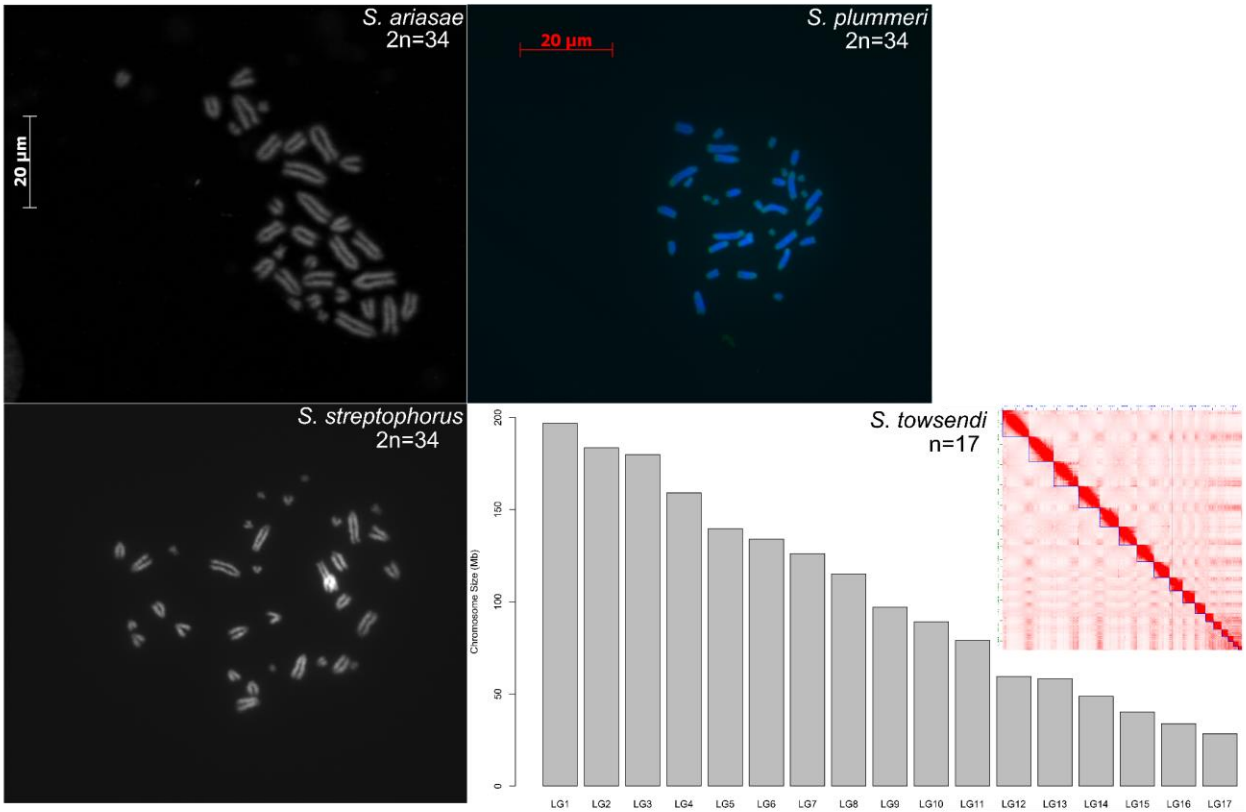
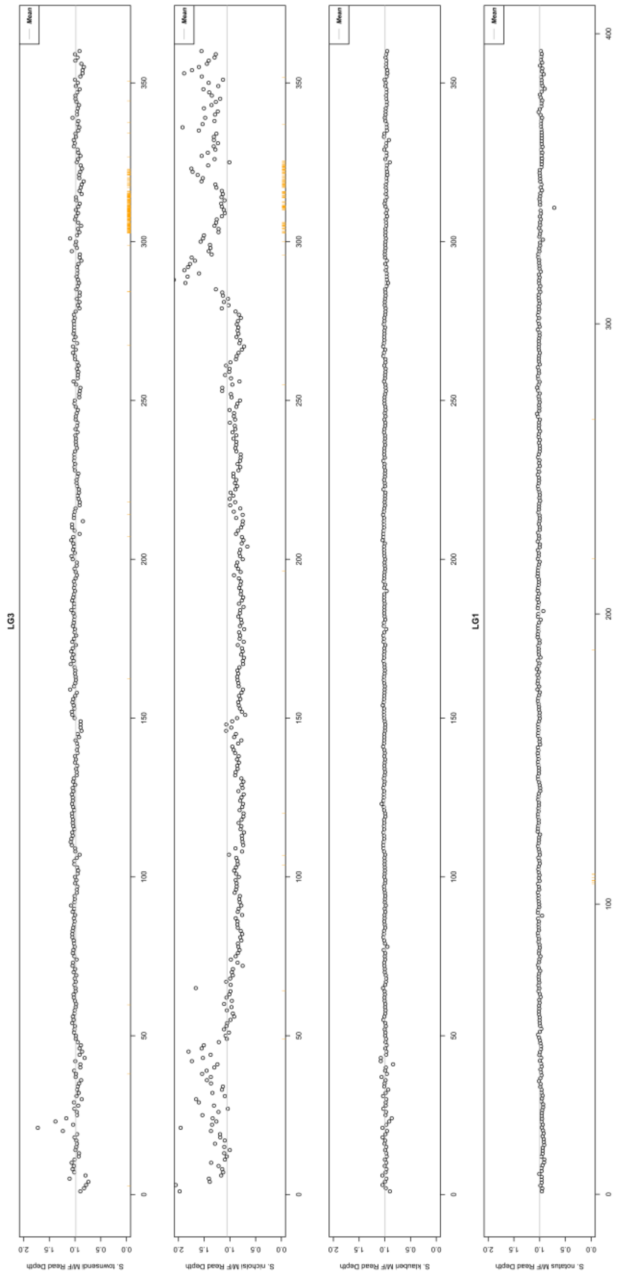
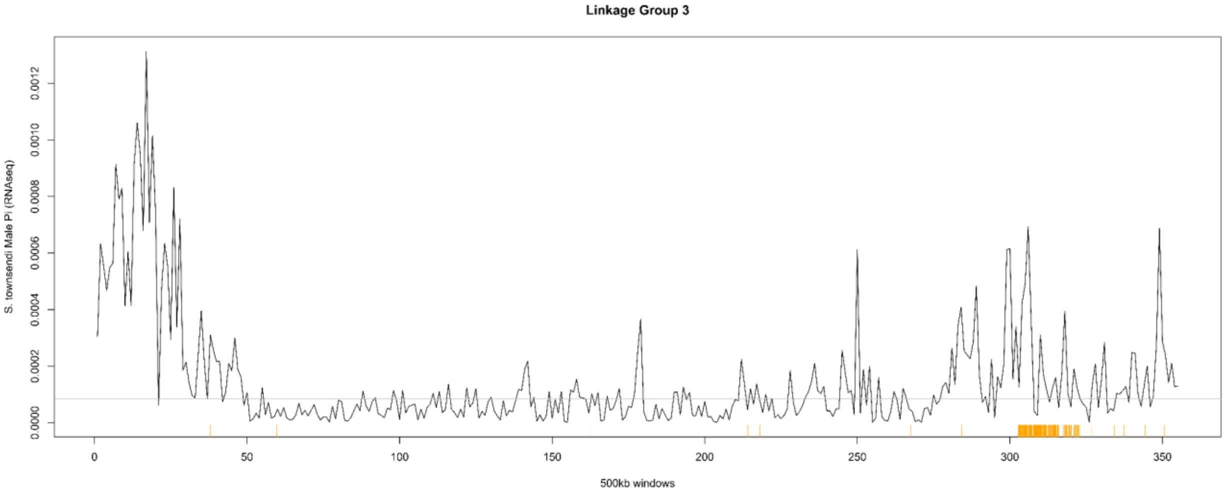
Supplemental Figures:



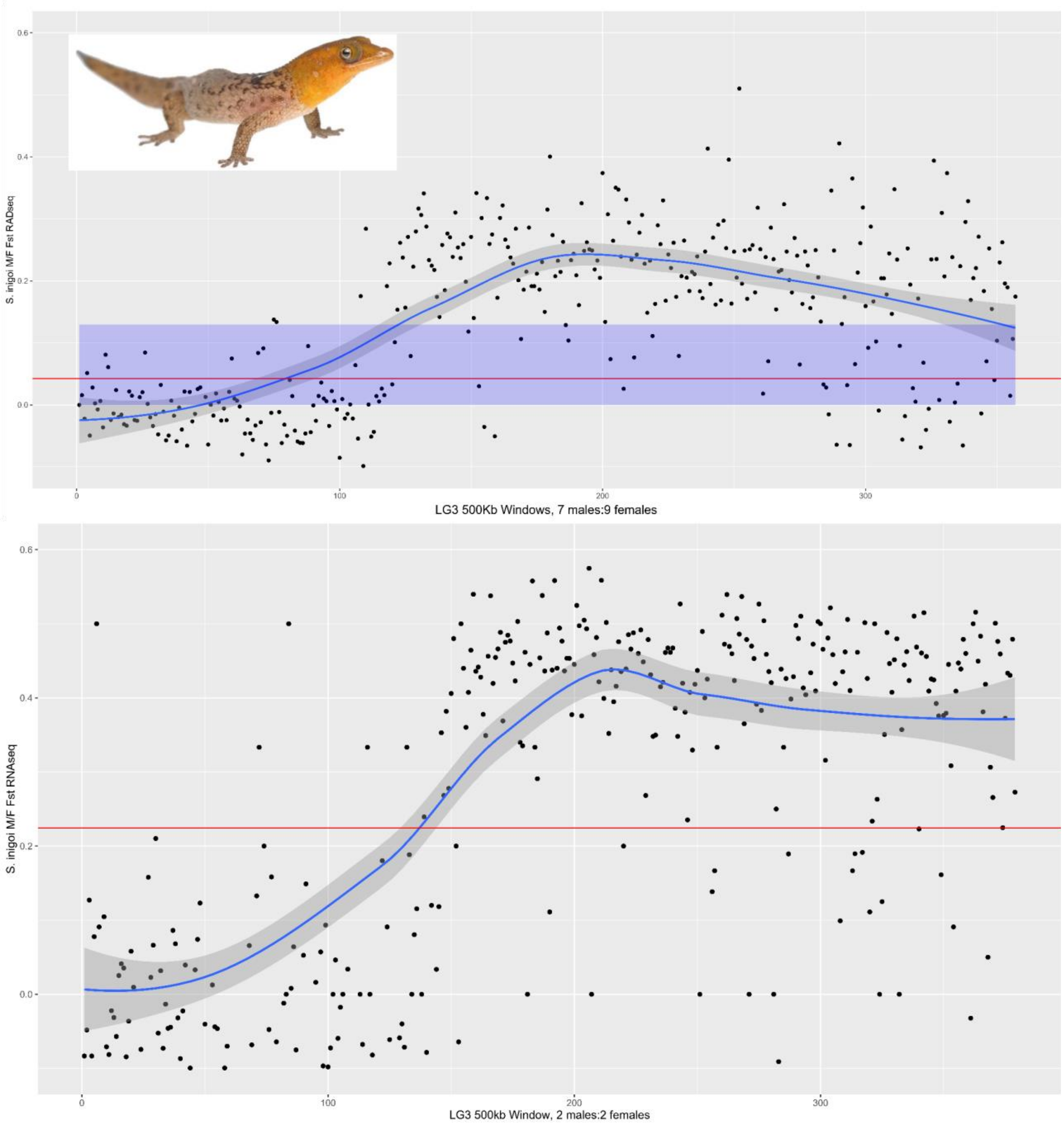
Supplemental Figure 1: Karyotypes for *S. ariasae* (female, TG1241), *S. plummeri* (male, TG1244), and *S. streptophorus* (female, TG1247)—relative to the 17 assembled linkage groups in the *S. townsendi* genome—chromosome length bar chart and HiC contact map shown. Karyotype methods outlined in Main et al. 2012.



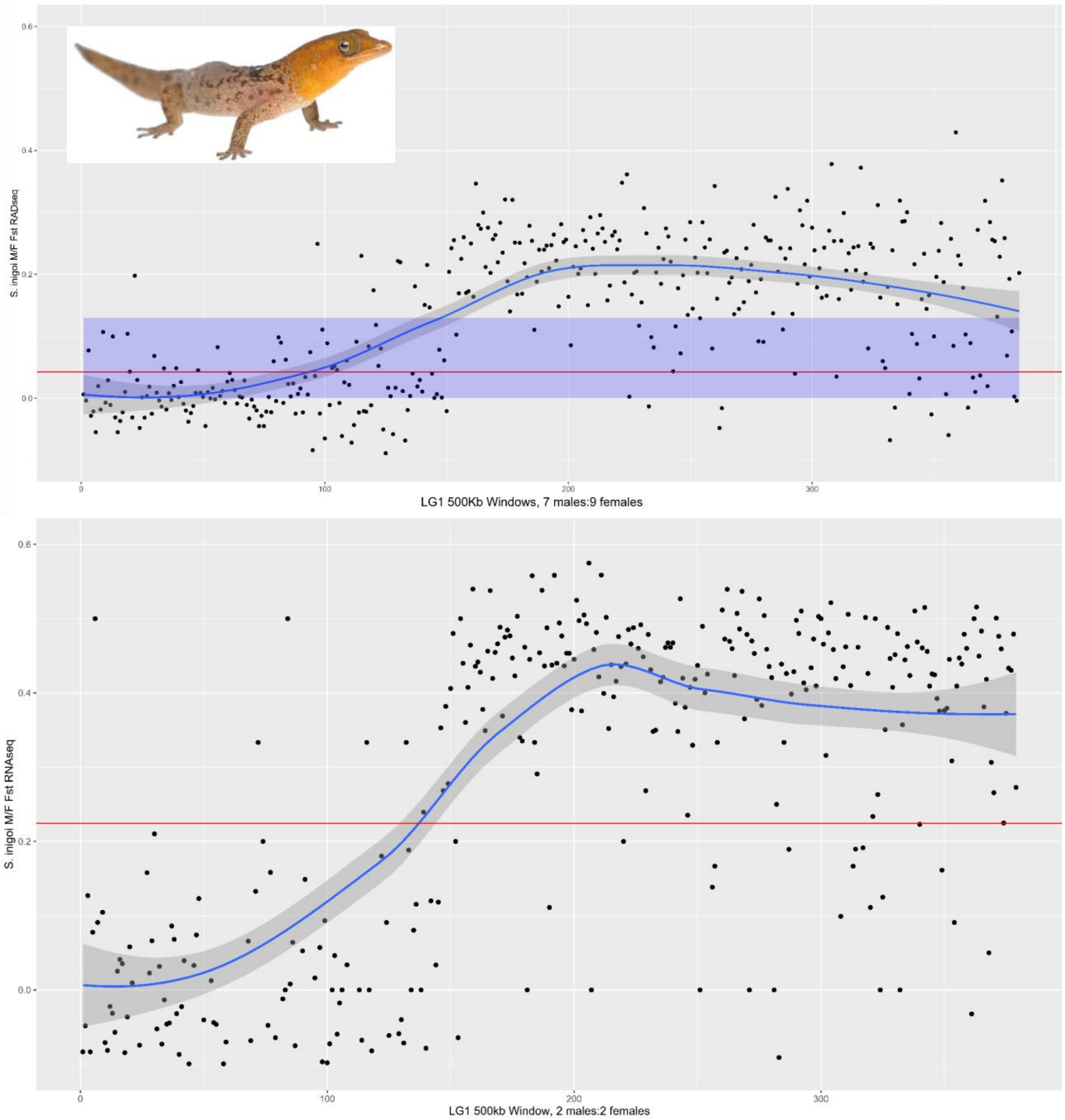
Supplemental Figure 2: *Sphaerodactylus* species with M/F WGS data. Comparing M/F read depth across sex chromosome linkage groups in each species LG3: *S. townsendi*, *S. nicholsi*, and *S. klauberi*; then LG1 for *S. notatus*, respectively.



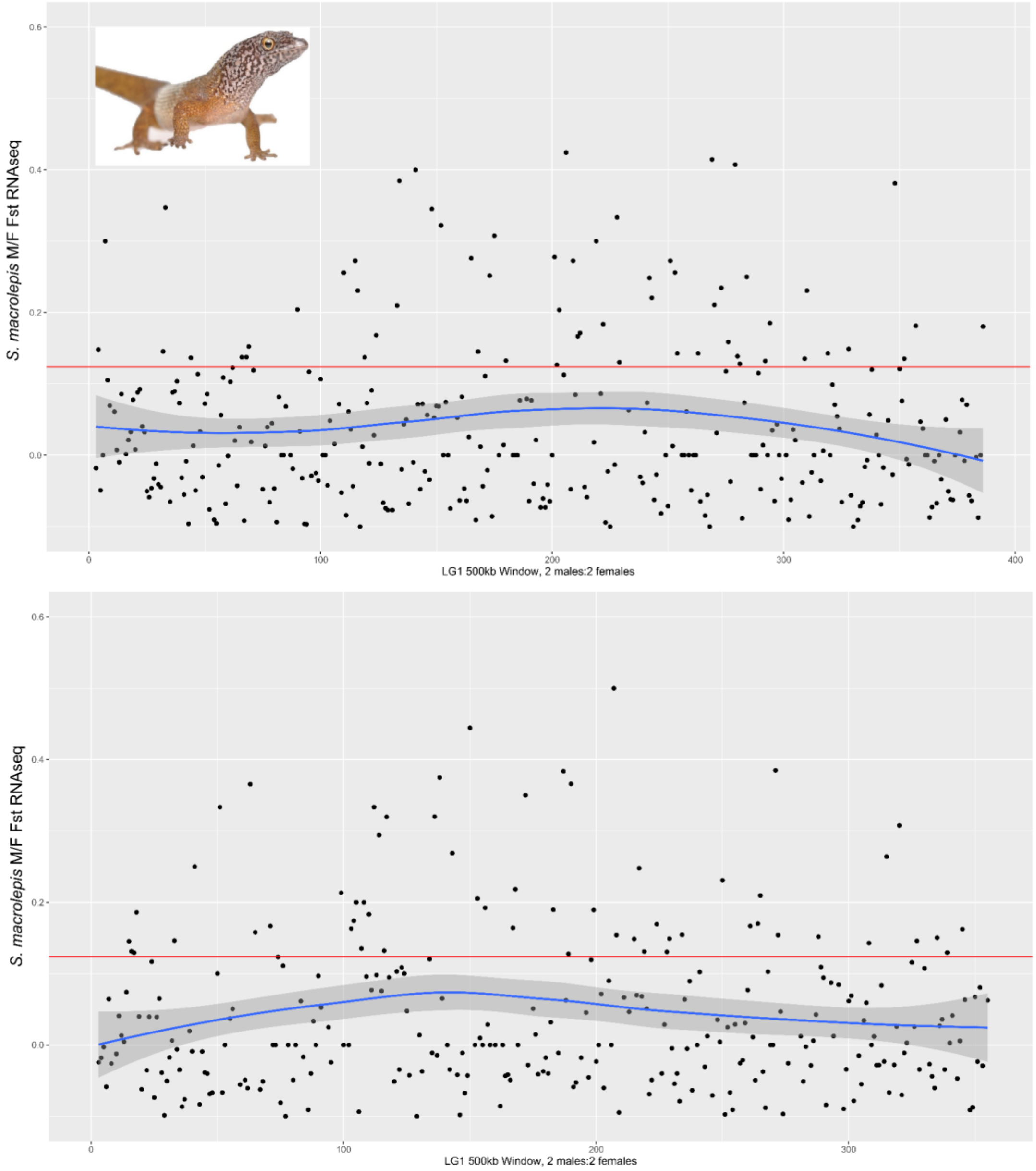
Supplemental Figure 3: *Sphaerodactylus townsendi*, male nucleotide diversity calculated from RNAseq data using vcftools. *S. townsendi* male-specific RADtags mapped to LG3 are denoted by orange ticks along the bottom of each graph. Grey horizontal line indicates the genomic mean value.



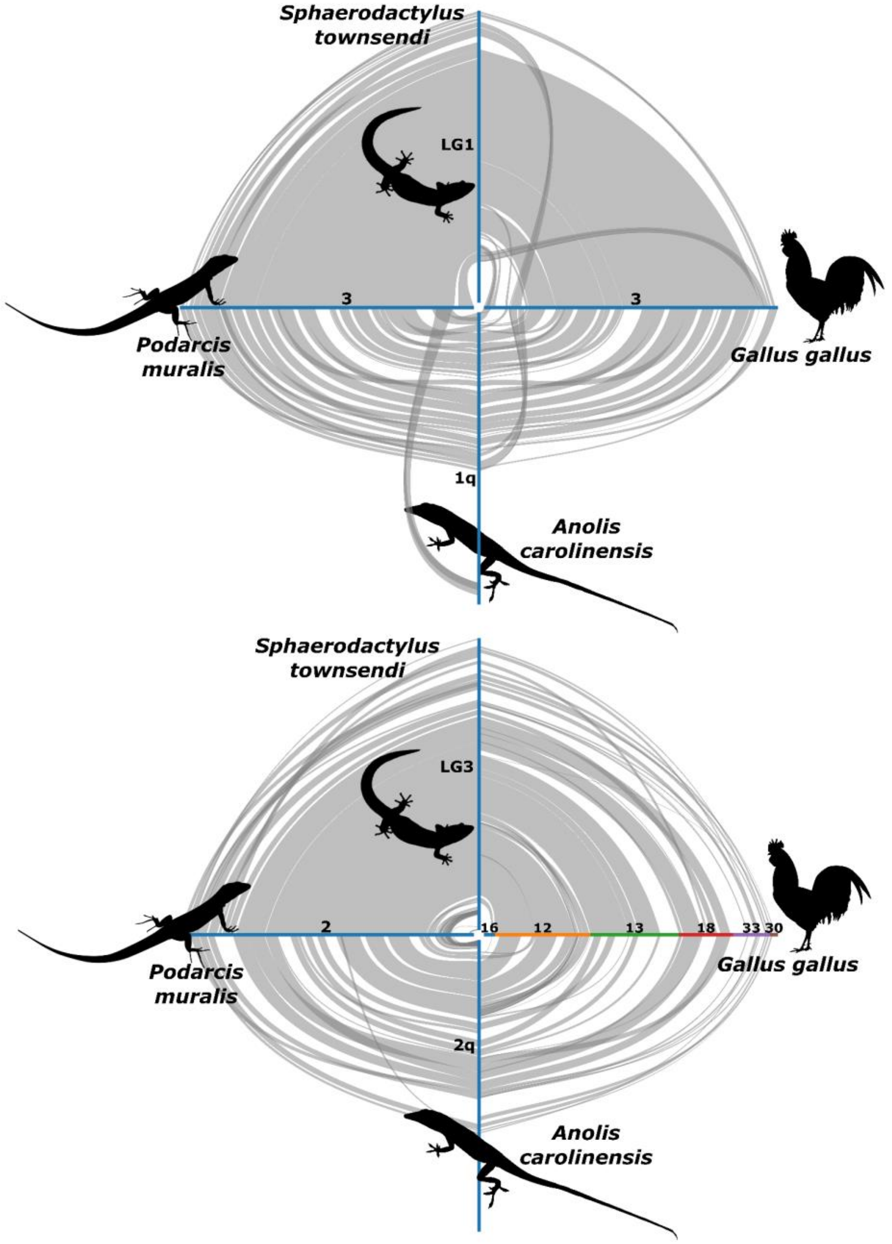
Supplemental Figure 4: *Sphaerodactylus inigoi*, comparison between M/F Fst values with RADseq (top) and RNAseq (bottom) data for *S. townsendi* LG3. Red line indicates the ‘autosomal’ mean (all other chromosomes except LG1 and LG3). Blue ribbon (top only) indicates the 95% confidence interval (μ + 2*σ).



Supplemental Figure 5: *Sphaerodactylus inigoi*, comparison between M/F Fst values with RADseq (top) and RNAseq (bottom) data for *S. townsendi* LG1. Red line indicates the ‘autosomal’ mean (all other chromosomes except LG1 and LG3). Blue ribbon (top only) indicates the 95% confidence interval (μ + 2*σ).



Supplemental Figure 6: *Sphaerodactylus macrolepis*, examination of M/F Fst values with RNAseq data across *S. townsendi* LG1 (top) and LG3 (bottom). Red line indicates the ‘autosomal’ mean (all other chromosomes except LG1 and LG3).



Supplemental Figure 7: Synteny of Sphaerodactylus sex chromosomes LG1 + LG3, the sex chromosomes linkage group in *Sphaerodactylus townsendi* relative to other squamates *Podarcis* and *Anolis*, and an outgroup, *Gallus*. Synteny plot generated using MCScanX and SynVisio, and silhouettes for *Podarcis*, *Anolis*, and *Gallus* were obtained from PhyloPic (*Anolis* by Sarah Werning and *Gallus* by Steven Traver).

Supplemental Table 1: Assembly stats

Version	Tool Used at Each Step	N50	L50
Assembly v1.1	SuperNova	12,629,056	37
Assembly v1.2	Tigmint	6,460,730	69
Assembly v1.3	ARCS	7,457,274	57
Assembly v1.4	TGS-Gapcloser	7,468,733	57
Assembly v1.5	Nextpolish	7,605,248	57
Assembly v1.6	3D-DNA	126,215,344	7
Assembly v1.7	Redundancy-filter	134,006,883	6
Assembly v1.8-2.1	10kb cutoff and annotation	134,006,883	6

Transcriptomes/Annotation			
Embryo transcriptome	runDRAP	3,092	14,803
Head transcriptome	runDRAP	2,750	15,526
Meta transcriptome	RunMeta	2,320	14,981
Annotated transcripts	Funannotate	1,380	6,110

N60	L60	N70	L70	N80	L80	N90	L90	N100
7,566,236	57	3,411,059	97	1,078,884	199	9,661	5,442	1,000
3,625,198	110	2,037,326	181	695,281	348	9,554	5,915	1
4,431,877	91	2,343,670	152	754,193	305	9,607	5,655	1,001
4,422,213	91	2,338,521	153	758,562	307	9,734	5,654	498
4,460,106	91	2,371,533	153	770,247	306	9,918	5,462	498
115,113,985	8	79,121,915	11	48,733,625	14	9,921	2,971	498
115,113,985	8	89,269,862	10	59,421,808	12	33,799,320	16	498
126,215,344	7	97,096,400	9	79,121,915	11	48,733,625	14	10,002

L100	N-count	Gaps	Mean	Largest	Size	CVG (C)
58,149	182,966,760	109,249	34,448	86,754,586	2,003,095,602	93.6%
59,469	179,364,669	109,143	33,622	52,059,470	1,999,493,511	93.6%
58,603	179,365,680	109,299	34,116	72,975,442	1,999,290,127	93.6%
58,603	67,505,130	10,696	34,374	73,487,924	2,014,429,111	94.9%
58,603	0	0	34,750	74,187,749	2,036,478,338	95.3%
56,114	248,900	2,489	36,296	196,877,829	2,036,727,238	95.7%
32,127	248,900	2,489	60,120	196,877,829	1,931,485,387	95.7%
1,823	248,800	2,488	997,312	196,877,829	1,818,099,321	95.7%

Tetra (C)	Tetra (C+P)	Tetra (S)	Tetra (D)	Tetra (F)	Tetra (M)
85.5%	92.7%	84.6%	0.9%	7.3%	7.3%
85.5%	92.7%	84.6%	0.9%	7.3%	7.3%
85.5%	92.7%	84.6%	0.9%	7.2%	7.3%
88.0%	93.4%	87.1%	0.9%	5.3%	6.7%
88.8%	93.7%	87.9%	0.9%	4.9%	6.3%
88.9%	93.8%	88.1%	0.8%	4.9%	6.2%
88.7%	93.2%	88.0%	0.7%	4.5%	6.8%
88.3%	92.2%	87.6%	0.7%	3.9%	7.8%

82.7%	85.4%	55.7%	27.0%	2.6%	14.7%
81.6%	86.9%	58.0%	23.6%	53.0%	13.1%
80.5%	83.8%	68.2%	12.3%	3.4%	16.1%
57.5%	76.3%	56.9%	0.6%	18.8%	23.7%

Supplemental Table 2: Comparative Lepidsaur genome information.

Common Name	Clade	Scaffold N50 (Mb)	Scaffold L50
King cobra	Elapidae	0.24	1,750
Eastern garter snake	Colubridae	0.65	639
Leopard gecko	Eublepharidae	0.66	796
Schlegel’s Japanese gecko	Gekkonidae	0.68	963
Glass lizard	Anguidae	1.3	408
Shaw’s Sea Snake	Elapidae	1.4	353
Chinese crocodile lizard	Shinisauridae	1.5	385
Five-pace viper	Viperidae	2.1	199
Central bearded dragon	Agamidae	2.3	219
Tuatara	Sphenodontidae	3.0	370
Madagascar ground gecko	Gekkonidae	4.1	93
Boa constrictor	Boidae	4.5	90
Eastern Brown Snake	Elapidae	14.7	31
Komodo dragon	Varanidae	23.8	17
European wall lizard	Lacertidae	92.4	7
Madagascar ground gecko	Gekkonidae	109.0	6
Townsend's dwarf gecko	**Sphaerodactylidae**	**134.0**	**6**
Green anole	Iguanidae	151.0	5
Brown anole	Iguanidae	253.6	4
Burmese python	Pythonidae	196.0	3
Prairie rattlesnake	Viperidae	197.9	3
Indian cobra	Elapidae	224.1	3
Desert horned lizard	Phrynosomatidae	273.2	3
Eastern Fence Lizard	Phrynosomatidae	275.0	3
Chinese crocodile lizard	Shinisauridae	297.0	4
Argentine black and white tegu	Teiidae	314.2	3

Total Scaffolds	Reference	Technology
296,399	Vonk et al. 2013	Illumina-only
7,930	Perry et al. 2018	Illumina-only
206,400	Xiong et al. 2016	Illumina-only
191,500	Liu et al. 2015	Illumina-only
6,715	Song et al. 2015	Illumina-only
3,139	Peng et al. 2020	Illumina, PacBio
1,257,129	Gao et al. 2017	Illumina-only
162,571	Yin et al. 2016	Illumina-only
545,310	Georges et al. 2015	Illumina-only
16,536	Gemmell et al. 2020	Illumina, mate pairs, Chicago
110,900	Hara et al. 2018	Illumina, mate pairs
19,927	Card et al. 2019	Illumina-only
28,550	University of New South Wales	10X
1,411	Lind et al. 2019	10X, ONT, PacBio, HiC
2,160	Andrade et al. 2019	Illumina, PacBio, HiC, Chicago
4,877	Yamaguchi et al. 2021	Illumina, mate pairs, HiC
1,823	**Pinto et al. 2021 (MPM_Stown_v2.2)**	**10X, ONT, Illumina, HiC**
6,457	Alfoldi et al. 2011	Sanger, physical mapped
3,738	Geneva et al. 2021	Illumina, PacBio, HiC, Chicago
29,190	Castoe et al. 2013	Illumina, HiC (DNAZoo)
7,043	Schield et al. 2019	Illumina, mate pairs, HiC, Chicago
1,897	Suryamohan et al. 2020	Everything
5,291	Koochekian et al. 2022	Illumina, Chicago, Hi-C
24	Westfall et al. 2021	10X, PacBio, HiC
1,553	Xie et al. 2022	PacBio, 10X, HiC
4,375	Roscito et al. 2018	Illumina, PacBio, OM, HiC (DNAZoo)

Assembly Link
https://www.ncbi.nlm.nih.gov/genome/10842
https://www.ncbi.nlm.nih.gov/genome/16688
http://dx.doi.org/10.5524/100246
https://www.ncbi.nlm.nih.gov/assembly/GCF_001447785.1
http://dx.doi.org/10.5524/100119
https://doi.org/10.6084/m9.figshare.11391606.v5
http://dx.doi.org/10.5524/100315
http://dx.doi.org/10.5524/100196
https://useast.ensembl.org/Pogona_vitticeps/Info/Index
https://useast.ensembl.org/Sphenodon_punctatus/Info/Index
https://doi.org/10.6084/m9.figshare.6220406.v1
http://darencard.net/boaCon/
https://www.ncbi.nlm.nih.gov/genome/?term=txid8673[orgn]
https://useast.ensembl.org/Varanus_komodoensis/Info/Index
https://useast.ensembl.org/Podarcis_muralis/Info/Index
https://www.ncbi.nlm.nih.gov/assembly/GCA_003118565.2/
https://www.ncbi.nlm.nih.gov/assembly/GCA_021028975.1
https://useast.ensembl.org/Anolis_carolinensis/Info/Index
https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/TTKBFU
https://www.dnazoo.org/assemblies/Python_bivittatus
https://www.ncbi.nlm.nih.gov/assembly/GCA_003400415.2
https://www.ncbi.nlm.nih.gov/genome/8395
https://www.ncbi.nlm.nih.gov/assembly/GCA_020142125.1
https://www.ncbi.nlm.nih.gov/genome?LinkName=nuccore_genome&from_uid=2064852242
https://www.ncbi.nlm.nih.gov/assembly/GCA_021292165.1
https://www.dnazoo.org/assemblies/Salvator_merianae

Supplemental Table 3: Sample information used in this study.

Species	**Individual**	**Sex**	**Experiment**	**Tissue**	**Bioproject**
S. townsendi	TG3544	M	10X	Blood/liver	PRJNA746057
S. townsendi	TG3544	M	ONT	Blood/liver	PRJNA746057
S. townsendi	TG3544	M	DNAseq	Tail	PRJNA746057
S. townsendi	TG3718	M	HiC	Blood/liver	PRJNA746057
S. townsendi	TG3828	F	DNAseq	Tail	PRJNA746057
S. nicholsi	TG1951	M	DNAseq	Tail	PRJNA746057
S. nicholsi	TG2115	F	DNAseq	Tail	PRJNA746057
S. klauberi	TG2759	M	DNAseq	Tail	PRJNA746057
S. klauberi	TG2760	F	DNAseq	Tail	PRJNA746057
S. macrolepis	TG2721	M	DNAseq	Tail	PRJNA746057
S. notatus	TG3846	M	DNAseq	Tail	PRJNA746057
S. notatus	TG3847	F	DNAseq	Tail	PRJNA746057
S. townsendi	TG3715	Unknown	RNAseq	Embryo	PRJNA746057
S. townsendi	TG3467	M	RNAseq	Head	PRJNA746057
S. inigoi	TG2751	M	RNAseq	Head	PRJNA746057
S. inigoi	TG2752	M	RNAseq	Head	PRJNA746057
S. inigoi	TG2754	F	RNAseq	Head	PRJNA746057
S. inigoi	TG2755	F	RNAseq	Head	PRJNA746057
S. macrolepis	TG2743	M	RNAseq	Head	PRJNA746057
S. macrolepis	TG2748	M	RNAseq	Head	PRJNA746057
S. macrolepis	TG2745	F	RNAseq	Head	PRJNA746057
S. macrolepis	TG2746	F	RNAseq	Head	PRJNA746057
S. townsendi	TG2020	M	RADseq	Tail	PRJNA746057
S. townsendi	TG2024	M	RADseq	Tail	PRJNA746057
S. townsendi	TG2025	M	RADseq	Tail	PRJNA746057
S. townsendi	TG2026	M	RADseq	Tail	PRJNA746057
S. townsendi	TG2028	M	RADseq	Tail	PRJNA746057
S. townsendi	TG3108	M	RADseq	Tail	PRJNA746057
S. townsendi	TG3869	M	RADseq	Tail	PRJNA746057
S. townsendi	TG3870	F	RADseq	Tail	PRJNA746057
S. townsendi	TG2021	F	RADseq	Tail	PRJNA746057
S. townsendi	TG2023	F	RADseq	Tail	PRJNA746057
S. townsendi	TG2059	F	RADseq	Tail	PRJNA746057
S. townsendi	TG3101	F	RADseq	Tail	PRJNA746057
S. townsendi	TG3110	F	RADseq	Tail	PRJNA746057
S. townsendi	TG3133	F	RADseq	Tail	PRJNA746057
S. nicholsi	TG1995	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG2003	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG2096	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG2100	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG3212	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG3213	M	RADseq	Tail	PRJNA746057
S. nicholsi	TG2102	F	RADseq	Tail	PRJNA746057
S. nicholsi	TG2115	F	RADseq	Tail	PRJNA746057

S. nicholsi	TG2666	F	RADseq	Tail	PRJNA746057
S. nicholsi	TG2672	F	RADseq	Tail	PRJNA746057
S. nicholsi	TG3214	F	RADseq	Tail	PRJNA746057
S. nicholsi	TG3216	F	RADseq	Tail	PRJNA746057
S. notatus	TG3777	M	RADseq	Tail	PRJNA746057
S. notatus	TG3778	M	RADseq	Tail	PRJNA746057
S. notatus	TG3818	M	RADseq	Tail	PRJNA746057
S. notatus	TG3820	M	RADseq	Tail	PRJNA746057
S. notatus	TG3821	M	RADseq	Tail	PRJNA746057
S. notatus	TG4158	M	RADseq	Tail	PRJNA746057
S. notatus	TG4160	M	RADseq	Tail	PRJNA746057
S. notatus	TG3815	M	RADseq	Tail	PRJNA746057
S. notatus	TG3779	F	RADseq	Tail	PRJNA746057
S. notatus	TG3780	F	RADseq	Tail	PRJNA746057
S. notatus	TG3781	F	RADseq	Tail	PRJNA746057
S. notatus	TG3819	F	RADseq	Tail	PRJNA746057
S. notatus	TG4159	F	RADseq	Tail	PRJNA746057
S. notatus	TG3816	F	RADseq	Tail	PRJNA746057
S. notatus	TG3817	F	RADseq	Tail	PRJNA746057
S. inigoi	TG2147	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2050	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1950	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2046	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1971	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2145	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2018	M	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2148	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1929	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2146	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1979	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1980	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2047	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG1981	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2048	F	RADseq	Tail	Gamble et al. 2015
S. inigoi	TG2049	F	RADseq	Tail	Gamble et al. 2015

SRA Accession
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