.334 | | KM383969 | KM383941 | | KM384017 | KM383887 | KM383830 | KM383803 |
| *H. occisa* 1 | La Palma | 18.550, -95.067 | | KM383972 | KM383933 | KM383906 | KM384001 | KM383866 | KM383848 | |
| *H. occisa* 2 | Benito Juarez | 18.359, -95.000 | | KM383971 | KM383934 | KM383904 | KM384002 | KM383868 | KM383846 | |
| *H. occisa* 3 | Benito Juarez | 18.359, -95.000 | | KM383973 | KM383935 | KM383911 | KM384004 | KM383869 | KM383847 | KM383809 |
| *H. occisa* 4 | La Palma | 18.550, -95.067 | KM383857 | KM383970 | KM383932 | KM383902 | KM384003 | KM383867 | KM383845 | KM383808 |
| *H. pilula* 1 | Cuetzalapan | 18.371, -95.001 | | KM383962 | KM383945 | KM383903 | KM384011 | KM383881 | | KM383804 |
| *H. pilula* 2 | Laguna Escondida | 18.592, -95.084 | | KM383964 | KM383943 | | KM384012 | KM383883 | KM383827 | KM383806 |
| *H. pilula* 3 | Laguna Escondida | 18.592, -95.084 | | KM383965 | KM383944 | | KM384010 | KM383882 | | KM383807 |
| *H. pilula* 4 | Balzapote | 18.614, -95.073 | KM383859 | KM383963 | | | KM384013 | | | KM383805 |
| *H. sempronia* 1 | Laguna Escondida | 18.592, -95.084 | KM383856 | KM383975 | KM383948 | KM383908 | KM384005 | KM383878 | | KM383814 |
| *H. sempronia* 2 | Laguna Escondida | 18.592, -95.084 | KM383855 | KM383974 | KM383946 | KM383901 | KM384006 | KM383877 | | KM383815 |
| *H. sempronia* 3 | Los Organos | 18.657, -95.151 | | KM383976 | KM383947 | KM383909 | KM384007 | KM383879 | | KM383816 |
| *H. sempronia* 4 | Upper Cuetzalapan | 18.367, -94.965 | | KM383977 | | KM383910 | KM384008 | KM383880 | | KM383817 |
| *H. titia* 1 | La Palma | 18.550, -95.067 | KM383863 | KM383959 | KM383937 | | KM384019 | KM383873 | KM383838 | KM383800 |
| *H. titia* 2 | Bartola | 10.989, -84.334 | | KM383960 | | KM383923 | KM384021 | KM383874 | KM383836 | KM383799 |
| *H. titia* 3 | Burr Ferry, LA | 31.076, -93.489 | | KM383958 | KM383936 | KM383907 | KM384018 | KM383875 | KM383837 | KM383798 |
| *H. titia* 4 | Armeria | 18.950, -103.934 | KM383860 | KM383961 | KM383938 | KM383912 | KM384020 | KM383876 | KM383835 | KM383797 |
| *H. vulnerata* 1 | Pixquiac | 19.467, -96.95 | KM383851 | KM383989 | KM383952 | KM383921 | KM383993 | KM383896 | KM383839 | KM383822 |
| *H. vulnerata* 2 | Pixquiac | 19.467, -96.95 | KM383852 | KM383990 | | KM383924 | KM383995 | KM383897 | KM383844 | KM383823 |
| *H. vulnerata* 3 | Sierra Vista, AZ | 31.480, -110.337 | KM383862 | KM383988 | KM383931 | KM383920 | KM383994 | KM383895 | KM383843 | KM383821 |

**Table S7.** Loci and primers used to generate sequence data for the phylogeny

| locus | primers (5'→3' forward top, 5'→3' reverse bottom) | total alignment length | min/max length of sequenced product (bp) | reference |
|---|---|---|---|---|
| *mitochondrial* | | | | |
| 1. cytochrome oxidase I | GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA | 664 | 611/664 | [58] |
| 2. 16s rRNA[p] | GCTCCGDTTTGAACTCAGAT AGTTCTCGCCTGTTTATCAAA | 534 | 512/523 | [59] |
| 3. 12s rRNA[p], tRNA-valine[c], 16s rRNA[p] | GATCTGATGAAGGTGGATTT TAGCTCTTCTGAAATCGAGA | 421 | 363/411 | [59] |
| 4. 16s rRNA[p], tRNA-leucine[c], NADH dehydrogenase I[p] | TTCAAACCGGTGTAAGCCAGG TAGAATTAGAAGATCAACCAG | 534 | 387/532 | [60] |
| *nuclear* | | | | |
| 5. elongation factor α[p] | GCYGARCGYGARCGTGGTATYAC CATGTTGTCGCCGTGCCAAC | 459 | 421/449 | [61] |
| 6. histone 3 | ATGGCTCGTACCAAGCAGACVGC ATATCCTTRGGCATRATRGTGAC | 328 | 328/328 | [62] |
| 7. tubulin alpha | GAAACCRGTKGGRCACCAGTC GARCCCTACAAYTCYATTCT | 155* | 447/466 | [63] |
| 8. 18s & 28s rRNA[p] 5.8s rRNA[c] | TAGAGGAAGTAAAAGTCG GCTTAAATTCAGCGG | 758 | 460/697 | [64] |

[p]=partial
[c]=complete
*only one codon was variable
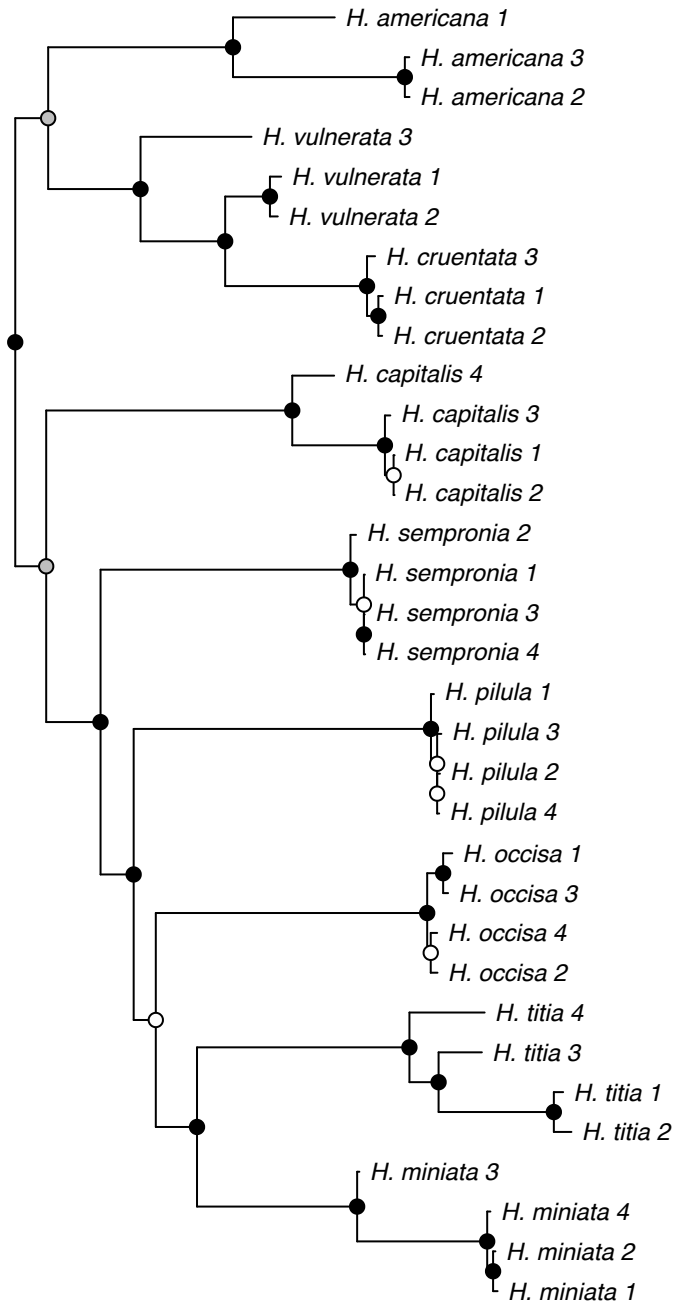
**Figure S4.** Maximum clade credibility tree calculated from partitioned data set using Bayesian tree inference. Black circles indicate mean posterior probability >0.95, gray circles >0.75 and < 0.95, and empty circles <0.75.

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