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Inter-chromosomal coupling between vision and pigmentation genes during genomic divergence

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Inter-chromosomal coupling between vision and pigmentation genes during genomic divergence (Supplementary Information)

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Supplementary Information

Supplementary Figures

Supplementary Figure 1: Broad-scale synteny between the hamlet and stickleback genomes. The comparison is based on a whole-genome alignment using last. Only alignments > 5000 bp are shown. Left: stickleback (*Gasterosteus aculeatus*, 21 LGs & mitochondria). Right: hamlet (*Hypoplectrus puella*, 24 LGs & mitochondria).

Supplementary Figure 2: Identification of putative backcrosses and hybrids. a, bars indicate the posterior probability of assignment to the different hybrid classes for each pairwise comparison. A total of nine individuals, highlighted in bold, were identified as putative hybrids or backcrosses, eight of which with high (>0.99) posterior probabilities (five in Belize, two in Honduras and one in Panama). Five of these involved butter hamlets (*H. unicolor*) from Belize, which is consistent with the fact that this species is rare in this location. The same individuals were often identified as putative hybrids or backcrosses in two pairwise comparisons, suggesting either multi-species exchanges or false positives in one species pair resulting from exchanges with a third species. **b**, putative hybrids and backcrosses (highlighted) were also intermediate (e.g. 18267) or clustered with other species (e.g. 18274) in the whole-genome PCA. Note that the sampling design explicitly excluded individuals with intermediate colour patterns, thereby reducing the probability of recovering F1 hybrids in our data set.

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Supplementary Figure 3: Genotype by phenotype (G × P) association among black (*H. nigricans*), barred (*H. puella*) and butter (*H. unicolor*) hamlets. Each species pair is represented by one colour, pooled across locations (Global) as well as within each location (Belize, Honduras & Panama). The *p* values are from the linear model with Wald test, transformed using the negative of the common logarithm and averaged across 50 kb window with 5 kb increments ($\overline{-\log_{10}(p)}$). The four genomic regions highlighted with a vertical line, included as reference, correspond to the four intervals identified in **Fig. 2**.

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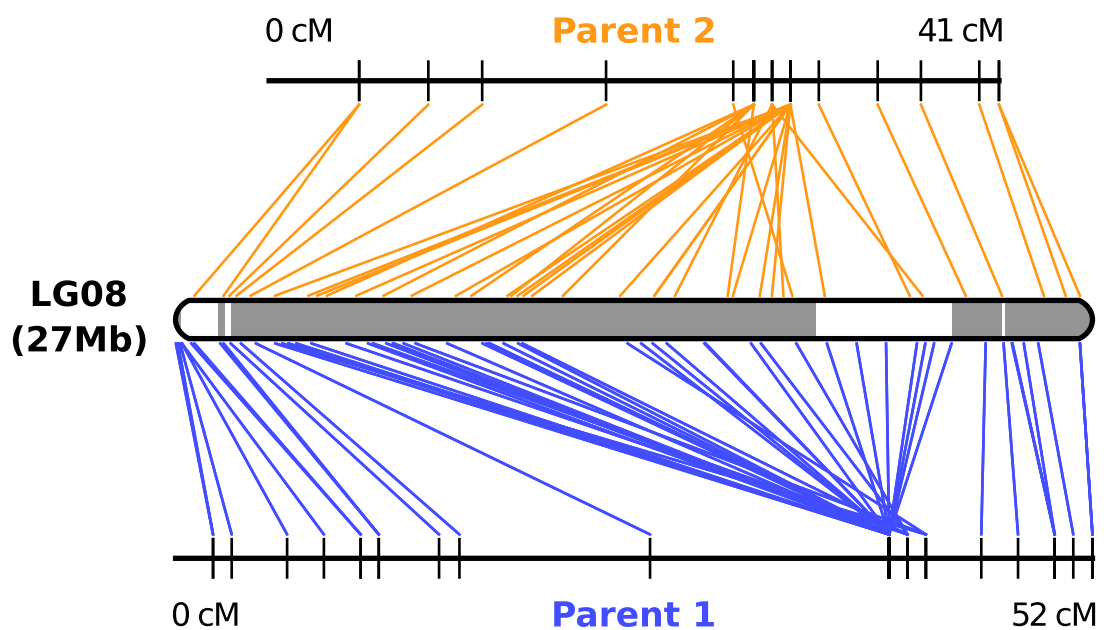
Supplementary Figure 4: Large low-recombining region on linkage group 08 (LG08). Top and bottom: linkage maps of the two parents used for the F_1 cross, from[26]. Middle: assembled linkage group 08. Lines connect individual RAD markers that are identified in both the linkage maps and the assembly. Gray and white blocks represent individual scaffolds. A large number of RAD markers that are in close proximity on the linkage maps are distributed over a wide region on the assembled linkage group, providing direct evidence of low recombination in this region.
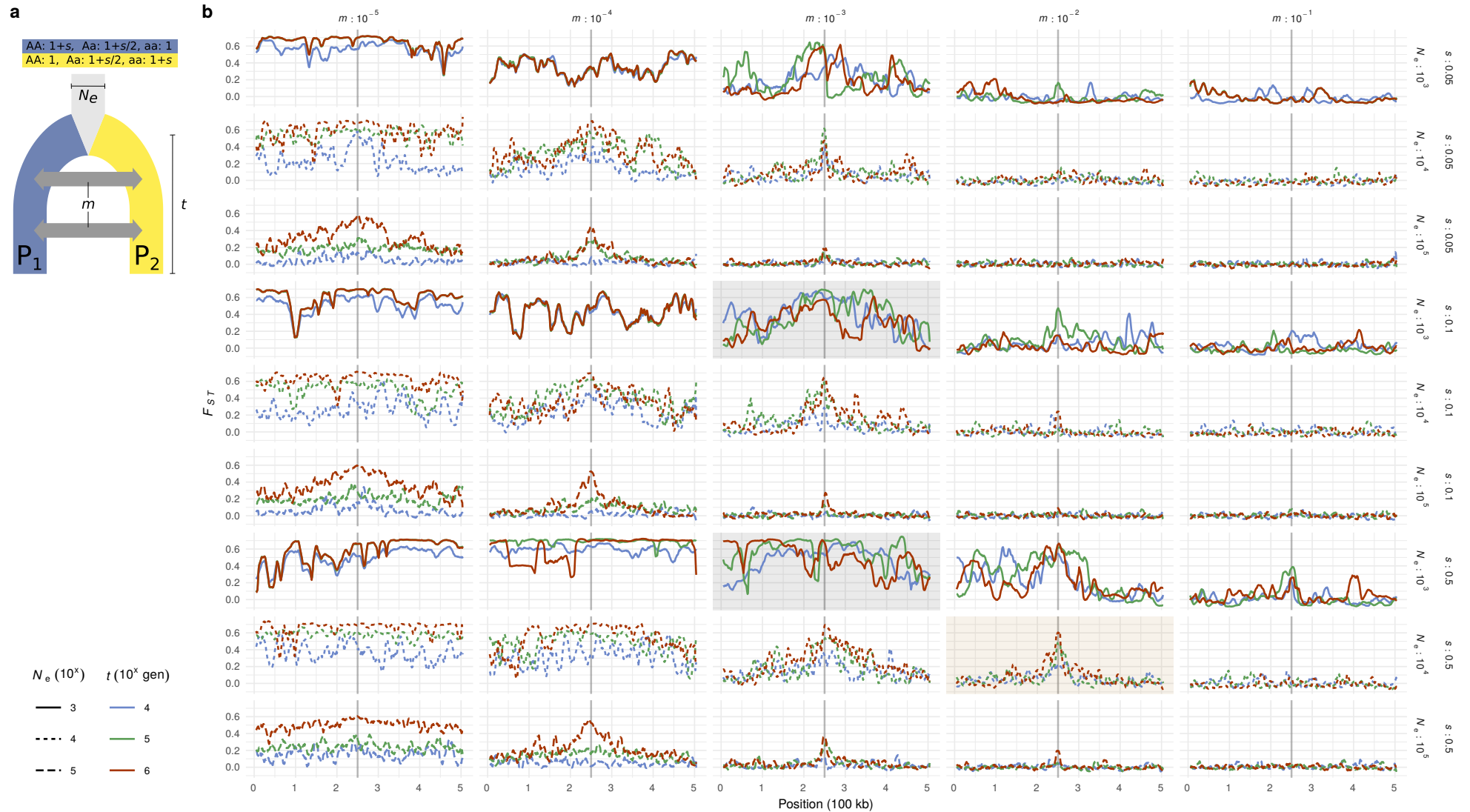
Supplementary Figure 5: Extent of differentiation under various simulated scenarios. The demographic history underlying the simulations consisted of two populations (1 & 2) of constant size N_e that split t generations ago and experienced constant and symmetrical migration (m) since then. A selected site was considered in the middle of a 500-kb chromosome, consisting of a codominant locus with two alleles A and a that are advantageous in population 1 and 2, respectively, with a fitness of $1+s$ for homozygotes and $1+s/2$ for heterozygotes where s is the selection coefficient. Scenarios highlighted in grey are similar to those explored by Charlesworth *et al.*[143]. Patterns similar to the ones observed in this study (highlighted in orange) were obtained with larger N_e (10,000) and higher m (0.01), which we suggest may be more representative of the situation in the hamlets.
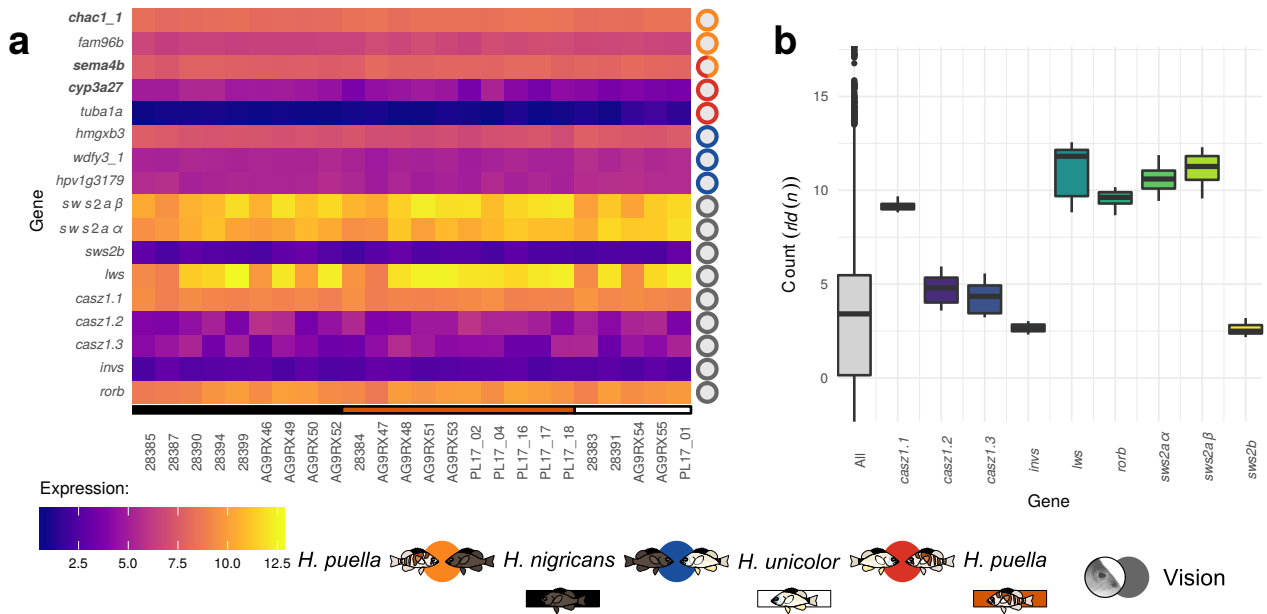
Supplementary Figure 6: Gene expression in the retinal tissue. a, only three genes, highlighted in bold, were significantly differentially expressed among species. Genes included in the figure correspond, from top to bottom, to the three most differentially expressed genes for each species pair (labelled by the colour-coded rings on the right) followed by the candidate genes related to vision identified in this study (grey rings, not differentially expressed). Data from 10 adult barred, 9 black and 5 butter hamlets from Panama (labelled by the colour bar on the bottom). **b**, albeit not differentially expressed, many of the candidate genes showed consistent above-average expression levels. Expression data transformed with regularised logarithm in both panels.
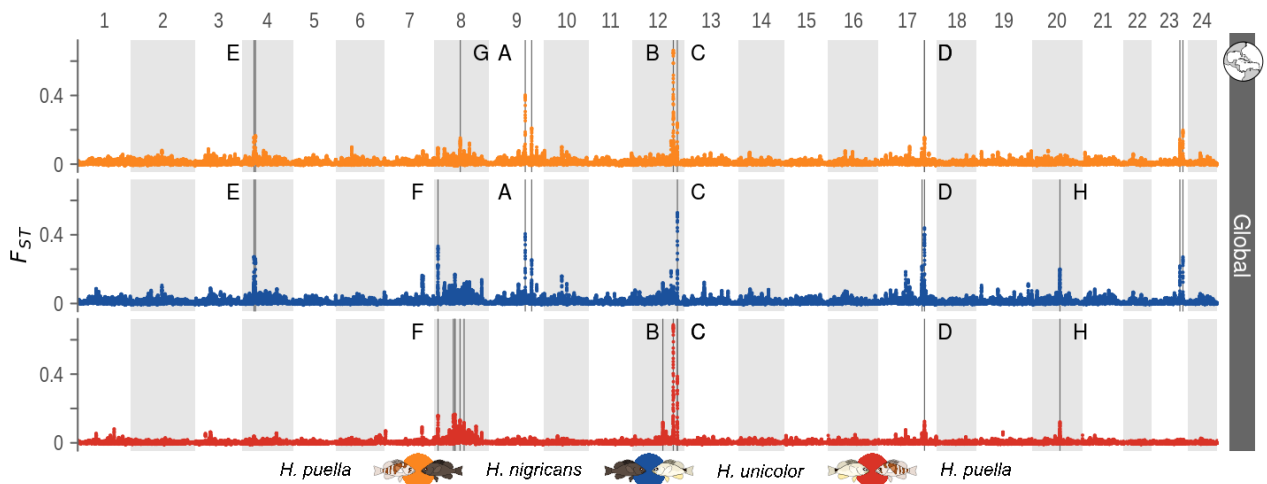
Supplementary Figure 7: Genomic intervals above the 99.90th F_{ST} percentile. F_{ST} values were estimated as the weighted mean per 50 kb window with 5 kb increments, considering all locations for each species. A total of 19 genomic intervals above the 99.90[th] F_{ST} percentile, highlighted with a vertical bar, were identified (Suppl. Tab. 1). Intervals containing candidate genes are labelled with capital letters. Peaks A − D are also above the 99.98[th] F_{ST} percentile, peaks E − H are not.
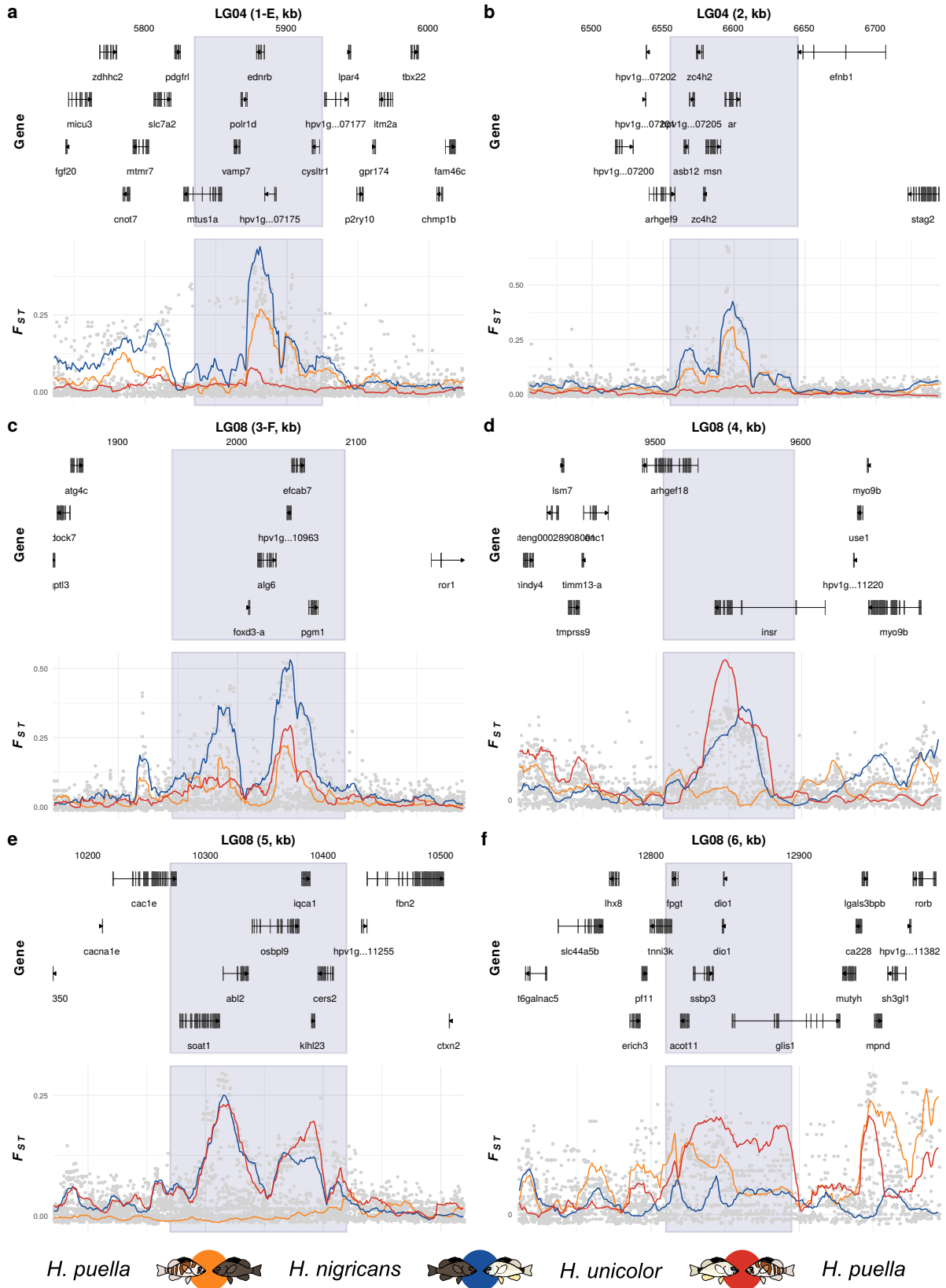
Supplementary Figure 8 (I): Close-up on all the intervals above the 99.90th F_{ST} percentile The panels (**a-r**, figure continued below) correspond to the 18 intervals above the 99.90th F_{ST} percentile. From top to bottom, each panel includes the respective linkage group (LG) and interval ID, the position on the LG, the gene model annotation and F_{ST} values. Gene models include the extent and direction of genes as well as exon boundaries. The F_{ST} plots show the pairwise comparisons among species (lines, weighted mean per 10 kb window with 1 kb increments). Additionally, the global F_{ST} values among the three species are shown as dots on a SNP basis. All comparisons with species samples pooled across the three locations. The highlighted area corresponds to the whole intervals as defined in Suppl. Tab. 1

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Supplementary Figure 8 (II): Close-up on all the intervals above the 99.90th F_{ST} percentile The panels (**a-r**, figure continued below) correspond to the 18 intervals above the 99.90[th] F_{ST} percentile. From top to bottom, each panel includes the respective linkage group (LG) and interval ID, the position on the LG, the gene model annotation and F_{ST} values. Gene models include the extent and direction of genes as well as exon boundaries. The F_{ST} plots show the pairwise comparisons among species (lines, weighted mean per 10 kb window with 1 kb increments). Additionally, the global F_{ST} values among the three species are shown as dots on a SNP basis. All comparisons with species samples pooled across the three locations. The highlighted area corresponds to the whole intervals as defined in Suppl. Tab. 1
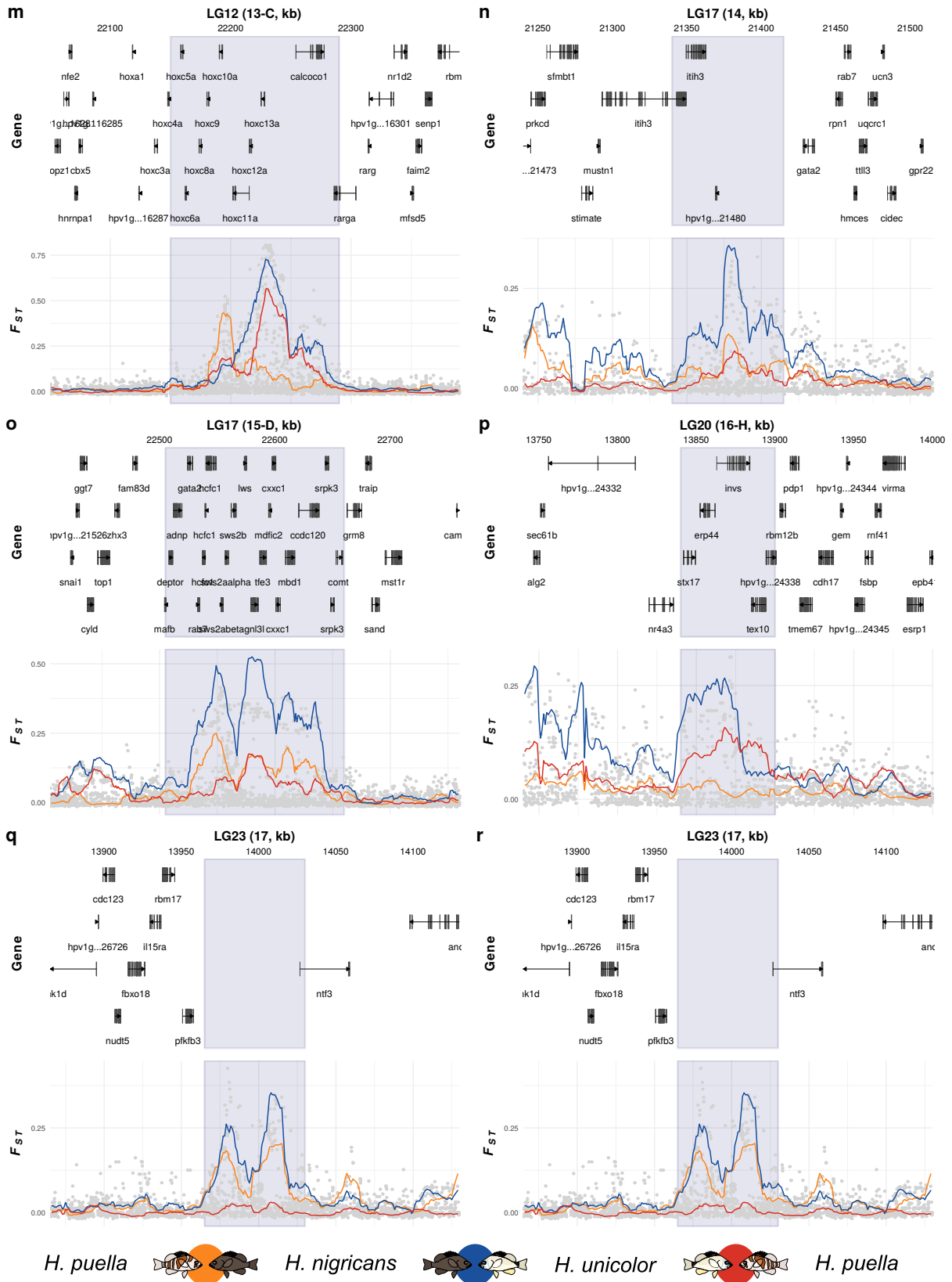
Supplementary Figure 8 (III): Close-up on all the intervals above the 99.90th F_{ST} percentile The panels (**a-r**) correspond to the 18 intervals above the 99.90[th] F_{ST} percentile. From top to bottom, each panel includes the respective linkage group (LG) and interval ID, the position on the LG, the gene model annotation and F_{ST} values. Gene models include the extent and direction of genes as well as exon boundaries. The F_{ST} plots show the pairwise comparisons among species (lines, weighted mean per 10 kb window with 1 kb increments). Additionally, the global F_{ST} values among the three species are shown as dots on a SNP basis. All comparisons with species samples pooled across the three locations. The highlighted area corresponds to the whole intervals as defined in Suppl. Tab. 1
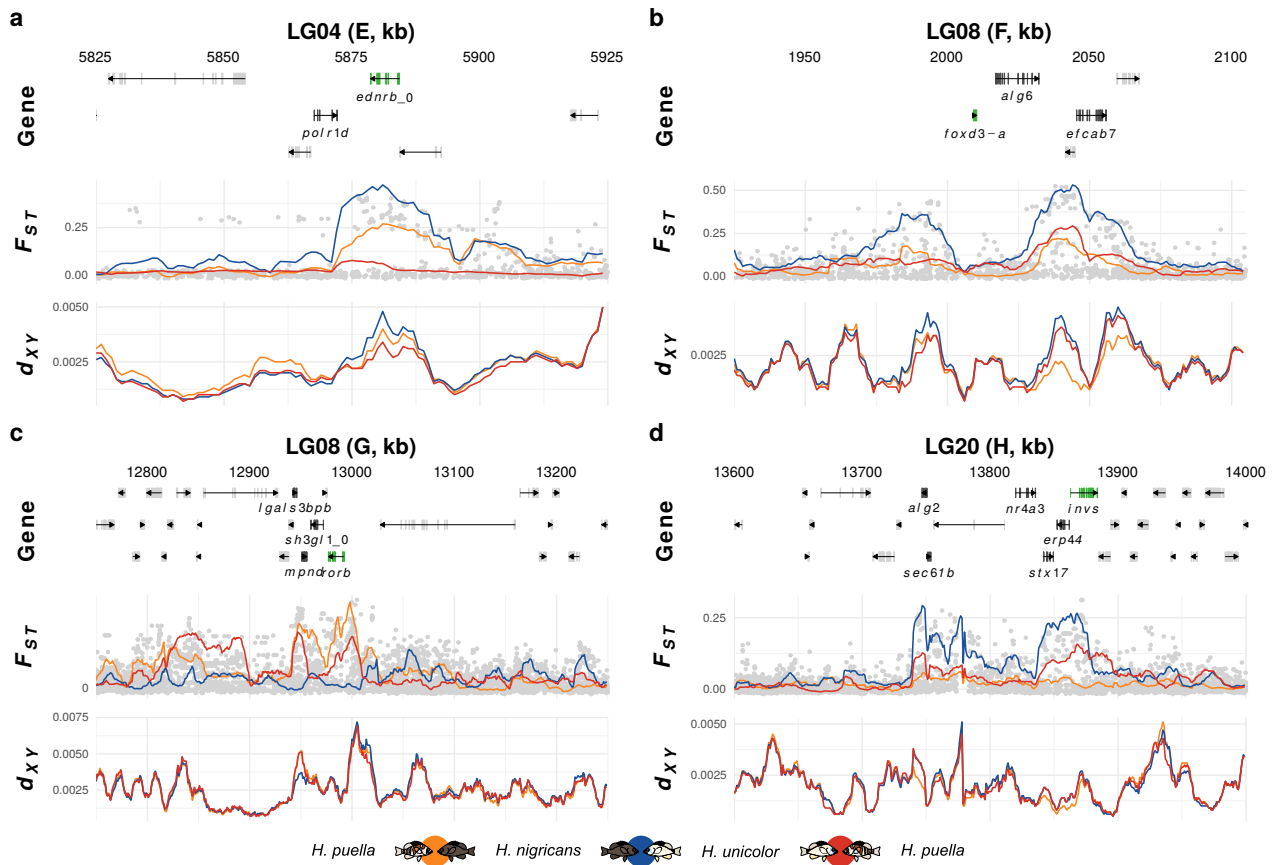
Supplementary Figure 9: Close-up on the four additional intervals containing candidate vision and pigmentation genes. The four panels (**a-d**) correspond to the four additional intervals above the 99.90[th] F_{ST} percentile (but not above the 99.98[th]) that include candidate vision and pigmentation genes. From top to bottom, each panel includes the respective linkage group (LG), the position on the LG, the gene model annotation, F_{ST} and d_{XY}. Gene models include the extent and direction of genes as well as exon boundaries. For clarity only the genes in high F_{ST} intervals are labelled, and candidate genes are highlighted in green. The F_{ST} plots show the pairwise comparisons among species (lines, weighted mean per 10 kb window with 1 kb increments). Additionally, the global F_{ST} values among the three species are shown as dots on a SNP basis. The d_{XY} values are also averaged over 10 kb windows with 1 kb increments. All comparisons with species samples pooled across the three locations.
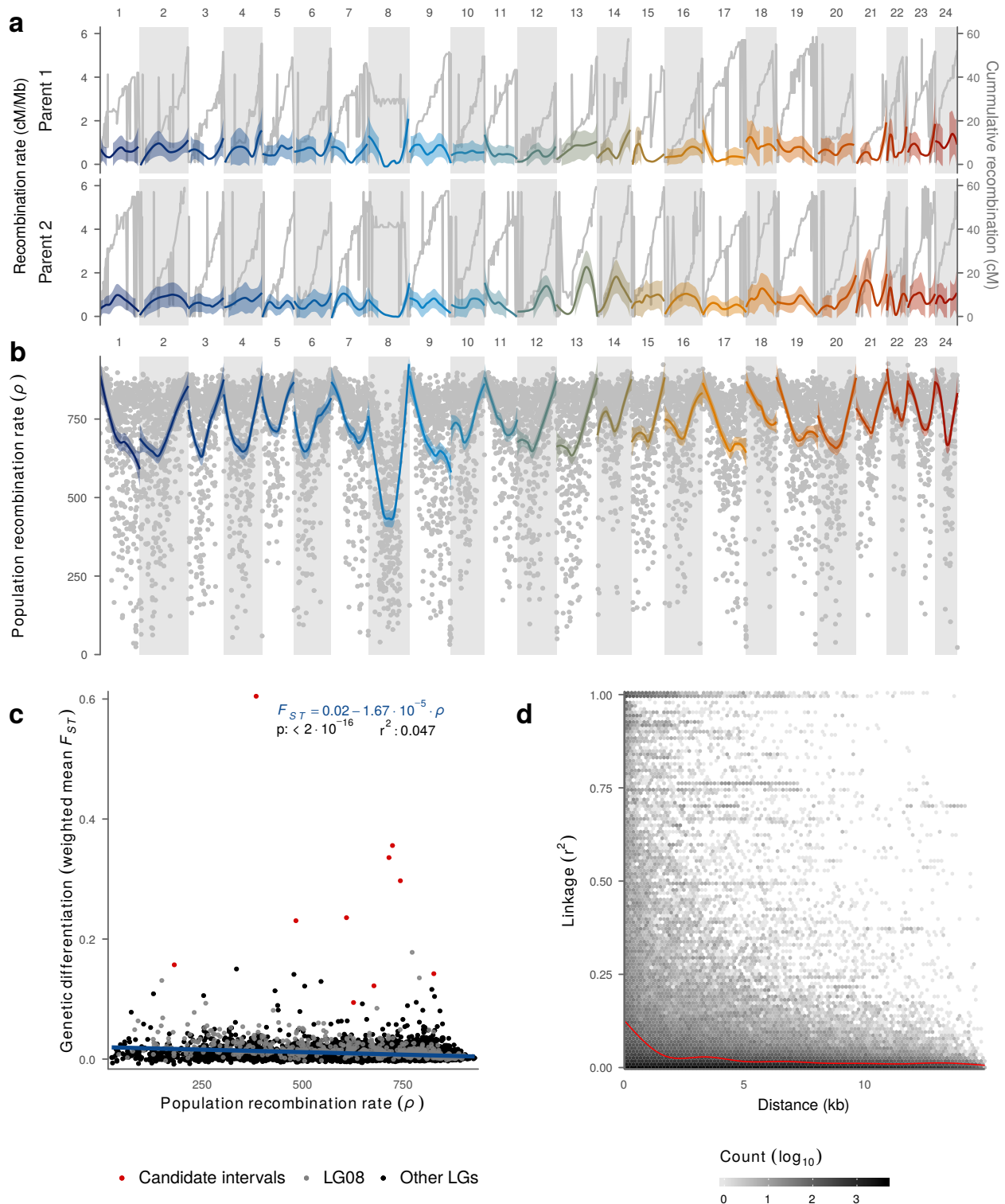
Supplementary Figure 10: Genome-wide recombination patterns. a, recombination landscape inferred from the combination of a within-species cross and the genome assembly. Recombination rate was inferred by mapping the linkage map[26] markers onto the genome assembly and dividing linkage (cM) by physical distance (Mb). **b**, recombination landscape inferred from population genomic data considering all species and locations. As expected due to the different data sets considered, the two types of recombination maps differ substantially. Yet both identify a large low-recombining region in LG08. **c**, correlation between population recombination rate and genetic differentiation among the three species considering non-overlapping 50-kb windows. As expected, a negative relationship is observed. The correlation and regression slope are nevertheless weak, indicating that recombination does not have a strong impact on differentiation at this stage of genomic divergence. Red dots correspond to the 50 kb windows that are within our four candidate regions; these windows do not show particularly low recombination rates. **d**, decay in linkage disequilibrium with physical distance, estimated over 20 randomly placed 15 kb windows. The shading of the hexagonal bins indicates the log_{10} count for each combination of distance and r^2 values. The red lines indicates a smoothing spline (gam, cubic regression spline) of the original data. Physical linkage decays rapidly within 2 kb.
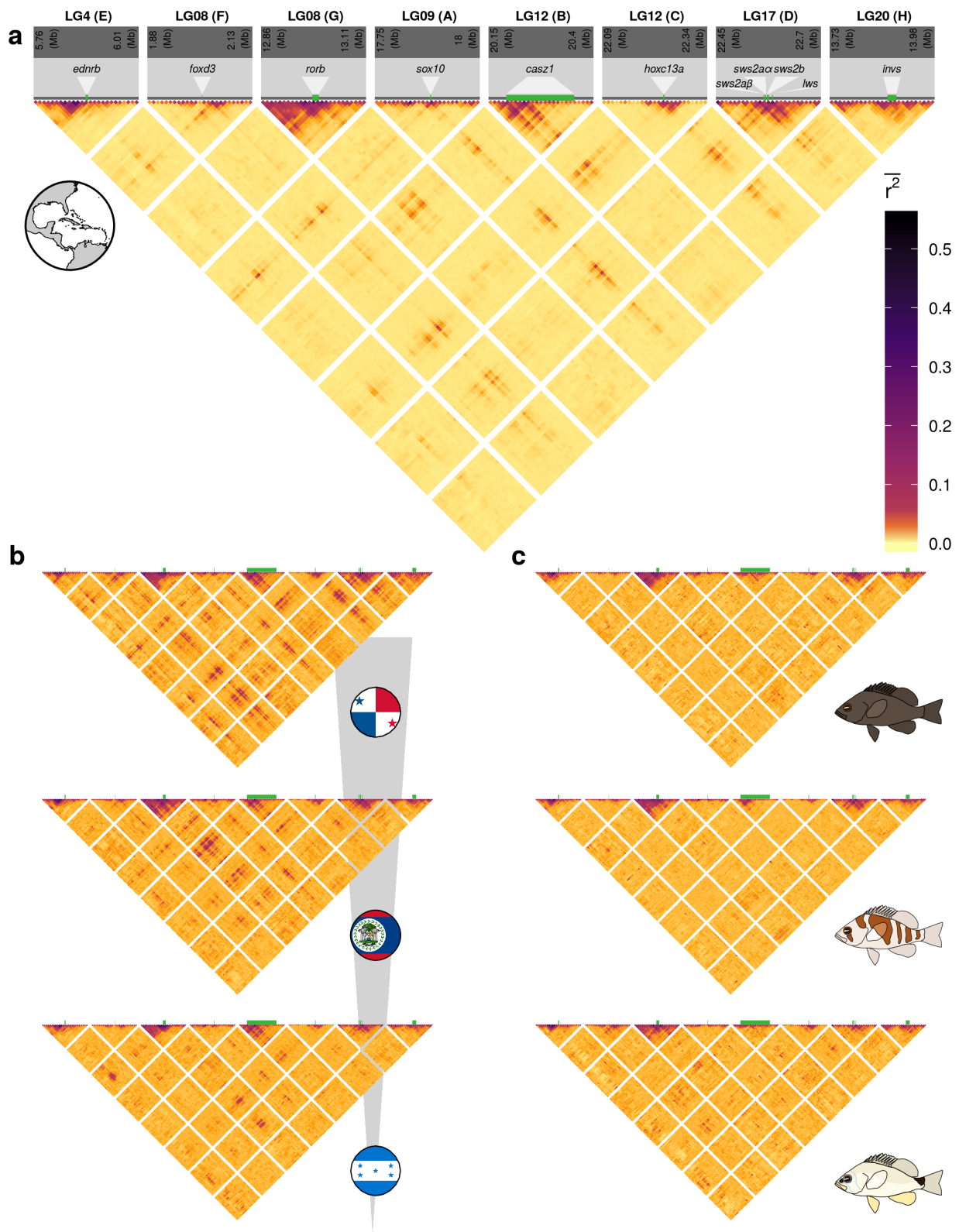
Supplementary Figure 11: Long-distance and inter-chromosomal linkage disequilibrium (LD) among the eight intervals containing candidate vision and pigmentation genes. a, the intervals identified in Fig. 2 & Suppl. Fig. 7 displayed increased long-distance and inter-chromosomal LD. LD was calculated between individual SNP pairs and averaged over 10 kb × 10 kb areas. **b**, LD among the eight intervals ordered by increasing differentiation among the three species (indicated by gray gradient, Fig. 1b,c, Suppl. Tab. 6). **c**, in contrast, LD was very low or absent within each of the three species.
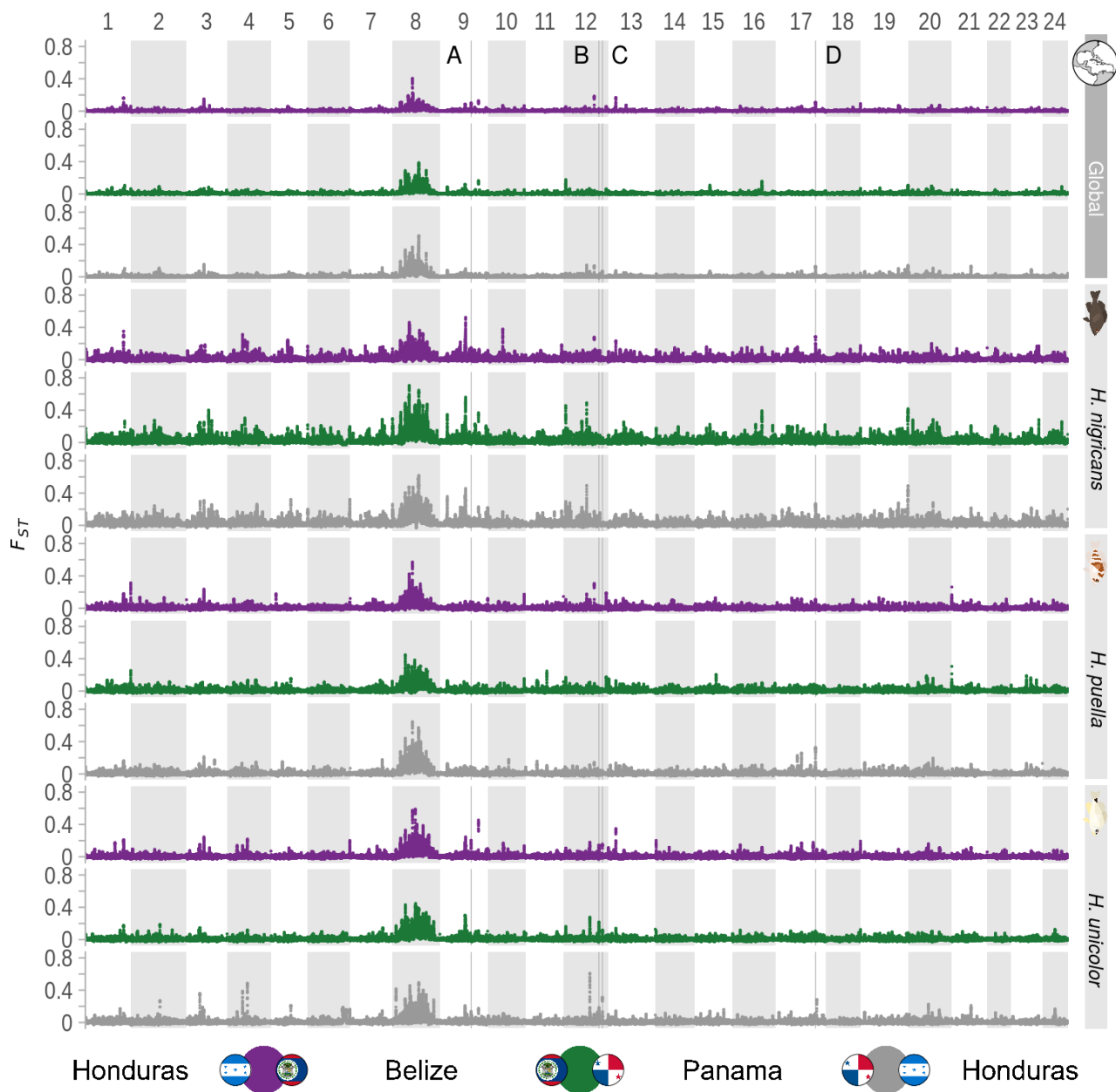
Supplementary Figure 12: Patterns of genomic differentiation among hamlets from Belize, Honduras and Panama. The alternating white and grey blocks represent the 24 linkage groups (LGs). Each population comparison is represented by one colour, pooled across species (Global) as well as within each species (*H. nigricans*, *H. puella* & *H. unicolor*). F_{ST} values were estimated as the weighted mean per 50 kb window with 5 kb increments. The four genomic regions highlighted with a vertical line, included as reference, correspond to the four intervals identified in **Fig. 2**.
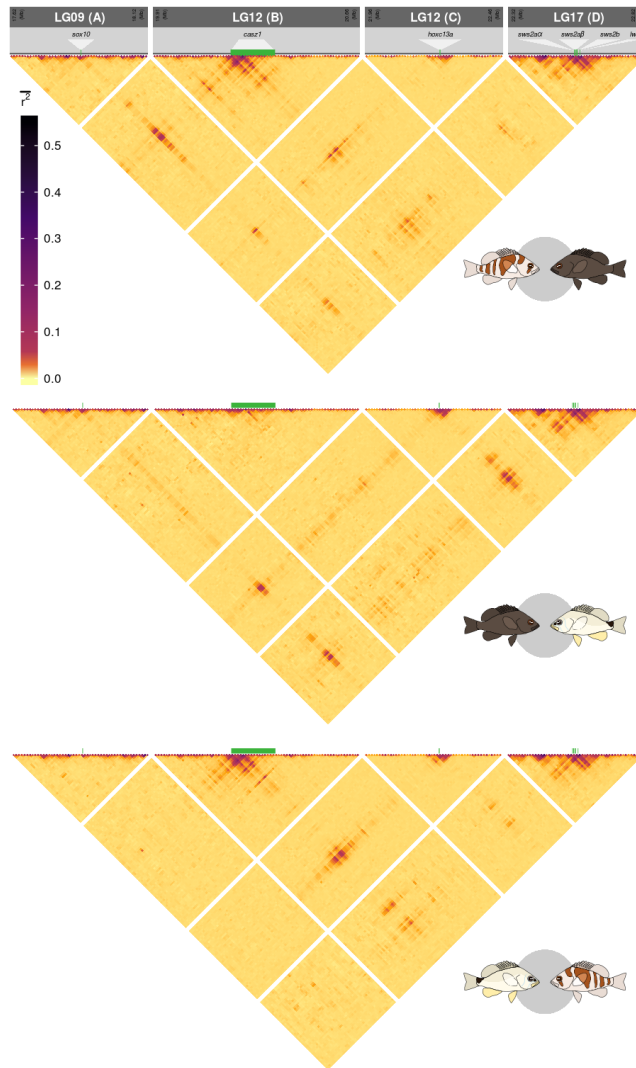
Supplementary Figure 13: Long-distance and inter-chromosomal linkage disequilibrium (LD) among the four candidate intervals for each species pair. The intervals identified in Fig. 2 displayed increased long-distance and inter-chromosomal LD, yet different pairs of intervals were in LD in each species pair. LD calculated between individual SNP pairs and averaged over 10 kb × 10 kb areas.
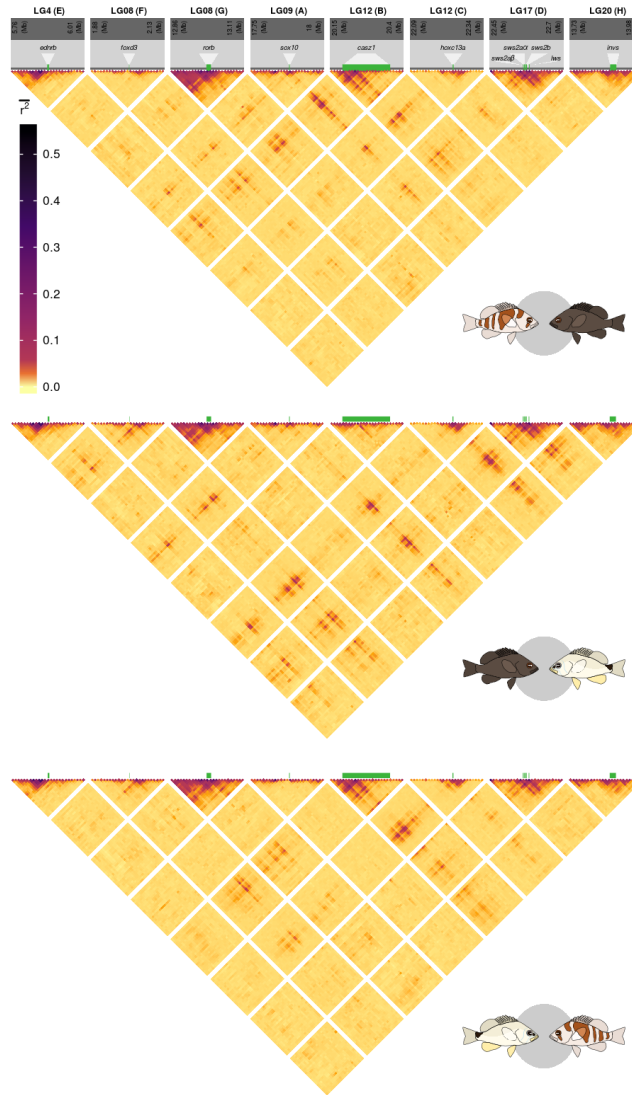
Supplementary Figure 14: Long-distance and inter-chromosomal linkage disequilibrium (LD) among the eight intervals containing vision and pigmentation candidate genes. The intervals identified in & Suppl. Fig. 7 displayed increased long-distance and inter-chromosomal LD, yet different pairs of genes were in LD in each species pair. LD was calculated between individual SNP pairs and averaged over 10 kb × 10 kb areas.
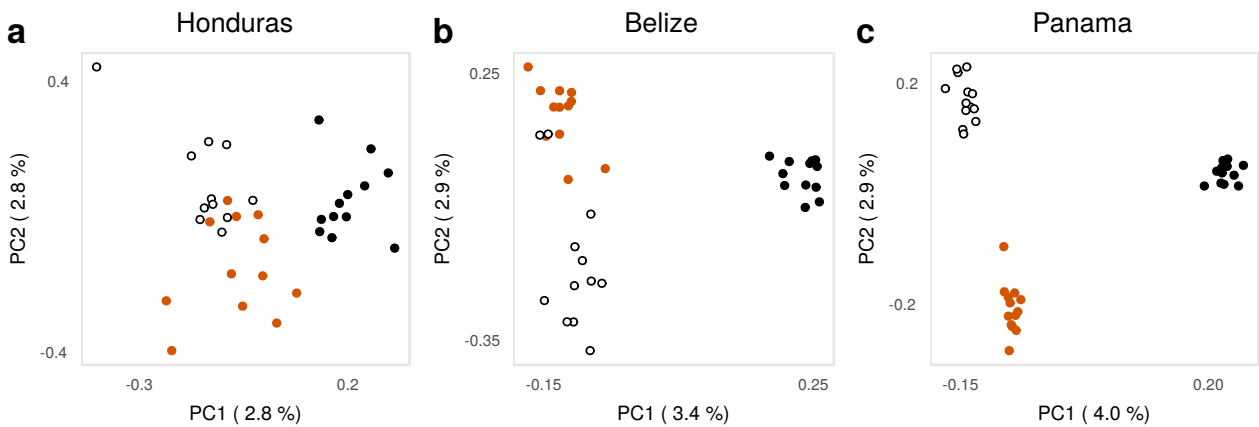
Supplementary Figure 15: PCA based on filtered data set. Principal Component Analysis (PCA) within each location. Genomic data was filtered for a minimum distance of 25 kb between SNPs to rule out physical linkage. After filtration 22,266 SNPs (0.3%) of the original data set remained.
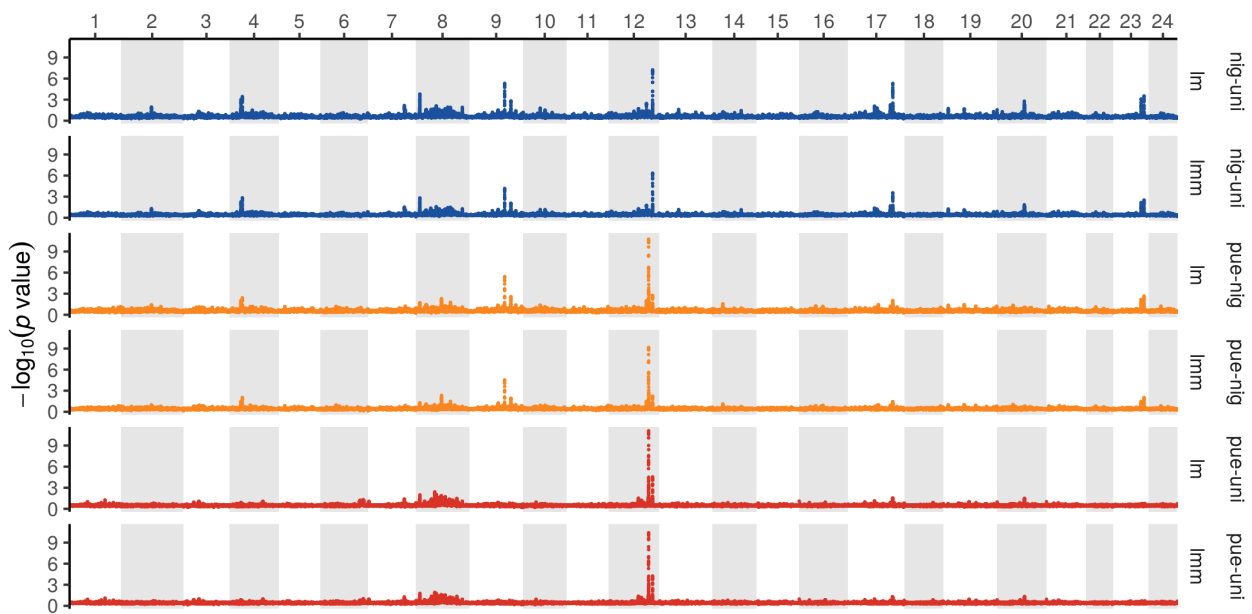
Supplementary Figure 16: Comparison of linear model and linear mixed model results of the genotype by phenotype (G × P) association among black (*H. nigricans*), barred (*H. puella*) and butter (*H. unicolor*) hamlets. Each species pair is represented by one colour, pooled across locations. Species comparisons are indicated on the right as well as model type (lm: linar model; lmm: linear mixed model). The *p* values are from the linear model with Wald test, transformed using the negative of the common logarithm and averaged across 50 kb window with 5 kb increments ($-\overline{\log_{10}(p)}$).
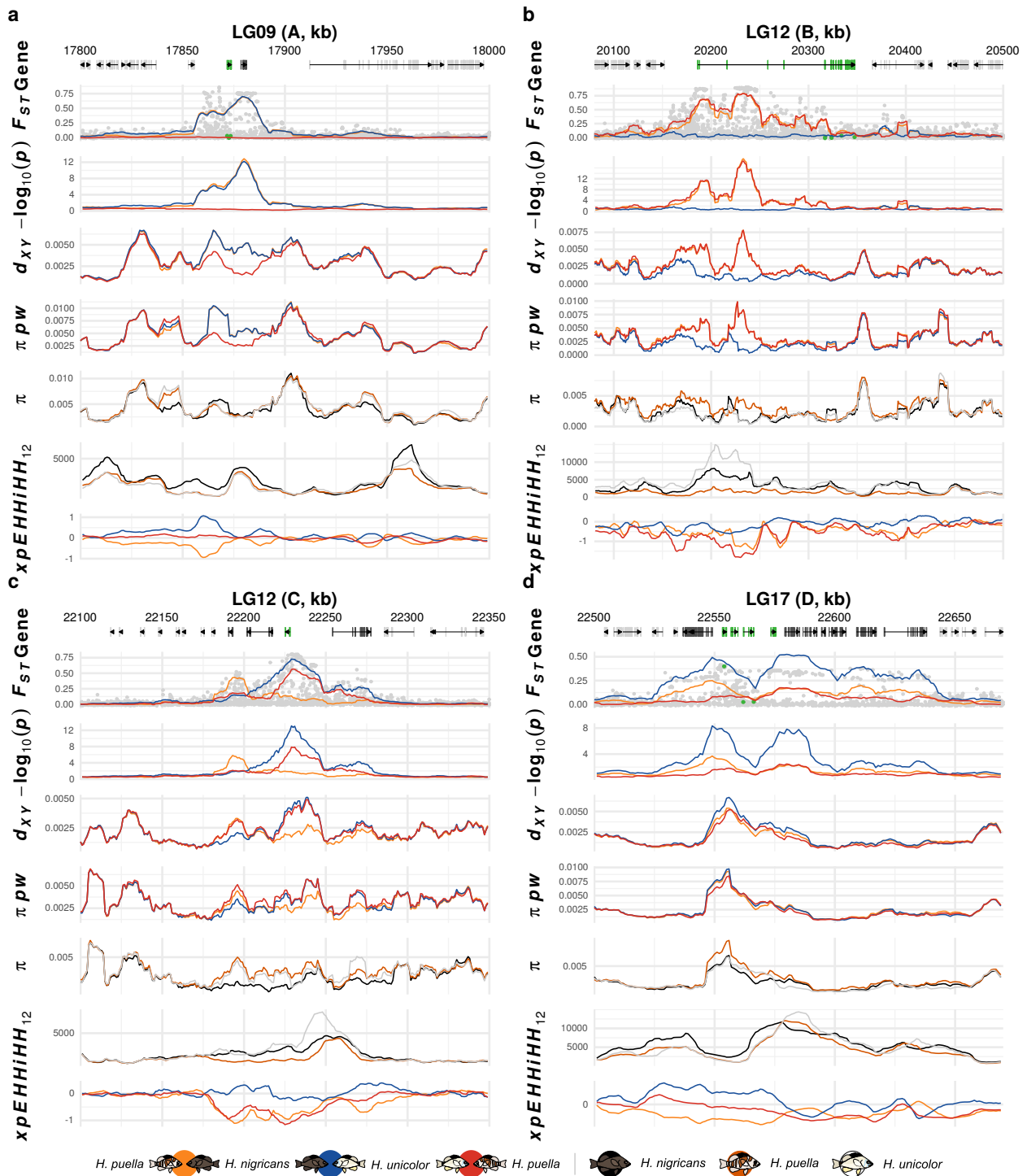
Supplementary Figure 17: Extended close-up on the four intervals above the 99.98th F_{ST} percentile. The four panels (**a-d**) correspond to the four intervals above the 99.98^{th} F_{ST} percentile identified in **Fig. 2** (A-D). From top to bottom, each panel includes the respective linkage group (LG), the position on the LG, the position of genes, F_{ST}, the $-\log_{10}(p)$ value from the G × P association, d_{XY}, π (per species and species pair), iHH$_{12}$ and xpEHH values. Candidate genes are highlighted in high green. All statistics show the pairwise comparisons among species (lines, averaged per 10 kb window with 1 kb increments). On the F_{ST} track, the global F_{ST} values among the three species are shown as dots on a SNP basis. Non-synonymous SNPs in candidate genes are highlighted in green. All comparisons with species samples pooled across the three locations.

Supplementary Tables

Supplementary Table 1: Genomic regions above the 99.90th F_{ST} percentile, based on sliding window analysis with 50 kb windows and 5 kb increments. The Comparison column refers to the specific pairs in which the region is above the 99.90th F_{ST} percentile: *H. nigricans vs. H. puella* (NP), *H. nigricans vs. H. unicolor* (NU) & *H. puella vs. H. unicolor* (NP). Regions containing candidate genes are labelled with capital letters. Regions A − D are also above the 99.98th F_{ST} percentile. The **Other genes** column includes all genes overlapping with the 50-kb windows above the 99.90th F_{ST} percentile. Note that this approach conservatively includes genes situated before and after peaks of differentiation at a 10-kb window resolution (Suppl. Fig. 8).

Nr	ID	LG	Start (kb)	End (kb)	Candidate genes	Other genes	Comparison
1	E	04	5835	5925	*ednrb*	*polr1d, hpv1g...7175, cysltr1, mtus1a, vamp7*	NP,NU
2		04	6555	6645		*asb12, zc4h2, hpv1g...7205, msn, ar, arhgef9, efnb1*	NP,NU
3	F	08	1945	2090	*foxd3*	*hpv1g...10963, alg6, efcab7, pgm1*	NU,PU
4		08	9505	9595		*arhgef18, insr*	PU
5		08	10270	10420		*klhl23, soat1, abl2, iqca1, cers2, cac1e, osbpl9*	PU
6		08	12810	12895		*fpgt, acot11, dio1, tnni3k, ssbp3, glis1*	PU
7	G	08	12945	13010	*rorb*	*mpnd, sh3gl1, hpv1g...11382, lgals3bpb*	NP
8		08	14825	14875		*ctdspl2a, arid3a, tmem79*	PU
9	A	09	17821	17930	*sox10*	*smdt1, hpv1g...12847, kcnj12, rnaseh2a, mast1, triobp*	NP,NU
10		09	20995	21085		*rnf24, smox, fbxo41*	NP,NU
11		12	15050	15105		*csf1, ren, eif2d, hpv1g...15947*	PU
12	B	12	20085	20355	*casz1*	*pgd, c1orf127, kif1c, kif1b, tardbp*	NP,PU
13	C	12	22150	22290	*hoxc13a*	*hoxc5a, hoxc6a, hoxc8a, hoxc9, hoxc10a, hoxc11a, hoxc12a, calcoco1, rarga*	NP,NU,PU
14		17	21340	21415		*itih3, hpv1g...21480*	NU
15	D	17	22505	22660	*lws, sws2aβ, sws2aα, sws2b*	*mafb, deptor, adnp, rab7, hcfc1, gnl3l, tfe3, mdfic2, cxxc1, srpk3, comt, gata2, mbd1, ccdc120*	NP,NU,PU
16	H	20	13840	13900	*invs*	*hpv1g...24338, stx17, erp44, tex10*	NU,PU
17		23	13965	14030		*ntf3*	NP,NU
18		23	15445	15530		*crys, glipr1l1, ache, nxpe3, krr1, st3gal1, gp2*	NP,NU

Supplementary Table 2: Software versions used in this study

Software	version	Software	version	Software	version
Allmaps	Version 1	LAST	737	proovread	2.13.13
bedtools	v2.27.1	Maker	v 3.0	R	3.4.1 (calculations)
Bowtie2	version 2.3.4.1	msa2vcf			3.4.3 (visualisations)
BUSCO	2	98d97d07d6101fab1b0bef757b4ceee279e171d9		RepeatMasker	Open-4.0.6
BWA	0.7.12-r1044	msms	3.2rc	RepeatModler	open-1-0-8
Circos	v 0.69	MultiQC	Version 0.8	SAMtools	1.7
FastQC	v0.11.3	NextClip	v1.3.1	selscan	v1.2.0a
GATK	v3.7-0-gcfedb67	NextFlow	0.31.1	SeqChunker	v0.22.2
GEMMA	0.97.2	NewHybrids	2.0+ Developmental	seq-gen	1.3.4
gffread	v0.9.12	PBjelly	v14.1	seqtk	1.2-r94
HISAT2	2.0.4	PGDSpider	2.1.1.5	SHAPEIT	v2.r837
Inkscape	0.91 r13725	Picard Tools	2.9.2-SNAPSHOT	Trimmomatic	0.33
kallisto	0.43.1	Platanus	1.2.4	Trinity	v2.2.0
Kraken	0.10.6-unreleased	plink	v1.90b4 64-bit	VCFtools	0.1.15

Supplementary Table 3a: Samples used for resequencing (Samples 1-50).

ID	Species	Location	Date	Latitude	Longitude	Cov.	Acces. Nr.
18151	*H. nigricans*	Belize	2004-07-25	16.7653	-088.1442	22.6	ERS2619600
18153	*H. nigricans*	Belize	2004-07-25	16.7653	-088.1442	23.7	ERS2619601
18155	*H. nigricans*	Belize	2004-07-25	16.8008	-088.0789	21.2	ERS2619602
18156	*H. nigricans*	Belize	2004-07-25	16.8008	-088.0789	26.5	ERS2619603
18157	*H. nigricans*	Belize	2004-07-25	16.8008	-088.0789	23.2	ERS2619604
18158	*H. nigricans*	Belize	2004-07-25	16.8008	-088.0789	21.6	ERS2619605
18159	*H. nigricans*	Belize	2004-07-25	16.8008	-088.0789	22.5	ERS2619606
18162	*H. nigricans*	Belize	2004-07-25	16.7653	-088.1442	19.1	ERS2619607
18165	*H. nigricans*	Belize	2004-07-25	16.7653	-088.1442	22.9	ERS2619608
18171	*H. nigricans*	Belize	2004-07-25	16.7653	-088.1442	26.4	ERS2619609
18185	*H. nigricans*	Belize	2004-07-26	16.8058	-088.0792	25.6	ERS2619610
18187	*H. nigricans*	Belize	2004-07-26	16.8058	-088.0792	26.6	ERS2619611
20599	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	24.2	ERS2619612
20600	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	25.6	ERS2619613
20601	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	22.8	ERS2619614
20602	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	23	ERS2619615
20603	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	26	ERS2619616
20604	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	20.3	ERS2619617
20605	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	22.9	ERS2619618
20606	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	22.6	ERS2619619
20607	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	25.9	ERS2619620
20608	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	24.6	ERS2619621
20609	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	26.2	ERS2619622
20610	*H. nigricans*	Honduras	2006-06-04	15.9558	-083.2931	22.9	ERS2619623
16_21-30	*H. nigricans*	Panama	2016	-	-	89.1	ERS2619624
18418	*H. nigricans*	Panama	2004-05-12	09.3775	-082.3039	18.7	ERS2619625
18424	*H. nigricans*	Panama	2004-05-12	10.2392	-083.1731	19.3	ERS2619626
18428	*H. nigricans*	Panama	2004-05-12	10.2392	-083.1731	19.1	ERS2619627
18436	*H. nigricans*	Panama	2004-05-12	09.3775	-082.3039	9.8	ERS2619628
18901	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	25.6	ERS2619629
18902	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	22.4	ERS2619630
18903	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	17.1	ERS2619631
18904	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	20.1	ERS2619632
18905	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	22.2	ERS2619633
18906	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	26.7	ERS2619634
18907	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	18.1	ERS2619635
18909	*H. nigricans*	Panama	2005-03-25	09.2983	-082.2894	21.9	ERS2619636
18152	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	23.4	ERS2619637
18154	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	24.6	ERS2619638
18161	*H. puella*	Belize	2004-07-26	16.8058	-088.0792	18.4	ERS2619639
18166	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	24.2	ERS2619640
18169	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	19.6	ERS2619641
18172	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	23.4	ERS2619642
18174	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	24.8	ERS2619643
18175	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	22.2	ERS2619644
18176	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	29	ERS2619645
18178	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	22.9	ERS2619646
18179	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	22.1	ERS2619647
18180	*H. puella*	Belize	2004-07-25	16.7653	-088.1442	21.7	ERS2619648
20551	*H. puella*	Honduras	2006-06-04	15.9558	-083.2931	23	ERS2619649

ID	Species	Location	Date	Latitude	Longitude	Cov.	Acces. Nr.
20552	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	24.5	ERS2619650
20553	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	26.2	ERS2619651
20554	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	22	ERS2619652
20555	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	23.2	ERS2619653
20556	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	29.1	ERS2619654
20558	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	25.5	ERS2619655
20559	*H. puella*	Honduras	2006-06-04	15.9558	-83.2931	26	ERS2619656
20625	*H. puella*	Honduras	2006-06-05	15.9558	-83.2931	21.8	ERS2619657
20633	*H. puella*	Honduras	2006-06-05	15.9558	-83.2931	27.6	ERS2619658
20635	*H. puella*	Honduras	2006-06-05	15.9558	-83.2931	26.3	ERS2619659
20638	*H. puella*	Honduras	2006-06-05	15.9558	-83.2931	21.7	ERS2619660
18419	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	19.8	ERS2619661
18421	*H. puella*	Panama	2004-05-12	22.7		22.7	ERS2619662
18422	*H. puella*	Panama	2004-05-12	9.3775	-82.3039	22.9	ERS2619663
18426	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	20.9	ERS2619664
18427	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	21.3	ERS2619665
18429	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	23.5	ERS2619666
18430	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	19.5	ERS2619667
18432	*H. puella*	Panama	2004-05-12	10.2392	-83.1731	21.6	ERS2619668
18434	*H. puella*	Panama	2004-05-12	9.3775	-82.3039	25.7	ERS2619669
18912	*H. puella*	Panama	2005-03-25	9.2983	-82.2894	23	ERS2619670
18915	*H. puella*	Panama	2005-03-25	9.2983	-82.2894	19.4	ERS2619671
18917	*H. puella*	Panama	2005-03-25	9.2983	-82.2894	18.8	ERS2619672
27678	*H. puella*	Panama	2013-04-12	9.3681	-82.2928	89.8	ERS2619673
18163	*H. unicolor*	Belize	2004-07-25	16.7653	-88.1442	24.5	ERS2619674
18261	*H. unicolor*	Belize	2004-07-24	-	-	21.8	ERS2619675
18267	*H. unicolor*	Belize	2004-07-24	-	-	28.4	ERS2619676
18274	*H. unicolor*	Belize	2004-07-24	-	-	24.4	ERS2619677
18276	*H. unicolor*	Belize	2004-07-25	16.7653	-88.1442	23.8	ERS2619678
19881	*H. unicolor*	Belize	2005-08-16	16.7078	-87.8598	23.7	ERS2619679
20092	*H. unicolor*	Belize	2005-08-15	16.8936	-88.1226	23.5	ERS2619680
20120	*H. unicolor*	Belize	2005-08-11	16.8008	-88.0789	30.3	ERS2619681
20126	*H. unicolor*	Belize	2005-08-12	16.8936	-88.1226	22.4	ERS2619682
20128	*H. unicolor*	Belize	2005-08-12	16.8936	-88.1226	22.6	ERS2619683
20135	*H. unicolor*	Belize	2005-08-12	16.8936	-88.1226	21.2	ERS2619684
20149	*H. unicolor*	Belize	2005-08-12	16.8936	-88.1226	23.5	ERS2619685
20560	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	21.3	ERS2619686
20561	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	24.2	ERS2619687
20562	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	25.4	ERS2619688
20563	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	23.1	ERS2619689
20564	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	28.9	ERS2619690
20565	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	25	ERS2619691
20566	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	27.4	ERS2619692
20567	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	27.3	ERS2619693
20568	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	30.1	ERS2619694
20571	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	24.6	ERS2619695
20572	*H. unicolor*	Honduras	2006-06-04	15.9558	-83.2931	23.8	ERS2619696
16_31-40	*H. unicolor*	Panama	2016	-	-	53.4	ERS2619697
18420	*H. unicolor*	Panama	2004-05-12	10.2392	-83.1731	20.3	ERS2619698
18435	*H. unicolor*	Panama	2004-06-23	9.3328	-82.2547	20.2	ERS2619699

Supplementary Table 3c: Samples used for resequencing (continued, Samples 101-110).

ID	Species	Location	Date	Latitude	Longitude	Cov.	Acces. Nr.
18439	*H. unicolor*	Panama	2004-06-25	9.3328	-82.2547	23.8	ERS2619700
18440	*H. unicolor*	Panama	2004-06-25	9.3328	-82.2547	19.7	ERS2619701
18441	*H. unicolor*	Panama	2004-06-25	9.3328	-82.2547	25.1	ERS2619702
18442	*H. unicolor*	Panama	2004-07-08	9.2983	-82.2894	20.6	ERS2619703
18445	*H. unicolor*	Panama	2004-06-28	9.3328	-82.2547	19	ERS2619704
18446	*H. unicolor*	Panama	2004-06-29	9.3328	-82.2547	26	ERS2619705
18447	*H. unicolor*	Panama	2004-07-09	9.2894	-82.2589	16.8	ERS2619706
18448	*H. unicolor*	Panama	2004-06-28	9.3328	-82.2547	26.1	ERS2619707
18450	*H. unicolor*	Panama	2004-06-25	9.3328	-82.2547	17.7	ERS2619708
18454	*H. unicolor*	Panama	2004-06-30	9.3481	-82.2633	22.7	ERS2619709

Supplementary Table 4: Overview of the sequencing data generated for the assembly of the *Hypoplectrus* genome (PE: paired end, MP: mate pair, PB: PacBio)

Nr	Tissue	Type	Type	Targeted insert size (bp)	Mapped insert size (bp, mean $\pm$ SD)	Read length (bp)	Coverage ($\times$)
1	gill	PE	DNA	300	264 $\pm$ 94	151	24
2	gill	PE	DNA	300	255 $\pm$ 80	151	144
3	gill	PE	DNA	500	299 $\pm$ 106	151	46
4	gill	PE	DNA	800	321 $\pm$ 123	151	67
5	muscle	PE (PCR-free)	DNA	550	579 $\pm$ 155	251	100
6	muscle	MP	DNA	3000	2457 $\pm$ 639	101	34
7	muscle	MP	DNA	6000	4329 $\pm$ 1110	101	31
8	muscle	PB	DNA	-	-	50$-$33680	16
9	gill	PE	RNA	-	-	251	-
10	muscle	PE	RNA	-	-	251	-
11	liver	PE	RNA	-	-	251	-

Supplementary Table 5: Samples used for RNA sequencing

ID	Date	Species	Latitude	Longitude	Raw reads (n, 10^6)	Filtered	Alignment rate (%)	Acession Number
28385	2017-02-06	*H. nigricans*	09.318	-082.222	2×7.4	2×4.7	60.84	ERS2619746
28387	2017-02-06	*H. nigricans*	09.318	-082.222	2×6.3	2×4.7	59.34	ERS2619747
28390	2017-02-06	*H. nigricans*	09.318	-082.222	2×5.9	2×4.4	61.72	ERS2619748
28394	2017-02-07	*H. nigricans*	09.301	-082.294	2×4.8	2×3.0	63.55	ERS2619749
28399	2017-02-07	*H. nigricans*	09.301	-082.294	2×7.3	2×5.2	62.11	ERS2619750
AG9RX46	2017-02-06	*H. nigricans*	09.318	-082.222	2×7.1	2×5.3	60.73	ERS2619751
AG9RX49	2017-02-07	*H. nigricans*	09.301	-082.294	2×7.1	2×5.3	63.38	ERS2619752
AG9RX50	2017-02-07	*H. nigricans*	09.301	-082.294	2×6.8	2×4.8	60.68	ERS2619753
AG9RX52	2017-02-07	*H. nigricans*	09.301	-082.294	2×6.7	2×4.9	59.98	ERS2619754
28384	2017-02-06	*H. puella*	9.318	-082.222	2×6.1	2×4.2	63.13	ERS2619755
AG9RX47	2017-02-06	*H. puella*	09.318	-082.222	2×5.5	2×4.0	68.03	ERS2619756
AG9RX48	2017-02-07	*H. puella*	09.301	-082.294	2×6.2	2×4.5	66.079	ERS2619757
AG9RX51	2017-02-07	*H. puella*	09.301	-082.294	2×6.7	2×5.0	61.41	ERS2619758
AG9RX53	2017-02-07	*H. puella*	09.301	-082.294	2×6.3	2×4.5	62	ERS2619759
PL17_02	2017-02-07	*H. puella*	09.301	-082.294	2×6.1	2×4.6	62.61	ERS2619760
PL17_04	2017-02-07	*H. puella*	09.301	-082.294	2×6.5	2×4.7	63.17	ERS2619761
PL17_16	2017-02-09	*H. puella*	09.367	-082.291	2×7.5	2×5.3	62.1	ERS2619762
PL17_17	2017-02-09	*H. puella*	09.367	-082.291	2×7.3	2×5.3	61.72	ERS2619763
PL17_18	2017-02-09	*H. puella*	09.367	-082.291	2×6.3	2×4.6	63.68	ERS2619764
28383	2017-02-06	*H. unicolor*	09.318	-082.222	2×6.8	2×4.7	61.04	ERS2619765
28391	2017-02-07	*H. unicolor*	09.301	-082.294	2×7.1	2×5.2	61.75	ERS2619766
AG9RX54	2017-02-06	*H. unicolor*	09.318	-082.222	2×5.5	2×3.9	59.2	ERS2619767
AG9RX55	2017-02-07	*H. unicolor*	09.301	-082.294	2×6.3	2×4.7	61.54	ERS2619768
PL17_01	2017-02-07	*H. unicolor*	09.301	-082.294	2×8.1	2×5.9	62.11	ERS2619769

Supplementary Table 6: Whole-genome weighted mean F_{ST} estimates among *H. nigricans*, *H. puella* & *H. unicolor*.

Location	*H. nigricans* *H. puella*	*H. nigricans* *H. unicolor*	*H. puella* *H. unicolor*	All
Global	0.0079	0.0098	0.0027	0.0068
Belize	0.0168	0.0153	0.0047	0.0123
Honduras	0.0033	0.0051	0.0030	0.0038
Panama	0.0274	0.0348	0.0125	0.0249

Supplementary Table 7: Anchoring statistics. Size selection refers to the removal of contigs smaller than 501 bp, which was done after anchoring.

	pre size selection			post size selection (> 500 bp)		
	Anchored	Oriented	Unplaced	Anchored	Oriented	Unplaced
Markers (unique)	2602	1991	0	2658	2012	0
Markers per Mb	4.6	4.5	0	4.7	4.6	0
N50 Scaffolds	50	48	0	38	36	0
Scaffolds	376	131	502438	380	127	14341
with 1 marker	128	0	0	131	0	0
with 2 markers	64	17	0	65	17	0
with 3 markers	33	12	0	32	11	0
⩾ 4 markers	151	102	0	152	99	0
Total bases	559 614 477	437 616 820	140 002 979	561 222 590	434 765 886	51 030 271
percent	80.0	62.6	20.0	91.7	71.0	8.3

Supplementary Table 8: Assembly statistics

	Super-Scaffolds	Scaffolds	Contigs	BUSCO	n	%
Sequences (n)	14 375	14 721	58 827	Complete Single-Copy	2597	85
Min (bp)	501	501	28	Complete Duplicated	61	2.0
Median (bp)	902	919	3 972	Fragmented	218	7.2
Mean (bp)	42 594	41 590	10 240	Missing	208	6.8
Max (bp)	31 702 000	17 788 340	212 451	Total	3023	100
Total (bp)	612 290 098	612 252 861	602 376 265			
n50 (bp)	24 210 077	4 424 055	25 823			
n90 (bp)	13 699 534	304 599	5 215			
n95 (bp)	108 356	2 813	89 255			