Supplementary material

Anolis sex chromosomes are derived from a single ancestral pair

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Supplementary Figure 1 – Majority rule phylogenetic tree inferred for 216 *Anolis* species using BEAST. Node support (posterior probability) for Beast and MrBayes analyses are indicated by color coded circles. Karyotype data were available for species indicated by black text.

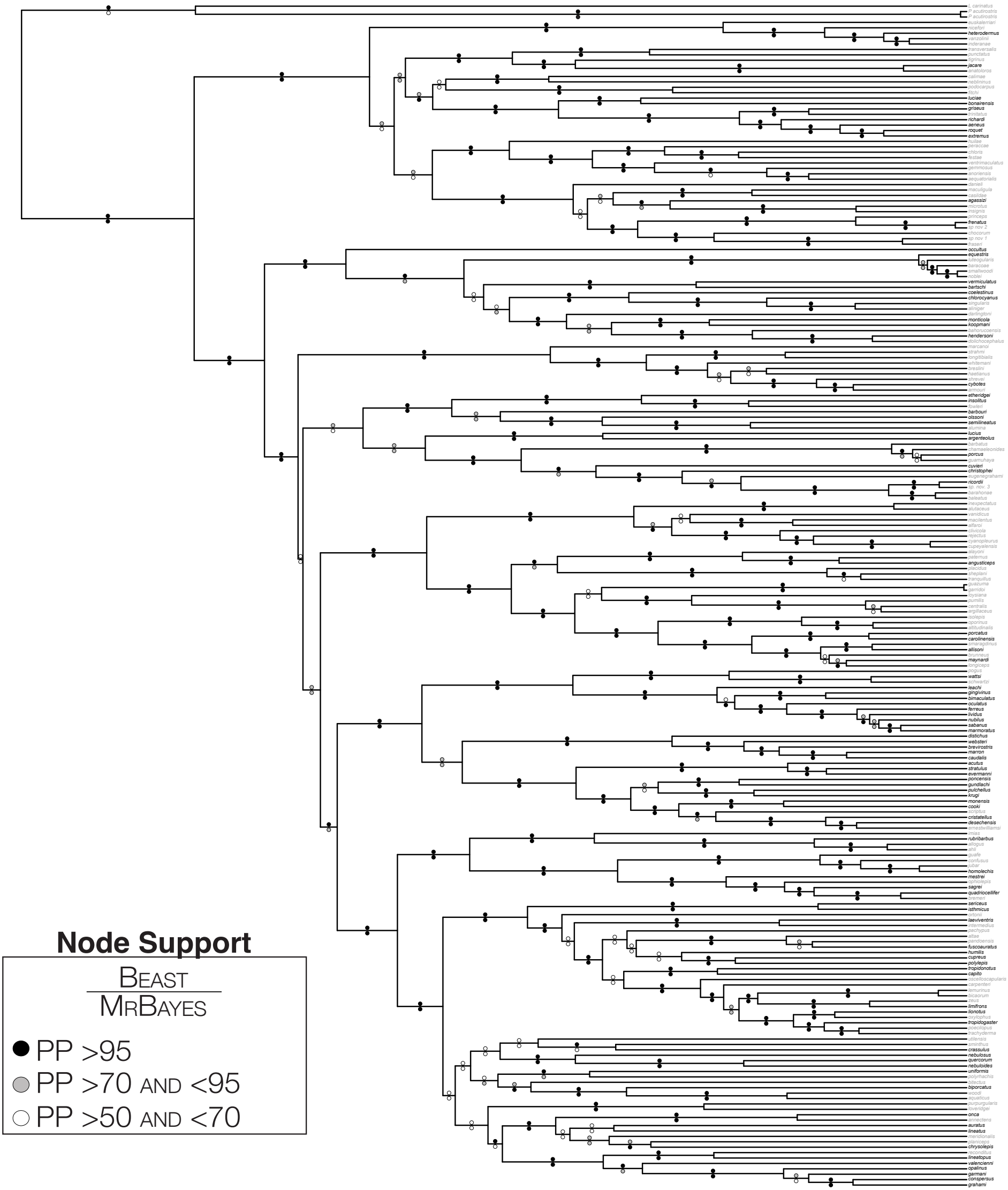
Supplementary Figure 2 – Ancestral state reconstruction of 1n female chromosome number onto the pruned phylogeny. Maximum likelihood estimate of ancestral chromosome number is indicated on each node. Female 1n chromosome number is indicated after each species name.

Supplementary Table 1 – Samples used in the phylogenetic and comparative evolutionary analyses.

Supplementary Table 2 – Samples used for qPCR.

Supplementary Table 3 – Primers used for qPCR.

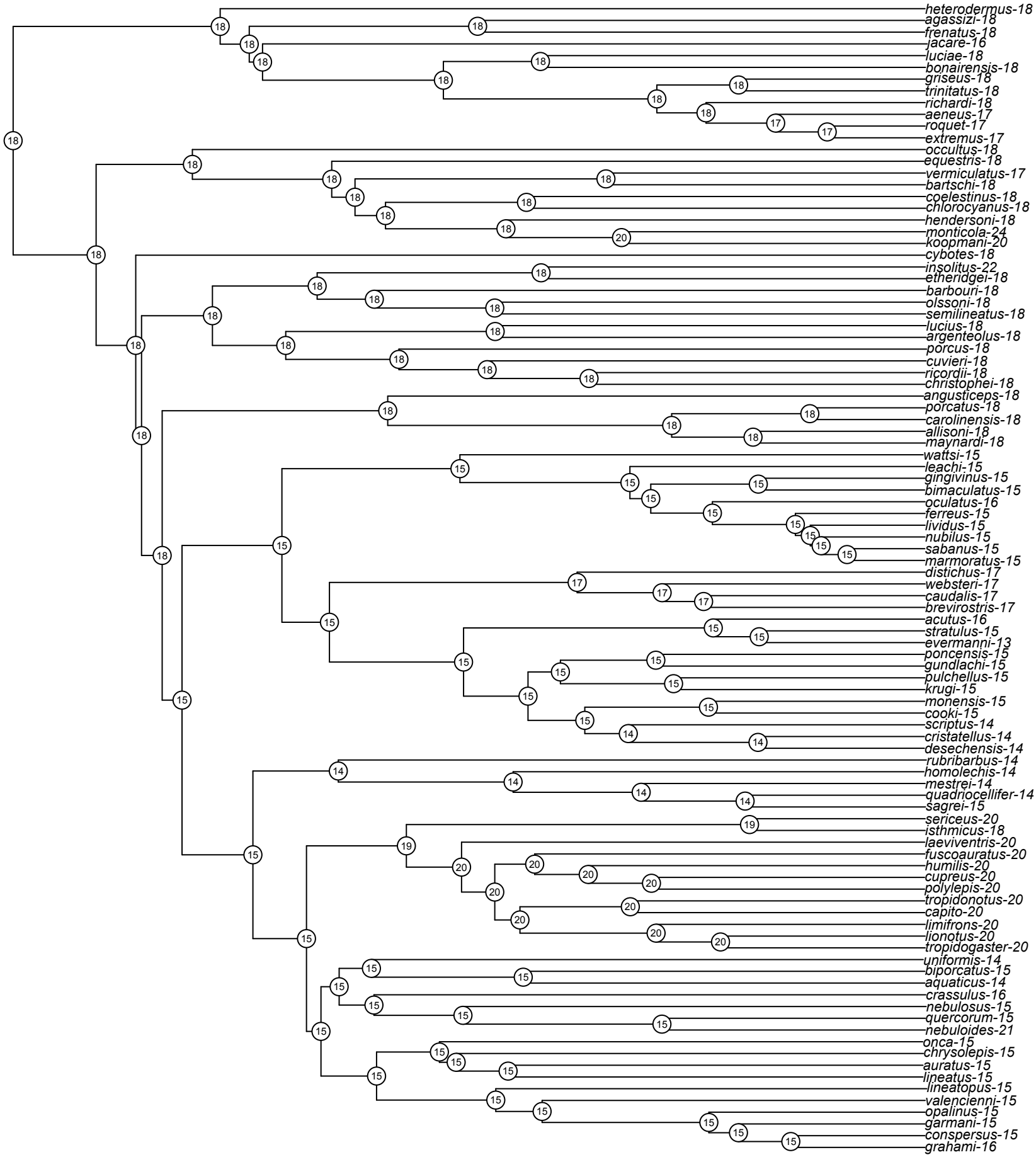
Supplementary Table 4 – qPCR results showing mean fold change in quantification of genes in male samples compared to female samples with standard error and 95% confidence intervals.



Node Support

BEAST / MRBAYES

- PP >95
- PP >70 AND <95
- PP >50 AND <70


heterodermus-18
agassizi-18
frenatus-18
jacare-16
luciae-18
bonairensis-18
griseus-18
trinitatus-18
richardi-18
aeneus-17
roquet-17
extremus-17
occultus-18
equestris-18
vermiculatus-17
bartschi-18
coelestinus-18
chlorocyanus-18
hendersoni-18
monticola-24
koopmani-20
cybotes-18
insolitus-22
etheridgei-18
barbouri-18
olssoni-18
semilineatus-18
lucius-18
argenteolus-18
porcus-18
cuvieri-18
ricordii-18
christophei-18
angusticeps-18
porcatus-18
carolinensis-18
allisoni-18
maynardi-18
wattsi-15
leachi-15
gingivinus-15
bimaculatus-15
oculatus-16
ferreus-15
lividus-15
nubilus-15
sabanus-15
marmoratus-15
distichus-17
websteri-17
caudalis-17
brevirostris-17
acutus-16
stratulus-15
evermanni-13
poncensis-15
gundlachi-15
pulchellus-15
krugi-15
monensis-15
cooki-15
scriptus-14
cristatellus-14
desechensis-14
rubribarbus-14
homolechis-14
mestrei-14
quadriocellifer-14
sagrei-15
sericeus-20
isthmicus-18
laeviventris-20
fuscoauratus-20
humilis-20
cupreus-20
polylepis-20
tropidonotus-20
capito-20
limifrons-20
lionotus-20
tropidogaster-20
uniformis-14
biporcatus-15
aquaticus-14
crassulus-16
nebulosus-15
quercorum-15
nebuloides-21
onca-15
chrysolepis-15
auratus-15
lineatus-15
lineatopus-15
valencienni-15
opalinus-15
garmani-15
conspersus-15
grahami-16

Supplementary table 1. Sources of data used in phylogenetic and comparative evolutionary analyses. Genbank accession number for ND2 sequences used in phylogenetic analyses. Cytogenetic data consists of chromosome number (1N) and the presence/absence of heteromorphic sex chromosomes as well as the source of that data ("no" means the species lacks heteromorphic sex chromosomes). *Anolis woodi*, labeled "n/a", was not included in the comparative analyses because there were no data concerning the occurrence of heteromorphic sex chromosomes in the original citation.

Species	ND2 GenBank	Female 1N	Sex Chromosome Complement	Source
Liocephalus carinatus	AF049864.1			
Polychrus acutirostris (1)	AF055925.2			
Polychrus *acutirostris* (2)	AF528737.1			
A. acutus	AF055926.2	16	XXY	(Gorman and Atkins 1969)
A. aeneus	AF055949.2	17	no	(Gorman and Atkins 1967)
A. aequatorialis	JN112663.1			
A. agassizi	AF055952	18	no	(Stamm and Gorman 1975)
A. ahli	KF819775			
A. alayoni	AY296149			
A. alfaroi	AY296150			
A. aliniger	EF531487.1			
A. allisoni	AY296151.1	18	no	(Gorman et al. 1967)
A. allogus	KF819776			
A. altae	AY090735			
A. altitudinalis	AY654203			
A. alumina	AY296153			
A. alutaceus	AF055971			
A. anatoloros	JN112668.1			
A. angusticeps	AF055967	18	no	(Gorman and Atkins 1968b)
A. annectens	AY909736			
A. anoriensis	JN112664.1			
A. aquaticus	AY909738	14	no	(Lieb 1981)
A. argenteolus	AY296154	18	no	(Gorman and Atkins 1968b)
A. argillaceus	AY909739			
A. armouri	AY263012.1			
A. auratus	AY909740	15	no	(Gorman et al. 1967)
A. bahorucoensis	AF055932			
A. baleatus	AY296155			
A. baracoae	AY296156			
A. barahonae	AF055972			
A. barbatus	AY296146			
A. barbouri	AF055946	18	no	(Paull et al. 1976)
A. bartschi	AF055960	18	no	(Gorman and Atkins 1968b)
A. bicaorum	AY909741			
A. bimaculatus	KF819777	15	XXY	(Gorman 1965)
A. biporcatus	KF819778	15	XXY	(Gorman and Atkins 1966)

Species	ND2 GenBank	Female 1N	Sex Chromosome Complement	Source
A. bitectus	AY909743			
A. bonairensis	AF317070.1	18	no	(Gorman 1965)
A. bremeri	AF296157.1			
A. breslini	AY263017			
A. brevirostris	AY296158	17	XXY	(Williams 1977)
A. brunneus	KF819779			
A. calimae	JN112669.1			
A. capito	AY909744	20	no	(Gorman 1973)
A. carolinensis	AF294279.1	18	no	(Porter et al. 1994)
A. carpenteri	AY296160			
A. casildae	AY909745			
A. caudalis	AY296161	17	XXY	(Williams 1977)
A. centralis	AY296162			
A. chamaeleonides	AF055975			
A. chloris	JN112671.1			
A. chlorocyanus	EF531535	18	no	(Gorman et al. 1967)
A. chocorum	JN112674.1			
A. christophei	AF055957	18	no	(Webster et al. 1972)
A. chrysolepis	AF294281.1	15	no	(Gorman 1965)
A. clivicola	AY909746			
A. coelestinus	AY296164.1	18	no	(Gorman et al. 1967)
A. confusus	AY909787			
A. conspersus	AF294304	15	XY	(Gorman and Atkins 1966, 1968a)
A. cooki	AY909747	15	XXY	(Gorman et al. 1968)
A. crassulus	AY909748	16	XY	(Lieb 1981)
A. cristatellus	EF531400	14	XXY	(Gorman et al. 1968)
A. cupeyalensis	AY909749			
A. cupreus	AY909750	20	no	(Gorman 1973)
A. cuvieri	AF055973	18	no	(Gorman and Atkins 1969)
A. cyanopleurus	AY909751			
A. cybotes	AY263138.1	18	no	(Gorman and Atkins 1966)
A. danieli	JN112677.1			
A. darlingtoni	AY367137			
A. desechensis	AY296167.1	14	XXY	(Brandley et al. 2006)
A. distichus	AY296168	17	XXY	(Gorman and Atkins 1969)
A. dolichocephalus	AY296169.1			
A. equestris	AF055978	18	no	(Gorman 1965)
A. ernestwilliamsi	AY296170.1			
A. etheridgei	AF055934	18	no	(Webster et al. 1972)
A. eugenegrahami	AY296171			
A. euskalerriari	JN112678.1			
A. evermanni	AY296172.1	13	XY	(Gorman and Atkins 1968a)
A. extremus	AF317065.1	17	no	(Gorman and Atkins 1967)
A. ferreus	AY296173.1	15	XXY	(Gorman and Atkins 1969)

Species	ND2 GenBank	Female 1N	Sex Chromosome Complement	Source
A. festae	JN112680.1			
A. fitchi	JN112681.1			
A. fowleri	AY296174			
A. fraseri	JN112683.1			
A. frenatus	AY909752	18	no	(Stamm and Gorman 1975)
A. fuscoauratus	AF337792.1	20	no	(Gorman 1973)
A. garmani	AF055936.2	15	no	(Gorman and Atkins 1968b)
A. garridoi	AY296175.1			
A. gemmosus	JN112686.1			
A. gingivinus	AY909753	15	XXY	(Gorman and Atkins 1966)
A. grahami	AF294299.1	16	no	(Blake 1986)
A. griseus	AY296176.1	18	no	(Gorman and Atkins 1967)
A. guafe	AY909788			
A. guamuhaya	AF055974			
A. guazuma	AY909754			
A. gundlachi	AY296177.1	15	XXY	(Gorman et al. 1968)
A. haetianus	AY263042.1			
A. hendersoni	AY296178.1	18	no	(Gorman et al. 1967)
A. heterodermus	AY296144	18	no	(Gorman et al. 1969)
A. homolechis	AY296179.1	14	no	(Gorman and Atkins 1968b)
A. huilae	JN112692.1			
A. humilis	KF819780	20	no	(Gorman 1973)
A. imias	KF819781			
A. inderanae	AY296145			
A. inexpectatus	AY296180.1			
A. insignis	JN112693.1			
A. insolitus	AF055933.2	22	no	(Webster et al. 1972)
A. intermedius	AY909755	20	n/a	(Lieb 1981)
A. isolepis	AY65022			
A. isthmicus	AY909762	18	XY	(Lieb 1981)
A. jacare	JN112694	16	no	(Williams et al. 1970)
A. jubar	KF819782			
A. koopmani	KF819783	20	no	(Webster et al. 1972)
A. krugi	AF055928.2	15	XXY	(Gorman and Atkins 1969)
A. laeviventris	AY909756	20	no	(Lieb 1981)
A. leachi	AY296183.1	15	XXY	(Gorman and Atkins 1966)
A. lemurinus	AF337782	20	n/a	(Lieb 1981)
A. limifrons	AF337783.1	20	no	(Gorman 1973)
A. lineatopus	AF055937.2	15	no	(Gorman 1965)
A. lineatus	AF337784.1	15	no	(Gorman and Atkins 1967)
A. lionotus	AY909757	20	no	(Gorman 1973)
A. lividus	AY909758	15	XXY	(Gorman and Atkins 1969)
A. longiceps	AY902407			
A. longitibialis	AY263009.1			
A. loveridgei	AY909759			
A. loysiana	AF055964.2			
A. luciae	AF055951.2	18	no	(Gorman 1965)

Species	ND2 GenBank	Female 1N	Sex Chromosome Complement	Source
A. lucius	AF055962.2	18	no	(Gorman and Atkins 1968b)
A. luteogularis	AF055977.2			
A. macilentus	AY296185.1			
A. maculigula	JN112699.1			
A. marcanoi	AY263006.1			
A. marmoratus	AY296186.1	15	XXY	(Gorman and Atkins 1966)
A. marron	AY296187			
A. maynardi	AY902409	18	no	(Gorman and Atkins 1968b)
A. meridionalis	AY909760			
A. mestrei	AF337779.1	14	no	(Gorman and Atkins 1968b)
A. microtus	AF055947.2			
A. monensis	AY296188.1	15	XXY	(Gorman and Stamm 1975)
A. monticola	AY296189.1	24	no	(Webster et al. 1972)
A. neblininus	JN112700.1			
A. nebuloides	AY909763	21	XY	(Lieb 1981)
A. nebulosus	HM236483.1	15	XY	(Gorman 1973)
A. nicefori	AF337768			
A. noblei	AY296190.1			
A. nubilus	AY909764	15	XXY	(Gorman and Stamm 1975)
A. occultus	AF055976.2	18	no	(Gorman and Atkins 1969)
A. oculatus	AY296191.1	16	XXY	(Gorman and Atkins 1967)
A. olssoni	AF055945.2	18	no	(Gorman et al. 1967)
A. onca	AY909765	15	XY	(Gorman 1969)
A. opalinus	AF294309.1	15	XY	(Gorman 1969)
A. ophiolepis	AF055942			
A. oporinus	AY909766			
A. ortonii	AF337799.1			
A. oscelloscapularis	AY909767			
A. oxylophus	AY909768			
A. pachypus	AY909769			
A. pandoensis	AY909770			
A. paternus	U82679.1			
A. peraccae	JN112701.1			
A. placidus	AY296192.1			
A. planiceps	AF337805			
A. podocarpus	JN112703.1			
A. poecilopus	AY909771			
A. pogus	AY296193.1			
A. polylepis	AY909772	20	no	(Gorman 1973)
A. polyrhachis	AY909773			
A. poncensis	AY296194.1	15	XXY	(Gorman and Atkins 1969)
A. porcatus	AY296195	18	no	(Gorman and Atkins 1968b)
A. porcus	AY296147	18	no	(Gorman et al. 1969)
A. princeps	JN112704.1			
A. pulchellus	AY296196.1	15	XXY	(Gorman et al. 1968)
A. pumilis	AF055963.2			

Species	ND2 GenBank	Female 1N	Sex Chromosome Complement	Source
A. punctatus	AF337777.1			
A. purpurgularis	AY909774			
A. quadriocellifer	AY655169	14	no	(Gorman and Atkins 1968b)
A. quercorum	AY909775	15	XY	(Lieb 1981)
A. reconditus	AY296198.1			
A. rejectus	AY909761			
A. richardi	AF055949.2	18	no	(Gorman and Atkins 1967)
A. ricordii	AF055949.2	18	no	(Gorman et al. 1967)
A. roquet	AY296199.1	17	no	(Gorman and Atkins 1967)
A. rubribarbus	AY909789	14	no	(Gorman and Atkins 1968b)
A. sabanus	AY909776	15	XXY	(Gorman and Atkins 1969)
A. sagrei	AF337778.1	15	XXY	(De Smet 1981)
A. schwartzi	AY909777			
A. scriptus	AY296200.1	14	XXY	(Gorman et al. 1968)
A. semilineatus	AY296201.1	18	no	(Gorman et al. 1967)
A. sericeus	AY909778	20	no	(Lieb 1981)
A. sheplani	AF055966.2			
A. shrevei	AY263037.1			
A. singularis	EF531478.1			
A. smallwoodi	AY296203.1			
A. smaragdinus	AY902424			
A. sminthus	AY909779			
A. sp nov 1	AY909737			
A. sp nov 2	AY909742			
A. sp nov. 3	KF819784			
A. strahmi	AY263007.1			
A. stratulus	AF055929.2	15	XXY	(Gorman and Atkins 1969)
A. tigrinus	JN112710.1			
A. trachyderma	AF337785.1			
A. tranquillus	AY909780			
A. transversalis	AF337769.1			
A. trinitatus	AY909781	18	no	(Gorman and Atkins 1967)
A. tropidogaster	AY909782	20	no	(Gorman 1973)
A. tropidonotus	AY909783	20	no	(Gorman 1973)
A. uniformis	AY909784	14	no	(Lieb 1981)
A. utilensis	AY909785			
A. valencienni	AF055939.2	15	no	(Gorman and Atkins 1968b)
A. vanidicus	AF055970.2			
A. vanzolinii	JN112712.1			
A. ventrimaculatus	JN112713.1			
A. vermiculatus	AF055961.1	17	no	(Gorman and Atkins 1968b)
A. wattsi	AF055931	15	XXY	(Gorman and Atkins 1969)
A. websteri	AY296205.1	17	XXY	(Williams 1977)
A. whitemani	AY263023.1			
A. woodi	AF337780.1	15	n/a	(Lieb 1981)
A. zeus	AY909786			

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Supplementary Table 2. *Anolis* samples used in qPCR experiments.

Species	ID	Sex	Locality
A. aeneus	TG1660	Female	Cambleton, Tobago, Trinidad & Toabago
A. aeneus	TG1687	Female	Arima, Trinidad, Trinidad & Tobago
A. aeneus	TG1831	Female	Arima, Trinidad, Trinidad & Tobago
A. aeneus	TG1832	Female	Arima, Trinidad, Trinidad & Tobago
A. aeneus	TG1726	Male	St. Joseph, Trinidad, Trinidad & Tobago
A. aeneus	TG1830	Male	Arima, Trinidad, Trinidad & Tobago
A. carolinensis	TG1437	Female	unknown
A. carolinensis	TG1607	Female	Kailua-Kona, Hawai'i County, Hawaii, USA
A. carolinensis	TG1608	Female	Kailua-Kona, Hawai'i County, Hawaii, USA
A. carolinensis	TG1436	Male	unknown
A. carolinensis	TG1578	Male	Kailua-Kona, Hawai'i County, Hawaii, USA
A. carolinensis	TG1609	Male	Kailua-Kona, Hawai'i County, Hawaii, USA
A. chlorocyanus	Glor5555	Female	Loc 486, Barrera, Dominican Republic
A. chlorocyanus	Glor5605	Female	Loc 488, west of Puerto Viejo, Dominican Republic
A. chlorocyanus	Glor5869	Female	Loc 528, northwest of San Juan de la Maguana, Dominican Republic
A. chlorocyanus	Glor6310	Male	Loc 567, east of Hato Mayor del Rey, Dominican Republic
A. chlorocyanus	Glor6583	Male	Loc 580, Cape Cana, Dominican Republic
A. chlorocyanus	Glor7299	Male	Loc 634, north of Bani, Dominican Republic
A. distichus ravitergum	Glor5522	Female	Loc 402, north of Bani, Dominican Republic
A. distichus ravitergum	Glor5537	Female	Loc 402, north of Bani, Dominican Republic
A. distichus ravitergum	Glor5542	Female	Loc 402, north of Bani, Dominican Republic
A. distichus ravitergum	Glor4245	Male	Loc 396, east of Bani, Dominican Republic
A. distichus ravitergum	Glor4246	Male	Loc 396, east of Bani, Dominican Republic
A. distichus ravitergum	Glor5527	Male	Loc 402, north of Bani, Dominican Republic
A. grahami	TG1490	Female	near Mahoe Bay, Saint Mary Parish, Jamaica

Species	ID	Sex	Locality
A. grahami	TG1492	Female	near Mahoe Bay, Saint Mary Parish, Jamaica
A. grahami	TG1493	Male	near Mahoe Bay, Saint Mary Parish, Jamaica
A. grahami	TG1494	Male	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatopus	TG1485	Female	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatopus	TG1486	Female	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatopus	TG1487	Female	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatopus	TG1488	Male	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatopus	TG1489	Male	near Mahoe Bay, Saint Mary Parish, Jamaica
A. lineatus	MCZ25767	Female	Downtown Oranjestad, Aruba
A. lineatus	MCZ25772	Female	Downtown Oranjestad, Aruba
A. lineatus	MCZ29307	Female	Downtown Oranjestad, Aruba
A. lineatus	C15	Male	Lodge Kura Hulanda, near Westpunt, Curaçao
A. lineatus	MCZ25719	Male	Downtown Oranjestad, Aruba
A. lineatus	MCZ29306	Male	Downtown Oranjestad, Aruba
A. richardii	TG1646	Female	near Charlotteville, Tobago, Trinidad & Tobago
A. richardii	TG1866	Female	near Charlotteville, Tobago, Trinidad & Tobago
A. richardii	TG1868	Female	near Charlotteville, Tobago, Trinidad & Tobago
A. richardii	TG1644	Male	near Charlotteville, Tobago, Trinidad & Tobago
A. richardii	TG1647	Male	near Charlotteville, Tobago, Trinidad & Tobago
A. richardii	TG1865	Male	near Charlotteville, Tobago, Trinidad & Tobago
A. sagrei	TG1461	Female	Tarpon Springs, Pinellas County, Florida, USA
A. sagrei	TG1462	Female	Tarpon Springs, Pinellas County, Florida, USA
A. sagrei	TG1463	Female	Gainesville, Alachua County, Florida, USA
A. sagrei	TG1454	Male	Lake Placid, Highlands County, Florida, USA
A. sagrei	TG1459	Male	Tarpon Springs, Pinellas County, Florida, USA
A. sagrei	TG1464	Male	Gainesville, Alachua County, Florida, USA

Supplementary Table 3. Primers used for qPCR. KANK1 primers AcF2 and AcR2 were used only with *A. aeneus* and *A. richardii* samples.

Primer name	Primer sequence (5' to 3')	Chromosome
NGFB		Chr 4
AnolisF1	ATCTGAAGATAATGCGCCTTTG	
AnolisR1	CCACCATCACAGTCACCTCTT	
KANK1		Chr 2
AcF	CCTTCCTTTGTAGGATCCAGTG	
AcR	GGAGCACAGGGATAGTTTTGAC	
AcF2	TCTTCTTTGGGTAGTTCCATCC	
AcR2	TACCTGGAGCACAGGGATAGTT	
CLTCL1		Chr X
epic-F	CAGACGTATTGCTGCTTACCTG	
epic-R	GCGAAACACTCCTTCTTGTCTT	
PI4KA		Chr X
epicA-F	GAAGGAAATCCACGATTTTGTC	
epicA-R	ATGATGACTTTGCTGGAGGTCT	

Supplementary Table 4. qPCR results showing fold difference in gene quantity in male *Anolis* compared to females for one autosomal gene and two genes that are X-linked in *Anolis carolinensis*. The autosomal gene *ngfb* (Chr 4) was used as a reference for all loci. Standard error and 95% confidence intervals were calculated using 5,000 bootstrap replicates. Sample size and specimen data are listed in Supplementary Table 2. Primers for each locus are listed in Supplementary Table 3.

Species	Gene	Chromosome	Fold difference	Std. Error	95% C. I.
A. aeneus	*kank1*	Chr 2	1.385	1.007 - 2.172	0.654 - 2.463
	cltcl1	Chr X	0.538	0.387 - 0.799	0.338 - 1.051
	pi4ka	Chr X	0.575	0.450 - 0.751	0.423 - 0.944
A. carolinensis	*kank1*	Chr 2	1.103	0.967 - 1.318	0.895 - 1.395
	cltcl1	Chr X	0.464	0.430 - 0.519	0.419 - 0.534
	pi4ka	Chr X	0.539	0.433 - 0.714	0.396 - 0.811
A. chlorocyanus	*kank1*	Chr 2	0.983	0.902 - 1.058	0.864 - 1.086
	cltcl1	Chr X	0.913	0.634 - 1.239	0.532 - 1.596
	pi4ka	Chr X	0.606	0.468 - 0.745	0.450 - 0.795
A. distichus	*kank1*	Chr 2	1.033	0.813 - 1.291	0.719 - 1.399
	cltcl1	Chr X	0.465	0.343 - 0.603	0.329 - 0.638
	pi4ka	Chr X	0.639	0.433 - 0.924	0.355 - 1.287
A. grahami	*kank1*	Chr 2	1.144	1.066 - 1.228	1.058 - 1.238
	cltcl1	Chr X	0.505	0.482 - 0.530	0.468 - 0.545
	pi4ka	Chr X	0.441	0.417 - 0.466	0.414 - 0.469
A. lineatopus	*kank1*	Chr 2	0.932	0.865 - 0.986	0.862 - 1.017
	cltcl1	Chr X	0.515	0.481 - 0.543	0.473 - 0.552
	pi4ka	Chr X	0.392	0.334 - 0.479	0.329 - 0.486
A. lineatus	*kank1*	Chr 2	1.117	1.035 - 1.185	1.009 - 1.272
	cltcl1	Chr X	0.477	0.432 - 0.522	0.411 - 0.541
	pi4ka	Chr X	0.437	0.403 - 0.464	0.390 - 0.493

Species	Gene	Chromosome	Fold difference	Std. Error	95% C. I.
A. richardii	*kank1*	Chr 2	1.558	0.929 - 2.968	0.629 - 3.781
	cltcl1	Chr X	0.474	0.376 - 0.573	0.334 - 0.632
	pi4ka	Chr X	0.412	0.338 - 0.511	0.322 - 0.524
A. sagrei	*kank1*	Chr 2	1.019	0.955 - 1.077	0.948 - 1.140
	cltcl1	Chr X	0.494	0.429 - 0.584	0.396 - 0.630
	pi4ka	Chr X	0.478	0.378 - 0.559	0.366 - 0.578