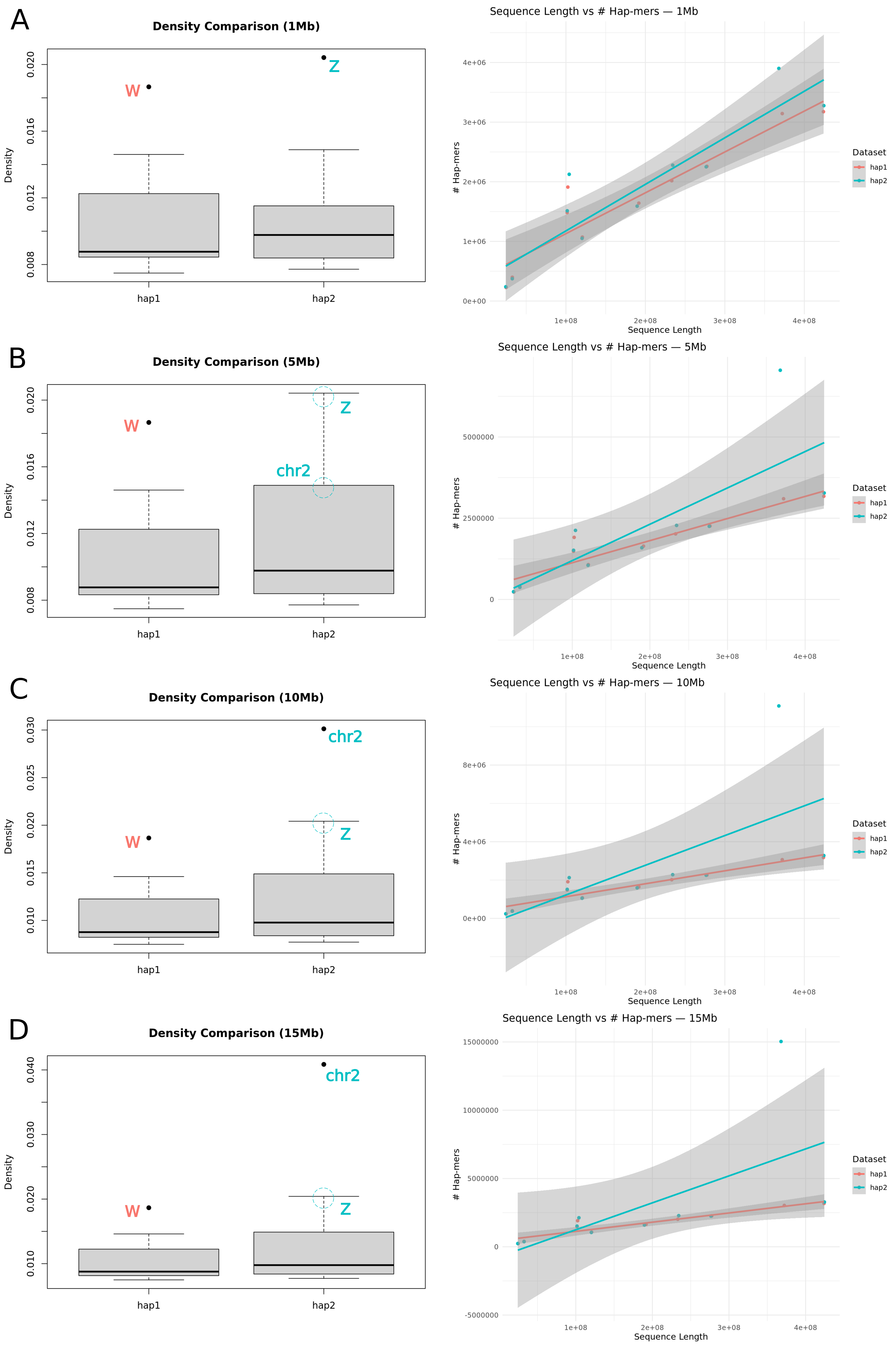
Supplemental materials

Supplemental Table 1: Sample list and accession numbers.

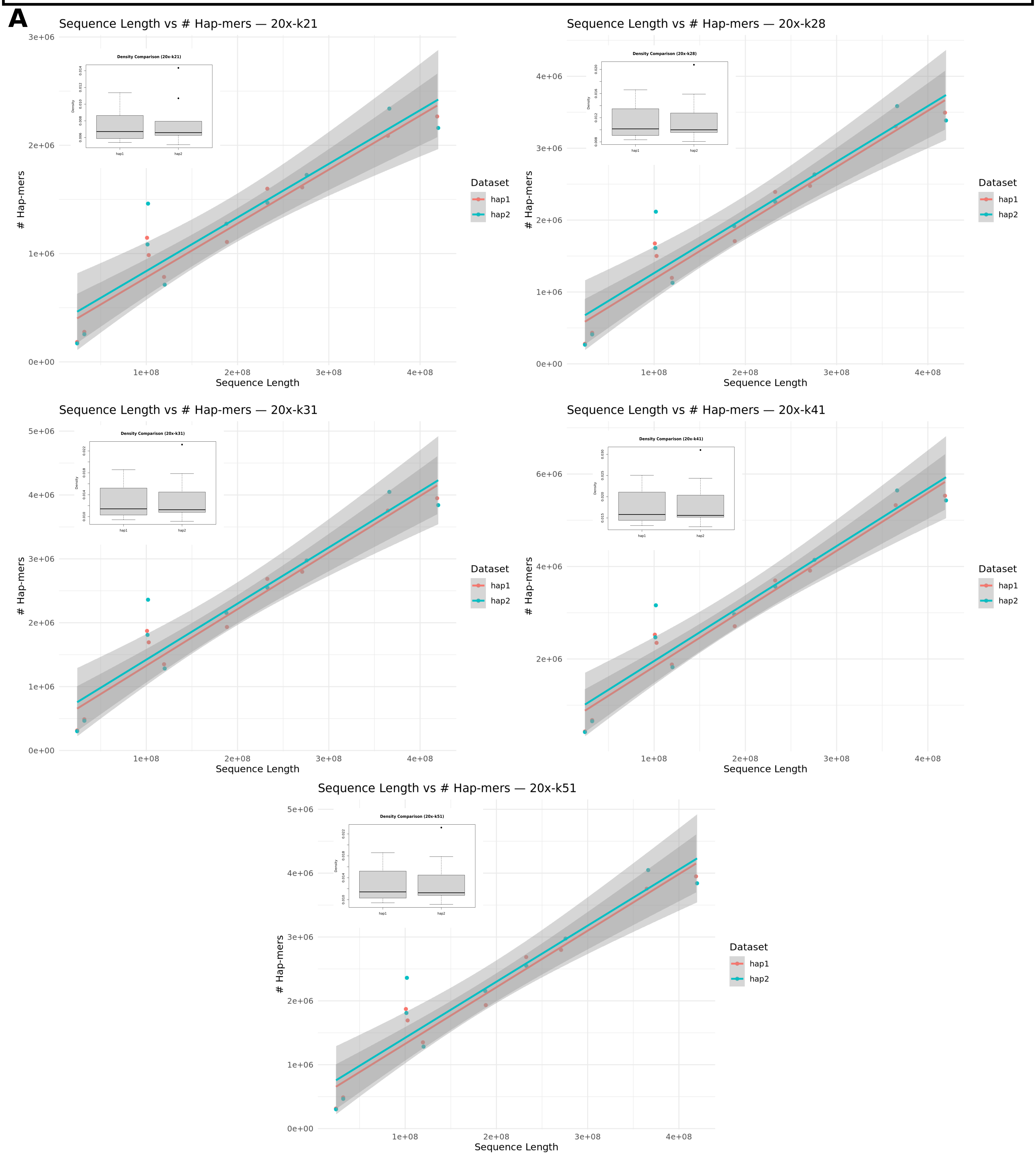
Group	**Species**	**Genotype**	**Type**	**Cov.**	**Database Accessions**
Squamata; Gekkota; Sphaerodactylidae	*Sphaerodactylus notatus*	XY	PacBio HiFi	20x	SAMN48008689
Squamata; Gekkota; Sphaerodactylidae	*Sphaerodactylus notatus*	XY	HiC	N/A.	SAMN48008690
Squamata; Gekkota; Diplodactylidae	*Correlophus ciliatus*	ZW	PacBio HiFi	30x	SRR28508818-22
Squamata; Gekkota; Diplodactylidae	*Correlophus ciliatus*	ZW	HiC	N/A.	SRR29774324
Squamata; Gekkota; Eublepharidae	*Eublepharis macularius*	N/A.	PacBio HiFi	30x	SRR21708437
Squamata; Gekkota; Eublepharidae	*Eublepharis macularius*	N/A.	HiC	N/A.	SRR21708436
Squamata; Gekkota; Gekkonidae	*Lepidodactylus listeri*	XY	PacBio HiFi	30x	DA149208, DA149210, DA162797
Squamata; Gekkota; Gekkonidae	*Lepidodactylus listeri*	XY	HiC	N/A.	HG5YLDMXY
Squamata; Scincomorpha; Scincidae	*Cryptoblepharus egeriae*	XY	PacBio HiFi	40x	DA149190, DA149222
Squamata; Scincomorpha; Cordylidae	*Hemicordylus capensis*	XY	PacBio HiFi	43x	SRR22311010-12, SRR22311021
Squamata; Scincomorpha; Cordylidae	*Hemicordylus capensis*	XY	HiC	N/A.	SRR22311007-8

Mammalia	*Pan troglodytes*	XY	Genome	60x	GCF_028858775.2, GCA_028858805.2
Mammalia; Carnivora	*Vulpes vulpes*	ZZ	Genome	28x	GCA_964106825.2, GCA_964106925.2
Aves; Columbidae	*Caloenas nicobarica*	ZZ	Genome	44x	GCA_036010745.1, GCA_036013445.1
Aves; Columbidae	*Guaruba guarouba*	ZW	Genome	52x	GCA_045345405.1, GCA_045519325.1
Squamata; Lacertidae	*Podarcis gaigeae*	ZW	Genome	44x	GCA_964106915.2, GCA_964106785.1
Squamata; Lacertidae	*Podarcis cretensis*	ZZ	Genome	N/A.	GCA_951804945.1
Squamata; Lacertidae	*Podarcis cretensis*	ZZ	PacBio HiFi	27x	ERR11413981, ERR11413982
Chondrichthyes; Narcinidae	*Narcine bancroftii*	XY	Genome	50x	GCF_036971445.1, GCA_036971175.1

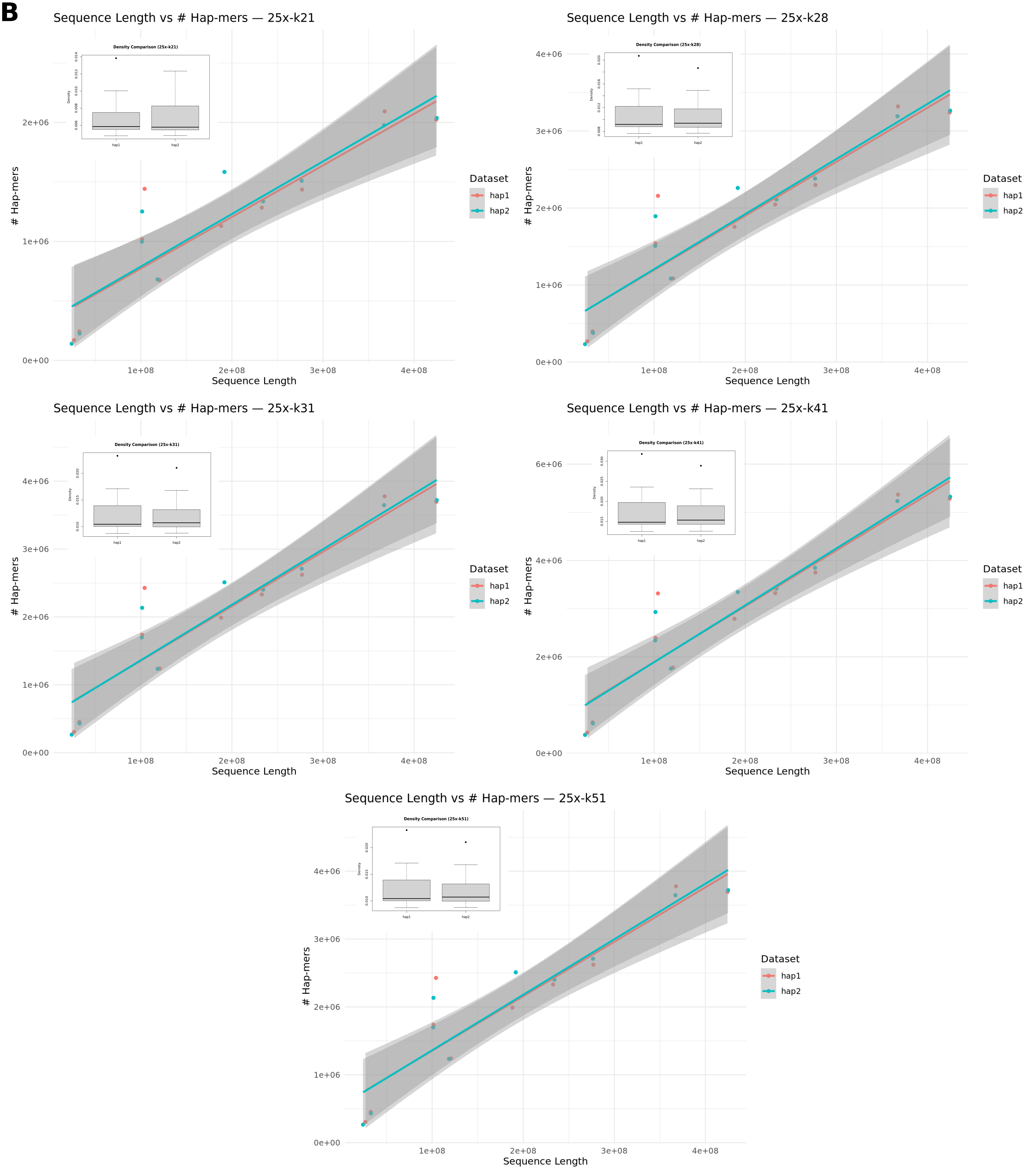
Supplemental Figure 1: Using the updated reference assembly for *Anniella stebbinsi* detailed in the main text. We systematically hard-masked a region on chromosome 2 in haplotype 1 of varying sizes to mimic a collapsed homozygous region in the assembly. (A) a 1Mb region increases noise, but retains the original sex chromosome signal, (B) a 5Mb region significantly increases noise, but qualitative assessment may still anticipate the original sex chromosome signal, (C) by 10Mb the original signal is nearly completely lost, and (D) further exacerbates the effects observed in panel C. Control analysis present in Figure 7.

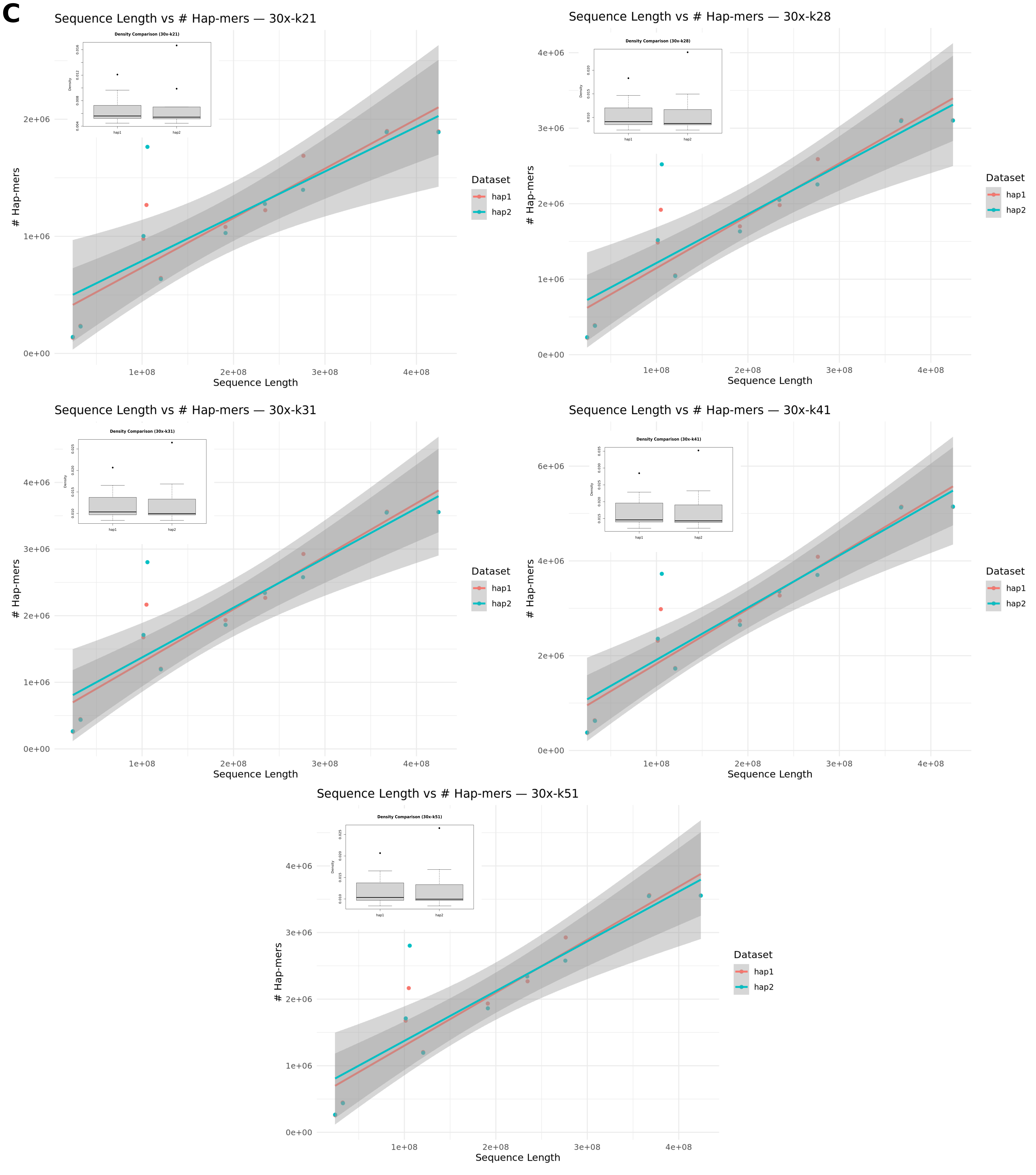


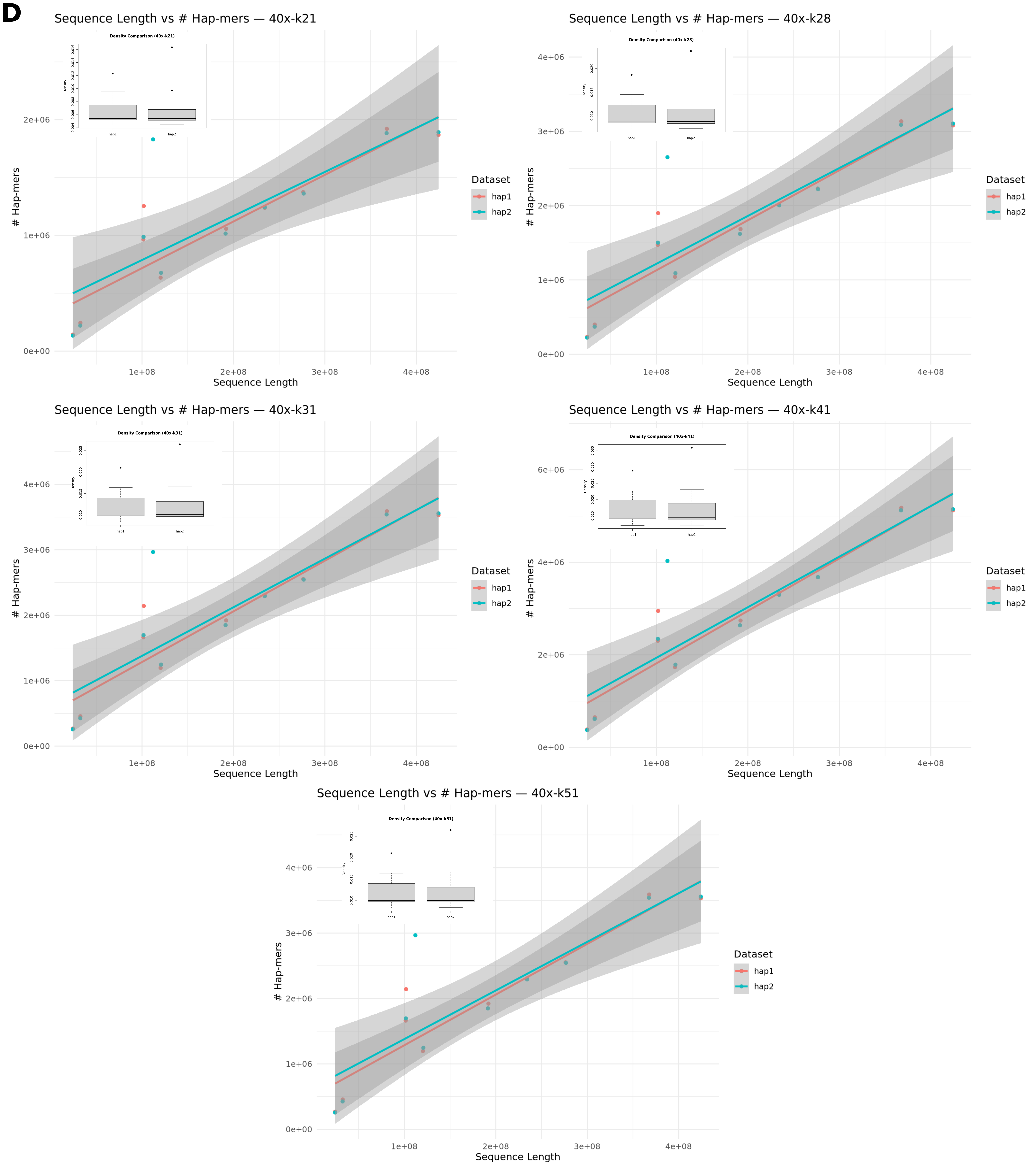
Supplemental Figure 2: Co-exploration of the effects of varying average sequencing depth of the assembly and kmer lengths used by SCINKD in *Anniella stebbinsi*. (A) 20x coverage, (B) 25x coverage, (C) 30x coverage, (D) 40x coverage. At 20x coverage, it’s difficult to definitively observe signals from one of the two haplotypes at any kmer length, while at k21 at all sequencing depths, sex chromosome signal is obscured. The anticipated SCINKD signal remains robust at ≥25x and ≥k28.



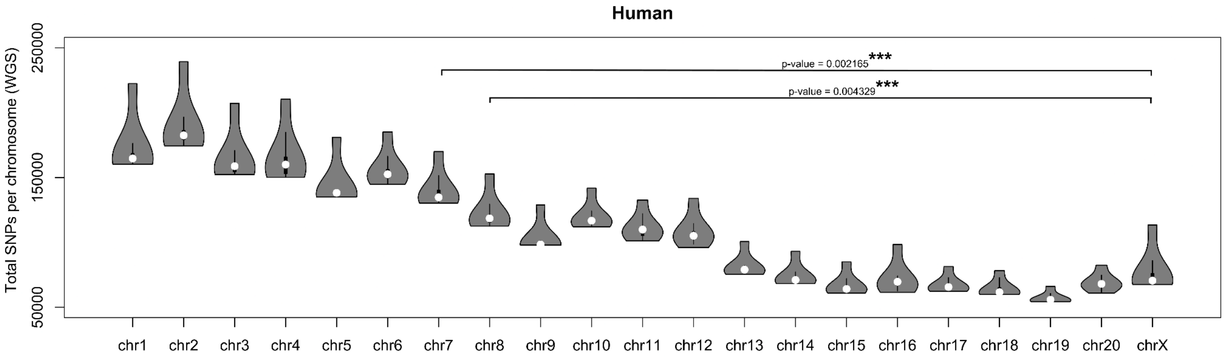
B


Sequence Length vs # Hap-mers — 25x-k21
Density Comparison (25x-k21)
Density
hap1
hap2
Hap-mers
Sequence Length
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 25x-k28
Density Comparison (25x-k28)
Sequence Length vs # Hap-mers — 25x-k31
Density Comparison (25x-k31)
Sequence Length vs # Hap-mers — 25x-k41
Density Comparison (25x-k41)
Sequence Length vs # Hap-mers — 25x-k51
Density Comparison (25x-k51)
0e+00
1e+06
2e+06
3e+06
4e+06
6e+06
1e+08
2e+08
3e+08
4e+08

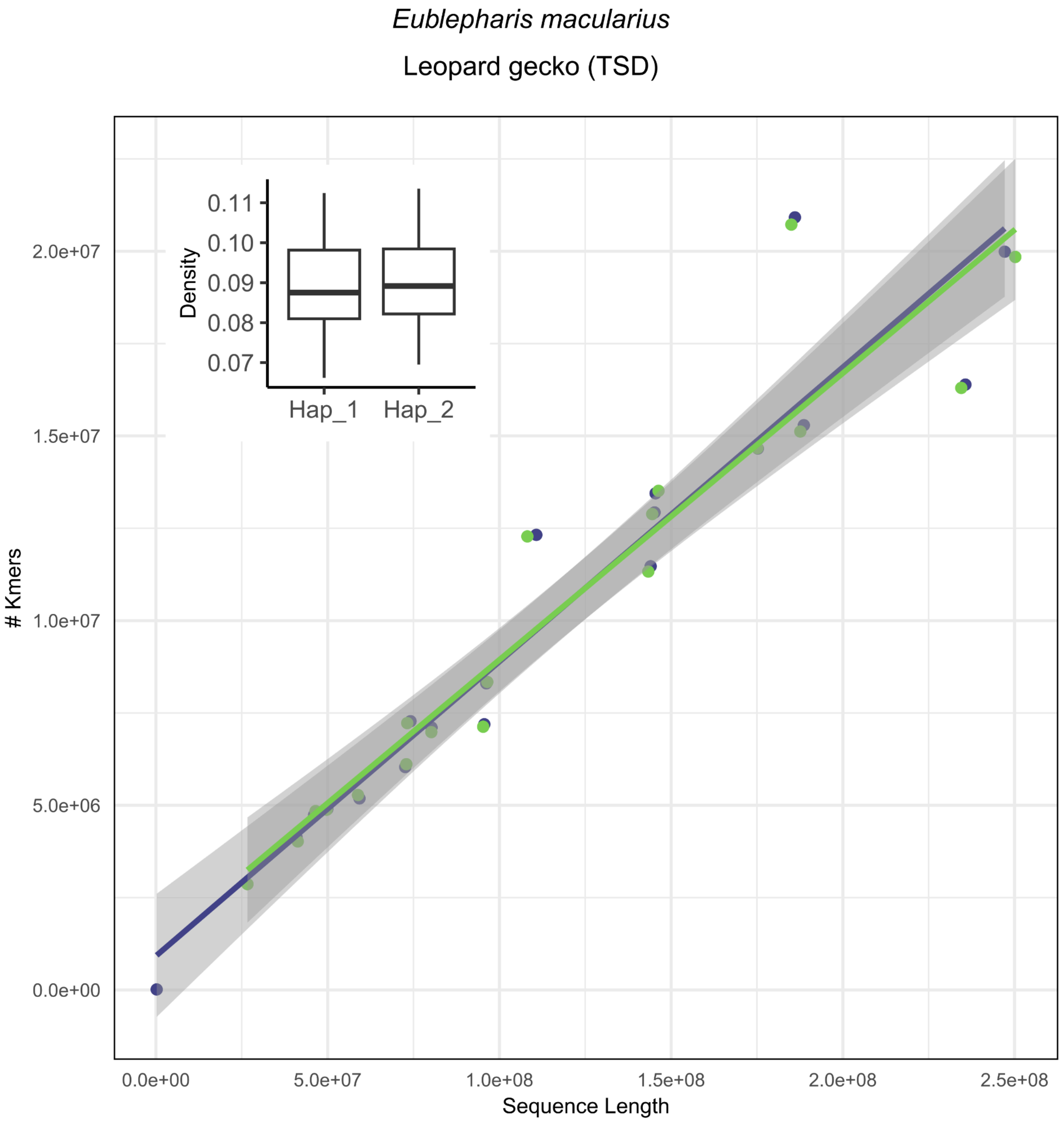
C
Sequence Length vs # Hap-mers — 30x-k21
Density Comparison (30x-k21)
Density
0.004
0.008
0.012
0.016
hap1
hap2
Hap-mers
0e+00
1e+06
2e+06
1e+08
2e+08
3e+08
4e+08
Sequence Length
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 30x-k28
Density Comparison (30x-k28)
Density
0.010
0.015
0.020
hap1
hap2
Hap-mers
0e+00
1e+06
2e+06
3e+06
4e+06
1e+08
2e+08
3e+08
4e+08
Sequence Length
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 30x-k31
Density Comparison (30x-k31)
Density
0.010
0.015
0.020
0.025
hap1
hap2
Hap-mers
0e+00
1e+06
2e+06
3e+06
4e+06
1e+08
2e+08
3e+08
4e+08
Sequence Length
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 30x-k41
Density Comparison (30x-k41)
Density
0.015
0.020
0.025
0.030
0.035
hap1
hap2
Hap-mers
0e+00
2e+06
4e+06
6e+06
1e+08
2e+08
3e+08
4e+08
Sequence Length
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 30x-k51
Density Comparison (30x-k51)
Density
0.010
0.015
0.020
0.025
hap1
hap2
Hap-mers
0e+00
1e+06
2e+06
3e+06
4e+06
1e+08
2e+08
3e+08
4e+08
Sequence Length
Dataset
hap1
hap2


D
Sequence Length vs # Hap-mers — 40x-k21
Density Comparison (40x-k21)
Density
hap1
hap2
Hap-mers
0e+00
1e+06
2e+06
Sequence Length
1e+08
2e+08
3e+08
4e+08
Dataset
hap1
hap2
Sequence Length vs # Hap-mers — 40x-k28
Density Comparison (40x-k28)
0e+00
1e+06
2e+06
3e+06
4e+06
Sequence Length vs # Hap-mers — 40x-k31
Density Comparison (40x-k31)
0e+00
1e+06
2e+06
3e+06
4e+06
Sequence Length vs # Hap-mers — 40x-k41
Density Comparison (40x-k41)
0e+00
2e+06
4e+06
6e+06
Sequence Length vs # Hap-mers — 40x-k51
Density Comparison (40x-k51)
0e+00
1e+06
2e+06
3e+06
4e+06

Supplemental Figure 3: Preliminary data suggesting that the number of biallelic SNPs across individuals roughly correlates with chromosome size in humans using whole genome re-sequencing (WGS) in GTEx data. In addition, the X chromosome contains fewer SNPs than expected based on its length, which is the approximate length of chr7 in GRCh38.



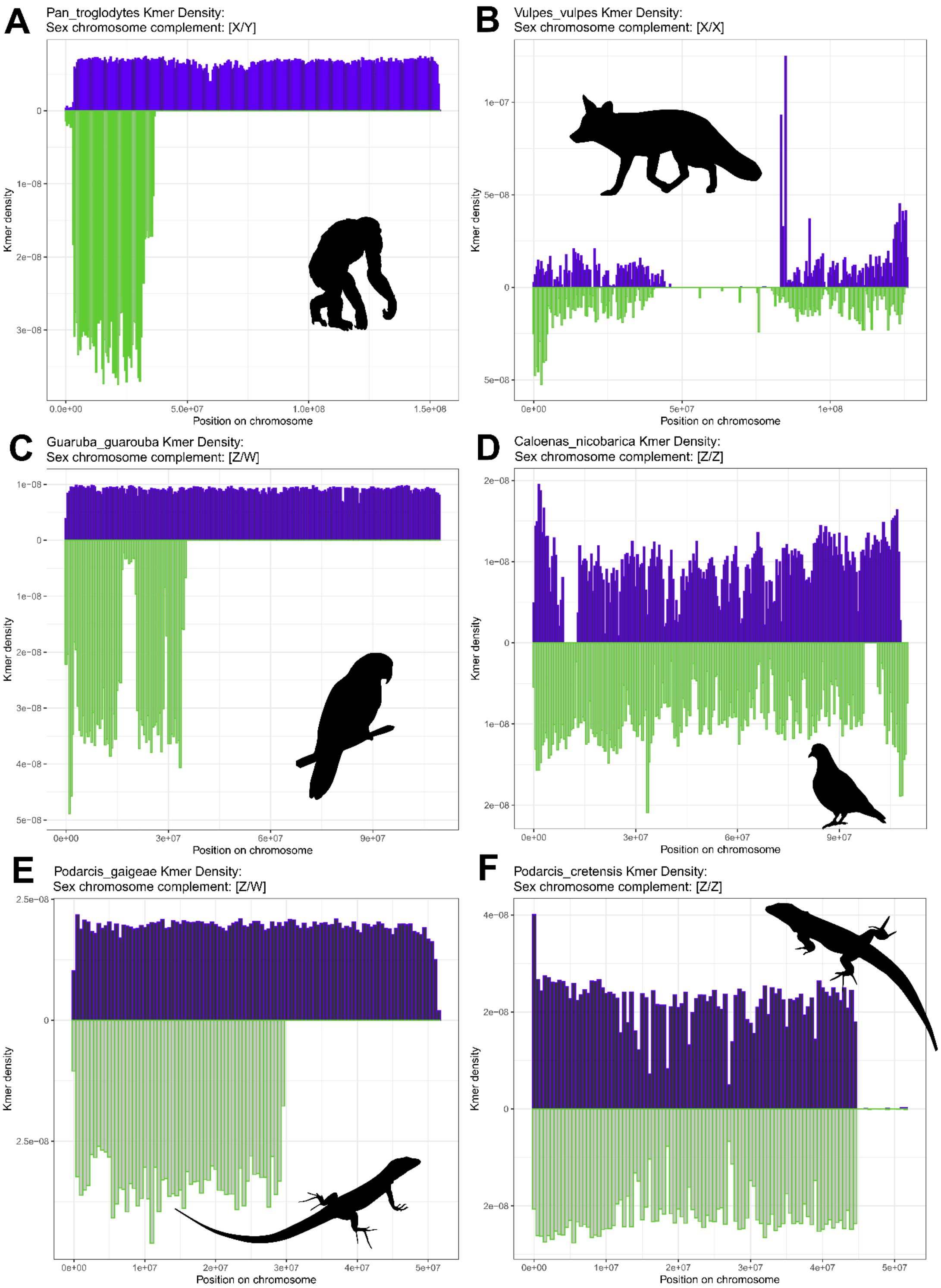
Supplemental Figure 4: Confirmation of a strong correlation (R^2 == 0.901) between hap-mers and chromosome length in a temperature-dependent species, the leopard gecko (*Eublepharis macularius*). Subtle variations between haplotypes are thought to be driven by poor phasing performance driven by low heterozygosity and/or sub-optimal coverage (30x) of PacBio HiFi data (Pinto et al. 2023).



Eublepharis macularius

Leopard gecko (TSD)

Supplemental Figure 5: Mirror plots for both sexes of the heteromorphic systems detailed in Figure 2.



A Pan_troglodytes Kmer Density:
Sex chromosome complement: [X/Y]

B Vulpes_vulpes Kmer Density:
Sex chromosome complement: [X/X]

C Guaruba_guarouba Kmer Density:
Sex chromosome complement: [Z/W]

D Caloenas_nicobarica Kmer Density:
Sex chromosome complement: [Z/Z]

E Podarcis_gaigeae Kmer Density:
Sex chromosome complement: [Z/W]

F Podarcis_cretensis Kmer Density:
Sex chromosome complement: [Z/Z]

Supplemental Figure 6: Single sex-only full mirror plots (summarized in Figures 3-10).