Current Biology, Volume *27*

Supplemental Information

The Discovery of XY Sex Chromosomes in a *Boa* and *Python*

Tony Gamble, Todd A. Castoe, Stuart V. Nielsen, Jaison L. Banks, Daren C. Card, Drew R. Schield, Gordon W. Schuett, and Warren Booth

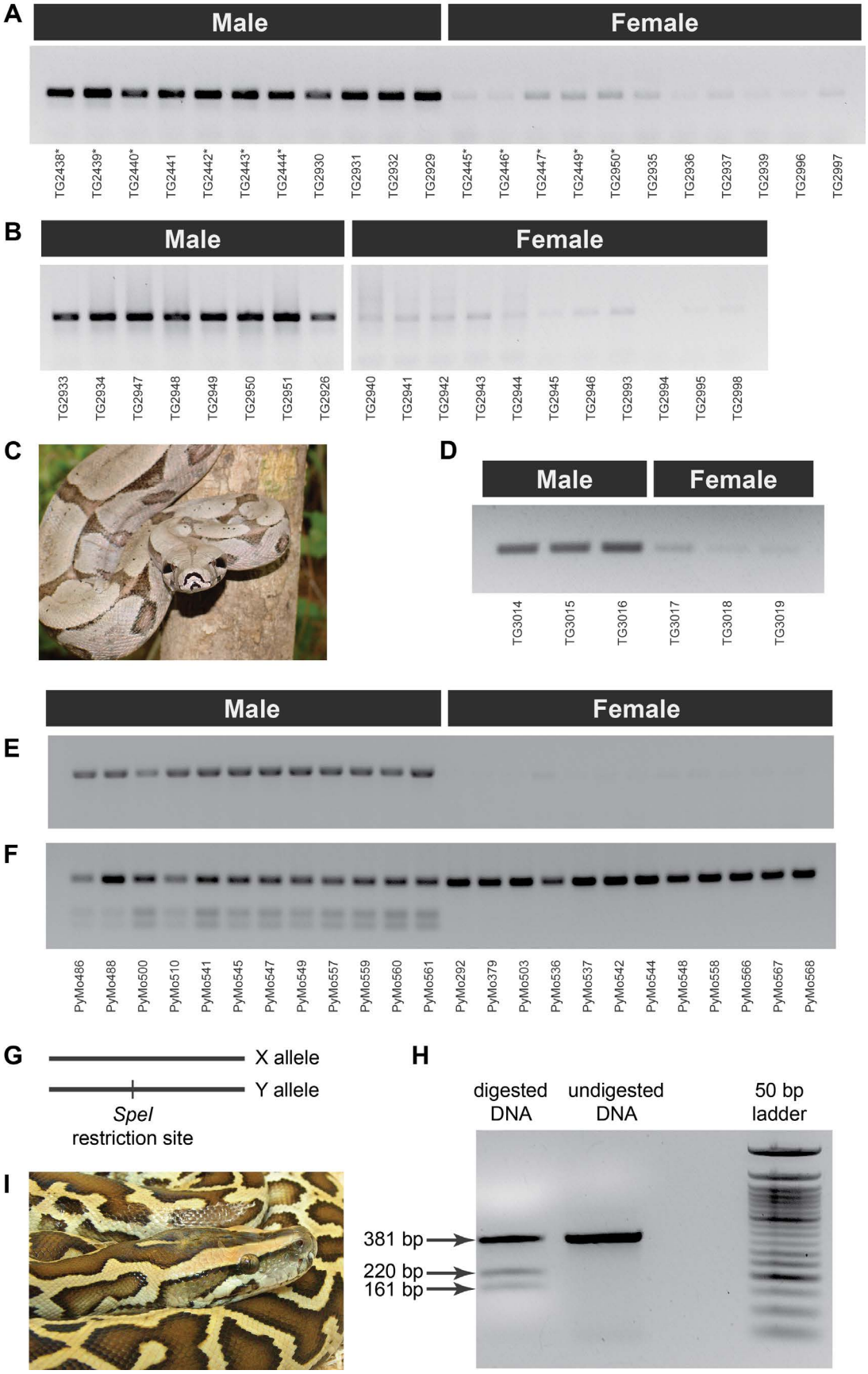
A
Male
Female
TG2438*
TG2439*
TG2440*
TG2441
TG2442*
TG2443*
TG2444*
TG2930
TG2931
TG2932
TG2929
TG2445*
TG2446*
TG2447*
TG2449*
TG2950*
TG2935
TG2936
TG2937
TG2939
TG2996
TG2997
B
Male
Female
TG2933
TG2934
TG2947
TG2948
TG2949
TG2950
TG2951
TG2926
TG2940
TG2941
TG2942
TG2943
TG2944
TG2945
TG2946
TG2993
TG2994
TG2995
TG2998
C
D
Male
Female
TG3014
TG3015
TG3016
TG3017
TG3018
TG3019
Male
Female
E
F
PyMo486
PyMo488
PyMo500
PyMo510
PyMo541
PyMo545
PyMo547
PyMo549
PyMo557
PyMo559
PyMo560
PyMo561
PyMo292
PyMo379
PyMo503
PyMo536
PyMo537
PyMo542
PyMo544
PyMo548
PyMo558
PyMo566
PyMo567
PyMo568
G
X allele
Y allele
SpeI
restriction site
H
digested DNA
undigested DNA
50 bp ladder
381 bp
220 bp
161 bp
I

Figure S1. PCR validation of male-specific RAD markers in boa and python. Related to Figure 1. A. Male-biased amplification of RAD marker TCBoa_2918 in eleven male and eleven female *Boa imperator*. Individual specimen ID numbers are listed below the gel image. **B.** Male-biased amplification of RAD marker TCBoa_2918 in an additional eight male and eleven female *Boa imperator*. Individual specimen ID numbers are listed below the gel image. **C.** Photograph of South American Boa (*Boa constrictor*) from Goiás, Brazil. **D.** Male-biased amplification of RAD marker TCBoa_2918 in three male and three female *Boa constrictor*. Individual specimen ID numbers are listed below the gel image. **E.** Male-biased amplification of RAD marker M3 in twelve male and twelve female *Python bivittatus*. Individual specimen ID numbers are listed below the gel image in panel F. **F.** Male-specific restriction digest of PCR amplicon (PCR-RFLP) from RAD marker M10 in twelve male and twelve female *Python bivittatus*. Individual specimen ID numbers are listed below the gel image. **G.** Cartoon illustrating PCR amplicons from the python RAD marker M10. The X and Y alleles are illustrated including the approximate position of the Y chromosome-specific *SpeI* restriction site. **H.** Gel image of python RAD marker M10 showing difference between digested and undigested PCR amplicons from a male *Python bivittatus*. The *SpeI* digested amplicons separate into a 381bp uncut X allele and the two digested Y fragments that are 220bp and 161bp. The undigested DNA retains a single band at 381bp, which is also the expected female pattern**. I.** Photograph of Burmese python (*Python bivittatus*), captive specimen. Primers for all loci are listed in Supplemental Experimental Procedures. Table 1. In all gel images, ID numbers with an asterisk (*) represent individuals that were also used to generate the RADseq dataset. Individuals lacking an asterisk were used solely for PCR validation and not used in the RADseq experiment (see Supplemental Experimental Procedures. Table 1).

Family	Species	Karyotype	Heteromorphic sex chromsomes	Citation(s)
Anomochilidae	*Anomochilus leonardi*	no	n/a	
Anomochilidae	*Anomochilus weberi*	no	n/a	
Boidae	*Acrantophis dumerili*	yes	yes	[S1]
Boidae	*Acrantophis madagascariensis*	no	n/a	
Boidae	*Boa constrictor*	yes	no	[S2-S5]
Boidae	*Boa imperator*	no	n/a	
Boidae	*Calabaria reinhardtii*	no	n/a	
Boidae	*Candoia aspera*	no	n/a	
Boidae	*Candoia bibroni*	yes	no	[S6]
Boidae	*Candoia carinata*	no	n/a	
Boidae	*Candoia paulsoni*	yes	no	[S6]
Boidae	*Candoia superciliosa*	no	n/a	
Boidae	*Charina bottae*	yes	no	[S7]
Boidae	*Charina umbratica*	no	n/a	
Boidae	*Chilabothrus angulifer*	no	n/a	
Boidae	*Chilabothrus chrysogaster*	no	n/a	
Boidae	*Chilabothrus exsul*	no	n/a	
Boidae	*Chilabothrus fordii*	no	n/a	
Boidae	*Chilabothrus gracilis*	no	n/a	
Boidae	*Chilabothrus granti*	no	n/a	
Boidae	*Chilabothrus inornatus*	no	n/a	
Boidae	*Chilabothrus monensis*	no	n/a	
Boidae	*Chilabothrus striatus*	yes	no	[S7]
Boidae	*Chilabothrus strigilatus*	no	n/a	
Boidae	*Chilabothrus subflavus*	no	n/a	
Boidae	*Corallus annulatus*	no	n/a	
Boidae	*Corallus batesii*	no	n/a	
Boidae	*Corallus blombergi*	no	n/a	
Boidae	*Corallus caninus*	yes	no	[S2]
Boidae	*Corallus cookii*	no	n/a	
Boidae	*Corallus cropanii*	no	n/a	
Boidae	*Corallus grenadensis*	yes	no	[S7]
Boidae	*Corallus hortulanus*	yes	no	[S5]
Boidae	*Corallus ruschenbergerii*	no	n/a	
Boidae	*Epicrates alvarezi*	no	n/a	
Boidae	*Epicrates assisi*	yes	no	[S5]
Boidae	*Epicrates cenchria*	yes	no	[S5]
Boidae	*Epicrates crassus*	yes	no	[S2, S5, S8]
Boidae	*Epicrates maurus*	no	n/a	

Family	Species	Karyotype	Heteromorphic sex chromsomes	Citation(s)
Boidae	*Eryx borrii*	no	n/a	
Boidae	*Eryx colubrinus*	no	n/a	
Boidae	*Eryx conicus*	yes	no	[S9-S12]
Boidae	*Eryx elegans*	no	n/a	
Boidae	*Eryx jaculus*	yes	no	[S13]
Boidae	*Eryx jayakari*	no	n/a	
Boidae	*Eryx johnii*	yes	no	[S10, S14, S15]
Boidae	*Eryx miliaris*	no	n/a	
Boidae	*Eryx muelleri*	no	n/a	
Boidae	*Eryx somalicus*	no	n/a	
Boidae	*Eryx tataricus*	no	n/a	
Boidae	*Eryx whitakeri*	no	n/a	
Boidae	*Eunectes beniensis*	no	n/a	
Boidae	*Eunectes deschauenseei*	no	n/a	
Boidae	*Eunectes murinus*	yes	no	[S2, S5]
Boidae	*Eunectes notaeus*	yes	no	[S5]
Boidae	*Exiliboa placata*	yes	no	[S6]
Boidae	*Lichanura orcutti*	yes	no	[S7]
Boidae	*Lichanura trivirgata*	no	n/a	
Boidae	*Sanzinia madagascariensis*	yes	no	[S1]
Boidae	*Sanzinia volontany*	no	n/a	
Boidae	*Ungaliophis continentalis*	no	n/a	
Boidae	*Ungaliophis panamensis*	no	n/a	
Cylindrophiidae	*Cylindrophis aruensis*	no	n/a	
Cylindrophiidae	*Cylindrophis boulengeri*	no	n/a	
Cylindrophiidae	*Cylindrophis burmanus*	no	n/a	
Cylindrophiidae	*Cylindrophis engkariensis*	no	n/a	
Cylindrophiidae	*Cylindrophis isolepis*	no	n/a	
Cylindrophiidae	*Cylindrophis jodiae*	no	n/a	
Cylindrophiidae	*Cylindrophis lineatus*	no	n/a	
Cylindrophiidae	*Cylindrophis maculatus*	no	n/a	
Cylindrophiidae	*Cylindrophis melanotus*	no	n/a	
Cylindrophiidae	*Cylindrophis mirzae*	no	n/a	
Cylindrophiidae	*Cylindrophis opisthorhodus*	no	n/a	
Cylindrophiidae	*Cylindrophis ruffus*	no	n/a	
Cylindrophiidae	*Cylindrophis subocularis*	no	n/a	
Cylindrophiidae	*Cylindrophis yamdena*	no	n/a	
Loxocemidae	*Loxocemus bicolor*	yes	no	[S16]
Pythonidae	*Antaresia childreni*	yes	no	[S6]

Family	Species	Karyotype	Heteromorphic sex chromsomes	Citation(s)
Pythonidae	*Antaresia maculosa*	no	n/a	
Pythonidae	*Antaresia perthensis*	yes	no	[S6]
Pythonidae	*Antaresia stimsoni*	no	n/a	
Pythonidae	*Aspidites melanocephalus*	yes	no	[S6]
Pythonidae	*Aspidites ramsayi*	no	n/a	
Pythonidae	*Bothrochilus albertisii*	yes	no	[S6]
Pythonidae	*Bothrochilus biakensis*	no	n/a	
Pythonidae	*Bothrochilus boa*	no	n/a	
Pythonidae	*Bothrochilus fredparkeri*	no	n/a	
Pythonidae	*Bothrochilus huonensis*	no	n/a	
Pythonidae	*Bothrochilus meridionalis*	no	n/a	
Pythonidae	*Bothrochilus montanus*	no	n/a	
Pythonidae	*Liasis fuscus*	yes	no	[S17]
Pythonidae	*Liasis mackloti*	yes	no	[S6]
Pythonidae	*Liasis olivaceus*	yes	no	[S1]
Pythonidae	*Liasis papuana*	no	n/a	
Pythonidae	*Malayopython reticulatus*	yes	no	[S6]
Pythonidae	*Malayopython timoriensis*	no	n/a	
Pythonidae	*Morelia bredli*	no	n/a	
Pythonidae	*Morelia carinata*	no	n/a	
Pythonidae	*Morelia spilota*	yes	no	[S6]
Pythonidae	*Morelia viridis*	no	n/a	
Pythonidae	*Python anchietae*	no	n/a	
Pythonidae	*Python bivittatus*	yes	no	[S18]
Pythonidae	*Python breitensteini*	no	n/a	
Pythonidae	*Python brongersmai*	no	n/a	
Pythonidae	*Python curtus*	yes	no	[S16]
Pythonidae	*Python kyaiktiyo*	no	n/a	
Pythonidae	*Python molurus*	yes	no	[S14]
Pythonidae	*Python natalensis*	no	n/a	
Pythonidae	*Python regius*	no	n/a	
Pythonidae	*Python sebae*	no	n/a	
Pythonidae	*Simalia amethistina*	yes	no	[S1]
Pythonidae	*Simalia boeleni*	yes	no	[S1]
Pythonidae	*Simalia clastolepis*	no	n/a	
Pythonidae	*Simalia kinghorni*	no	n/a	
Pythonidae	*Simalia nauta*	no	n/a	
Pythonidae	*Simalia oenpelliensis*	yes	no	[S6]
Pythonidae	*Simalia tracyae*	no	n/a	
Uropeltidae	*Brachyophidium rhodogaster*	no	n/a	

Family	Species	Karyotype	Heteromorphic sex chromsomes	Citation(s)
Uropeltidae	*Melanophidium bilineatum*	no	n/a	
Uropeltidae	*Melanophidium khairei*	no	n/a	
Uropeltidae	*Melanophidium punctatum*	no	n/a	
Uropeltidae	*Melanophidium wynaudense*	no	n/a	
Uropeltidae	*Platyplectrurus madurensis*	no	n/a	
Uropeltidae	*Platyplectrurus trilineatus*	no	n/a	
Uropeltidae	*Plectrurus aureus*	no	n/a	
Uropeltidae	*Plectrurus canaricus*	no	n/a	
Uropeltidae	*Plectrurus guentheri*	no	n/a	
Uropeltidae	*Plectrurus perroteti*	no	n/a	
Uropeltidae	*Pseudotyphlops philippinus*	no	n/a	
Uropeltidae	*Rhinophis blythii*	no	n/a	
Uropeltidae	*Rhinophis dorsimaculatus*	no	n/a	
Uropeltidae	*Rhinophis drummondhayi*	no	n/a	
Uropeltidae	*Rhinophis erangaviraji*	no	n/a	
Uropeltidae	*Rhinophis fergusonianus*	no	n/a	
Uropeltidae	*Rhinophis goweri*	no	n/a	
Uropeltidae	*Rhinophis homolepis*	no	n/a	
Uropeltidae	*Rhinophis lineatus*	no	n/a	
Uropeltidae	*Rhinophis oxyrhynchus*	no	n/a	
Uropeltidae	*Rhinophis philippinus*	no	n/a	
Uropeltidae	*Rhinophis porrectus*	no	n/a	
Uropeltidae	*Rhinophis punctatus*	no	n/a	
Uropeltidae	*Rhinophis sanguineus*	no	n/a	
Uropeltidae	*Rhinophis travancoricus*	no	n/a	
Uropeltidae	*Rhinophis tricolorata*	no	n/a	
Uropeltidae	*Rhinophis zigzag*	no	n/a	
Uropeltidae	*Teretrurus sanguineus*	no	n/a	
Uropeltidae	*Uropeltis arcticeps*	no	n/a	
Uropeltidae	*Uropeltis beddomii*	no	n/a	
Uropeltidae	*Uropeltis bicatenata*	no	n/a	
Uropeltidae	*Uropeltis broughami*	no	n/a	
Uropeltidae	*Uropeltis ceylanicus*	no	n/a	
Uropeltidae	*Uropeltis dindigalensis*	no	n/a	
Uropeltidae	*Uropeltis ellioti*	no	n/a	
Uropeltidae	*Uropeltis liura*	no	n/a	
Uropeltidae	*Uropeltis macrolepis*	no	n/a	

Family	Species	Karyotype	Heteromorphic sex chromsomes	Citation(s)
Uropeltidae	*Uropeltis macrorhyncha*	no	n/a	
Uropeltidae	*Uropeltis maculata*	no	n/a	
Uropeltidae	*Uropeltis madurensis*	no	n/a	
Uropeltidae	*Uropeltis melanogaster*	no	n/a	
Uropeltidae	*Uropeltis myhendrae*	no	n/a	
Uropeltidae	*Uropeltis nitida*	no	n/a	
Uropeltidae	*Uropeltis ocellata*	no	n/a	
Uropeltidae	*Uropeltis petersi*	no	n/a	
Uropeltidae	*Uropeltis phillipsi*	no	n/a	
Uropeltidae	*Uropeltis phipsonii*	no	n/a	
Uropeltidae	*Uropeltis pulneyensis*	no	n/a	
Uropeltidae	*Uropeltis rubrolineata*	no	n/a	
Uropeltidae	*Uropeltis rubromaculatus*	no	n/a	
Uropeltidae	*Uropeltis ruhunae*	no	n/a	
Uropeltidae	*Uropeltis shorttii*	no	n/a	
Uropeltidae	*Uropeltis smithi*	no	n/a	
Uropeltidae	*Uropeltis woodmasoni*	no	n/a	
Xenopeltidae	*Xenopeltis hainanensis*	no	n/a	
Xenopeltidae	*Xenopeltis unicolor*	yes	no	[S19]
Xenophidiidae	*Xenophidion schaeferi*	no	n/a	

Table S2. List of *Boa* genome scaffolds (assembly SGA) with sex-specific variants identified from RADseq datasets. Related to Figure 1. The single digest RADseq dataset is indicated by "RADseq" while the double-digest RADseq dataset is indicated by "ddRADseq."

Scaffold	Gene(s)	Synteny - Anole	Synteny - Human	Dataset(s)	# of male-specific variants
scaffold-0				RADseq	1
scaffold-103	EML2, VASP, RTN2	GL343870	19q	ddRADseq / RADseq	4 / 1
scaffold-1132				RADseq	2
scaffold-1141				ddRADseq	4
scaffold-1171	AKT2	Lgf	19q	RADseq	1
scaffold-1327				RADseq	1
scaffold-1588				ddRADseq	1
scaffold-1619	FOSB, PPP1R13L, ERCC2, KLC3, CKM, MARK4	GL344338, GL344965	19q	RADseq	2
scaffold-1631				RADseq	1
scaffold-1654	PLEKHG2	GL344052	19q	ddRADseq / RADseq	2 / 3
scaffold-1714				RADseq	2
scaffold-1758				RADseq	2
scaffold-1826	CLIP3,ALKBH6	GL344980	19q	RADseq	5
scaffold-1932	SYCN, PAK4	GL343755	19q	RADseq	1
scaffold-1974	BLVRB	Lgf	19q	RADseq	2
scaffold-2296	CPNS1	GL343994	19q	RADseq	5
scaffold-2352				RADseq	2
scaffold-2383	SIPA1L3, DPF1, NUMBL	Lgf	19q	RADseq	1
scaffold-2411	GMFG, SAMD4B	GL343496	19q	RADseq	4
scaffold-2551				RADseq	5
scaffold-2575	ATP4A, GAPDHS, LSR, ETV2, RBM42, PSMC4, GRAMD1A, SCN1B, FNDC8	GL343281, GL343514	19q, 17q	RADseq	5
scaffold-2806				RADseq	1
scaffold-2833				ddRADseq	1
scaffold-2918				ddRADseq	1 / PCR validated
scaffold-3007	DHX34	Lgf	19q	RADseq	1
scaffold-3281				RADseq	2
scaffold-3444	EXOC3L2	GL344489	19q	RADseq	1
scaffold-3453	SMG9, KCNN4, HAS1, PPP2R1A, VSIG10L, ETFB	Lgf, GL343604, GL343281	19q	RADseq	1
scaffold-3462	PRX, HIPK4, PLD3	Lgf	19q	ddRADseq / RADseq	1 / 1
scaffold-3468	ANKRD22, STAMBPL1, CH25H	GL343246	10q	RADseq	1
scaffold-3612				ddRADseq	2
scaffold-3621				ddRADseq	2
scaffold-3876				RADseq	1

Scaffold	Gene(s)	Synteny - Anole	Synteny - Human	Dataset(s)	# of male-specific variants
scaffold-3904				RADseq	1
scaffold-424				RADseq	5
scaffold-4244				RADseq	1
scaffold-4353				RADseq	1
scaffold-4355				ddRADseq	1
scaffold-444				ddRADseq	1
scaffold-4541				RADseq	2
scaffold-4598	SPTBN4	Lgf	19q	ddRADseq	2
scaffold-756				ddRADseq	1
scaffold-782	KCNK6, LTBP4	Lgf	19q	ddRADseq	2
scaffold-831				RADseq	1
scaffold-85				RADseq	1
scaffold-92	NRG3	GL343199	10q	RADseq	1

Table S3. Table. List of *Python* transcripts with sex-specific SNPs, based on RNAseq data from intestinal tissue from six males and two females. Related to Figure 1.

Transcript	Reference	Allele	Zygosity	P-value (Bonferroni corrected)	Sample frequency (male)	Sample frequency (female)	*Anolis* chromosome	*Gallus* chromosome	Human chromosome
XM007423441 EFTUD2	G	A	Heterozygous	0.0357	100	0	6	27	17
XM007428540 PDIA4	G	C	Heterozygous	0.0357	100	0	6	2	7
XM015887663 GOLGA4	C	T	Heterozygous	0.0357	100	0	6	2	3
XM015888085 ZMYM6NB	G	A	Unknown	0.0357	100	0	GL343558	23	1
XM007430524 PINX1	-	T	Unknown	0.0357	100	0	GL343553	unk	8

Species	Purpose	Sex	ID	Locality	SRA Accession
Boa constrictor	PCR validation	Female	TG3017	Commercial breeder	n/a
Boa constrictor	PCR validation	Female	TG3018	Commercial breeder	n/a
Boa constrictor	PCR validation	Female	TG3019	Commercial breeder	n/a
Boa constrictor	PCR validation	Male	TG3014	Commercial breeder	n/a
Boa constrictor	PCR validation	Male	TG3015	Commercial breeder	n/a
Boa constrictor	PCR validation	Male	TG3016	Commercial breeder	n/a
Boa imperator	Double Digest RADseq	Female	Boco14	Lagoon Cay, Belize	SRR5434514
Boa imperator	Double Digest RADseq	Female	Boco15	West Snake Cay, Belize	SRR5434513
Boa imperator	Double Digest RADseq	Female	Boco18	West Snake Cay, Belize	SRR5434512
Boa imperator	Double Digest RADseq	Female	Boco20	Lagoon Cay, Belize	SRR5434511
Boa imperator	Double Digest RADseq	Female	Boco21	Lagoon Cay, Belize	SRR5434510
Boa imperator	Double Digest RADseq	Female	Boco26	Lagoon Cay, Belize	SRR5434505
Boa imperator	Double Digest RADseq	Female	Boco27	Belize District, Belize	SRR5434504
Boa imperator	Double Digest RADseq	Female	Boco28	Belize District, Belize	SRR5434503
Boa imperator	Double Digest RADseq	Female	Boco32	West Snake Cay, Belize	SRR5434502
Boa imperator	Double Digest RADseq	Male	Boco11	Cayo District, Belize	SRR5434516
Boa imperator	Double Digest RADseq	Male	Boco12	Belize District, Belize	SRR5434515
Boa imperator	Double Digest RADseq	Male	Boco22	Lagoon Cay, Belize	SRR5434509
Boa imperator	Double Digest RADseq	Male	Boco23	Lagoon Cay, Belize	SRR5434508
Boa imperator	Double Digest RADseq	Male	Boco24	West Snake Cay, Belize	SRR5434507
Boa imperator	Double Digest RADseq	Male	Boco25	West Snake Cay, Belize	SRR5434506
Boa imperator	PCR validation	Female	TG2935	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2936	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2937	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2939	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2940	Commercial breeder	n/a

Species	Purpose	Sex	ID	Locality	SRA Accession
Boa imperator	PCR validation	Female	TG2941	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2942	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2943	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2944	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2945	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2946	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2993	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2994	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2995	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2996	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2997	Commercial breeder	n/a
Boa imperator	PCR validation	Female	TG2998	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2441	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2926	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2929	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2930	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2931	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2932	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2933	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2934	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2947	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2948	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2949	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2950	Commercial breeder	n/a
Boa imperator	PCR validation	Male	TG2951	Commercial breeder	n/a
Boa imperator	Single Digest RADseq, PCR validation	Female	TG2445	Commercial breeder	SAMN07159337

Species	Purpose	Sex	ID	Locality	SRA Accession
Boa imperator	Single Digest RADseq, PCR validation	Female	TG2446	Commercial breeder	SAMN07159338
Boa imperator	Single Digest RADseq, PCR validation	Female	TG2447	Commercial breeder	SAMN07159339
Boa imperator	Single Digest RADseq, PCR validation	Female	TG2449	Commercial breeder	SAMN07159340
Boa imperator	Single Digest RADseq, PCR validation	Female	TG2450	Commercial breeder	SAMN07159341
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2438	Commercial breeder	SAMN07159342
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2439	Commercial breeder	SAMN07159343
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2440	Commercial breeder	SAMN07159344
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2442	Commercial breeder	SAMN07159345
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2443	Commercial breeder	SAMN07159346
Boa imperator	Single Digest RADseq, PCR validation	Male	TG2444	Commercial breeder	SAMN07159347
Crotalus atrox	Double Digest RADseq	Female	CA0194	Palo Pinto Co., Texas	SRR1706442
Crotalus atrox	Double Digest RADseq	Female	CA0237	Pima Co., Arizona	SRR4344143
Crotalus atrox	Double Digest RADseq	Female	CA0304	Cochise Co., Arizona	SRR4344144
Crotalus atrox	Double Digest RADseq	Female	CA0342	Nueces Co., Texas	SRR4344148
Crotalus atrox	Double Digest RADseq	Female	CA0343	Kleburg Co., Texas	SRR4344149
Crotalus atrox	Double Digest RADseq	Female	CA0344	Kleburg Co., Texas	SRR4344150
Crotalus atrox	Double Digest RADseq	Female	CA0345	Kleburg Co., Texas	SRR4344151
Crotalus atrox	Double Digest RADseq	Male	CA0272	Pima Co., Arizona	SRR4344152
Crotalus atrox	Double Digest RADseq	Male	CA0278	Pima Co., Arizona	SRR4344153
Crotalus atrox	Double Digest RADseq	Male	CA0281	Pima Co., Arizona	SRR4344154
Crotalus atrox	Double Digest RADseq	Male	CA0300	Cochise Co., Arizona	SRR4344155
Crotalus atrox	Double Digest RADseq	Male	CA0306	Santa Cruz Co., Arizona	SRR4344145
Crotalus atrox	Double Digest RADseq	Male	CA0308	Cochise Co., Arizona	SRR4344146
Crotalus atrox	Double Digest RADseq	Male	CA0335	Yavapai Co., Arizona	SRR4344147
Python bivittatus	Double Digest RADseq	Female	Genome	Commercial breeder	SRR5434226
Python bivittatus	Double Digest RADseq	Female	R17_F1	Commercial breeder	SRR5434232
Python bivittatus	Double Digest RADseq	Female	U25_F2	Commercial breeder	SRR5434231
Python bivittatus	Double Digest RADseq	Female	Y1_F3	Commercial breeder	SRR5434230

Species	Purpose	Sex	ID	Locality	SRA Accession
Python bivittatus	Double Digest RADseq	Male	Y7_M1	Commercial breeder	SRR5434229
Python bivittatus	Double Digest RADseq	Male	Y8_M2	Commercial breeder	SRR5434228
Python bivittatus	Double Digest RADseq	Male	Y9_M3	Commercial breeder	SRR5434227
Python bivittatus	mRNAseq	Female	Pymo216	Florida	SRR5434340
Python bivittatus	mRNAseq	Female	Pymo219	Florida	SRR5434338
Python bivittatus	mRNAseq	Male	Pymo208	Florida	SRR5434345
Python bivittatus	mRNAseq	Male	Pymo209	Florida	SRR5434344
Python bivittatus	mRNAseq	Male	Pymo210	Florida	SRR5434343
Python bivittatus	mRNAseq	Male	Pymo213	Florida	SRR5434342
Python bivittatus	mRNAseq	Male	Pymo215	Florida	SRR5434341
Python bivittatus	mRNAseq	Male	Pymo218	Florida	SRR5434339
Python bivittatus	PCR validation	Female	PyMo292	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo379	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo503	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo536	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo537	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo542	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo544	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo548	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo558	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo566	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo567	Florida	n/a
Python bivittatus	PCR validation	Female	PyMo568	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo486	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo488	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo500	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo510	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo541	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo545	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo547	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo549	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo557	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo559	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo560	Florida	n/a
Python bivittatus	PCR validation	Male	PyMo561	Florida	n/a

Supplemental References

S1. Mengden, G.A., and Stock, A.D. (1980). Chromosomal evolution in Serpentes: A comparison of G and C chromosome banding patterns of some colubrid and boid genera. Chromosoma *79*, 53–64.

S2. Becak, W. (1965). Constituição cromossômica e mecanismo de determinação do sexo em ofídios sul–americanos. I. Aspectos cariotipicos. Memórias do Instituto Butantan *32*, 37–78.

S3. Bianchi, N., Beçak, W., de Bianchi, M.S., Beçak, M.L., and Rabello, M.N. (1969). Chromosome replication in four species of snakes. Chromosoma *26*, 188–200.

S4. Beçak, M.L., Beçak, W., Roberts, F.L., Shoffner, R.N., Volpe, E.P., Benirschke, K., and Hsu, T. (1971). *Boa constrictor amarali* Stull. In Chromosome Atlas: Fish, Amphibians, Reptiles and Birds. (Springer), pp. 101–103.

S5. Viana, P.F., Ribeiro, L.B., Souza, G.M., de Menezes Chalkidis, H., Gross, M.C., and Feldberg, E. (2016). Is the karyotype of neotropical boid snakes really conserved? Cytotaxonomy, chromosomal rearrangements and karyotype organization in the Boidae family. PLoS ONE *11*, e0160274.

S6. Mengden, G.A. (1982). Chromosomal Evolution and the Phylogeny of Elapid Snakes, (Australian National University: Ph.D. Dissertation).

S7. Gorman, G.C., and Gress, F. (1970). Chromosome cytology of four boid snakes and a varanid lizard, with comments on the cytosystematics of primitive snakes. Herpetologica *26*, 308–317.

S8. Beçak, M.L., Beçak, W., Roberts, F.L., Shoffner, R.N., Volpe, E.P., Benirschke, K., and Hsu, T. (1971). *Epicrates cenchria crassus* Cope. In Chromosome Atlas: Fish, Amphibians, Reptiles and Birds. (Springer), pp. 105–107.

S9. Jones, K.W., and Singh, L. (1985). Snakes and the evolution of sex chromosomes. Trends Genet *1*, 55–61.

S10. Ray-Chaudhuri, S., Singh, L., and Sharma, T. (1971). Evolution of sex-chromosomes and formation of W-chromatin in snakes. Chromosoma *33*, 239–251.

S11. Singh, L. (1972). Evolution of karyotypes in snakes. Chromosoma *38*, 185–236.

S12. Parkash, S.O., and Kour, G. (2005). Karyology of four Indian snakes. Caryologia *58*, 380–386.

S13. Werner, Y.L. (1959). Chromosomes of primitive snakes from Israel. Bulletin of the Research Council of Israel *8B*, 197–198.

S14. Singh, L., Sharma, T., and Ray-Chaudhuri, S. (1968). Chromosomes and the classification of the snakes of the family Boidae. Cytogenet Genome Res *7*, 161–168.

S15. Sharma, O., and Kour, G. (2005). On the chromosomes of four species of Indian snakes. Cytologia *70*, 65–70.

S16. Fischman, H.K., Mitra, J., and Dowling, H. (1972). Chromosome characteristics of 13 species in the order Serpentes. Mamm Chromosomes Newsl *13*, 7–9.

S17. O'Meally, D., Patel, H.R., Stiglec, R., Sarre, S.D., Georges, A., Marshall Graves, J.A., and Ezaz, T. (2010). Non-homologous sex chromosomes of birds and snakes share repetitive sequences. Chromosome Res *18*, 787–800.

S18. Matsubara, K., Tarui, H., Toriba, M., Yamada, K., Nishida-Umehara, C., Agata, K., and Matsuda, Y. (2006). Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc Natl Acad Sci U S A *103*, 18190–18195.
S19. Cole, C., and Dowling, H. (1970). Chromosomes of the sunbeam snake, *Xenopeltis unicolor* Reinwardt (Reptilia: Xenopeltidae). Herpetol Rev *2*, 35–36.