Supplementary Table 1. Samples used for gecko RAD-seq.

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Aristelliger expectatus	Glor5825	F	Carrettera a Galindo, approx. 2.3km ENE of Galindo (by road) Azua Province (Loc497), Dominican Republic	842362	67307	9.8
Aristelliger expectatus	Scantle353	F	woods and oasis east of Parador approx. 21 km S from intersection of San Juan – Galvan Road with interstate 50 (to Vallenuevo), Bahoruco Province, Dominican Republic	5547791	97123	51.9
Aristelliger expectatus	Scantle448	F	Hotel Iguana, La Deschubierta, Dominican Republic	669999	20871	8.2
Aristelliger expectatus	Scantle667	F	Small Cay ESE of Colonia Juancho, Pedernales Province, Dominican Republic	5288918	10087	55.9

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Aristelliger expectatus	TG1447	F	Captive born (parents from Hotel Iguana, La Deschubierta, Dominican Republic)	3759834	95328	35.5
Aristelliger expectatus	TG1449	F	Captive born (parents from Hotel Iguana, La Deschubierta, Dominican Republic)	2839509	93644	27.8
Aristelliger expectatus	TG1450	F	Hotel Iguana, La Deschubierta, Dominican Republic	1752977	79062	19.5
Aristelliger expectatus	Glor5806	M	Glor loc490, Rancheria, Azua Province, Dominican Republic	3091364	93107	32
Aristelliger expectatus	Glor5839	M	Main entrance to Martín García (523), Barahona Province, Dominican Republic	5147983	94426	54.7
Aristelliger expectatus	Glor6231	M	Glor loc561, Playa Pedernales, Pedernales Province, Dominican Republic	891549	41964	15.7

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Aristelliger expectatus	Glor6232	M	Glor loc561, Playa Pedernales, Pedernales Province, Dominican Republic	2422314	60185	28.6
Aristelliger expectatus	Scantle352	M	DPS28, woods and oasis east of Parador approx. 21 km south from intersection of San Juan – Galvan Road with interstate 50 (to Vallenuevo), Bahoruco Province, Dominican Republic	9844696	96952	101.5
Aristelliger expectatus	TG1448	M	Captive born (parents from Hotel Iguana, La Deschubierta, Dominican Republic)	2165352	88450	24.3
Aristelliger expectatus	TG1451	M	Hotel Iguana, La Deschubierta, Dominican Republic	1132250	70576	15
Christinus marmoratus	TG1906	F	Canberra, ACT, Australia	1895057	115310	16
Christinus marmoratus	TG1907	F	Canberra, ACT, Australia	2780396	123132	22.5

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Christinus marmoratus	TG1909	F	Canberra, ACT, Australia	2467987	119235	20.5
Christinus marmoratus	TG1911	F	Canberra, ACT, Australia	2548547	119944	21.1
Christinus marmoratus	TG1914	F	Canberra, ACT, Australia	2410136	119412	20
Christinus marmoratus	TG1920	F	Canberra, ACT, Australia	2031735	116442	17.2
Christinus marmoratus	TG1828	M	Canberra, ACT, Australia	2367190	114326	20.4
Christinus marmoratus	TG1910	M	Canberra, ACT, Australia	2474761	118343	20.8
Christinus marmoratus	TG1912	M	Canberra, ACT, Australia	1974481	111736	17.4
Christinus marmoratus	TG1913	M	Canberra, ACT, Australia	1903248	110374	17.1
Christinus marmoratus	TG1915	M	Canberra, ACT, Australia	3277477	121993	27.3
Christinus marmoratus	TG1919	M	Canberra, ACT, Australia	2910569	120147	24.2
Correlophus ciliatus	tg1619	F	Captive born	3425841	81030	45.2
Correlophus ciliatus	tg1623	F	Captive born	1646378	61455	28

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Correlophus ciliatus	tg1625	F	Captive born	828941	43281	18.9
Correlophus ciliatus	tg1628	F	Captive born	6259044	100357	64.3
Correlophus ciliatus	tg1636	F	Captive born	1310445	59864	21.7
Correlophus ciliatus	tg1637	F	Captive born	3578854	91079	39.6
Correlophus ciliatus	tg2155	F	Captive born	7408843	98857	65.8
Correlophus ciliatus	tg1467	M	Captive born	4950357	98857	44.1
Correlophus ciliatus	tg1624	M	Captive born	1389314	65327	21.5
Correlophus ciliatus	tg1638	M	Captive born	4771095	99155	50.7
Correlophus ciliatus	tg1639	M	Captive born	3671950	94164	40.9
Correlophus ciliatus	tg1640	M	Captive born	2145819	79979	27.5
Correlophus ciliatus	tg1641	M	Captive born	4123482	84089	50.4
Gehyra mutilata	TG1015	F	Kailua-Kona, Hawaii, USA	1915156	103130	18.7

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Gehyra mutilata	TG1560	F	Kailua-Kona, Hawaii, USA	1520574	94625	15.6
Gehyra mutilata	TG1563	F	Kailua-Kona, Hawaii, USA	2950192	109433	27.2
Gehyra mutilata	TG1575	F	Kailua-Kona, Hawaii, USA	3300822	108971	30.6
Gehyra mutilata	TG2136	F	Kailua-Kona, Hawaii, USA	5041870	114620	43.8
Gehyra mutilata	TG2137	F	Kailua-Kona, Hawaii, USA	1923704	102194	18.9
Gehyra mutilata	TG772	F	Kailua-Kona, Hawaii, USA	3934011	111125	36
Gehyra mutilata	TG1039	M	Kailua-Kona, Hawaii, USA	2949645	88260	34.4
Gehyra mutilata	TG1040	M	Kailua-Kona, Hawaii, USA	2129865	101426	20.8
Gehyra mutilata	TG1559	M	Kailua-Kona, Hawaii, USA	5591215	112346	51.7
Gehyra mutilata	TG2133	M	Kailua-Kona, Hawaii, USA	2607285	108187	24.2
Gehyra mutilata	TG2134	M	Kailua-Kona, Hawaii, USA	2631479	105912	25.3
Gehyra mutilata	TG2135	M	Kailua-Kona, Hawaii, USA	2382040	105259	22.9

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Gehyra mutilata	TG774	M	Kailua-Kona, Hawaii, USA	3516054	108696	32.9
Hemidactylus frenatus	TG1526	F	Kailua-Kona, Hawaii, USA	4758066	115421	39.9
Hemidactylus frenatus	TG1527	F	Kailua-Kona, Hawaii, USA	5991345	117210	50.3
Hemidactylus frenatus	TG1529	F	Kailua-Kona, Hawaii, USA	1079418	76507	11.7
Hemidactylus frenatus	TG1574	F	Kailua-Kona, Hawaii, USA	3540107	111676	31.6
Hemidactylus frenatus	TG1880	F	Kailua-Kona, Hawaii, USA	1858709	98886	18.3
Hemidactylus frenatus	TG1886	F	Kailua-Kona, Hawaii, USA	4483923	113192	39.1
Hemidactylus frenatus	TG1887	F	Kailua-Kona, Hawaii, USA	3892831	113241	34.2
Hemidactylus frenatus	TG1524	M	Kailua-Kona, Hawaii, USA	4885177	110057	42.8
Hemidactylus frenatus	TG1528	M	Kailua-Kona, Hawaii, USA	2441300	106943	21.4
Hemidactylus frenatus	TG1530	M	Kailua-Kona, Hawaii, USA	1594915	96812	15
Hemidactylus frenatus	TG1531	M	Kailua-Kona, Hawaii, USA	5692400	117540	46.6

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Hemidactylus frenatus	TG1533	M	Kailua-Kona, Hawaii, USA	2723799	112561	22.4
Hemidactylus frenatus	TG1573	M	Kailua-Kona, Hawaii, USA	1500110	92243	15
Hemidactylus frenatus	TG1884	M	Kailua-Kona, Hawaii, USA	2974077	109058	26.6
Hemidactylus frenatus	TG1885	M	Kailua-Kona, Hawaii, USA	2409954	105430	22.1
Hemidactylus frenatus	TG1888	M	Kailua-Kona, Hawaii, USA	2203988	104519	20.3
Hemidactylus mabouia	TG1677	F	Aripo Station Office, Trinidad, Trinidad & Tobago	2618246	96290	27.3
Hemidactylus mabouia	TG1678	F	Aripo Station Office, Trinidad, Trinidad & Tobago	1637611	90335	17.9
Hemidactylus mabouia	TG1684	F	Chicken farm near Biche, Trinidad, Trinidad & Tobago	4487451	98788	44
Hemidactylus mabouia	TG1685	F	Chicken farm near Biche, Trinidad, Trinidad & Tobago	2930306	97955	29.5
Hemidactylus mabouia	TG1686	F	Chicken farm near Biche, Trinidad, Trinidad & Tobago	2605322	95965	26.7

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Hemidactylus mabouia	TG1728	F	Chicken farm near Biche, Trinidad, Trinidad & Tobago	3447337	98094	34.2
Hemidactylus mabouia	TG1674	M	Aripo Station Office, Trinidad, Trinidad & Tobago	3297832	97153	34.1
Hemidactylus mabouia	TG1675	M	Aripo Station Office, Trinidad, Trinidad & Tobago	1931058	91257	20.1
Hemidactylus mabouia	TG1676	M	Aripo Station Office, Trinidad, Trinidad & Tobago	3257571	98515	33
Hemidactylus mabouia	TG1679	M	Aripo Station Office, Trinidad, Trinidad & Tobago	3285772	97969	31.8
Hemidactylus mabouia	TG1680	M	Aripo Station Office, Trinidad, Trinidad & Tobago	3141631	97671	32.1
Hemidactylus mabouia	TG1683	M	Chicken farm near Biche, Trinidad, Trinidad & Tobago	3766436	99307	36.5
Hemidactylus turcicus	TG2126	F	Arlington, Texas, USA	2318113	105607	22.5
Hemidactylus turcicus	TG2127	F	Arlington, Texas, USA	4197927	113268	36.1

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Hemidactylus turcicus	TG2131	F	Arlington, Texas, USA	1472051	111389	35.9
Hemidactylus turcicus	TG2135	F	Arlington, Texas, USA	3843791	110787	36.9
Hemidactylus turcicus	TG2169	F	Arlington, Texas, USA	3946108	112154	41.2
Hemidactylus turcicus	TG2188	F	Arlington, Texas, USA	4526516	105621	16.7
Hemidactylus turcicus	TG2189	F	Arlington, Texas, USA	1911023	114559	65.5
Hemidactylus turcicus	TG2123	M	Arlington, Texas, USA	7583876	113770	65.4
Hemidactylus turcicus	TG2124	M	Arlington, Texas, USA	7175174	110087	35
Hemidactylus turcicus	TG2130	M	Arlington, Texas, USA	3708668	97488	14.8
Hemidactylus turcicus	TG2187	M	Arlington, Texas, USA	3663626	112758	32.7
Hemidactylus turcicus	TG2190	M	Arlington, Texas, USA	4045617	114070	34.9
Hemidactylus turcicus	TG2199	M	Arlington, Texas, USA	3074947	112323	28
Hemidactylus turcicus	TG2201	M	Arlington, Texas, USA	7028743	114712	61.9

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Heteronotia binoei	TG1781	F	near Silverton, NSW, Australia	3850905	130227	29.5
Heteronotia binoei	TG1782	F	near Silverton, NSW, Australia	4129595	131177	31
Heteronotia binoei	TG1783	F	near Silverton, NSW, Australia	3979667	129648	30
Heteronotia binoei	TG1785	F	near Silverton, NSW, Australia	3547666	131114	27.1
Heteronotia binoei	TG1790	F	near Silverton, NSW, Australia	4297636	116771	34.8
Heteronotia binoei	TG1791	F	near Silverton, NSW, Australia	4980333	129623	36.5
Heteronotia binoei	TG1794	F	Yathong State Forest, NSW, Australia	3391463	119262	26.5
Heteronotia binoei	TG1779	M	near Silverton, NSW, Australia	1729857	100942	15.3
Heteronotia binoei	TG1780	M	near Silverton, NSW, Australia	2611086	119393	20.5
Heteronotia binoei	TG1784	M	near Silverton, NSW, Australia	3806597	130572	28.7
Heteronotia binoei	TG1789	M	near Silverton, NSW, Australia	2052117	109990	17
Heteronotia binoei	TG1793	M	Yathong State Forest, NSW, Australia	4297078	122918	32
Lialis burtonis	TG2111	F	New Guinea	3557532	117622	29.1

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Lialis burtonis	TG2114	F	New Guinea	7342815	122497	58.1
Lialis burtonis	TG2116	F	New Guinea	2522415	108441	21.9
Lialis burtonis	TG2108	M	New Guinea	4400968	121463	35.2
Lialis burtonis	TG2109	M	New Guinea	6720135	123474	52.6
Lialis burtonis	TG2113	M	New Guinea	119753	2050	14.3
Lialis burtonis	TG2112	M	New Guinea	13460219	125542	105.6
Lialis burtonis	TG2117	M	New Guinea	1780206	92702	17
Sphaerodactylus macroplepis	TG1929	F	Flamenco Beach, Culebra, Puerto Rico, USA	6165027	146856	38.8
Sphaerodactylus macroplepis	TG1979	F	Flamenco Beach, Culebra, Puerto Rico, USA	4236918	136503	26.6
Sphaerodactylus macroplepis	TG1980	F	Flamenco Beach, Culebra, Puerto Rico, USA	1437574	67642	15.8
Sphaerodactylus macroplepis	TG1981	F	Flamenco Beach, Culebra, Puerto Rico, USA	3930211	124393	29.3
Sphaerodactylus macroplepis	TG2047	F	Flamenco Beach, Culebra, Puerto Rico, USA	3081487	108525	26.6
Sphaerodactylus macroplepis	TG2048	F	Flamenco Beach, Culebra, Puerto Rico, USA	4105614	121572	32

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Sphaerodactylus macroplepis	TG2049	F	Flamenco Beach, Culebra, Puerto Rico, USA	5144834	129995	37.1
Sphaerodactylus macroplepis	TG2146	F	Curva de sixto, Vieques, Puerto Rico, USA	3814022	127873	25.9
Sphaerodactylus macroplepis	TG2148	F	Curva de sixto, Vieques, Puerto Rico, USA	3010791	117501	21.6
Sphaerodactylus macroplepis	TG1950	M	Flamenco Beach, Culebra, Puerto Rico, USA	2044556	93975	17.5
Sphaerodactylus macroplepis	TG1971	M	Curva de sixto, Vieques, Puerto Rico, USA	2915957	106280	23.8
Sphaerodactylus macroplepis	TG2018	M	Mt. Pirata, Vieques, Puerto Rico, USA	4273549	121529	29.9
Sphaerodactylus macroplepis	TG2046	M	Flamenco Beach, Culebra, Puerto Rico, USA	2469479	98929	22.3
Sphaerodactylus macroplepis	TG2050	M	Flamenco Beach, Culebra, Puerto Rico, USA	1776989	85309	16.6
Sphaerodactylus macroplepis	TG2145	M	Curva de sixto, Vieques, Puerto Rico, USA	3104653	117898	22.4
Sphaerodactylus macroplepis	TG2147	M	Curva de sixto, Vieques, Puerto Rico, USA	1819755	82052	17.2

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Sphaerodactylus nicholsi	TG1998	F	Boqueron, Puerto Rico, USA	4994749	157592	29.7
Sphaerodactylus nicholsi	TG2098	F	Boqueron, Puerto Rico, USA	3908965	136811	26.8
Sphaerodactylus nicholsi	TG2099	F	Boqueron, Puerto Rico, USA	5355966	153666	33.2
Sphaerodactylus nicholsi	TG2101	F	Boqueron, Puerto Rico, USA	2510660	116112	18.5
Sphaerodactylus nicholsi	TG2102	F	Boqueron, Puerto Rico, USA	3445323	128775	24.4
Sphaerodactylus nicholsi	TG2115	F	Boqueron, Puerto Rico, USA	2192874	105410	17.8
Sphaerodactylus nicholsi	TG2151	F	Boqueron, Puerto Rico, USA	2139016	104756	17.4
Sphaerodactylus nicholsi	TG1994	M	Boqueron, Puerto Rico, USA	3478772	131996	23.7
Sphaerodactylus nicholsi	TG1995	M	Boqueron, Puerto Rico, USA	2755057	128156	19.1
Sphaerodactylus nicholsi	TG1996	M	Boqueron, Puerto Rico, USA	2928286	130117	19.8
Sphaerodactylus nicholsi	TG1997	M	Boqueron, Puerto Rico, USA	2918639	128818	20
Sphaerodactylus nicholsi	TG2096	M	Boqueron, Puerto Rico, USA	3299968	134166	22.6

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Sphaerodactylus nicholsi	TG2097	M	Boqueron, Puerto Rico, USA	4579553	154731	28.1
Sphaerodactylus nicholsi	TG2100	M	Boqueron, Puerto Rico, USA	3966063	147912	24.9
Thecadactylus rapicauda	TG1659	F	Little Tobago near Speyside, Tobago, Trinidad & Tobago	4123563	140664	29.9
Thecadactylus rapicauda	TG1717	F	Aripo Station Office, Trinidad, Trinidad & Tobago	3096260	133368	22.5
Thecadactylus rapicauda	TG1724	F	Little Tobago near Speyside, Tobago, Trinidad & Tobago	2308338	126864	18.1
Thecadactylus rapicauda	TG1725	F	Little Tobago near Speyside, Tobago, Trinidad & Tobago	1828641	123562	14.5
Thecadactylus rapicauda	TG1864	F	Aripo Station Office, Trinidad, Trinidad & Tobago	1472432	106705	12.5
Thecadactylus rapicauda	TG1656	M	Little Tobago near Speyside, Tobago, Trinidad & Tobago	7041193	142539	50.3
Thecadactylus rapicauda	TG1657	M	Little Tobago near Speyside, Tobago, Trinidad & Tobago	4449922	140952	31.8

Species	ID	Sex	Locality	# of reads	# of RAD-tags	Mean read depth
Thecadactylus rapicauda	TG1658	M	Little Tobago near Speyside, Tobago, Trinidad & Tobago	4946065	141333	35.7
Thecadactylus rapicauda	TG1682	M	South of Charlotteville, Tobago, Triniada & Tobago	2983418	131620	21.9
Thecadactylus rapicauda	TG1715	M	Aripo Station Office, Trinidad, Trinidad & Tobago	1589028	112295	12.7
Thecadactylus rapicauda	TG1716	M	Aripo Station Office, Trinidad, Trinidad & Tobago	1597970	113059	12.9
Thecadactylus rapicauda	TG1721	M	Little Tobago near Speyside, Tobago, Trinidad & Tobago	2185142	124729	17.3
Thecadactylus rapicauda	TG1723	M	Little Tobago near Speyside, Tobago, Trinidad & Tobago	1159727	94350	10.8
Thecadactylus rapicauda	TG1863	M	Aripo Station Office, Trinidad, Trinidad & Tobago	1703944	111461	14.1

Supplementary Table 2. RAD-seq adapters used in the paper. “P” denotes a phosphate, “*” denotes a phosphorothioate bond.

Name	Sequence (5' to 3')
P1-A1-bot	P-ACTCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-A1-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAGTTGC*A
P1-A2-bot	P-GACTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-A2-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGTCTGC*A
P1-A3-bot	P-GTGACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-A3-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCACTGC*A
P1-A4-bot	P-AGAGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-A4-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCTTGC*A
P1-B1-bot	P-TGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-B1-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACATGC*A
P1-B2-bot	P-CAAGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-B2-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTTGTGC*A
P1-B3-bot	P-CCCTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-B3-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGGTGC*A
P1-B4-bot	P-TTGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-B4-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAATGC*A
P1-Ado1-bot	P-CCTATCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Ado1-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATAGGTGC*A
P1-Ado2-bot	P-CTTCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Ado2-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGAAGTGC*A
P1-Ado3-bot	P-CAATTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Ado3-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAATTGTGC*A
P1-Ado4-bot	P-CATCATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Ado4-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATGATGTGC*A
P1-Ado5-bot	P-CTCTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC

Name	Sequence (5' to 3')
P1-Ado5-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGAGTGC*A
P1-Ado6-bot	P-CGATTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Ado6-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCAATCGTGC*A
P1-Wil1-bot	P-TTGGCGACACATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Wil1-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATGTGTCGCCAATGC*A
P1-Wil2-bot	P-CAAGTATCGTCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Wil2-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGACGATACTTGTGC*A
P1-Wil3-bot	P-GTCAGCATCTAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Wil3-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAGATGCTGACTGC*A
P1-Wil4-bot	P-ACATACGGTGTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Wil4-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGACACCGTATGTTGC*A
P1-Wil5-bot	P-TGTACGCTCAGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATC
P1-Wil5-top	GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGAGCGTACATGC*A
P2-topLONG	P-GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCAGAACAA
P2-longI3-bottom	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI4-bottom	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI5-bottom	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI6-bottom	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI11-bottom	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI19-bottom	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI22-bottom	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI23-bottom	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI27-bottom	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI29-bottom	CAAGCAGAAGACGGCATACGAGATTAGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P2-longI31-bottom	CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T
P1-PCR	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
P2-I3-PCR	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGT

Name	Sequence (5' to 3')
P2-I4-PCR	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGT
P2-I5-PCR	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGT
P2-I6-PCR	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGT
P2-I11-PCR	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGT
P2-I19-PCR	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGT
P2-I22-PCR	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGT
P2-I23-PCR	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGT
P2-I27-PCR	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGT
P2-I29-PCR	CAAGCAGAAGACGGCATACGAGATTAGTTGGTGACTGGAGTTCAGACGTGT
P2-I31-PCR	CAAGCAGAAGACGGCATACGAGATATCGTGGTGACTGGAGTTCAGACGTGT

Supplementary Table 3. PCR primers used to validate sex-specific RAD-seq markers.

Species	SDM	Pattern	Primer ID	Sequence (5'-3')
Lialis burtonis	XY	male only	contig4-F	GTTTGAGGTGCCAAGACCAT
			contig4-R	GCTGCTCACACTCCCATTCT
Correlophus ciliatus	ZW	female only	CC9771-F	TACAAAGGAGGAGGGGTACAAG
			CC9771-R	GCATAGAGCCAGAGCAGTTACC
		female only	CC0065-F	TTACAACGAGTGAGACCCACAG
			CC0065-R	GCATCCTTTTTAGGACTGGTCA
		female only	CC0006-F	GCAGGGAGCACAGAGTTTTT
			CC0006-R	GGTTCACAAGACATTGTTCAGC
		two bands in females, single band in males	CC0011-F	CCGTCCATTTCAAAACAAGG
			CC0011-R	GACTTGCAGGTATGCCCCTA
Aristelliger expectatus	ZW	female only	f001-F	GTTTGCCCAGTTGGAAACATT
			f001-R	AGAAGGCATCCCCATTACTCA
		female only	f007-F	TGCAGGAGGCCAAGTTACTCT
			f007-R	CGAAATGCAACGTAAGGATCA

Species	SDM	Pattern	Primer ID	Sequence (5'-3')
Sphaerodactylus nicholsi	XY	two bands in males, single band in females	m_0173-F	GGCTCCTGCAGTTCTGGTAAG
			m_0173-R	CCATCTCTTGCCTTGAAAACC
Sphaerodactylus macroplepis	XY	two bands in males, single band in females	SmVC_C3_3_72-F	GGTTATTTCCCCAAGGAAAGC
			SmVC_C3_3_72-R	ACCAACGAAGGGAACAACTCT
		male only	SmVC_C120-F	CATGGAGGTGAAGGGAGAATC
			SmVC_C120-R	CTGTTGGGTCGTCAGTGTGAT
Thecadactylus rapicauda	ZW	female only	Thrap_05-F	CTCGTATCCCATCAGCAAAAA
			Thrap_05-R	AAGCAATTGTGCCTCATTCAG
		female only	Thrap_06-F	AGAGGCTGCCAAGTTTCTAGC
			Thrap_06-R	AAGTGGTGCGTAAATGCTGTT
		single band in females multiple, non-specific bands in males	Thrap_09-F	AGTGGAAGCTCTGTTGTGAGG
			Thrap_09-R	ATAAGCAAGAGATGGGGGAAG
Gehyra mutilata	ZW	female only	GM074-F	ACCTCTTGAGGGATCTGTTCG
			GM074-R	AAGACCTGTGTGTCAGCATCC
		female only	GM021-F	TTCTCGTTTGTGAGCCACTG
			GM021-R	AATCGCCCTGTTAGGCTGTA
		female only	GM023-F	GGGAAGTTTTCCCGTTACCTA

Species	SDM	Pattern	Primer ID	Sequence (5'-3')
			GM023-R	CCCAGACAATCTGAAGATCCA
Hemidactylus mabouia	XY	two bands in males, single band in females	m018-F	CACCTTTCAGCTTGGAAGAGC
			m018-R	CCTGGACCAAGGAGCATTTAG
Hemidactylus turcicus	XY	male only	Ht_002-F	GCCGAGCAGTATTTTTCCTTT
			Ht_002-R	AGGGGCCTCCAGTATCAAAAT
		male only	Ht_015-F	TGCAGGACCTAACAAACCACT
			Ht_015-R	AGTTCAAATTTGGGGTTGAGC
		two bands in males, single band in females	Ht_024-F	CAGGTTGAGACATTCCTGGAG
			Ht_024-R	TTACCTCACTGCCACCAAGAG
		male only	Ht_025-F	ATAACCCTCTCTGGTGGCTGT
			Ht_025-R	GAGAAGGCCAATCCCAGTAAG
Hemidactylus frenatus	ZW	female only	hf_f1-F	ATCTGCAGGCTCCTGATCTTT
			hf_f1-R	TCCTCTATGCACCACTCTTGG
Heteronotia binoei	ZW	single band in females multiple, non-specific bands in males	f_206-F	CCCAAGATGTCGACTGCCTA
			f_206-R	CCTGCTCCTTCCCTTTTCTTT
Christinus marmoratus	ZW	female only	MC84-F	CCAGCTGAGCTAAGGCATTT

Species	SDM	Pattern	Primer ID	Sequence (5'-3')
			MC84-R	AGCGTCTTTTCAGTCTCCTCA
		female only	MC328-F	GCTCAAAAAGGTTGGGAATG
			MC328-R	GGTCTGTCTGGGGGAAAAAT

Supplementary Table 4. Squamate species used in phylogenetic comparative analysis. Sex-determining mechanisms (SDM) are listed and are categorized as temperature-dependent sex determination (TSD), male heterogamety (XY), or female heterogamety (ZW). In some cases the species with known sex determining mechanism from the literature was not included in the phylogeny and a closely related species was used instead. In these instances, the species included in the original tree are listed first and the species with known SDM is listed in brackets [ ]. Clade names, as shown in Figure 2, are abbreviated: Ac, Acrodonta; An, Anguimorpha; G, Gekkota; L, Lacertibaenia; P, Pleurodonta; Sc, Scincidae; Se, Serpentes; and T, Teiioidea.

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
-	Dibamidae	*Dibamus novaeguineae*	XY	(Cole and Gans 1997)
-	Sphenodontidae	*Sphenodon punctatus*	TSD	(Cree et al. 1995)
Ac	Agamidae	*Agama agama*	TSD	(Charnier 1966)
Ac	Agamidae	*Agama impalearis*	TSD	(El Mouden et al. 2001)
Ac	Agamidae	*Amphibolurus muricatus*	TSD	(Harlow 2004)
Ac	Agamidae	*Amphibolurus nobbi*	ZW	(Ezaz et al. 2009)
Ac	Agamidae	*Calotes versicolor*	TSD	(Inamdar et al. 2012)
Ac	Agamidae	*Chlamydosaurus kingii*	TSD	(Harlow and Shine 1999)
Ac	Agamidae	*Ctenophorus decresii*	TSD	(Harlow 2004)
Ac	Agamidae	*Ctenophorus fordi*	ZW	(Uller and Olsson 2006; Ezaz et al. 2009)
Ac	Agamidae	*Ctenophorus ornatus*	TSD	(Harlow 2004)
Ac	Agamidae	*Ctenophorus pictus*	TSD	(Harlow 2004)
Ac	Agamidae	*Intellagama lesueurii*	TSD	(Harlow 2004; Doody et al. 2006)
Ac	Agamidae	*Laudakia caucasia*	TSD	(Viets et al. 1994)
Ac	Agamidae	*Lophognathus gilberti*	TSD	(Harlow 2004)
Ac	Agamidae	*Lophognathus temporalis*	TSD	(Harlow 2004)
Ac	Agamidae	*Phrynocephalus mystaceus [P. vlangalii]*	ZW	(Zeng et al. 1997)

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
Ac	Agamidae	*Pogona barbata*	ZW	(Ezaz et al. 2009)
Ac	Agamidae	*Pogona vitticeps*	ZW	(Ezaz et al. 2005)
Ac	Chamaeleonidae	*Bradypodion ventrale*	ZW	Gordon, 1989, in (Olmo and Signorino 2005)
An	Helodermatidae	*Heloderma suspectum*	ZW	(Pokorná et al. 2014b)
An	Varanidae	*Varanus acanthurus*	ZW	(King et al. 1982)
An	Varanidae	*Varanus exanthematicus*	ZW	(King and King 1975)
An	Varanidae	*Varanus niloticus*	ZW	(King and King 1975)
An	Varanidae	*Varanus rosenbergi*	ZW	(Matsubara et al. 2014)
An	Varanidae	*Varanus varius*	ZW	(King and King 1975)
G	Carphodactylidae	*Underwoodisaurus milii*	ZW	(Pokorná et al. 2014a)
G	Diplodactylidae	*Correlophus ciliatus*	ZW	RAD-seq
G	Eublepharidae	*Coleonyx elegans*	XY	(Pokorná et al. 2011)
G	Eublepharidae	*Eublepharis macularius*	TSD	(Bull 1980; Viets et al. 1993)
G	Eublepharidae	*Hemitheconyx caudicinctus*	TSD	(Viets et al. 1994)
G	Gekkonidae	*Christinus marmoratus*	ZW	(King and Rofe 1976; King and King 1977); RADseq
G	Gekkonidae	*Cyrtodactylus philippinicus [C. pubisulcus]*	ZW	(Ota et al. 1992)
G	Gekkonidae	*Dixonius siamensis*	ZW	(Ota et al. 2001)
G	Gekkonidae	*Gehyra australis*	ZW	(King 1983)
G	Gekkonidae	*Gehyra mutilata*	ZW	RAD-seq
G	Gekkonidae	*Gehyra nana*	ZW	(Moritz 1986)
G	Gekkonidae	*Gehyra purpurascens*	ZW	(Moritz 1984a)
G	Gekkonidae	*Gekko gecko*	XY	(Solleder and Schmid 1984)
G	Gekkonidae	*Gekko hokouensis*	ZW	(Kawai et al. 2009)
G	Gekkonidae	*Gekko japonicus*	XY	(Yoshida and Itoh 1974)
G	Gekkonidae	*Hemidactylus frenatus*	ZW	RAD-seq
G	Gekkonidae	*Hemidactylus mabouia*	XY	RAD-seq

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
G	Gekkonidae	*Hemidactylus turcicus*	XY	RAD-seq
G	Gekkonidae	*Heteronotia binoei*	ZW	(Moritz 1984b; Moritz et al. 1990); RAD-seq
G	Gekkonidae	*Paroedura lohatsara*	ZW	(Koubová et al. 2014)
G	Gekkonidae	*Paroedura masobe*	ZW	(Koubová et al. 2014)
G	Gekkonidae	*Paroedura oviceps*	ZW	(Koubová et al. 2014)
G	Gekkonidae	*Paroedura stumpffi*	ZW	(Koubová et al. 2014)
G	Gekkonidae	*Phelsuma dubia*	TSD	(Osadnik 1987; Viets et al. 1994)
G	Gekkonidae	*Phelsuma guentheri*	TSD	(Bloxam and Tonge 1980; Viets et al. 1994)
G	Gekkonidae	*Phelsuma guimbeaui*	TSD	(Viets et al. 1994)
G	Gekkonidae	*Phelsuma madagascariensis*	TSD	(Viets et al. 1994)
G	Phyllodactylidae	*Phyllodactylus lanei*	ZW	(King 1991)
G	Phyllodactylidae	*Tarentola boettgeri*	TSD	(Nettmann and Rykena 1985)
G	Phyllodactylidae	*Tarentola mauritanica*	TSD	(Nettmann and Rykena 1985)
G	Phyllodactylidae	*Thecadactylus rapicauda*	ZW	(Schmid et al. In press); RAD-seq
G	Pygopodidae	*Aprasia parapulchella*	XY	(Matsubara et al. 2013)
G	Pygopodidae	*Delma butleri*	XY	(King 1990)
G	Pygopodidae	*Lialis burtonis*	XY	(Gorman and Gress 1970); RAD-seq
G	Sphaerodactylidae	*Aristelliger praesignis [A. expectatus]*	ZW	RAD-seq
G	Sphaerodactylidae	*Sphaerodactylus macrolepis*	XY	RAD-seq
G	Sphaerodactylidae	*Sphaerodactylus nicholsi*	XY	RAD-seq
L	Bipedidae	*Bipes canaliculatus*	ZW	(Cole and Gans 1987)
L	Lacertidae	*Acanthodactylus erythrurus*	ZW	(Olmo et al. 1987)
L	Lacertidae	*Darevskia valentini*	ZW	(Kupriyanova 1989)
L	Lacertidae	*Eremias velox*	ZW	(Ivanov and Federova 1973)

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
L	Lacertidae	*Gallotia galloti*	ZW	(Olmo et al. 1987)
L	Lacertidae	*Heliobolus lugubris*	ZW	(Odierna et al. 1990)
L	Lacertidae	*Iberolacerta cyreni*	ZW	(Odierna et al. 1996)
L	Lacertidae	*Lacerta agilis*	ZW	(De Smet 1981)
L	Lacertidae	*Lacerta viridis*	ZW	(De Smet 1981)
L	Lacertidae	*Meroles cuneirostris*	ZW	(Olmo et al. 1987)
L	Lacertidae	*Mesalina olivieri*	ZW	(Gorman 1969)
L	Lacertidae	*Ophisops elegans*	ZW	(Bhatnagar and Yoniss 1976)
L	Lacertidae	*Pedioplanis namaquensis*	ZW	(Odierna et al. 1990)
L	Lacertidae	*Podarcis erhardii*	ZW	(Stille et al. 1983)
L	Lacertidae	*Podarcis melisellensis*	ZW	(De Smet 1981)
L	Lacertidae	*Podarcis siculus*	ZW	(Olmo et al. 1987)
L	Lacertidae	*Psammodromus algirus*	ZW	(De Smet 1981)
L	Lacertidae	*Takydromus sexlineatus*	ZW	(Olmo et al. 1984)
L	Lacertidae	*Timon lepidus*	ZW	(De Smet 1981; Olmo et al. 1987)
P	Crotaphytidae	*Crotaphytus collaris*	XY	(Rovatsos et al. 2014b)
P	Dactyloidae	*Anolis aeneus*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis bimaculatus*	XY	(Gorman 1965)
P	Dactyloidae	*Anolis carolinensis*	XY	(Alföldi et al. 2011)
P	Dactyloidae	*Anolis chlorocyanus*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis cristatellus*	XY	(Gorman et al. 1968)
P	Dactyloidae	*Anolis distichus*	XY	(Gorman and Atkins 1969)
P	Dactyloidae	*Anolis equestris*	XY	(Rovatsos et al. 2014a)
P	Dactyloidae	*Anolis evermanni*	XY	(Gorman and Atkins 1968)
P	Dactyloidae	*Anolis grahami*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis lineatopus*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis lineatus*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis richardii*	XY	(Gamble et al. 2014)
P	Dactyloidae	*Anolis sagrei*	XY	(De Smet 1981)
P	Iguanidae	*Cyclura nubila*	XY	(Rovatsos et al. 2014b)
P	Iguanidae	*Iguana iguana*	XY	(Rovatsos et al. 2014b)

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
P	Leiocephalidae	*Leiocephalus carinatus*	XY	(Rovatsos et al. 2014b)
P	Liolaemidae	*Phymaturus palluma*	XY	Lamborot & Navarro-Suarez 1984
P	Opluridae	*Chalarodon madagascariensis*	XY	(Rovatsos et al. 2014b)
P	Opluridae	*Oplurus cyclurus [O. fierinensis]*	XY	(Rovatsos et al. 2014b)
P	Phrynosomatidae	*Petrosaurus thalassinus*	XY	(Rovatsos et al. 2014b)
P	Phrynosomatidae	*Sceloporus clarkii*	XY	(Cole 1970)
P	Phrynosomatidae	*Sceloporus graciosus*	XY	(Reed et al. 1990)
P	Phrynosomatidae	*Sceloporus grammicus*	XY	(Hall and Selander 1973)
P	Phrynosomatidae	*Sceloporus jarrovii*	XY	(Cole et al. 1967)
P	Phrynosomatidae	*Sceloporus magister*	XY	(Leaché and Sites 2010)
P	Phrynosomatidae	*Sceloporus melanorhinus*	XY	(Cole 1970)
P	Phrynosomatidae	*Sceloporus merriami*	XY	(Cole 1971)
P	Phrynosomatidae	*Sceloporus ornatus*	XY	(Hall 1973)
P	Phrynosomatidae	*Sceloporus poinsettii*	XY	(Cole et al. 1967)
P	Phrynosomatidae	*Sceloporus pyrocephalus*	XY	(Cole 1971)
P	Phrynosomatidae	*Sceloporus torquatus*	XY	(Leaché and Sites 2010)
P	Phrynosomatidae	*Sceloporus variabilis*	XY	(Leaché and Sites 2010)
P	Phrynosomatidae	*Uma inornata*	XY	(Kral 1969)
P	Phrynosomatidae	*Uta palmeri*	XY	(Pennock et al. 1969)
P	Phrynosomatidae	*Uta squamata*	XY	(Pennock et al. 1969)

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
P	Phrynosomatidae	*Uta stansburiana*	XY	(Pennock et al. 1969)
P	Polychrotidae	*Polychrus acutirostris*	XY	(Peccinini et al. 1971)
P	Polychrotidae	*Polychrus marmoratus*	XY	(Gorman et al. 1967)
P	Tropiduridae	*Tropidurus hispidus*	XY	(Kasahara et al. 1987)
P	Tropiduridae	*Uranoscodon superciliosus*	XY	(Rovatsos et al. 2014b)
Sc	Scincidae	*Bassiana duperreyi*	XY	(Shine et al. 2002)
Sc	Scincidae	*Ctenotus rawlinsoni*	XY	(Hutchinson and Donnellan 1992)
Sc	Scincidae	*Cyclodina lichenigera [C. olivieri]*	XY	(Hardy 1979)
Sc	Scincidae	*Eulamprus tympanum*	TSD	(Robert and Thompson 2001)
Sc	Scincidae	*Lampropholis guichenoti [L. adonis]*	XY	(Donnellan 1991)
Sc	Scincidae	*Niveoscincus ocellatus*	TSD	(Wapstra et al. 2004)
Sc	Scincidae	*Pseudemoia entrecasteauxii*	XY	(Hutchinson and Donnellan 1992)
Sc	Scincidae	*Pseudemoia pagenstecheri*	XY	(Hutchinson and Donnellan 1992)
Sc	Scincidae	*Saproscincus czechurai*	XY	(Donnellan 1991)
Sc	Scincidae	*Scincella lateralis*	XY	(Wright 1973)
Sc	Scincidae	*Sphenomorphus indicus*	TSD	(Ji et al. 2006)
Se	Boidae	*Acrantophis dumerili*	ZW	(Mengden and Stock 1980)
Se	Boidae	*Boa constrictor*	ZW	(Vicoso et al. 2013)
Se	Colubridae	*Ahaetulla nasuta*	ZW	(Sharma and Nakhasi 1979)
Se	Colubridae	*Masticophis flagellum*	ZW	(Baker et al. 1972)
Se	Colubridae	*Natrix natrix*	ZW	(Kobel 1967)
Se	Colubridae	*Pantherophis guttatus*	ZW	(Baker et al. 1972)
Se	Colubridae	*Storeria dekayi*	ZW	(Baker et al. 1972)
Se	Colubridae	*Thamnophis sirtalis*	ZW	(Vicoso et al. 2013)

Clade	Family	Species in phylogeny [species from literature]	SDM	Citation
Se	Colubridae	*Xenochrophis piscator*	ZW	(Singh et al. 1968)
Se	Elapidae	*Bungarus caeruleus*	ZW	(Singh et al. 1970b)
Se	Elapidae	*Bungarus fasciatus*	ZW	(Singh 1974)
Se	Elapidae	*Laticauda colubrina*	ZW	(Gorman 1981)
Se	Elapidae	*Micrurus fulvius*	ZW	(Graham 1977)
Se	Elapidae	*Micrurus surinamensis*	ZW	(Gutierrez et al. 1988)
Se	Elapidae	*Naja kaouthia*	ZW	(Singh et al. 1970a)
Se	Elapidae	*Naja naja*	ZW	(Singh et al. 1970a)
Se	Elapidae	*Notechis scutatus*	ZW	(Shine and Bull 1977)
Se	Lamprophiidae	*Heterodon nasicus*	ZW	(Baker et al. 1972)
Se	Pythonidae	*Python molurus*	ZW	(Matsubara et al. 2006)
Se	Viperidae	*Agkistrodon contortrix*	ZW	(Baker et al. 1972)
Se	Viperidae	*Bothriechis schlegelii*	ZW	(De Smet 1978)
Se	Viperidae	*Bothrops asper*	ZW	(Gutiérrez et al. 1979)
Se	Viperidae	*Crotalus atrox*	ZW	(Baker et al. 1972)
Se	Viperidae	*Lachesis muta*	ZW	(Beçak and Beçak 1969)
Se	Viperidae	*Sistrurus miliarius*	ZW	(Vicoso et al. 2013)
T	Gymnophthalmidae	*Calyptommatus leiolepis*	XY	(Yonenaga–Yassuda et al. 2005)
T	Gymnophthalmidae	*Calyptommatus nicterus*	XY	(Yonenaga–Yassuda et al. 2005)
T	Gymnophthalmidae	*Calyptommatus sinebrachiatus*	XY	(Yonenaga–Yassuda et al. 2005)
T	Gymnophthalmidae	*Gymnophthalmus pleei*	XY	(Cole et al. 1990)
T	Gymnophthalmidae	*Micrablepharus maximiliani*	XY	(Yonenaga-Yassuda and Rodrigues 1999)
T	Gymnophthalmidae	*Nothobachia ablephara*	XY	(Pellegrino et al. 1999)
T	Teiidae	*Aspidoscelis tigris*	XY	(Cole et al. 1969)

Supplementary Table 5. Sex-determining mechanisms (SDM) in geckos. The level of confidence in calling a particular SDM is listed. Confidence levels for species with genotypic sex determination (GSD), which includes both XY and ZW systems, are as follows: High confidence - Karyotype both sexes or validated genotyping; Medium confidence - Karyotype both sexes but differences minor, small sample size, or sexes sampled from different populations; Low confidence - Karyotype just one sex or species is parthenogenetic. Confidence levels for species with temperature-dependent sex determination (TSD) are as follows: High confidence - reported sample size with clearly skewed sex ratios; Medium confidence - Small sample size or only one incubation temperature used; Low confidence - No sample size reported for incubation experiments. Only species with sex-determining mechanisms known with high confidence were used in the comparative phylogenetic analyses (see Supp. Table 4). Gecko families are abbreviated: C, Carphodactylidae, D, Diplodactylidae, E, Eublepharidae; G, Gekkonidae; Ph, Phyllodactylidae; Py, Pygopodidae; S, Sphaerodactylidae.

Species	Family	SDM	Confidence	Citation
Underwoodisaurus milli	C	ZW	High	(Pokorná et al. 2014a)
Correlophus ciliatus	D	ZW	High	RAD-seq
Rhacodactylus leachianus	D	TSD	Low	(Seipp and Henkel 2000; de Vosjoli et al. 2003)
Coleonyx brevis	E	GSD	Medium	(Viets et al. 1994)
Coleonyx elegans	E	XY	High	(Pokorná et al. 2011)
Coleonyx mitratus	E	GSD	High	(Bragg et al. 2000; Kratochvil et al. 2008)
Coleonyx variegatus	E	GSD	High	(Viets et al. 1994) (Kratochvil et al. 2008)
Eublepharis macularius	E	TSD	High	(Bull 1980; Viets et al. 1993)
Goniurosaurus araneus	E	GSD	Low	(Seufer et al. 2005)
Goniurosaurus kuroiwae	E	TSD	Low	(Seufer et al. 2005)
Goniurosaurus lichtenfelderi	E	GSD	Low	(Seufer et al. 2005; Pokorná et al. 2011)
Goniurosaurus luii	E	GSD	Low	(Seufer et al. 2005)
Goniurosaurus orientalis	E	TSD	Low	(Seufer et al. 2005)

Species	Family	SDM	Confidence	Citation
Goniurosaurus splendens	E	TSD	Low	(Seufer et al. 2005)
Hemitheconyx caudicinctus	E	TSD	High	(Viets et al. 1994)
Christinus marmoratus	G	ZW	High	(King and Rofe 1976; King and King 1977); RADseq
Cyrtodactylus pubisulcus	G	ZW	High	(Ota et al. 1992)
Dixonius siamensis	G	ZW	High	(Ota et al. 2001)
Gehyra australis	G	ZW	High	(King 1983)
Gehyra mutilata	G	ZW	High	RAD-seq
Gehyra nana	G	ZW	High	(Moritz 1986)
Gehyra purpurascens	G	ZW	High	(Moritz 1984a)
Gekko gecko	G	XY	High	(Solleder and Schmid 1984)
Gekko hokouensis	G	ZW	High	(Kawai et al. 2009)
Gekko japonicus	G	XY	High	(Yoshida and Itoh 1974)
Gekko japonicus	G	TSD	High	(Tokunaga 1985)
Hemidactylus frenatus	G	ZW	High	RAD-seq
Hemidactylus mabouia	G	XY	High	RAD-seq
Hemidactylus platyurus	G	ZW	Medium	(Trifonov et al. 2011)
Hemidactylus turcicus	G	XY	High	RAD-seq
Hemidactylus vietnamensis	G	ZW	Low	(Darevsky et al. 1984)
Heteronotia binoei	G	ZW	High	(Moritz 1984b; Moritz et al. 1990); RAD-seq
Lepidodactylus lugubris	G	ZW	Low	(Volobouev and Pasteur 1988)
Lygodactylus picturatus	G	OW?	Medium	(Castiglia 2004)
Paroedura karstophila	G	ZW	High	(Koubová et al. 2014)
Paroedura	G	ZW	High	(Koubová et al. 2014)

Species	Family	SDM	Confidence	Citation
lohatsara				
Paroedura masobe	G	ZW	High	(Koubová et al. 2014)
Paroedura oviceps	G	ZW	High	(Koubová et al. 2014)
Paroedura picta	G	GSD	High	(Blumberg et al. 2002; Kratochvil et al. 2008)
Paroedura stumpffi	G	ZW	High	(Koubová et al. 2014)
Phelsuma dubia	G	TSD	High	(Osadnik 1987; Viets et al. 1994)
Phelsuma grandis	G	TSD	High	(Viets et al. 1994)
Phelsuma guentheri	G	TSD	High	(Bloxam and Tonge 1980; Viets et al. 1994)
Phelsuma guimbeaui	G	TSD	High	(Viets et al. 1994)
Phelsuma laticauda	G	TSD	Low	(Viets et al. 1994)
Phelsuma madagascariensis	G	TSD	Medium	(Viets et al. 1994)
Phelsuma pusilla	G	TSD	Medium	(Viets et al. 1994)
Phyllodactylus cf. lanei	Ph	ZW	High	(King 1991)
Tarentola angustimentalis	Ph	TSD	Low	(Nettmann and Rykena 1985)
Tarentola annularis	Ph	TSD	Low	(Nettmann and Rykena 1985)
Tarentola boettgeri hierrensis	Ph	TSD	High	(Nettmann and Rykena 1985)
Tarentola delalandii	Ph	TSD	Low	(Nettmann and Rykena 1985)
Tarentola gomerensis	Ph	TSD	Low	(Nettmann and Rykena 1985)
Tarentola mauritanica	Ph	TSD	High	(Nettmann and Rykena 1985)
Thecadactylus rapicauda	Ph	ZW	High	(Schmid et al. In press); RAD-seq
Aprasia parapulchella	Py	XY	High	(Matsubara et al. 2013)
Delma inornata	Py	XY	High	(King 1990)
Lialis burtonis	Py	XXY	High	(Gorman and Gress 1970); RAD-seq

Species	Family	SDM	Confidence	Citation
Aristelliger expectatus	S	ZW	High	RAD-seq
Euleptes europaea	S	XY	Medium	(Gornung et al. 2013)
Gonatodes ceciliae	S	XY	Low	(McBee et al. 1987)
Sphaerodactylus macrolepis	S	XY	High	RAD-seq
Sphaerodactylus nicholsi	S	XY	High	RAD-seq

Literature Cited

Alföldi J, Di Palma F, Grabherr M, et al. 2011. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature 477: 587–591.

Baker RJ, Mengden GA and Bull JJ. 1972. Karyotype studies of thirty–eight species of North American Snakes. Copeia 1972: 257–265.

Beçak W and Beçak ML. 1969. Cytotaxonomy and chromosomal evolution in Serpentes. Cytogenetics 8: 247–262.

Bhatnagar A and Yoniss YT. 1976. A proof of female heterogamety in a lacertid lizard, *Ophisops elegans*. Cytologia (Japan) 41: 507–511.

Bloxam Q and Tonge S. 1980. Maintenance and breeding of *Phelsuma guentheri* (Boulenger 1885). In: Townson S, Millichamp NJ, Lucas D, Millwood AJ, editors. The Care and Breeding of Reptiles. London: The British Herpetological Society. p. 51–62.

Blumberg MS, Lewis SJ and Sokoloff G. 2002. Incubation temperature modulates post–hatching thermoregulatory behavior in the Madagascar ground gecko, *Paroedura pictus*. J Exp Biol 205: 2777–2784.

Bragg WK, Fawcett JD, Bragg TB and Viets BE. 2000. Nest-site selection in two eublepharid gecko species with temperature-dependent sex determination and one with genotypic sex determination. Biol J Linn Soc 69: 319–332.

Bull JJ. 1980. Sex determination in reptiles. Q Rev Biol 55: 3–21.

Castiglia R. 2004. First chromosomal analysis for the genus *Lygodactylus* (Gray, 1864): the karyotype of *L. picturatus* (Squamata, Gekkonidae, Gekkoninae). Afr J Herpetol 53: 95–97.

Charnier M. 1966. Action de la température sur le sex-ratio chez l'embryon d'*Agama agama* (Agamidae, Lacertilien). C R Seances Soc Biol Fil 160: 620–622.

Cole CJ. 1970. Karyotypes and evolution of the *spinosus* group of lizards in the genus *Sceloporus*. Am Mus Novit: 1–47.

Cole CJ. 1971. Karyotypes and relationships of the *pyrocephalus* group of lizards in the genus *Sceloporus*. Herpetologica 27: 1–8.

Cole CJ, Desauer HC, Townsend CR and Arnold MG. 1990. Unisexual lizards of the genus *Gymnophthalmus* (Reptilia: Teiidae) in the neotropics: Genetics, origin, and systematics. Am Mus Novit 2994: 1–29.

Cole CJ and Gans C. 1987. Chromosomes of Bipes, Mesobaena, and other amphisbaenians (Reptilia), with comments on their evolution. Am Mus Novit 2869: 1–9.

Cole CJ and Gans C. 1997. The karyotype of *Dibamus novaeguineae* (Squamata: Dibamidae). Herpetologica 53: 229–232.

Cole CJ, Lowe CH and Wright JW. 1967. Sex chromosomes in lizards. Science 155: 1028–1029.

Cole CJ, Lowe CH and Wright JW. 1969. Sex chromosomes in Teiid whiptail lizards (Genus *Cnemidophorus*). Am Mus Novit: 1–14.

Cree A, Thompson MB and Daugherty CH. 1995. Tuatara sex determination. Nature 375: 543.

Darevsky IS, Kupriyanova LA and Roshchin VV. 1984. A new all–female triploid species of gecko and karyological data on the bisexual *Hemidactylus frenatus* from Vietnam. J Herpetol 18: 277–284.
De Smet WHO. 1978. The chromosomes of 23 species of snakes. Acta Zool Pathol Antverp 70: 85–118.
De Smet WHO. 1981. Description of the orcien stained karyotypes of 27 lizard species (Lacertilia: Reptilia) belonging to the families Iguanidae, Agamidae Chameleontidae and Gekkonidae (Ascalabota). Acta Zool Pathol Antverp 76: 35–72.
de Vosjoli P, Fast F and Repashy A. 2003. *Rhacodactylus*: The complete guide to their selection and care. Vista, California: Advanced Visions, Inc.
Donnellan SC. 1991. Chromosomes of Australian lygosomine skinks (Lacertilia, Scincidae) II. The genus *Lampropholis*. Genetica 83: 223–234.
Doody JS, Guarino E, Georges A, Corey B, Murray G and Ewert M. 2006. Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. Evol Ecol 20: 307–330.
El Mouden, Hassan E, Znari M and Pieau C. 2001. Effects of incubation temperature on embryonic development and sex determination in the North African agamid lizard, *Agama impalearis*. Herpetol J 11: 101–108.
Ezaz T, Quinn AE, Miura I, Sarre SD, Georges A and Marshall Graves JA. 2005. The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. Chromosome Res 13: 763–776.
Ezaz T, Quinn AE, Sarre SD, O'Meally D, Georges A and Graves JAM. 2009. Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. Chromosome Res 17: 91–98.
Gamble T, Geneva AJ, Glor RE and Zarkower D. 2014. *Anolis* sex chromosomes are derived from a single ancestral pair. Evolution 68: 1027–1041.
Gorman GC. 1965. Interspecific karyotypic variation as a systematic character in the genus *Anolis* (Sauria: Iguanidae). Nature 208: 95–97.
Gorman GC. 1969. New chromosome data for 12 species of lacertid lizards. J Herpetol 3: 49–54.
Gorman GC. 1981. The chromosomes of *Laticauda* and a review of karyotypic evolution in Elapidae. J Herpetol 15: 225–233.
Gorman GC and Atkins L. 1968. Confirmation of an X-Y sex determining mechanism in lizards (*Anolis*). Copeia 1968: 159–160.
Gorman GC and Atkins L. 1969. The zoogeography of Lesser Antillean *Anolis* lizards - An analysis based upon chromosomes and lactic dehydrogenases. Bull Mus Comp Zool 138: 53–80.
Gorman GC, Atkins L and Holzinger T. 1967. New karyotypic data on 15 genera of lizards in the family Iguanidae, with a discussion of taxonomic and cytological implications. Cytogenetics 6: 286–299.
Gorman GC and Gress F. 1970. Sex chromosomes of a pygopodid lizard, *Lialis burtonis*. Experientia 26: 206–207.
Gorman GC, Thomas R and Atkins L. 1968. Intra- and interspecific chromosome variation in the lizard *Anolis cristatellus* and its closest relatives. Breviora: 1–13.

Gornung E, Mosconi F, Annesi F and Castiglia R. 2013. The first cytogenetic description of *Euleptes europaea* (Gené, 1839) from Northern Sardinia reveals the highest diploid chromosome number among sphaerodactylid geckos (Sphaerodactylidae, Squamata). Comparative cytogenetics 7: 153–161.

Graham GL. 1977. The karyotype of the Texas coral snake, *Micrurus fulvius tenere*. Herpetologica 33: 345–348.

Gutierrez JM, Solórzano A, Cerdas L and Vannini JP. 1988. Karyotypes of Five Species of Coral Snakes (*Micrurus*). J Herpetol 22: 109–112.

Gutiérrez JM, Taylor RT and Bolaños-Herrera R. 1979. Cariotipos de diez especies de serpientes costarricenses de la familia Viperidae. Rev Biol Trop 27: 309–319.

Hall WP. 1973. Comparative Population Cytogenetics, Speciation, and Evolution of the Iguanid Lizard Genus *Sceloporus*. [PhD]. [Cambridge, MA]: Harvard University.

Hall WP and Selander RK. 1973. Hybridization of karyotypically differentiated populations in the *Sceloporus grammicus* complex (Iguanidae). Evolution: 226–242.

Hardy GS. 1979. The karyotypes of two scincid lizards, and their bearing on relationships in genus *Leiolopisma* and its relatives (Scincidae: Lygosominae). N Z J Zool 6: 609–612.

Harlow PS. 2004. Temperature-dependent sex determination in lizards. In: Valenzuela N, Lance V, editors. Temperature-dependent sex determination in vertebrates. Washington D. C., USA: Smithsonian Books. p. 42–52.

Harlow PS and Shine R. 1999. Temperature-dependent sex determination in the frillneck lizard, *Chlamydosaurus kingii* (Agamidae). Herpetologica 55: 205–212.

Hutchinson MN and Donnellan SC. 1992. Taxonomy and genetic variation in the Australian lizards of the genus *Pseudemoia* (Scincidae, Lygosominae). J Nat Hist 26: 215–264.

Inamdar LS, Vani V and Seshagiri PB. 2012. A tropical oviparous lizard, *Calotes versicolor*, exhibiting a potentially novel FMFM pattern of temperature-dependent sex determination. J Exp Zool Part A Ecol Genet Physiol 317: 32–46.

Ivanov V and Federova T. 1973. Heterochromosomes in the karyotype of *Eremias arguta* Pall. Tsitologiia 15: 762–765.

Ji X, Lin LH, Luo LG, Lu HL, Gao JF and Han J. 2006. Gestation temperature affects sexual phenotype, morphology, locomotor performance, and growth of neonatal brown forest skinks, *Sphenomorphus indicus*. Biol J Linn Soc 88: 453–463.

Kasahara S, Yonenaga-Yassuda Y and Rodrigues MT. 1987. Karyotype and evolution of the *Tropidurus nanuzae* species group (Sauria, iguanidae). Rev Bras Genet 10: 185–197.

Kawai A, Ishijima J, Nishids C, Kosaka A, Ota H, Kohno S and Matsuda Y. 2009. The ZW sex chromosomes of *Gekko hokouensis* (Gekkonidae, Squamata) represent highly conserved homology with those of avian species. Chromosoma 118: 43–51.

King M. 1983. Karyotypic evolution in *Gehyra* (Gekkonidae: Reptilia) III: The *Gehyra australis* complex. Aust J Zool 31: 723–741.
King M. 1990. Chromosomal and immunogenetic data: A new perspective on the origin of Australia's reptiles. In: Olmo E, editor. Cytogenetics of Amphibians and Reptiles. Basel: Birkhäuser. p. 153–180.
King M. 1991. Chromosome change and speciation in lizards. In: Atchley WR, Woodruff DS, editors. Evolution and Speciation. Cambridge, England: Cambridge University Press. p. 262–285.
King M and King D. 1975. Chromosomal evolution in the lizard genus *Varanus* (reptilia). Aust J Biol Sci 28: 89–108.
King M and King D. 1977. An additional chromosome race of *Phyllodactylus marmoratus* (Gray) (Reptilia: Gekkonidae) and its phylogenetic implications. Aust J Zool 25: 667–672.
King M, Mengden G and King D. 1982. A pericentric-inversion polymorphism and s ZZ/ZW sex-chromosome system in *Varanus acanthurus* Boulenger analyzed by G-and C-banding and Ag staining. Genetica 58: 39–45.
King M and Rofe R. 1976. Karyotypic Variation in Australian Gekko *Phyllodactylus marmoratus* (Gray) (Gekkonidae, Reptilia). Chromosoma 54: 75–87.
Kobel HR. 1967. Morphometrische karyotypanalyse einiger schalangen-arten. Genetica 38: 1–31.
Koubová M, Pokorná MJ, Rovatsos M, Farkačová K, Altmanová M and Kratochvíl L. 2014. Sex determination in Madagascar geckos of the genus *Paroedura* (Squamata: Gekkonidae): Are differentiated sex chromosomes indeed so evolutionary stable? Chromosome Res: 1–12.
Kral B. 1969. A karyotypic study of the fringe-toed lizard *Uma inornata* Cope, 1895. Zool List 18: 185–194.
Kratochvil L, Kubicka L and Landova E. 2008. Does the mechanism of sex determination constrain the potential for sex manipulation? A test in geckos with contrasting sex–determining systems. Naturwissenschaften 95: 209–215.
Kupriyanova LA. 1989. Cytogenetic evidence for genome interaction in hybrid lacertid lizards. In: Dawley RM, Bogart JP, editors. Evolution and Ecology of Unisexual Vertebrates. p. 236–240.
Leaché A and Sites J. 2010. Chromosome evolution and diversification in North American spiny lizards (genus *Sceloporus*). Cytogenet Genome Res 127: 166–181.
Matsubara K, Knopp T, Sarre SD, Georges A and Ezaz T. 2013. Karyotypic analysis and FISH mapping of microsatellite motifs reveal highly differentiated XX/XY sex chromosomes in the pink-tailed worm-lizard (*Aprasia parapulchella*, Pygopodidae, Squamata). Mol Cytogenet 6: 60.
Matsubara K, Sarre SD, Georges A, Matsuda Y, Graves JAM and Ezaz T. 2014. Highly differentiated zw sex microchromosomes in the Australian *Varanus* species evolved through rapid amplification of repetitive sequences. PLoS ONE 9: e95226.
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.
McBee K, Bickham JW and Dixon JR. 1987. Male heterogamety and chromosomal variation in Caribbean geckos. J Herpetol 21: 68–71.
Mengden GA and Stock AD. 1980. Chromosomal evolution in Serpentes; A comparison of G and C chromosome banding patterns of some colubrid and boid genera. Chromosoma 79: 53–64.
Moritz C. 1984a. The evolution of highly variable sex chromosomes in *Gehyra purpurascens* (Gekkonidae). Chromosoma 90: 111–119.
Moritz C. 1984b. The origin and evolution of parthenogenesis in *Heteronotia binoei* (Gekkonidae) I. Chromosome banding studies. Chromosoma 89: 151–162.
Moritz C. 1986. The population biology of *Gehyra* (Gekkonidae): Chromosome change and speciation. Syst Zool 35: 46–67.
Moritz C, Adams M, Donnellan S and Baverstock P. 1990. The origin and evolution of parthenogenesis in *Heteronotia binoei* (Gekkonidae): Genetic diversity among bisexual populations. Copeia: 333–348.
Nettmann HK and Rykena S. 1985. Verhaltens und fortpflanzungsbiologische Notizen über kanarische un nordafrikanische *Tarentola*-Arten. Bonn Zool Beitr 36: 287–305.
Odierna G, Aprea G, Arribas OJ, Capriglione T, Caputo V and Olmo E. 1996. The karyology of the Iberian rock lizards. Herpetologica 52: 542–550.
Odierna G, Capriglione T, Olmo E, Cardone A and Rosati C. 1990. The karyology of some South African lacertids belonging to the genera *Heliobolus*, *Meroles* and *Pedioplanis*. Rev Zool Afr 104: 541–547.
Olmo E, Cobror O, Morescalchi A and Odierna G. 1984. Homomorphic sex chromosomes in the lacertid lizard *Takydromus sexlineatus*. Heredity 53: 457–459.
Olmo E, Odierna G and Capriglione T. 1987. Evolution of sex-chromosomes in lacertid lizards. Chromosoma 96: 33–38.
Olmo E and Signorino GG. Chromorep: A reptiles chromosomes database [Internet]. 2005 [cited 2006 20 March]. Available from: http://chromorep.univpm.it
Osadnik G. 1987. Untersuchungen zur Reproduktionsbiologie des madagassischen Taggeckos *Phelsuma dubia*. [PhD]. [Bochum]: Ruhr–Universität Bochum.
Ota H, Hikida T, Matsui M and Mori A. 1992. Karyotypes of two species of the genus *Cyrtodactylus* (Squamata: Gekkonidae) from Sarawak, Malaysia. Caryologia 45: 43–49.
Ota H, Hikida T, Nabhitabhata J and Panha S. 2001. Cryptic taxonomic diversity in two broadly distributed lizards of Thailand (*Mabuya macularia* and *Dixonius siamensis*) as revealed by chromosomal investigations (Reptilia: Lacertilia) The Natural History Journal of Chulalongkorn University 1: 1–7.
Peccinini D, Frota-Pessoa O and Ferrari I. 1971. Sex determination of the "pseudo-XO/XX" type in the Brazilian lizard *Polychrus* sp. (Sauria, Iguanidae). Caryologia 24: 129–139.
Pellegrino KCM, Rodrigues MT and Yonenaga-Yassuda Y. 1999. Chromosomal polymorphisms due to supernumerary chromosomes and pericentric

inversions in the eyelidless microteiid lizard *Nothobachia ablephara* (Squamata, Gymnophthalmidae). Chromosome Res 7: 247–254.
Pennock L, Tinkle D and Shaw M. 1969. Minute Y chromosome in the lizard genus *Uta* (family Iguanidae). Cytogenet Genome Res 8: 9–19.
Pokorná M, Rábová M, Ráb P, Ferguson-Smith M, Rens W and Kratochvíl L. 2011. Differentiation of sex chromosomes and karyotypic evolution in the eye-lid geckos (Squamata: Gekkota: Eublepharidae), a group with different modes of sex determination. Chromosome Res 18: 809–820.
Pokorná M, Rens W, Rovatsos M and Kratochvíl L. 2014a. A ZZ/ZW sex chromosome system in the thick-tailed gecko (*Underwoodisaurus milii*; Squamata: Gekkota: Carphodactylidae), a member of the ancient gecko lineage. Cytogenet Genome Res 142: 190–196.
Pokorná MJ, Rovatsos M and Kratochvíl L. 2014b. Sex Chromosomes and Karyotype of the (Nearly) Mythical Creature, the Gila Monster, *Heloderma suspectum* (Squamata: Helodermatidae). PLoS ONE 9: e104716.
Reed KM, Sudman PD, Sites JW, Jr. and Greenbaum IF. 1990. Synaptonemal complex analysis of sex chromosomes in two species of *Sceloporus*. Copeia 1990: 1122–1129.
Robert KA and Thompson MB. 2001. Viviparous lizard selects sex of embryos. Nature 412: 698–699.
Rovatsos M, Altmanová M, Pokorná M and Kratochvíl L. 2014a. Conserved sex chromosomes across adaptively radiated *Anolis* lizards. Evolution 68: 2079–2085.
Rovatsos M, Pokorná M, Altmanová M and Kratochvíl L. 2014b. Cretaceous park of sex determination: Sex chromosomes are conserved across iguanas. Biol Lett 10: 20131093.
Schmid M, Steinlein C, Haaf T and Mijares-Urrutia A. In press. Nascent ZW Sex Chromosomes in *Thecadactylus rapicauda* (Reptilia, Squamata, Phyllodactylidae). Cytogenet Genome Res.
Seipp R and Henkel FW. 2000. *Rhacodactylus*: Biology, Natural History & Husbandry. Frankfurt: Edition Chimaira.
Seufer H, Kaverkin Y and Kirschner A. 2005. The Eyelash Geckos: Care, Breeding and Natural History. Karlsruhe, Germany: Kirschner and Seufer Verlag.
Sharma G and Nakhasi U. 1979. Karyological studies on six species of Indian snakes (Colubridae: Reptilia). Cytobios 27: 177–192.
Shine R and Bull JJ. 1977. Skewed sex ratios in snakes. Copeia 1977: 228–234.
Shine R, Elphick MJ and Donnellan S. 2002. Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. Ecol Lett 5: 486–489.
Singh L. 1974. Chromosomes of six species of Indian snakes. Herpetologica: 419–429.
Singh L, Sharma T and Ray-Chaudhuri S. 1968. W chromosome in the Indian water snake (checkered keel back) *Natrix piscator* (Colubridae). Cellular and Molecular Life Sciences 24: 79–80.

Singh L, Sharma T and Ray-Chaudhuri S. 1970a. Chromosome numbers and sex chromosomes in few Indian species of amphibia and reptiles. Mammal Chrom Newslett 11: 91–94.
Singh L, Sharma T and Ray-Chaudhuri S. 1970b. Multiple sex-chromosomes in the common Indian Krait, *Bungarus caeruleus* Schneider. Chromosoma 31: 386–391.
Solleder E and Schmid M. 1984. XX/XY sex chromosomes in *Gekko gecko* (Sauria, Reptilia). Amphib-Reptilia 5: 339–345.
Stille B, Persson A and Niklasson M. 1983. The karyotypes of *Podarcis erhardii* (Bedriaga) and *Podarcis milensis* (Bedriaga)(Reptilia, Lacertidae). Genet Pol 24: 371–372.
Tokunaga S. 1985. Temperature-dependent sex determination in *Gekko japonicus* (Gekkonidae, Reptilia). Development Growth and Differentiation 27: 117–120.
Trifonov VA, Giovannotti M, O'Brien PC, Wallduck M, Lovell F, Rens W, Parise-Maltempi PP, Caputo V and Ferguson-Smith MA. 2011. Chromosomal evolution in Gekkonidae. I. Chromosome painting between *Gekko* and *Hemidactylus* species reveals phylogenetic relationships within the group. Chromosome Res 19: 843–855.
Uller T and Olsson M. 2006. No seasonal sex-ratio shift despite sex-specific fitness returns of hatching date in a lizard with genotypic sex determination. Evolution 60: 2131–2136.
Vicoso B, Emerson JJ, Zektser Y, Mahajan S and Bachtrog D. 2013. Comparative sex chromosome genomics in snakes: Differentiation, evolutionary strata, and lack of global dosage compensation. PLoS Biol 11: e1001643.
Viets BE, Ewert MA, Talent LG and Nelson CE. 1994. Sex–determining mechanisms in squamate reptiles. J Exp Zool 270: 45–56.
Viets BE, Tousignant A, Ewert MA, Nelson CE and Crews D. 1993. Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. J Exp Zool 265: 679–683.
Volobouev V and Pasteur G. 1988. Presumptive sex chromosomes of a unisexual homomorphic species of lizards, *Lepidodactylus lugubris*. Heredity 60: 463–467.
Wapstra E, Olsson M, Shine R, Edwards A, Swain R and Joss JMP. 2004. Maternal basking behaviour determines offspring sex in a viviparous reptile. Proc R Soc Biol Sci Ser B 271: S230–S232.
Wright J. 1973. Evolution of the X1X2Y sex chromosome mechanism in the scincid lizard *Scincella laterale* (Say). Chromosoma 43: 101–108.
Yonenaga-Yassuda Y and Rodrigues MT. 1999. Supernumerary chromosome variation, heteromorphic sex chromosomes and banding patterns in microteiid lizards of the genus *Micrablepharus* (Squamata, Gymnophthalmidae). Chromosome Res 7: 21–29.
Yonenaga–Yassuda Y, Rodrigues MT and Pellegrino KCM. 2005. Chromosomal banding patterns in the eyelid–less microteiid lizard radiation: The X1X1X2X2 : X1X2Y sex chromosome system in *Calyptommatus* and the

karyotypes of *Psilophthalmus* and *Tretioscincus* (Squamata, Gymnophthalmidae). Genet Mol Biol 28: 700–709.
Yoshida M and Itoh M. 1974. Karyotype of the gecko, *Gekko japonicus*. Chromosome Information Service 17: 29 –31.
Zeng M, Wang Y, Liu Z, Fang Z, Wu G, Theodore JP and Macey RJ. 1997. Karyotypes of nine species in the genus *Phrynocephalus*, with discussion of karyotypic evolution of Chinese *Phrynocephalus*. Acta Zool Sin 43: 399–410.

Supplementary Figures

Supplementary Figure 1. Results of the PCR validation of sex-specific RAD-seq markers. Phylogenetic relationships among sampled geckos and inferred sex-determining systems are shown on the left.

Supplementary Figure 2. Evolution of sex-determining systems in lepidosaurs. Dataset includes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate maximum likelihood ancestral state reconstructions using the TRAP model.

Supplementary Figure 3. Evolution of sex-determining systems in lepidosaurs. Dataset includes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate posterior probabilities of ancestral states calculated with 1000 stochastic mapping simulations using the ARD model.

Supplementary Figure 4. Evolution of sex-determining systems in lepidosaurs. Dataset includes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate posterior probabilities of ancestral states calculated with 1000 stochastic mapping simulations using the TRAP model.

Supplementary Figure 5. Evolution of sex-determining systems in lepidosaurs. Dataset excludes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate posterior probabilities of ancestral states calculated with 1000 stochastic mapping simulations using the ARD model.

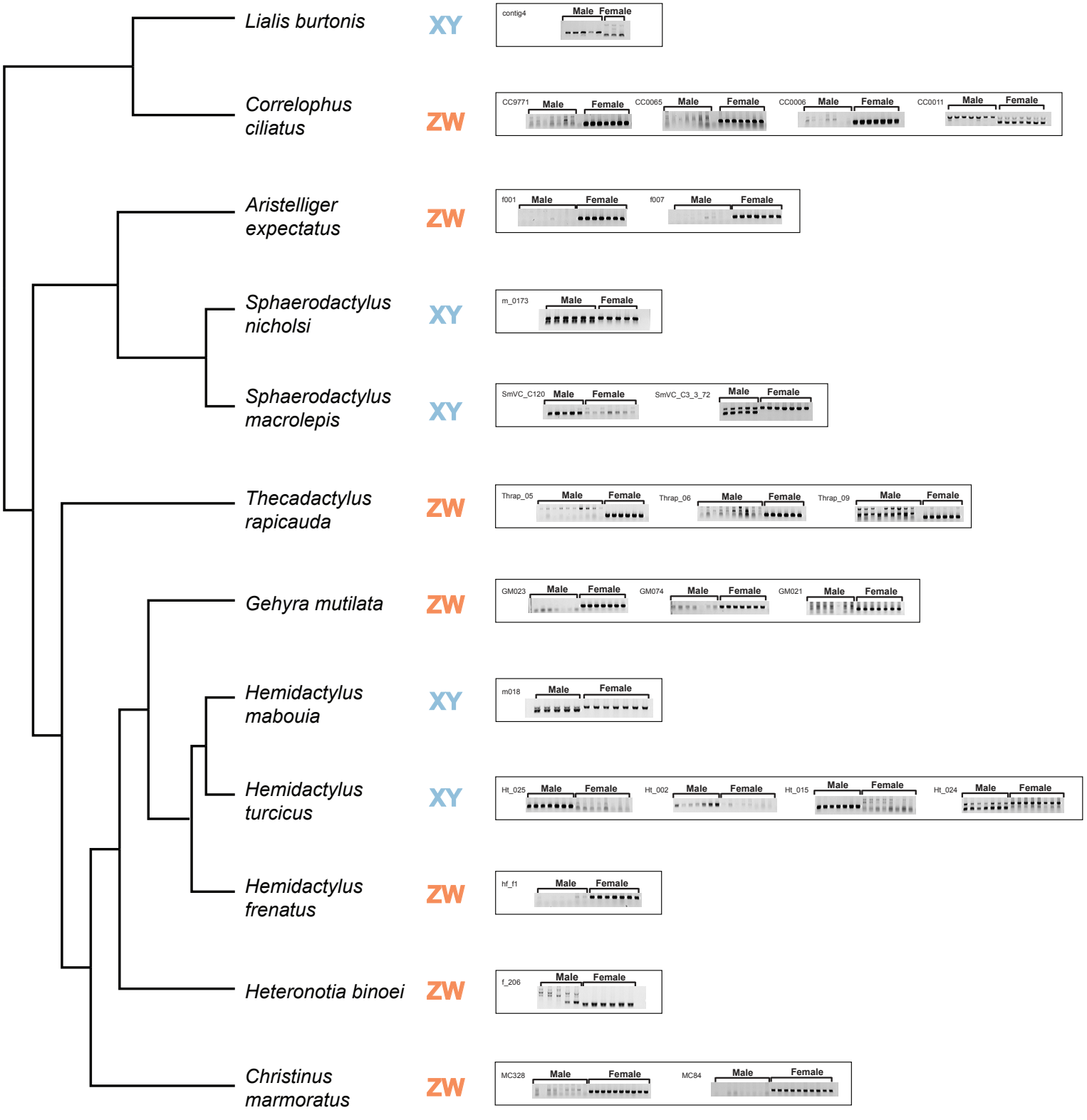
Supplementary Figure 6. Evolution of sex-determining systems in lepidosaurs. Dataset excludes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate posterior probabilities of ancestral states calculated with 1000 stochastic mapping simulations using the TRAP model.

Supplementary Figure 7. Evolution of sex-determining systems in lepidosaurs with data coded as a binary trait, TSD and GSD. Dataset includes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-determining systems of sampled species. Circles at internal nodes indicate maximum likelihood ancestral state reconstructions using the ARD model.

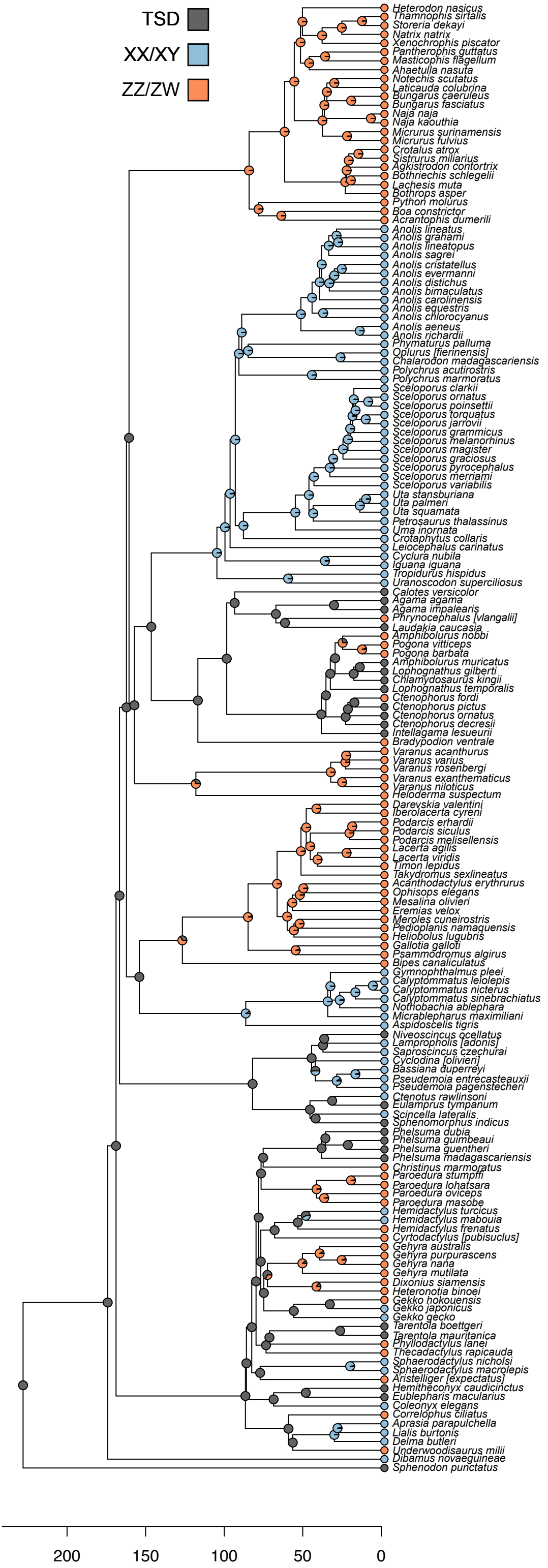
Supplementary Figure 8. Evolution of sex-determining systems in lepidosaurs with data coded as a binary trait, TSD and GSD. Dataset excludes skinks with putative TSD. Colored circles at the tips of the phylogenetic tree indicate sex-

determining systems of sampled species. Circles at internal nodes indicate maximum likelihood ancestral state reconstructions using the ARD model.

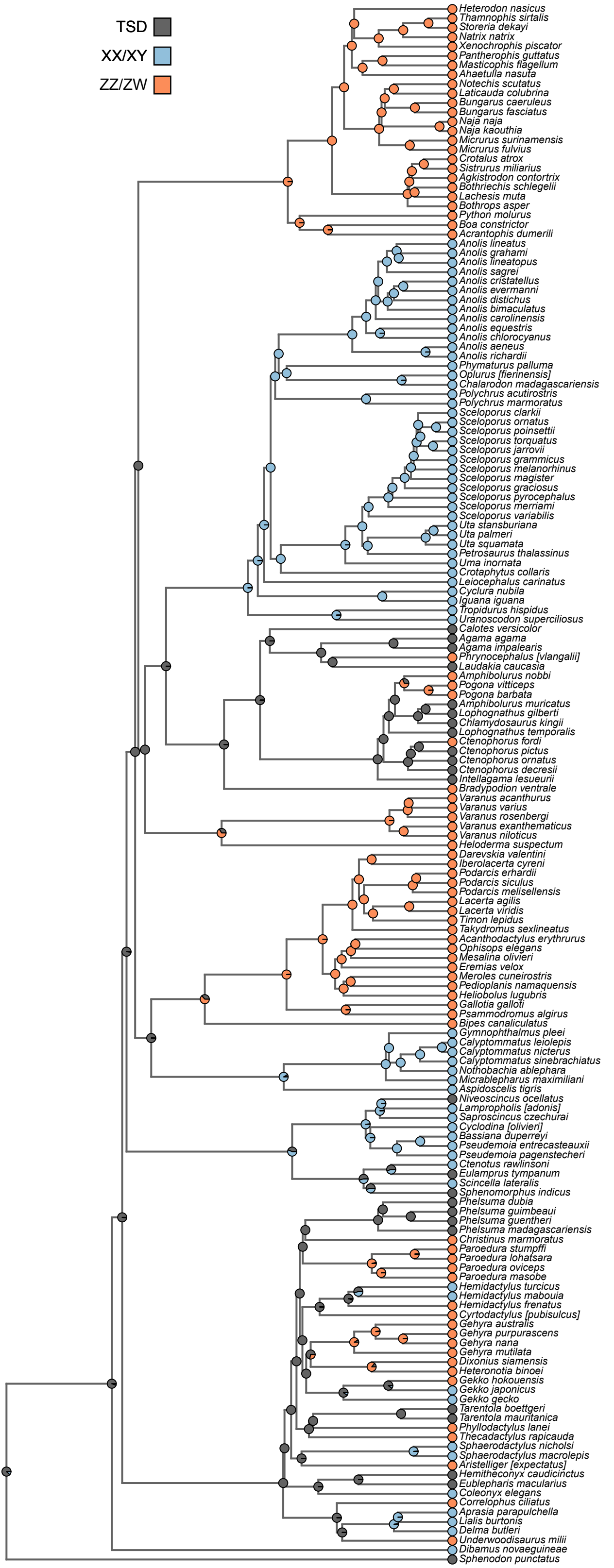
Supplementary Figure 1



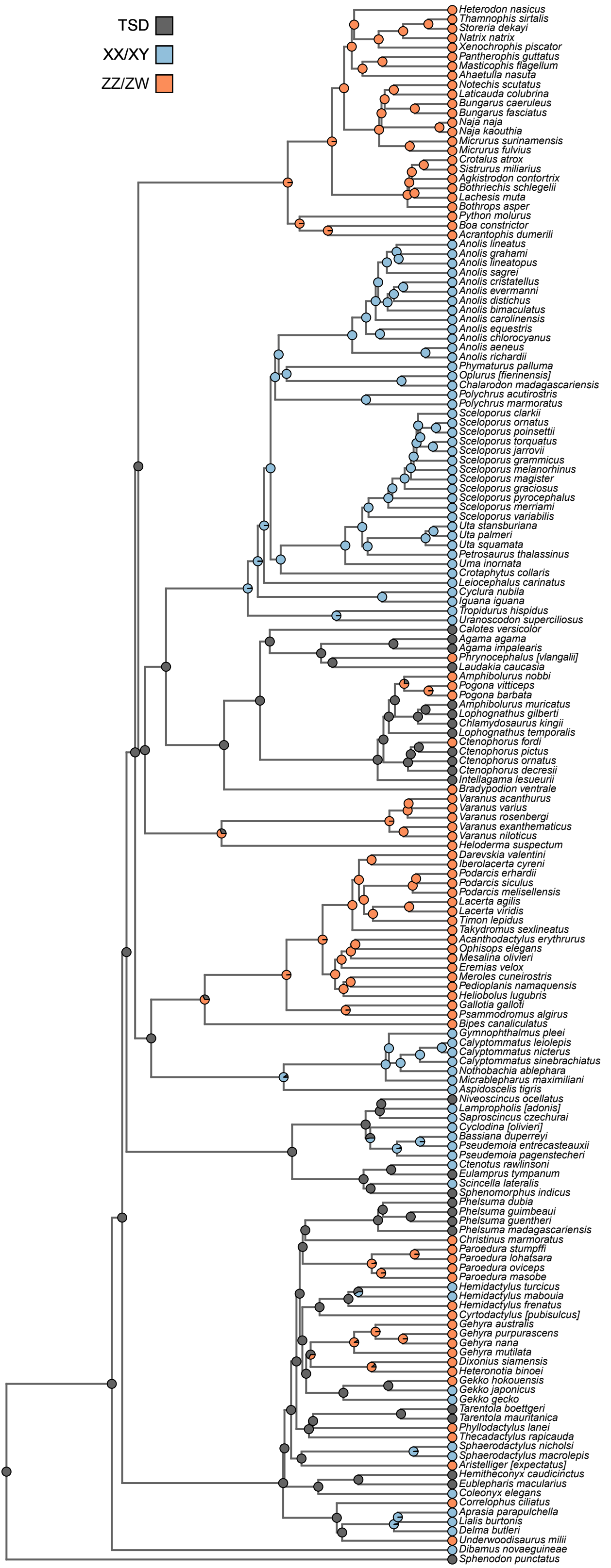
Supplementary Figure 2



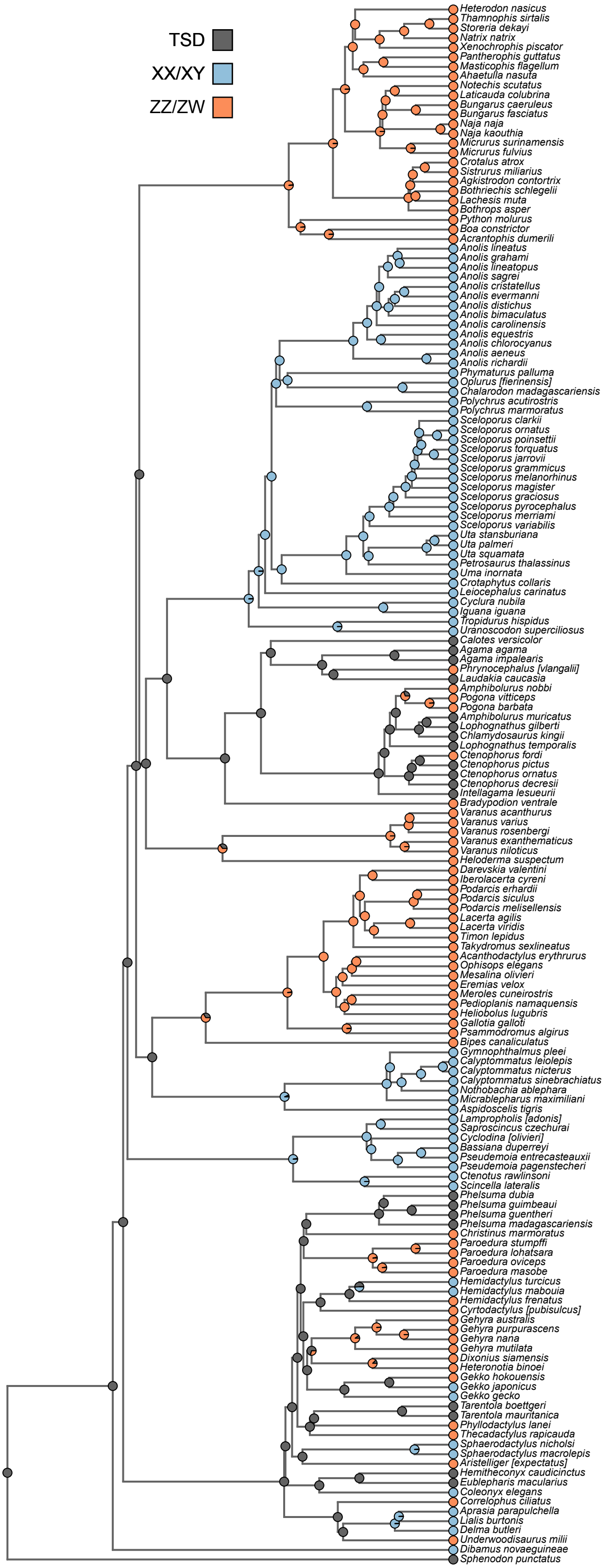
Supplementary Figure 3



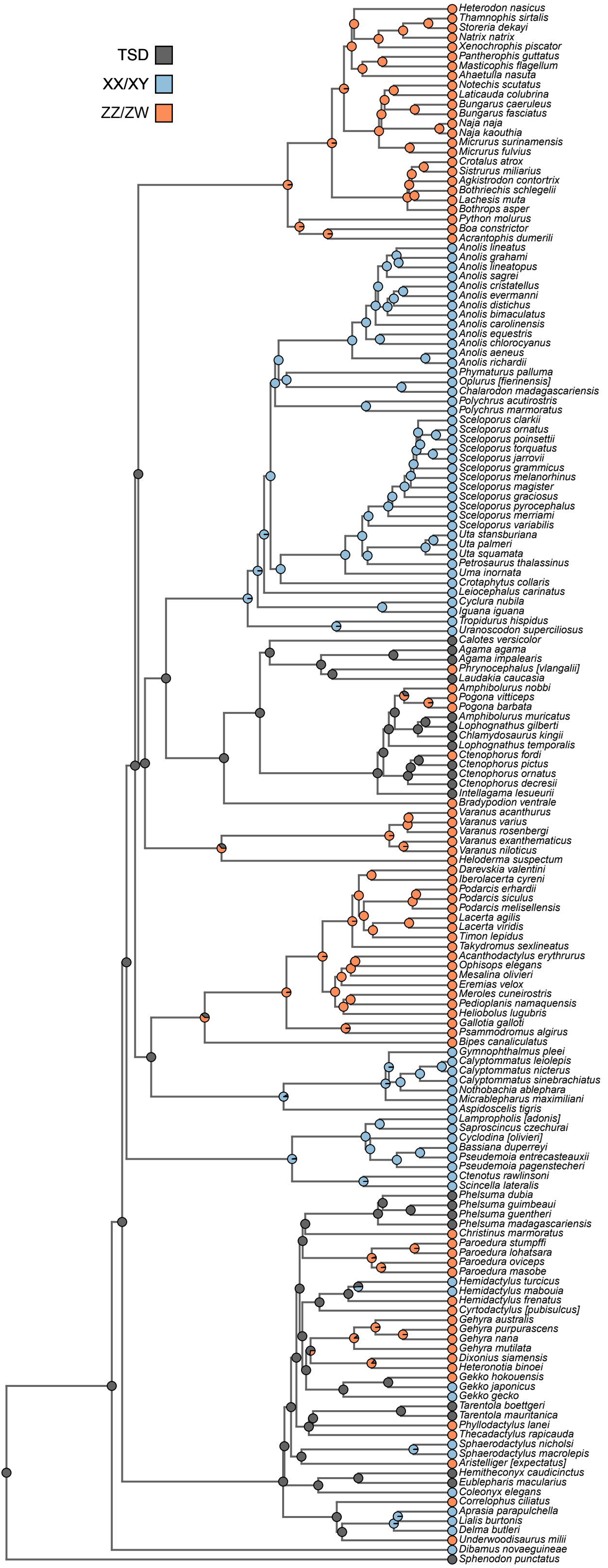
Supplementary Figure 4



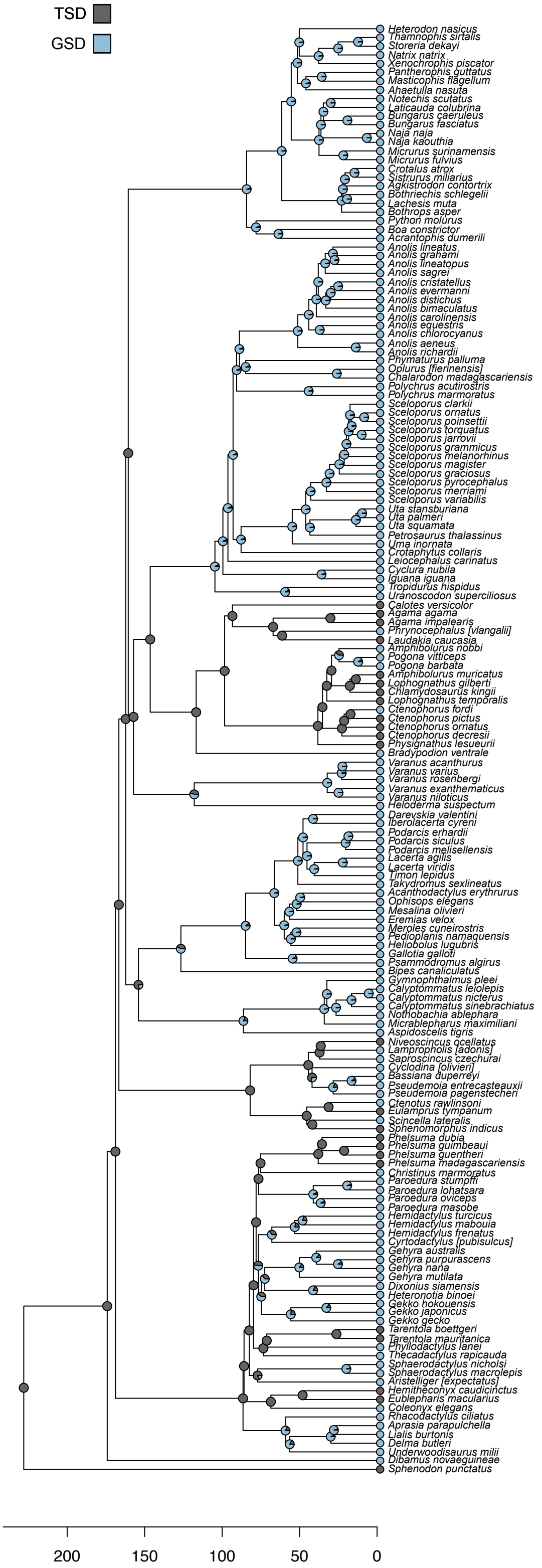
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8