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A hybrid phylogenetic-phylogenomic approach for species tree estimation in African *Agama* lizards with applications to biogeography, character evolution, and diversification



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ABSTRACT

Africa is renowned for its biodiversity and endemicity, yet little is known about the factors shaping them across the continent. African Agama lizards (45 species) have a pan-continental distribution, making them an ideal model for investigating biogeography. Many species have evolved conspicuous sexually dimorphic traits, including extravagant breeding coloration in adult males, large adult male body sizes, and variability in social systems among colorful versus drab species. We present a comprehensive time-calibrated species tree for Agama, and their close relatives, using a hybrid phylogenetic-phylogenomic approach that combines traditional Sanger sequence data from five loci for 57 species (146 samples) with anchored phylogenomic data from 215 nuclear genes for 23 species. The Sanger data are analyzed using coalescent-based species tree inference using ^{*}BEAST, and the resulting posterior distribution of species trees is attenuated using the phylogenomic tree as a backbone constraint. The result is a time-calibrated species tree for Agama that includes 95% of all species, multiple samples for most species, strong support for the major clades, and strong support for most of the initial divergence events. Diversification within Agama began approximately 23 million years ago (Ma), and separate radiations in Southern, East, West, and Northern Africa have been diversifying for >10 Myr. A suite of traits (morphological, coloration, and sociality) are tightly correlated and show a strong signal of high morphological disparity within clades, whereby the subsequent evolution of convergent phenotypes has accompanied diversification into new biogeographic areas.

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1. Introduction

African Agama lizards are among the most diverse and widespread terrestrial squamates in Africa, making them an ideal group for investigating biogeography and conducting comparative ecological and evolutionary studies (Leaché et al., 2009; Geniez et al., 2011; Gonçalves et al., 2012; Mediannikov et al., 2012). Some Agama exhibit extreme sexual dimorphism, and extravagant adult male breeding coloration is among the most conspicuous traits in the genus (Wagner et al., 2011). Species with brilliantly colored males are also usually larger in size compared to females, but adult male body sizes in Agama can vary widely in maximum snout-vent length (SVL) from small A. gracilimembris (47 mm) in West Africa and the Sahel to large A. caudospinosa (133 mm) in East Africa. Social systems are also variable within Agama, with some species forming colonies composed of a single male with many females, whereas males of some species are solitary. This suite of traits is assumed to be the result of sexual selection, and in this study we quantify the correlations among these characters, and investigate the evolution of these traits in relation to phylogeny, biogeography and diversification.

Comparative genomics data are becoming increasingly easy to obtain for molecular phylogenetic studies of non-model organisms (reviewed by Lemmon and Lemmon, 2013; McCormack et al., 2013). Sequence capture approaches (also referred to as hybrid enrichment) are emerging as a popular option for obtaining phylogenomic data, because they can provide data capable of resolving difficult phylogenetic problems at deep (Crawford et al., 2012) and shallow levels (Smith et al., 2014). Sequence capture methods use short probes (60–120 base pairs) to hybridize to specific genomic regions that are then isolated and sequenced using next-generation sequencing (Gnirke et al., 2009). Competing techniques for sequence capture exploit different aspects of the genome for probe hybridization, including ultraconserved elements (Faircloth et al., 2012), conserved regions (Lemmon et al., 2012), or protein-coding genes (Li et al., 2013). Regardless of which genomic regions are exploited for hybridization, the approach offers genome-wide sampling of large numbers of loci.

A current challenge in molecular phylogenetics is the integration of phylogenomic data with traditional multilocus data ("Sanger" data). The dimensions of the data matrices are typically transposed in terms of numbers of samples and numbers of loci, with the phylogenomic data containing hundreds of loci for relatively few samples, whereas Sanger data generally contain relatively few loci with dense taxonomic sampling. Combining these different types of data introduces extensive levels of missing data, and this precludes the application of coalescent-based species tree inference methods that require independent loci to be sampled for each species. Even if data were present for all species at every locus, large numbers of loci impose computational limitations that prevent the application of most species tree inference methods.

In this study, we estimate the phylogenetic relationships among African *Agama* species using traditional multilocus data obtained using Sanger sequencing and with phylogenomic data obtained using sequence capture (Lemmon et al., 2012). The Sanger data contain 146 specimens (representing 57 species) and five independent loci (four nuclear genes and mitochondrial DNA), whereas the phylogenomic data include 23 species and 215 loci. We use a hybrid phylogenetic–phylogenomic approach to obtain a species tree for *Agama* that benefits from the properties of both types of data. The Sanger data offer the dense taxonomic sampling necessary for a comprehensive species-level phylogeny, and the phylogenomic data provide dense locus sampling for strengthening the backbone of the phylogenetic tree.

2. Materials and methods

2.1. Sanger data

2.1.1. Molecular methods

Our taxonomic sampling within *Agama* includes 95% of the described valid taxa (43 of 45 taxa, including described species and subspecies) and multiple samples for most species (116 specimens total; average = 2.7 samples/species). Outgroups include six genera belonging to the African/West Asian Agamidae clade (*Acanthocercus, Laudakia* [recently recognized as *Stellagama*], *Phrynocephalus, Pseudotrapelus, Trapelus,* and *Xenagama*), and *Calotes versicolor* from the South Asian sister clade (Macey et al., 2000, 2006) is used to root phylogenetic trees when necessary. A total of 146 specimens representing 57 species are included in the phylogenetic analyses (Table 1).

We collected traditional multilocus data using Sanger sequencing to obtain nearly complete taxonomic coverage with multiple samples within species. The Sanger data includes five loci: mitochondrial DNA (mtDNA) and four single copy protein coding nuclear genes. The mtDNA data (1207 aligned base pairs) includes fragments of the 16S rRNA gene (*16S*), the ND4 protein-coding gene (*ND4*), and the adjacent histidine, serine, and leucine tRNA genes (*tRNAs*). The nuclear genes (2793 aligned base pairs) include neurotrophin-3 (*NT3*), oocyte maturation factor Mos (*CMOS*), pinin gene (*PNN*), and RNA fingerprint protein 35 (*R35*). Molecular lab protocols for Sanger sequencing follow Leaché et al. (2009), and primer sequences are provided in Table 2.

2.1.2. Alignment and partitioning

Multiple sequence alignments were estimated for the indel-rich 16S and *tRNAs* using SATé v2.0.3 (Liu et al., 2011). SATé uses maximum likelihood (ML) to estimate phylogenetic trees and multiple sequence alignments simultaneously using a divide-and-conquer realignment technique, which can boost alignment accuracy substantially (Liu et al., 2009). Initial alignments were generated using ClustalW v2.0.12 (Thompson et al., 1994), and subsequent alignment refinement steps in SATé used MUSCLE v3.8 (Edgar, 2004a, 2004b) in conjunction with ML trees estimated with RAxML v7.2.6 (Stamatakis, 2006) under the GTRGAMMA model. SATé was run for 12 h with default parameter values.

The molecular genetic data were partitioned into 17 distinct data blocks including 15 blocks for the 1st, 2nd, and 3rd codon positions for each of the five protein-coding genes (e.g., ND4, CMOS, PNN, NT3, and R35) and two blocks for the non-coding 16S gene and tRNAs (treated as one block). Nucleotide substitution models for each data block were selected using jModelTest v0.1.1 (Posada, 2008). Three substitution model types were evaluated on a fixed BIONJ-JC tree, and model selection was conducted using the Bayesian information criteria (BIC). We used PartitionFinder v0.9 (Lanfear et al., 2012) to identify the optimal partitioning scheme for the 17 data blocks, and the best-fit nucleotide substitution model for each partition. We ran PartitionFinder twice with the models of molecular evolution restricted to those that are available in either MrBayes v3.2.1 (Ronquist et al., 2012) or RAxML. All PartitionFinder analyses used the greedy search algorithm, linked branch lengths in calculations of likelihood scores, and the BIC for selecting among alternative partitioning strategies.

2.1.3. Gene tree estimation

Gene trees were inferred using maximum likelihood and Bayesian inference. Gene trees were estimated from the Sanger data for each nuclear locus separately, the combined mtDNA data, the combined nuclear data, and the concatenated mtDNA and nuclear data. Analyses of protein-coding genes used codon partitioning.

Voucher specimen information and Genbank accession numbers for Sanger sequences. The complete Sanger and anchored phylogenomics data are available on Dryad. Anchored phylogenomics data were collected for the 23 specimens highlighted in bold.

Species	Voucher	16S	ND4	CMOS	NT3	R35	PNN
Acanthocercus annectans	CAS 227508	JX668128	JX857621	JX838886	JX839175	JX839078	JX838995
Acanthocercus annectans	MVZ 242740	IX668129	IX857561	IX838887	IX839176	IX839079	IX838996
Acanthocercus atricollis	CAS 201727	IX668130	IX857596	IX838888	IX839177	IX839080	IX838997
Acanthocercus atricollis	EBG 1761	IX668131	IX857574	IX838889	IX839178	_	IX838998
Acanthocercus atricollis	EBG 2167	JX668132	JX857631	JX838890	JX839179	JX839081	_
Acanthocercus atricollis	MVZ 265804	JX668133	JX857555	JX838891	-	JX839082	JX838999
Acanthocercus atricollis	ZFMK 61662	JX668134	JX857632	_	-	_	_
Acanthocercus cyanogaster	MVZ 257904	JX668135	JX857609	JX838892	-	-	JX839000
Acanthocercus cyanogaster	MVZ 257924	JX668136	JX857562	JX838893	-	-	_
Acanthocercus cyanogaster	MVZ 257928	JX668137	JX857548	JX838894	JX839180	[X839083	JX839001
Acanthocercus cyanogaster	MVZ 257937	JX668138	JX857628	JX838895	_	JX839084	_
Acanthocercus cyanogaster	MVZ 257938	JX668139	JX857602	JX838896	-	JX839085	-
Acanthocercus yemenensis	MVZ 236454	JX668140	JX857608	JX838897	JX839181	JX839086	JX839002
Acanthocercus yemenensis	MVZ 236455	JX668141	JX857559	JX838898	JX839182	JX839087	-
Agama aculeata	AMNH 141775	JX668142	JX857573	JX838899	JX839183	JX839088	JX839003
Agama aculeata	MCZ Z37841	JX668143	JX857566	JX838900	JX839184	JX839089	JX839004
Agama aculeata	MVZ 198076	GU128430	GU128467	JX838901	JX839185	JX839090	JX839005
Agama africana	ULM 200	GU128440	GU128477	JX838902	JX839186	JX839091	JX839006
Agama agama	MCZ 184560	JX668144	JX857595	JX838903	JX839187	JX839092	JX839007
Agama agama	ZFMK 15222	GU133323	-	-	-	-	-
Agama anchietae	AMB 4906	JX668145	JX857610	JX838904	JX839188	JX839093	JX839008
Agama anchietae	AMB 7582	GU128446	GU128483	JX838905	JX839189	JX839094	JX839009
Agama anchietae	MCZ Z37865	JX668146	JX857592	JX838906	JX839190	-	-
Agama anchietae	ZFMK 72906	JX668147	-	JX838907	JX839191	JX839095	JX839010
Agama armata	AMB 8317	JX668148	JX857615	JX838908	JX839192	JX839096	JX839011
Agama armata	AMB 8350	JX668149	JX857598	-	JX839193	JX839097	JX839012
Agama armata	CAS 198929	JX668150	JX857620	JX838909	JX839194	JX839098	JX839013
Agama armata	CHI 201	JX668151	JX857580	JX838910	JX839195	JX839099	-
Agama armata	ZFMK 84990	GU128447	GU128484	-	-	JX839100	-
Agama atra	AMB 4487	JX668152	JX857616	JX838911	JX839196	JX839101	JX839014
Agama atra	AMB 4826	JX668153	JX857567	JX838912	JX839197	-	-
Agama atra	ZFMK 41744	JX668154	JX857569	JX838913	JX839198	JX839102	-
Agama boensis	KU 291845	JX668155	JX857589	JX838914	-	JX839103	JX839015
Agama boensis	TR 496	JX668156	JX857575	JX838915	-	JX839104	JX839016
Agama bottegi	CAS 227496	JX668157	JX857587	JX838916	JX839199	JX839105	-
Agama boueti	6251X	JX668158	-	JX838917	JX839200	JX839106	-
Agama boueti	6253X	JX668159	JX857557	JX838918	JX839201	JX839107	JX839017
Agama boueti	FMNH 262261	JX668160	JX857623	JX838919	JX839202	JX839108	JX839018
Agama boueti	MNCN 43869	JN665051	-	-	-	-	-
Agama boueti	MNHN IV	JX668161	JX857551	JX838920	JX839203	JX839109	JX839019
Agama boueti	MVZ 238892	JX668162	JX857613	JX838921	JX839204	JX839110	JX839020
Agama boueti	ZFMK 80057	GU133313	-	-	-	-	-
Agama boulengeri	MNHN I	GU133324	JX857619	JX838989	JX839205	JX839169	JX839021
Agama boulengeri	MVZ 235763	JX668163	JX857603	JX838923	JX839206	JX839112	JX839022
Agama boulengeri	MVZ 235764	GU128449	GU128486	JX838924	-	-	-
Agama boulengeri	ZFMK 76868	JX668164	-	-	-	JX839113	-
Agama castroviejoi	MNCN 41779	AY522929	-	-	-	-	-
Agama castroviejoi	MVZ 235766	GU128454	GU128491	JX838925	-	JX839114	JX839023
Agama cauaospinosa	ZFIVIK 83662	GU128450	GU128487	JX838926	-	JX839115	JX839024
Agama cf. benueensis	FK 2235X	JX668165	JX857588	-	-	-	-
Agama of homeomotic	FK 2820X	JX008100	JX857001	-	-	-	-
Agama cristata	TR 4210A	GU126451	GU126466	-	-	-	-
Agama cristata	TR 3449	JE520717	_	_	_	_	_
Agama dodomae	7FMK 8/083	JF520718 IX668167	-	-	_	-	-
Agama doriga	MUZ 257067	JX008107	JX0J7JJ2 IV957614	12020020	-	JX859110	JX859025
Agama doriao	MVZ 257907	JX008108	17857620	12020020	JX859207	-	-
Agama doriae	MVZ 257500	IX668170	JX857600	1X838030		JX055117	
Agama doriae	MVZ 257570 MVZ 257071	JX008170 IX668171	12857622	12838031			12830036
Agama etoshae	7FMK 21066	JX008171 JX668172	JX857544	12838033			12830020
Agama finchi	ZEMK 83653	CU128452	CU128480	1X838033		-	12830028
Agama gracilimembris	LIWBM 5576	IX668173	IX857617	IX838934	_	IX839119	IX839029
Agama gracilimembris	UWBM 5577	IX668174	IX857563	IX838935	_	IX839120	IX839030
Agama gracilimembris	UWBM 5578	IX668175	JX857611	JX838936	_	JX839120	JX839031
Agama hartmanni	ZFMK 27598	IX668176	IX857590	_	_	_	_
Agama hispida	AMB 4800	GU128453	GU128490	IX838937	IX839208	IX839122	IX839032
Agama hispida	AMB 5625	IX668177	IX857594	IX838938	IX839209	IX839123	IX839033
Agama impalearis	2934I	IX668178	JX857625	IX838939	JX839210	IX839124	IX839034
Agama impalearis	AJ414684	AJ414684	_	-	_	_	_
Agama insularis	KU 291843	IX668179	[X857593	_	_	[X839125	_
Agama insularis	TR 500	JX668180	[X857583	JX838940	JX839211	JX839126	[X839035
Agama insularis	TR 554	JX668181	JX857550	JX838941	JX839212	JX839127	JX839036
Agama insularis	TR 555	GU133325	_	JX838942	JX839213	JX839128	JX839037
Agama insularis	ZFMK 88247	JX668182	JX857633	JX838943	-	-	-
-		-	-	-			

(continued on next page)

Table 1 (continued)

Species	Voucher	16S	ND4	CMOS	NT3	R35	PNN
Agama kaimosae	ZFMK 82075	IX668183	IX857630	_	_	_	_
Agama kirkii	MVZ 265806	JX668184	JX857624	JX838944	-	[X839129	[X839038
Agama kirkii	MVZ 265812	IX668185	IX857549	IX838945	-	IX839130	IX839039
Agama kirkii	MVZ 265827	_	IX857570	IX838946	-	IX839131	IX839040
Agama kirkii	MVZ 265828	IX668186	IX857558	IX838947	_	-	IX839041
Agama kirkii	ZFMK 54533	IX668187	_	-	-	-	-
Agama knobeli	AMB 4305	IX668188	IX857546	IX838948	IX839214	IX839132	1X839042
Agama knobeli	AMB 4670	JX668189	JX857547	1X838949	JX839215	JX839132	_
Agama knobeli	AMB 4850	IX668190	JX857556	1X838950	IX839216	IX839134	12839043
Agama knobeli	CAS 103/35	CU128448	CU128485	JX050550 JX838051	18830217	18830135	JX055045
Agama knobeli	MC7 A38433	IX668191	120405	1X838952	IX839218	IX839136	1X839044
Agama lebretoni	CAS 207957	JX668197	JX857627	1X838953	JX839210	JX839137	1X839045
Agama lebretoni	MV7 253099	CU128444	GU128481	IX838954	1X839220	IX839138	1X839046
Agama lionotus	CAS 100008	12668103	120401	12838055	JX055220	JX055150	JX055040
Agama lionotus	NMK 1/2720	12668104	JX037337	JX050555			
Agama lionotus	7FMK 82064	JX668195	_				_
Agama lionotus	ZEMIK 83646	CU128456	CU128/03	12838056	12830221	12830130	12830047
Agama makarikarica	ZEMIK 18369	IX668196	-	1X838957	1X839222	IX839140	1X839048
Agama makarikarica	ZEMIK 18300	JX008150 JX668107		12838058	JX0J3222	JX833140 JX8301/1	1X830040
Agama montana	CAS 168011	12668108	12857570	12838050	-	18830147	12830050
Agama montana	EMNU 251224	JX000150	12057500	12626922	12020224	JX033142	12220051
Agama mossambica	7EMIX 12/170	17668200	JN0J7J02	JV929200	JX039224	JX859145	JV022021
Agama mwanzao	ZEMIK 13479	CU129457	-	-	-	-	-
Agama narafricana	MV7 240605	IV662201	- IV957610	12020001	-	-	-
Agama paragama	NIVE 249005	CU122221	JN037012	JN020902	JN059225	JX659144	JV922025
Agama picticauda	20011	17662202	-	-	-	-	-
Agama picticauda	AMNH 100700	CU129441	JX857020 CU128478	12020001	12820227	JX839143	12020024
Agama nicticauda	MU7 22001	CU120441	CU120470	12020065	12020220	12020147	12020055
Agama picticauda	NCSM 76790	17662202	GU120400	12020000	12820220	12020147	Jv922022
Agama picticauda	7ENAL 7294E	JX008203		12020007	JN039229	JX039140	-
Agama picticauda	ZEMIX 75045	JA008204	JA657005	12020000	-	JA659149	-
Agama picticauda	ZEIVIN 70000 7MD 71577	GU126442 IV669205	GU126479 IV957572	176269060	JA659250 IV920221	JV928120	12020057
Agama planicens	AMB 7638	CU128458	CU128404	18838070	18830333	- IX830151	12830028
Agama planicops	MC7 A29009	126436	GU120494 IV957571	12020071	12029232	12020152	12020020
Agama robocchii	7EMIC 27912	12668200	JX657571 IX857607	J70309/1	Jv9232222	JX039132	JV022022
Agama monnalli	LFIVIN 57612	JA006207	JA657007	-	-	JA039133	-
Agama monnalli	NVZ 241550	JA008208	JA657599	JA030972	JA039234	JX659154	Jv922000
Agama monnalli	IVIVZ 241557	JA006209	JA037004	JA030973	JN039233	-	-
Agama rueppelli	IVIVZ 241338	JX008210	JA85/381	JX838974	JX839230	-	-
Agama canliaranica	NVZ 241540	GU128459	GU128495	JA030973	JV0297221	JA659155	JV02200C2
Agama sankaranisa	7EN/K 84002	GU120400	GU120490	JV020210	-	JX839130	JX859002
Agama spinosa	LINK 04992	IN665065	JV07/200	-	-	-	-
Agama spinosa	INCCEOCC	INCCEDEC	-	-	-	-	-
Agama spinosa	JIN003000	CU129461	- CU129407	-	-	-	-
Agama spinosa	NUZ 230430	GU120401	GU126497	JA030977	JN039230	JA659157	-
Agama minosa	NVZ 230439	JA006211	JA657505	JA030970	JV020240	JA039130	JV029002
Agama spinosa	MV7 241334	JX008212	12057545	12020000	JX839240	12820160	12820065
Agama tassilionsis	INIG65061	JX008213	JV021242	JV020200	JA039241	JX859100	Jv922002
Agama tassilionsis	JN005001	JN005001	-	-	-	-	-
Agama tassilionsis	IN665063	IN665062	_	_	_	_	-
Agama tassilionsis	IN665064	IN665064	_	_	_	_	-
Agama turuensis	7FMK 7/020	IX668214		_	_	_	_
Agama weidholzi	AMNH 100810	IX668215	IX857501	1X838981	18830242	IX839161	1X830066
Agama weidholzi	TR 481	CU128462	CU128498	1X838982	1X839242	IX839162	1X839067
Agama weidholzi	TR 482	IX668216	IX857554	1X838982	JX839245	IX839163	IX830068
Agama weidholzi	7FMK 75001	CU133328	JX857564	1X838984	1X839245	IX839164	_
Calotes versicolor	MV7 248401	12668217	18857560	12838085	18830245	18830165	12830060
Laudakia stellio	MVZ 230213	CU128464	GU128500	1X838986	1X839240	IX839166	1X839070
Phrynocenhalus mystaceus	MVZ 245941	IX668218	IX857553	1X838980	JX839247 JX839248	JX839167	JX833070 JX839071
Pseudotranelus sinaitus	SRFD 11271	JX668219	JX857606	1X838988	1X839240	IX839168	1X839072
Tranelus agnetae	ZFMK 86579	IX668220	_	_	-	_	_
Trapelus hoehmei	MNHN II	IX668221	IX857584	1X838922	IX839250	IX839111	IX839073
Trapelus boehmei	ZFMK49664	H0901112	_	_	_	_	_
Trapelus mutabilis	ZFMK 64395	H0901114	GU128501	_	_	IX839170	_
Xenagama hatillifera	ZFMK 83411	IX668222	-	_	_	_	_
Xenagama batillifera	ZFMK 83412	IX668223	-	_	_	_	_
Xenagama batillifera	ZFMK 84370	IX668224	IX857572	1X838990	_	_	_
Xenagama sp. nov	AMNH 105545	IX668225	IX857585	IX838991	IX839251	IX839171	IX839074
Xenagama sp. nov	AMNH 105546	IX668226	IX857576	IX838992	IX839252	IX839172	JX839075
Xenagama tavlori	MVZ 241356	GU128466	GU128502	IX838993	IX839253	IX839173	IX839076
Xenagama tavlori	MVZ 241361	IX668227	IX857578	IX838994	IX839254	IX839174	IX839077
Xenagama taylori	ZFMK 75072	JX668228	JX857568	-	-	_	-
		-	-				

Table 2Primers used for PCR and Sanger sequencing.

Locus	Primer Name: Sequence (5'-3')	Citation
CMOS	F: GCGGTAAAGCAGGTGAAGAAA	Saint et al. (1998)
	R: TGAGCATCCAAAGTCTCCAAT	
NT3	F: ATATTTCTGGCTTTTCTCTGTGGC	Noonan and
	R: GCGTTTCATAAAAATATTGTTTGACCGG	Chippindale (2006)
PNN	F: ACAGGTAATCAGCACAATGAYGTAGA	Townsend
	R: TCTYYTGCCTGAYCGACTACTYTCTGA	et al. (2008)
R35	F: GACTGTGGAYGAYCTGATCAGTGTGGTGCC	Leaché (2009)
	R: GCCAAAATGAGSGAGAARCGCTTCTGAGC	
16S	F: CGCCTGTTTAACAAAAACAT	Leaché et al. (2009)
	R: CCGGTCTGAACTCAGATCACGT	
ND4	F: CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)
	R: CATTACTTTACTTGGATTTGCACCA	

Analyses of the concatenated data used the partitions and models identified with PartitionFinder. Maximum likelihood analyses were conducted with RAxML. All ML analyses executed 100 rapid bootstrap replicates followed by a thorough ML search under the specified model. Bayesian analyses used a modified version of MrBayes v3.2.1 that includes compound Dirichlet priors for branch lengths (Rannala et al., 2012). These branch length priors reduce the strong assumptions about the tree length imposed by the default branch length priors (i.e., exponential distributions) that cause trees to grow too long (Brown et al., 2010; Marshall, 2010). We implemented a gamma prior on the tree length, with shape (α_T) and rate $(\beta_{\rm T})$ parameters = 1 (using GammaDir(1,1,1,1); Zhang et al., 2012). All nucleotide substitution model parameters were unlinked across partitions and the different partitions were allowed to evolve at different rates using the "prset ratepr = variable" command. For each Bayesian analysis we ran four concurrent chains (one cold and three heated) for 10 million generations and recorded samples every 1000 generations. The first 2000 samples were discarded as burn-in, and the remaining 8000 samples were used to summarize the posterior probability distributions for parameters. Maximum likelihood bootstrap values and Bayesian posterior probability values were joined and mapped to the Bayesian tree (i.e., the 50% majority-rule consensus tree calculated from the posterior distribution of trees) using SumLabels, a phylogenetic tree label concatenation utility in the python package DendroPy (Sukumaran and Holder, 2010).

2.1.4. Species tree estimation

A time-calibrated species tree for Agama was estimated with the Sanger data using ^{*}BEAST v1.7.1 (Heled and Drummond, 2010). The species tree analysis contained 146 samples belonging to 57 species (including non-Agama outgroup species). The site models, clock models, and gene trees were unlinked across loci. The ploidy type for each locus was specified to account for the fourfold smaller effective population size of the mtDNA relative to the nuclear genes resulting from the haploid and maternal inheritance of mtDNA (Ballard and Whitlock, 2004). The nucleotide substitution models selected using jModelTest were applied; however, difficulty in estimating overly complex models prompted us to exclude the invariant sites parameter (I) from models that also included the among-site rate variation parameter (Γ). The uncorrelated lognormal relaxed clock was applied to each locus. Twenty replicate analyses were conducted with random starting seeds and chain lengths of 400 or 600 million generations with parameters sampled every 100,000 steps. Long chains were necessary for achieving high effective sample sizes (ESS) for parameters, and ESS values \ge 200 were used as a proxy for convergence of parameters. Species trees were summarized after discarding the first 25% of trees as burn-in. The species trees obtained across replicate runs were compared for congruence by examining their topology and posterior probability values in Are We There Yet? (AWTY; Nylander et al., 2008). The post-burn-in samples from analyses were combined and used to summarize the posterior probability distribution of parameters.

We estimated species trees under two tree priors, a Yule prior and a birth-death prior, and compared the posterior distributions using the harmonic mean likelihood in conjunction with Bayes factors and Akaike's information criterion through Markov chain Monte Carlo simulation (AICM; Baele et al., 2012). Under the AICM, an increase in the number of parameters penalizes more complex models, and models with lower AICM values are preferred over models with higher values.

Two calibrations were used to obtain divergence dates on the species tree following the method outlined by McCormack et al. (2010): (1) the divergence between *Calotes* and *Phrvnocephalus* occurred 62.5 Ma. a date obtained by a study of squamate divergence times using 11 fossil calibrations (Wiens et al., 2006). Uncertainty in this date was accommodated using a normal prior probability distribution with a mean of 62.5 Ma and a standard deviation of 3.0 Ma, which results in 5% and 95% quantiles at 57.6 Ma and 67.4 Ma, respectively. (2) The divergence between Xenagama and Pseudotrapelus occurred between 16.4 Ma and 19.6 Ma (normal distribution, mean = 18 Ma and stdev = 1.0 Ma), which encompasses the estimates obtained using pairwise sequence divergence calculations for mtDNA (Macey et al., 2006). These two calibration points are derived from previous studies and not specific fossil calibrations, which may lead to compounded inaccuracy of estimated divergence times.

2.2. Anchored phylogenomics data

2.2.1. Molecular methods

The anchored phylogenomics data include 23 agamid lizards representing all major clades of Agama identified in the timecalibrated species tree, as well as two outgroups (Table 1). Anchored phylogenomics (Lemmon et al., 2012) utilizes hybrid enrichment via sequence capture to enrich for a set of 512 loci that have been pre-screened for properties amenable to phylogenetic analysis (e.g., single-copy, low repetitive DNA, few indels, etc.). Indexed libraries were prepared following a protocol modified from Kircher et al. (2011). Libraries were pooled (8 per pool) and then enriched using the v.1.0 probe set for vertebrates (Lemmon et al., 2012) through the Agilent Custom SureSelect kit. Enriched libraries were sequenced via single-end 50 bp sequencing on an Illumina HiSeq 2000 at the Florida State University Biology Core Facility. Raw sequencing reads were processed using a bioinformatics workflow that de-multiplexes and removes low quality reads, merges overlapping reads, and removes PCR duplicates (Lemmon et al., 2012). Reads were mapped to each locus, and a consensus sequence was made for each individual for each locus.

2.2.2. Alignment and partitioning

Alignments were generated for each locus using MUSCLE. The appropriate nucleotide substitution model was selected for each locus using jModelTest. This search was limited to models with three substitution schemes, estimated base frequencies, and rate variation under a gamma parameter with four rate categories. The base tree was estimated using ML optimization and nearestneighbor interchange branch swapping. Models within the 95% confidence interval based on the BIC were retained and the most parameter rich model out of this set was chosen. The proportions of variable and informative sites were compared to the estimated model of evolution to determine if there is a correlation between model complexity and site variability. The site proportion was regressed against the number of parameters in the estimated model using the linear model function (Chambers, 1992) in R (R Development Core Team, 2011). Uncorrected pairwise sequence divergence of the concatenated anchored phylogenomic loci and the number of variable and parsimony informative sites for each locus was estimated using PAUP^{*} v.4.0b10 (Swofford, 2003).

2.2.3. Gene tree estimation

Maximum likelihood trees for each of the anchored phylogenomics loci were estimated using the GTRGAMMA model in RAx-ML. We also conducted phylogenetic analysis on the concatenated phylogenomic data. We estimated a maximum likelihood phylogeny using RAxML using the GTRGAMMA model (without locus partitioning) with 1000 bootstrap replicates. We also calculated genetic distance trees for the concatenated data using UPGMA and NJ in PAUP^{*}.

2.2.4. Species tree estimation

We used the summary statistic approaches STEAC and STAR (Liu et al., 2009) to generate species trees from the individual ML gene trees estimated for each locus. The ML gene trees were combined into a single file and analyzed using the phybase package in R to generate the STEAC and STAR trees. We used ^{*}BEAST to estimate species trees using a subset of the genes with the largest proportion of informative sites (the top 10 and top 20 loci). For the ^{*}BEAST analyses, the substitution models were based on jModelTest, and all genes were assigned an uncorrelated log-normal clock. The species trees used a Yule prior and the population size parameter was set to constant linear. The ^{*}BEAST analyses were run with four replicates for 100 million (10 genes) or 200 million (20 genes) generations. Convergence across replicate runs was assessed using Tracer and AWTY, and retained trees were summarized using the TreeAnnotator utility in BEAST (Drummond and Rambaut, 2007).

2.3. Hybrid phylogenetic-phylogenomic approach

The final combined posterior distribution of time-calibrated species trees obtained from the ^{*}BEAST analyses of the Sanger data was filtered (based on topology only) using the preferred phylogenomic tree as a backbone constraint in PAUP^{*}. The phylogenomic tree included a 4-way polytomy to reflect uncertainty in relationships found at the base of the tree. The filtering step retained only those topologies in the posterior distribution of time-calibrated species trees that were in agreement with the backbone constraint imposed by the phylogenomic tree. A maximum clade credibility (MCC) tree was estimated from the resultant filtered posterior distribution of species trees using TreeAnnotator. The hybrid phylogenetic-phylogenomic posterior distribution and MCC tree were used in subsequent analyses of biogeography, character evolution, and diversification.

2.4. Biogeography

The dispersal-extinction-cladogenesis model of geographic range evolution was implemented using Lagrange v0.1 (Ree et al., 2005; Ree and Smith, 2008). Information on the timing of lineage divergences was incorporated using the hybrid phylogenetic– phylogenomic species tree. Seven major biogeographic areas were defined based on a recent cluster analysis of thousands of plant and animal species (Linder et al., 2012). These include (1) Northern Africa (north of the Sahel), (2) Sahel (transition zone between the Sahara and savannas), (3) Horn of Africa, (4) West Africa (west of Cameroon), (5) South Africa (south of the Zambezi and Cunene Rivers), (6) East Africa (including the Great Rift Valley), and (7) Central Africa (core of the continent). The availability of connections between areas (dispersal routes) were unconstrained.

2.5. Trait correlations and morphological evolution

Data on body size, coloration, and mating systems of Agama were taken from the literature (Grandison, 1968; Joger, 1979; Moody and Böhme, 1984; Branch, 1998; Wagner et al., 2008a,b; Wagner, 2010a; Geniez et al., 2011; Wagner and Bauer, 2011; Mediannikov et al., 2012), scored from museum specimens, or made from personal observations in the field (Supplemental Appendix 1). We recorded maximum male body size observed for each species, measured in SVL (to the nearest mm) for our primary body size trait. Because lizards have indeterminate growth, we used log-transformed maximum SVL data for all analyses that included body size. Color and sociality traits were coded as discrete characters with two states. Social system was scored as either (0) solitary, or (1) colonial breeding. Male territoriality states included (0) only during breeding season. and (1) territoriality all year. Female coloration (and male throat coloration) was coded as absent (0) or present (1). Finally, male breeding coloration was coded as either (0) minor/seasonal, or (1) extensive.

Correlations among pairs of morphological and sociality traits were tested using maximum likelihood and Bayesian methods in BayesTraits v1.0 (Pagel et al., 2004; Pagel and Meade, 2006). The posterior distribution of hybrid phylogenetic-phylogenomic species trees was used in all analyses. Ancestral states and models of trait evolution were estimated using BayesMultistate (Pagel et al., 2004). Four replicate analyses were conducted for one million generations each (retaining 900 samples). Tests for correlated evolution between two binary traits were conducted using maximum likelihood and Bayesian implementations of BayesDiscrete (Pagel and Meade, 2006). The independent and discrete models were compared using likelihood ratio tests for the maximum likelihood results, and Bayes factors for the Bayesian posterior distributions. Phylogenetic ANOVA (Garland et al., 1993) was performed on log-transformed body size for each of our five discrete groupings. We used phy.anova in the Geiger R package (Harmon et al., 2008) with 1000 simulations to determine a phylogenetic *p*-value.

Morphological disparity through time (Harmon et al., 2003) was examined using the delta-disparity test (Burbrink et al., 2012). We used the R function Badbrains (Burbrink et al., 2012) to produce a distribution of Δ -MDI values that quantify morphological disparity in body size across the posterior distribution of species trees. Negative Δ -MDI values indicate low within-clade disparity, distributions centered on zero are no different from the null model of Brownian motion, and positive Δ -MDI values suggest high within-clade disparity.

2.6. Diversification

To test the null hypothesis that per-lineage speciation and extinction rates have remained constant through time, we applied the γ statistic (Pybus and Harvey, 2000), which measures whether internal nodes of a phylogeny are closer to the root than would be expected under a model of constant diversification rates ($\gamma = 0$). Significant *P* values for negative values of γ are indicative of early burst diversification followed by a deceleration in lineage accumulation. Theory predicts that gene trees will produce earlier divergences compared to species trees (Pamilo and Nei, 1988), and that gene trees should therefore be biased towards strongly negative γ values (Burbrink and Pyron, 2011). We contrasted γ values calculated for the species tree and concatenated Sanger tree (which is essentially a gene tree) to determine whether the earlier branching times expected for the concatenated tree support early burst diversification.

3. Results

3.1. Sanger data

3.1.1. Data characteristics

The nucleotide substitution models selected for each data partition are provided in Table 3, and the best-fit partitioning scheme for the 17 data blocks estimated using PartitionFinder includes seven partitions (Table 4). The codon partition blocks for the mtDNA data are in different partitions, with the exception of *ND4* 1st positions grouping with the *tRNAs*. The 3rd codon positions for the four nuclear genes grouped into the same partition. The 1st and 2nd codon positions for the nuclear genes were placed into two partitions, one contained *PNN*, and the other contained *CMOS*, *NT3*, and *R35*.

3.1.2. Gene trees

The Bayesian and ML analyses of the concatenated nuclear and mtDNA data (Fig. 1) contains several notable relationships, and most of these relationships are supported by the phylogenetic analyses of the independent loci and concatenated nuclear genes (Supplemental Appendix 1). First, the genus Acanthocercus is paraphyletic, and contains Pseudotrapelus sinaitus, Agama robecchii, and Xenagama. Second, Agama robecchii and Acanthocercus annectans form a clade, a result that is restricted to phylogenetic analyses containing the mtDNA data. Third, Acanthocercus yemenensis is the sister taxon to the remaining members of "Acanthocercus." Fourth, Acanthocercus atricollis is the sister taxon to the genus Xenagama. Fifth, the initial divergences within Agama are weakly supported, which renders the order of relationships of the major clades tenuous. Finally, population samples for some species within Agama do not form exclusive groups, including A. agama, A. lionotus, and A. boueti.

3.1.3. Species tree

The BEAST species tree analyses using the Yule tree prior were favored over the birth-death tree prior. The Bayes factor (2logBF) calculated from the harmonic mean of the marginal likelihoods

Table 3

Nucleotide substitution models (selected out of 24 candidate models) for the nuclear and mitochondrial gene data blocks based on the Bayesian information criterion using jModelTest.

Data blocks	Model
Nuclear genes	GTR+I+G
PNN	GTR+I+G
1st positions	GTR+G
2nd positions	GTR+G
3rd positions	K80+G
R35	GTR+I+G
1st positions	SYM+I+G
2nd positions	K80+I+G
3rd positions	HKY+G
NT3	GTR+I+G
1st positions	GTR+I+G
2nd positions	K80+I+G
3rd positions	SYM+G
CMOS	HKY+I+G
1st positions	K80+I+G
2nd positions	K80+I+G
3rd positions	GTR+I+G
Mitochondrial genes	GTR+I+G
16S	GTR+I+G
tRNA	GTR+G
ND4	GTR+I+G
1st positions	GTR+I+G
2nd positions	GTR+I+G
3rd positions	GTR+G

Table 4

Best-fit partitioning scheme for the	17	data	blocks	using	the	Bayesian	informati	on
criterion in PartitionFinder.								

Partition	Best Model		Data blocks in partition
	MrBayes	RAxML	
1	GTR+I+G	GTR+I+G	ND4-1st, tRNA
2	GTR+I+G	GTR+I+G	ND4-2nd
3	GTR+G	GTR+G	ND4-3rd
4	GTR+I+G	GTR+I+G	16S
5	K80+G	GTR+G	CMOS-3rd, NT3-3rd, PNN-3rd, R35-3rd
6	GTR+G	GTR+G	PNN-1st, PNN-2nd
7	SYM+I+G	GTR+I+G	CMOS-1st, CMOS-2nd, NT3-1st, NT3-2nd, R35-1st, R35-2nd

was 8.2, or 82 times more likely in favor of the Yule model versus the birth–death model. The model comparison based on Akaike's information criterion through MCMC simulation ranked the Yule model (-86,548.4) over the birth–death model (-86,611.1).

The final combined posterior distribution of time-calibrated species trees from the separate ^{*}BEAST analyses contained 16,382 trees. The MCC species tree (Fig. 2) supports the same notable relationships outlined for the concatenated phylogeny. These relationships include the paraphyly of *Acanthocercus*, and the placement of *Agama robecchii* in the "*Acanthocercus*" clade. The species tree does not provide evidence for the exclusivity of *Agama agama*, *A. lionotus*, or *A. boueti*, because we assumed that these species were independent lineages prior to conducting coalescent-based species tree inference.

The time-calibrated species tree indicates that diversification within *Agama* began approximately 23 Ma (95% HPD = 18.6–27.4 Ma), a timeframe that is contingent upon the calibration priors (Fig. 2). The species tree indicates that *Agama* is partitioned into at least five regional species assemblages, including, West, East, Sahel, Southern, and Northern clades. The support for each of these clades is typically strong (posterior probability values \ge 0.95), but the support for the interrelationships among these major groups is weak (posterior probabilities <0.95).

Achieving convergence between ^{*}BEAST analyses with the multilocus data, which included missing data for some species across multiple loci, proved difficult. While most of the 20 independent chains produced similar likelihood estimates, four of the analyses did not converge on similar posterior probabilities (e.g., the likelihood and model parameters), and were trapped on local optima for up to 300 million generations. Convergence diagnostics are provided in Supplemental Appendix 1.

3.2. Anchored phylogenomics data

3.2.1. Data characteristics

The anchored phylogenomics approach provided 215 loci with complete sampling for the 21 Agama and two outgroup species. The loci were trimmed to maximize coverage, resulting in alignments with no missing data. The 215 loci totaled 71,614 bp with an average length of 333 bp (range 200–643 bp). We note that both the number and lengths of compete loci could be increased substantially by increasing sequencing effort (i.e., 150 bp paired-end reads instead of 50 bp single-end reads). These data contained an average of 7.5% parsimony informative (range 0–16.9%) and 14.5% variable (range 0.8-26.5%) sites within loci (Fig. 3), and uncorrected pairwise sequence divergence between species averaged 2.5% within Agama and 4.5% from Agama to the outgroup taxa. Model testing preferred models with gamma to accommodate rate variation (Fig. 4). The K80 model was selected most often, followed by the HKY model, suggesting that the genomic regions associated with these loci have equal base frequency and a transition/transversion bias (Fig. 4). A



Fig. 1. Concatenated data phylogeny (mtDNA + four nuclear genes) for African Agama based on a Bayesian phylogenetic analysis using MrBayes. Posterior probability values \geq 0.50 and RAxML bootstrap values \geq 50% are shown on branches.

regression of the proportion of variable and informative sites against models selected by jModelTest indicated model selection is not correlated with site variability (Fig. 4).

3.2.2. Gene trees

The concatenated ML tree of the 215 loci recovered 100 percent bootstrap support for all nodes, with the exception of the two



Fig. 2. Time-calibrated Bayesian species tree for African Agama estimated using ^{*}BEAST (mtDNA + four nuclear genes) for the Markov chains that reached apparent stationarity. Numbers on nodes are posterior probability values. Horizontal bars indicate the 95% HPD for divergence times (in millions of years).



Fig. 3. Site variability in the 215 anchored phylogenomics loci, four nuclear loci, and two mitochondrial genes, ordered by proportion of informative sites.

nodes supporting the initial divergences within Agama (Fig. 5). These two nodes have bootstrap values of 72 and 48, and the ML topology differs from the species tree analysis of the Sanger data (Fig. 2). To explore how each of the 215 loci supported the base of the Agama phylogeny, we conducted ML analyses for each locus. First, we collapsed the phylogeny into a four-way polytomy at the base of Agama, which has 15 possible rooted solutions. Backbone constraint trees for each of these 15 possible topologies were used to determine which was supported by each of the 215 loci. For each locus, we maximized the likelihood for each of the 15 topologies in PAUP^{*}, using the preferred model selected from jModelTest. We enforced a molecular clock to keep the trees rooted. The ML topologies for each of the 215 loci recovered all 15 possible alternate topologies for these basal nodes, with some genes supporting multiple topologies (Table 5). Of the 15 possible solutions, the topologies placing the clade containing Agama bottegi, A. boueti, and A. spinosa at the base were recovered most frequently (Table 5).



Fig. 4. The proportion of informative sites (A) and proportion of variable sites (B) for the 215 anchored phylogenomics loci, arranged by substitution model (selected using jModelTest). Twelve substitution models were evaluated, and models incorporating the gamma parameter were preferred (C).

3.2.3. Species trees

The species tree topologies estimated from the phylogenomic data differ from the ML tree estimated for the concatenated data (Fig. 5). The topological differences concern the placement of *Agama boulengeri* in relation to the two nodes with low bootstrap support in the ML tree. The ML tree places *A. boulengeri* as the sister taxon to all other *Agama*. The STAR tree places *A. boulengeri* as the sister taxon to a clade containing *A. bottegi*, *A. boueti*, *A. spinosa*, *A. gracilimembris*, *A. insularis*, and *A. weidholzi*. The STEAC and ^{*}BEAST topology (the same topology as UPGMA and NJ) places the clade containing *A. bottegi*, *A. boueti*, and *A. spinosa* as sister to the rest of *Agama*, and *A. boulengeri* sister to a clade containing *A. gracilimembris*, *A. insularis*, and *A. weidholzi*. The ^{*}BEAST analyses using the top 10 or 20 most informative loci recovered the same topology, but the analysis with 20 loci provided increased support (Fig. 5).

3.3. Hybrid phylogenetic-phylogenomic approach

The Sanger data did not provide strong support for the relationships among the major clades, but the support for the backbone of the phylogeny was increased after filtering the posterior distribution of species trees (16,382 trees) for those that were congruent with the backbone constraint imposed by the topology from the phylogenomic data (Fig. 6). The filtered posterior distribution of species trees retained 206 trees, and the MCC tree calculated from this reduced posterior distribution is referred to as the hybrid phylogenetic–phylogenomic species tree (Fig. 6). This MCC species tree and/or the reduced posterior distribution was used for the analyses of biogeography, character evolution, and diversification. This species tree places *A. boulengeri* sister to all other *Agama* with weak support (posterior probability = 0.67; Fig. 6). The remaining species are divided into five major clades arranged asymmetrically (posterior probability ≥ 0.95 ; Fig. 6). At shallow levels of divergence (i.e., $\leqslant 5$ mya), relationships are weak among species within the East African *A. lionotus* complex and the West Africa *A. agama* complex (Fig. 6), two understudied groups that may harbor additional cryptic species diversity.

3.4. Biogeography

The dispersal-extinction-cladogenesis model of geographic range evolution suggests that monophyletic radiations of Agama have been established in Southern, East, West, and Northern Africa for approximately 10 mya (Fig. 6). The biogeographic origin of Agama is confined to Northern or West Africa; a Northern origin follows intuition since the biogeographic distribution of the clade sister to Agama is mostly North and Northeastern African and Middle Eastern. However, the low support for the placement of the West African endemic A. cristata near the base of the tree introduces uncertainty into this inference. Many instances of dispersal between adjacent biogeographic regions are evident, and these movements inform the history of zoogeographic connections across Africa (Supplemental Appendix 1). For instance, the Western clade contains species distributed as far as East Africa (A. finchi) and southern Africa (A. planiceps). Thus, the once widespread Guineo-Congolian rainforest, which is now confined to fragments in West and Central Africa, retains a foothold as far to the east as the Kakamega Forest in Kenya (Wagner et al., 2008a,b). These data also implicate Angola as a biogeographic corridor that links Central and Southern Africa. In general, the Angolan lizard fauna is characterized by an extremely abrupt turnover, but with some leakage of equatorial taxa into the south (e.g., A. planiceps).



Fig. 5. Phylogenetic trees for *Agama* based on the 215 anchored phylogenomics loci. The STEAC, UPGMA, NJ, and ^{*}BEAST topologies are identical. The STAR topology differs from the others by placing the clade containing *A. spinosa*, *A. bottegi*, and *A. bouteti* sister to the rest of *Agama*. The RAXML phylogeny placed *A. boulengeri* sister to remaining *Agama*. Numbers on nodes are support values (posterior probabilities or bootstrap values), and black dots indicate posterior probability values ≥ 0.95 or bootstraps = 100.

The maximum likelihood topologies for the 215 phylogenomic loci support all 15 possible rooted topologies depicting the initial divergences within *Agama*. Some anchored phylogenomic loci supported more than one topology with the same ML score. Abbreviations in gene trees are as follows: A = *Agama boulengeri*; B = *A. bottegi*, *A. boueti*, *A. spinosa* clade; C = *A. gracilimembris*, *A. insularis*, *A. weidholzi* clade; D = remaining *Agama*.

Gene tree	Rank	Count
(B, (D, (C, A)))	1	29
(B, (C, (D, A)))	2	27
(B, (A, (D, C)))	3	26
(C, (B, (B, D)))	4	24
(A, (B, (D, C)))	5	21
(A, (C, (D, B)))	6	20
(C, (D, (B, A)))	7	17
(A, (D, (C, B)))	8	16
(D, (B, (C, B)))	9	16
(D, (C, (B, A)))	10	15
(C, (A, (D, B)))	11	14
((B, D), (C, A))	12	13
((B, A), (D, C))	13	12
(D, (B, (C, A)))	14	10
((A, D), (C, B))	15	4

3.5. Trait correlations and morphological evolution

Tests for correlated evolution in the binary traits (i.e., male breeding coloration and mating system) support the correlated evolution of these traits under maximum likelihood and Bayesian methods (Table 6). Male throat coloration and female coloration are not correlated with male breeding color using ML, but using Bayesian estimation there is a significant correlation (posterior probability = 0.99) between female coloration and male breeding color (Table 6). The instantaneous rates for forward and backward changes in traits are nearly equal for male breeding color, mating systems, and male throat color; however, rates of change in female coloration are five times higher for gains than losses (Table 7). The posterior probabilities for trait evolution models favor the one rate (equal rate) model for male breeding color, mating systems, and male throat color (posterior probabilities ≥ 0.8), but for female coloration the equal rate model has a low posterior probability = 0.43 (Table 7). The phylogenetic ANOVA supports correlations between male body size and male breeding color, mating system, and male territoriality (P < 0.0001), but body size correlations with female color or male throat pattern are non-significant (P > 0.5).

Within each biogeographic region, we see the repeated association among a suite of sexually dimorphic traits (Fig. 7). Specifically, large males typically have colorful secondary sexual characteristics, whereas small males are drab and tend to lack extensive coloration.

Relative morphological disparity through time in *Agama* is higher than that predicted under a Brownian motion model (Fig. 8). This indicates that most variation is found within clades with a burst of morphological disparity starting approximately 20 mya coinciding with the diversification of *Agama* into different biogeographic regions of Africa. High within-clade morphological diversification is also indicated by a positive Δ -MDI distribution (Fig. 8).

3.6. Diversification

A constant rate diversification model cannot be rejected for *Agama* using the γ statistic, which supports a steady increase in lineage accumulation through time (Table 8). This result is robust when using the MCC species tree ($\gamma = -1.013$, P = 0.155; Table 8). However, the Sanger concatenation tree rejects the constant rate diversification model in favor of an early burst model with a slowdown in lineage accumulation through time ($\gamma = -1.754$; P = 0.039; Table 8). A large portion of the posterior distribution for the concatenated



Fig. 6. Hybrid phylogenetics-phylogenomic species tree for African *Agama*, and biogeographic relationships across the continent. The Sanger data (mtDNA and four nuclear genes) were used to estimate a time-calibrated species tree using ^{*}BEAST (Fig. 2). This posterior distribution of species trees was then filtered using the anchored phylogenomics tree (215 loci; Fig. 5 "Filter Tree") as a backbone constraint. The branches connecting the 23 species with anchored phylogenomics data are highlighted in bold. Bayesian posterior probabilities for clades \geq 0.95 are not shown.

Trait correlations with male breeding color in Agama lizards tested with maximum likelihood and Bayesian methods in BayesTraits. Correlations with male breeding color indicated in bold exceed standard statistical significance levels.

Male breeding color	Maximum likelihood ^a			Bayesian estimation ^e			
	Dependent model ^b	Independent model ^c	LRT ^d	Dependent model	Independent model	BF ^f	
+♂ throat coloration	-42.39	-43.78	2.77	0.92	0.08	5.9	
+♀ coloration	-34.37	-39.07	9.41	0.99	0.01	1.8	
+mating system	-26.85	-43.53	33.36	1.0	0.0	39.5	

^a Marginal log likelihood averaged across 10 runs.

^b Number of model parameters = 8.

^c Number of model parameters = 4.

^d Likelihood ratio test.

^e Averaged over the posterior distribution of trees.

f Bayes factor.

Table 7

Bayesian estimation of trait evolution in Agama. Estimated values are averages over the posterior probability distribution of the hybrid phylogenetic-phylogenomic species tree.

Trait	$\ln(L)^{a}$	q_{01}	q_{10}^{c}	Pr(model) ^d				
				ZO	10	00	01	0Z
Male breeding coloration 0 = minor/seasonal 1 = extensive	-25.48	0.24	0.22	0.14	0.01	0.82	0.00	0.03
Mating system ^e 0 = solitary male 1 = colony	-26.04	0.72	0.71	0.17	0.00	0.80	0.00	0.02
Female coloration 0 = absent 1 = present	-22.72	0.36	1.54	0.18	0.14	0.43	0.18	0.07
Male throat color 0 = absent 1 = present	-26.33	0.86	0.86	0.00	0.00	0.94	0.00	0.06

^a Marginal log likelihood.

^b The mean of the instantaneous rate of forward change integrated over all models.

^c The mean of the instantaneous rate of backward change integrated over all models.

^d Posterior probability for trait evolution models: Z0 (1 rate, $q_{ij} > 0$, $q_{ji} = 0$); 00 (1 rate, $q_{ij} = q_{ji}$); 0Z (1 rate, $q_{ij} = 0$, $q_{ji} > 0$); 10 (2 rates, $q_{ij} > q_{ji}$); 01 (2 rates, $q_{ij} < q_{ji}$);

^e Mating system and male territoriality are linked in *Agama*.



Fig. 7. Phenograms of *Agama* male body size show high levels of morphological disparity within clades occupying different biogeographic regions of Africa: (A) Southern clade, (B) West clade, (C) Northern clade, (D) East clade, (E) West/Sahel Clade. The repeated evolution of sexually selected traits (branches and species are color coded by absolute number of traits) accompanies dispersal into new biogeographic areas. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Sanger data supports significant negative γ values (66.6%; Table 8). This contrasts with the posterior distribution of species trees, in which only 1.4% support significant negative γ values.

4. Discussion

4.1. Phylogenomics

The hybrid phylogenetic-phylogenomic approach taken here accomplishes the task of combining dense taxonomic sampling for time-calibrated species tree inference, while also including the valuable information gained from the phylogenomic data. However, the hybrid approach does have some limitations. The final tree is driven by the initial posterior distribution of trees generated by the Sanger data, and the utility of the phylogenomic data is limited to that of a filtering device. Another approach that could be taken would be to conduct species tree inference with topological constraints that enforce the phylogenomic relationships. This would allow the MCMC analysis to sample from the stationary distribution without any need for post hoc tree filtering. In our study, the estimated divergence times for the hybrid species tree are based solely on the Sanger data, and do not include the coalescent



Fig. 8. (A) Relative subclade disparity through time for morphological traits in *Agama* (solid line) is higher than that predicted under a Brownian motion model (gray indicates results from 10,000 simulations; dashed line is simulation median). Most variation is found within clades with a burst of morphological disparity coinciding with diversification into different areas of Africa starting approximately 20 mya. (B) Histogram of the relative disparity through time estimates represented as Δ -MDI scores calculated over the posterior probability distribution of *Agama* species trees.

Summary of the γ statistic for the hybrid phylogenetic–phylogenomic species tree and the concatenation tree. Results are provided for the maximum clade credibility trees (MCC) and the posterior distributions. A significant *P* value (*P* < 0.05) for negative γ is indicative of early burst diversification followed by a deceleration in lineage accumulation.

Tree type	γ Statistic MCC tree	P value	Trees with significant negative γ across the posterior distribution (%)
Species tree ^a	-1.013	0.155	1.4
Concatenation ^b	-1.754	0.039	66.6

^a Hybrid phylogenetic-phylogenomic species tree (Fig. 6).

^b Concatenated phylogeny from the Sanger sequence data (Fig. 1).

time information from the 215 phylogenomic loci. The addition of 215 loci improved the support among the major clades of *Agama*, but these data did not resolve the topological conflict near the base of the *Agama* tree.

Analyzing large numbers of loci remains a challenge in phylogenetics. Coalescent-based summary statistic approaches such as STEAC and STAR (Liu et al., 2009) offer alternative strategies for estimating species trees from phylogenomic data that do not have the computational limitations of ^{*}BEAST. However, the summary statistic approaches are tenable at the sacrifice of information content (they use the gene trees as primary data), and therefore they typically require more data to obtain accurate results (Liu et al., 2009). Newer implementations of these methods use bootstrapping of gene trees to provide measures of clade support for the species tree, which might make it easier to contrast different summaries of the data. Other potential solutions for injecting support measures into these summary statistic approaches include analyzing random subsets of loci and quantifying support for branches across a set of results (Liu et al., 2009), or running the methods many times while sampling gene trees from their posterior distributions (Faircloth et al., 2012).

4.2. Systematics of African agamid lizards

The number of molecular systematic investigations of Agama lizards has grown in recent years, and most studies have focused on specific geographic regions instead of monophyletic groups (Geniez et al., 2011; Gonçalves et al., 2012; Mediannikov et al., 2012). Our emphasis on continental-wide diversification patterns enables us to estimate the relationships among almost all described Agama species and therefore identify natural groupings, which are not necessarily constrained to geographic regions. We find moderate support for the monophyly of Agama with respect to a sister clade of African genera that includes Acanthocercus, Pseudotrapelus, and Xenagama. Agama robecchii is included in the sister clade of Agama (Figs. 1 and 2), and is currently being reallocated to a different genus.

The West African species Agama agama has been the most challenging species to define (Wagner et al., 2009), which has previously contained more than ten subspecies. Refining the species limits within A. agama began with the realization that several East African populations with similar adult male coloration (e.g., blue bodies with orange heads) were in fact different species (Böhme et al., 2005). We now recognize these big, blue-bodied, orangeheaded Agama species from all regions of Africa as belonging to deeply divergent clades (Figs. 6 and 7). Herein, we follow the definition of A. agama according to (Wagner et al., 2009) and recognize A. wagneri (Mediannikov et al., 2012) as a synonym of A. agama. For many of the remaining populations found across West Africa, the name A. picticauda Peters, 1877 is available. The deep genetic splits separating many Agama species are masked when considering only morphology and coloration, and this leads to a high potential for discovering additional cryptic diversity as populations are investigated in greater detail using multilocus genetic data.

4.3. Biogeography

The geographic limits of the major biogeographic clades of Agama show some correspondence to the seven biogeographical regions for sub-Saharan Africa identified by a recent cluster analysis of thousands of plant and animal species (Wagner, 2010b; Linder et al., 2012). Our biogeographic analysis of Agama supports a close relationship between East and Southern African species (Fig. 6), and an arid corridor between these groups is expected in birds, snakes, and amphibians, but not necessarily in mammals (Linder et al., 2012). The Cunene and Zambezi Rivers are traditional boundaries separating these regions, but they do not appear to have acted as natural dispersal barriers in the genus Agama. For example, the Southern Africa clade contains three colorful and rupicolous or arboreal species (e.g., A. kirkii, A. mossambica, and A. montana) that occur north of the Zambezi River and are arguably components of the East African fauna. In addition, their placement within the Southern African clade further illustrates that morphology, coloration, and behavior are labile traits in Agama that can mislead morphology-based species relationships.

Today, African savannas, the predominant habitat type for *Agama* lizards, are among the most understudied biomes in the world (Kier et al., 2005; Lorenzen et al., 2012). Although *Agama* species only inhabit the margins of rainforests, their diversification is influenced by long-term historical fluctuations in the size and

location of this biome. A major decline in rainforest cover about 10 mya (Kissling et al., 2012), corresponding to a time of savanna expansion, coincides with the time when radiations of Agama were diversifying throughout most geographic areas of Africa. Among these regional clades of Agama, those with the highest diversification occur in topographic heterogeneous areas, such as Southern, West, and East Africa. The relatively young species complexes in East Africa (e.g., the A. lionotus complex) and West Africa (e.g., the A. agama complex) contain many species that are restricted to small geographic areas. This pattern contrasts with that found in the Sahel corridor and Africa north of the Sahara where we find some of the most geographically widespread species that are relatively older. Southern Africa is a region of diversification and glacial refuge for other arid adapted reptiles (Barlow et al., 2013; Bauer and Lamb, 2005; Stanley et al., 2011). Two ecologically similar and range-restricted species in Southern Africa. A. etoshae and A. makarikarica, are associated with geologically young habitats bordering the Etosha and Makgadikgadi pans, respectively, which were separated by the Kalahari dune system in the Pleistocene (Heine, 1989).

4.4. Diversification and character evolution

Diversification within *Agama* began approximately 23 Ma, and separate radiations in Southern, East, West, and Northern Africa have been diversifying for >10 Myr. The repeated evolution of a suite of sexually selected traits has resulted in regional *Agama* assemblages comprised of (a) sexually dimorphic species with large, colorful males that control harems of drab females, and (b) sexually monomorphic species with small, drab or only seasonally colorful males that are not easily distinguishable from females and are solitary for most of the year. The repeated evolution of these traits creates a signal of high morphological variation within clades.

Species diversification analyses using molecular phylogenies typically report patterns of early bursts of diversification (Burbrink et al., 2012; Reddy et al., 2012; Schenk et al., 2013). Our study provides new empirical evidence demonstrating that species trees and gene trees support different diversification patterns. An early burst pattern of diversification is not supported for *Agama* when using a species tree, but using gene concatenation yields the common pattern of early burst diversification (Table 8). Therefore, the method of inference used to estimate divergence times (i.e., gene tree versus species tree) may account for the diversification patterns reported in some studies. It is possible that the common reporting of early burst speciation is a methodological artifact that could be avoided by using species trees, or that constant processes of diversification, while important, are simply underreported (Moen and Morlon, 2014).

Data archival locations

Dryad DOI information (http://dx.doi.org/10.5061/dryad.4kt16) will be available for the following data files after acceptance:

- Anchored phylogenomic data (nexus format).
- Sanger data alignment, concatenated (nexus format).

NCBI Genbank: Nucleotide sequences for new Sanger sequences include Accession Nos. JX668128–JX668228, JX838886–JX839254, JX857543–JX857633.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014. 06.013.

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