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Phylogeny and biogeography of the African burrowing snake subfamily Aparallactinae (Squamata: Lamprophiidae)

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ABSTRACT

Members of the snake subfamily Aparallactinae occur in various habitats throughout sub-Saharan Africa. The monophyly of aparallactine snakes is well established, but relationships within the subfamily are poorly known. We sampled 158 individuals from six of eight aparallactine genera in sub-Saharan Africa. We employed concatenated gene-tree analyses, divergence dating approaches, and ancestral-area reconstructions to infer phylogenies and biogeographic patterns with a multi-locus data set consisting of three mitochondrial (*16S*, *cyt b*, and *ND4*) and two nuclear genes (*c-mos* and *RAG1*). As a result, we uncover several cryptic lineages and elevate a lineage of *Polemon* to full species status. Diversification occurred predominantly during the Miocene, with a few speciation events occurring subsequently in the Pliocene and Pleistocene. Biogeographic analyses suggested that the Zambezian biogeographic region, comprising grasslands and woodlands, facilitated radiations, vicariance, and dispersal for many aparallactines. Moreover, the geographic distributions of many forest species were fragmented during xeric and cooler conditions, which likely led to diversification events. Biogeographic patterns of aparallactine snakes are consistent with previous studies of other sub-Saharan herpetofauna.

1. Introduction

Species of the subfamily Aparallactinae, endemic to sub-Saharan Africa, have been the center of a great deal of taxonomic confusion because many species share similar morphological characters (e.g., Spawls and Branch, 1995; Greene, 1997; Branch, 1998; Shine et al.,

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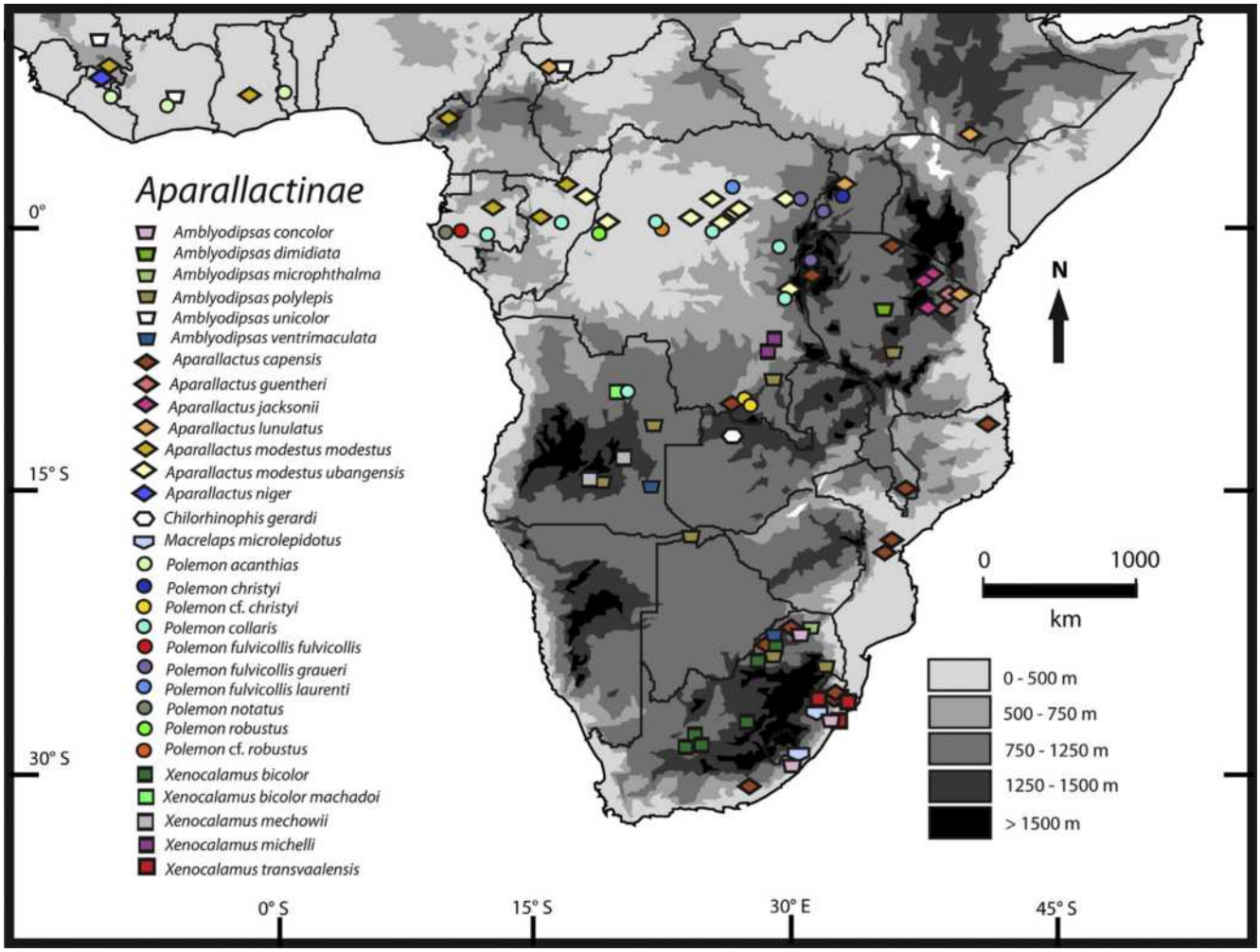

Fig. 1. Map of sub-Saharan Africa showing sampling localities for aparallactines used in this study.

2006). The majority of aparallactines are burrowers found in savannas, desert scrublands and lowland rainforests, making them difficult to find during opportunistic surveys. Because of their fossorial nature, it is likely that aparallactines and their close atractaspidine relatives have retained or evolved similar morphological characters that are adaptations for burrowing (Moyer and Jackson, 2011).

The content and phylogenetic relationships among aparallactines are not well known (Kelly et al., 2011; Moyer and Jackson, 2011; Pyron et al., 2013; Figueroa et al., 2016). Following Bogert's (1940) early studies, several genera were usually assigned to the aparallactines, including *Amblyodipsas, Aparallactus, Brachyophis, Chilorhinophis, Elapotinus, Hypoptophis, Macrelaps, Micrelaps, Poecilopholis, Polemon*, and *Xenocalamus* (Underwood and Kochva 1993; Spawls and Branch, 1995; Branch, 1998), which are distributed broadly in Africa, with a limited occurrence in the Middle East. Of these, *Elapotinus picteti* was recently shown to be related to Malagasy pseudoxyrhophines, and not the Aparallactinae (Kucharzewski et al., 2014). The East African and Middle Eastern genus *Micrelaps*, considered to be an aparallactine until recently, is currently classified as *incertae sedis*, because recent phylogenetic evidence suggests the genus shows no affinities with aparallactines (Rasmussen, 2002; Underwood and Kochva, 1993; Kelly et al., 2009; Pyron et al., 2013; Figueroa et al., 2016). Inclusion of the rarer genera (e.g. *Brachyophis, Hypoptophis,* and *Poecilopholis*) within the Aparallactinae has not been rigorously assessed with molecular data.

Many phylogenetic studies that included aparallactines have been limited by factors that are inherent to rare species, including low sample sizes and limited taxon sampling (Nagy et al., 2005; Kelly et al., 2011; Moyer and Jackson, 2011; Pyron et al., 2011, 2013; Figueroa et al., 2016). Kelly et al. (2009) provided a dating and biogeographic analysis of the superfamily Elapoidea, including aparallactines, but detailed biogeographic information (e.g. dating estimates) within the subfamily was not provided. Currently, Aparallactinae is composed of eight genera (*Amblyodipsas*, *Aparallactus*, *Brachyophis*, *Chilorhinophis*, *Hypoptophis*, *Macrelaps*, *Polemon*, and *Xenocalamus*). Several studies (Pyron et al., 2013; Figueroa et al., 2016; Weinell and Brown, 2017) have demonstrated that *Xenocalamus transvaalensis* was strongly nested within *Amblyodipsas*, rendering the latter genus polyphyletic. Figueroa et al. (2016) synonymized *Xenocalamus* with *Amblyodipsas*, but the placement of *A. concolor* in our study renders *Amblyodipsas* paraphyletic, which could be resolved by reviving *Choristocalamus* Witte & Laurent, 1957, for *A. concolor*. However, this conclusion was inconclusive due to the low aparallactine taxon sampling, and species relationships within Aparallactinae remain poorly known.

In this study, we include multiple aparallactine genera and species from several localities throughout sub-Saharan Africa. We employ phylogenetic analyses in conjunction with temporal biogeographic information to gain a more comprehensive understanding of the evolutionary history of aparallactines. Specifically, we evaluate the following questions: (1) Are genera and species monophyletic? (2) When did the different species and genera of aparallactines diverge from one another? (3) Can estimated divergence times be correlated with biogeographic events? (4) How did formation of the major biogeographic regions of sub-Saharan Africa shape diversification within

Table 1
Voucher numbers, localities, and GenBank accession numbers for genetic samples. DRC = Democratic Republic of the Congo, SA = South Africa; GNP = herpetological collection of the E.O. Wilson Biodiversity Center, Gorongosa National Park, Mozambique.

Species	Collection no.	Field no.	Locality	*16S*	*ND4*	*cyt b*	*c-mos*	*RAG1*
Eutropis longicaudata	SAMA R38916	–	Malaysia	–	AY169645	DQ239139	DQ238979	–
Rena humilis	CAS 190589	–	–	–	–	–	–	–
Boa constrictor	–	–	–	–	–	AF471036	AF471115	–
Acrochordus granulatus	–	–	–	–	U49296	AF217841	AF471124	–
Agkistrodon piscivorus	–	–	–	–	AF156578	AF471074	AF471096	–
Atheris nitschei	–	–	–	–	AY223618	AF471070	AF471125	–
Crotalus viridis	–	–	–	–	AF194157	AF471066	AF471135	–
Diadophis punctatus	–	–	–	–	AF258910	AF471094	AF471122	–
Hypsiglena torquata	–	–	–	–	U49309	AF471038	AF471159	–
Natrix natrix	–	–	–	–	AY873710	AF471059	AF471121	–
Thamnophis sirtalis	–	–	–	–	AF420196	AF402929	DQ902094	–
Boiga dendrophila	–	–	–	–	U49303	AF471089	AF471128	–
Coluber dorri	–	–	–	–	AY487042	AY188040	AY188001	–
Hierophis jugularis	–	–	–	–	AY487046	AY376740	AY376798	–
Dendroaspis polylepis	–	–	–	–	AY058974	AF217832	AY058928	–
Naja kaouthia	–	–	–	–	AY058982	AF217835	AY058938	–
Naja annulata	–	–	–	–	AY058970	AF217829	AY058925	–
Bothrolycus ater	–	–	–	–	–	–	–	–
Gonionotophis brussauxi	IRSNB 16266	–	Gabon: Ogooué-Lolo Province: Offoué-Onoy Department: Mount Iboundji	–	FJ404358	AY612043	AY611952	–
Lycophidion capense	PEM R22890	CMRK 275	Botswana	–	DQ486320	DQ486344	DQ486168	–
Bothrophthalmus lineatus	–	–	Uganda	–	–	AF471090	AF471090	–
Lycodonomorphus laevissimus	PEM R5630	–	SA: Eastern Cape Province, Grahamstown District	–	DQ486314	DQ486338	DQ486162	–
Lycodonomorphus rufulus	PEM R22892	CMRK 236	SA: Eastern Cape Province, Hole in the Wall	–	HQ207153	HQ207111	HQ207076	–
Boaedon upembae	UTEP 21002	ELI 205	DRC: Katanga Province: Kyolo	–	KM519681	KM519700	KM519734	KM519719
Boaedon upembae	UTEP 21003	ELI 208	DRC: Katanga Province: Kyolo	–	KM519680	KM519699	KM519733	KM519718
Boaedon fuliginosus 1	–	–	Burundi	–	FJ404364	FJ404302	AF544686	–
Boaedon fuliginosus 2	PEM R5639	–	Rwanda: Butare District	–	HQ207147	HQ207105	HQ207071	–
Boaedon fuliginosus 3	PEM R5635	–	Rwanda: Nyagatare District	–	HQ207148	HQ207106	HQ207072	–
Psammophylax variabilis	IPMB J296	–	Burundi	–	FJ404328	AY612046	AY611955	–
Atractaspis aterrima	–	CI 267	Ivory Coast: Allakro	–	–	–	–	MG775793
Atractaspis bibronii	–	–	Zimbabwe	–	U49314	AY188008	AY187969	–
Atractaspis bibronii	–	MB 21703	SA	–	–	–	MG775900	MG775791
Atractaspis boulengeri	IPMB J355	–	Gabon: Ogooué-Maritime Province: Rabi	AY611833	FJ404334	AY612016	AY611925	–
Atractaspis congica	–	633	Angola: Soyo	–	–	–	–	MG775788
Atractaspis corpulenta	IPMB J369	–	Gabon: Ogooué-Maritime Province: Rabi	AY611837	FJ404335	AY612020	AY611929	–
Atractaspis corpulenta	PEM R22707	MBUR 03936	Republic of Congo: Niari	–	–	–	–	MG775790
Atractaspis duerdeni	–	MB 21346	SA: North Cape Province	–	–	–	–	MG775789
Atractaspis engaddensis	R 16542	–	Israel: Hare Gilboa	–	–	–	MG775901	MG775792
Atractaspis irregularis	UTEP 21658	EBG 1190	DRC: South Kivu Province, Lwiro	–	MG776014	MG746785	MG775898	–
Atractaspis irregularis	UTEP 21654	ELI 1208	Burundi: Kibira Forest	–	–	–	–	MG775787
Atractaspis irregularis	UTEP 21655	ELI 1635	DRC: South Kivu Province, Lwiro	MG746901	MG776015	–	MG775899	MG775786
Atractaspis micropholis	IPMB J283	–	Togo	AY611823	FJ404336	AY612006	AY611915	–
Atractaspis microlepidota	–	–	–	–	–	AF471046	AF471127	–
Atractaspis cf. microlepidota	–	3258WW	Saudi Arabia: Algassim	MG746902	–	–	–	–
Homoroselaps lacteus 1	LSUMZ 55386	–	–	–	AY058976	–	AY058931	–
Homoroselaps lacteus 2	PEM R17097	–	SA: Eastern Cape Province	–	FJ404339	–	FJ404241	–
Amblyodipsas concolor	–	634	SA: KwaZulu-Natal	–	MG775916	MG746801	MG775806	MG775720
Amblyodipsas concolor	–	618	SA: KwaZulu-Natal	–	MG775917	MG746802	MG775807	MG775721
Amblyodipsas concolor	NMB R11375	MBUR 01624	SA: Limpopo Province	MG746916	MG775920	MG746804	MG775810	MG775724
Amblyodipsas concolor	NMB R11376	MBUR 01659	SA: Limpopo Province	–	MG775918	MG746803	MG775808	MG775722
Amblyodipsas concolor	NMB R11377	MBUR 01660	SA: Limpopo Province	MG746915	MG775919	–	MG775809	MG775723
Amblyodipsas concolor	PEM R19437	WC 373	SA: Eastern Cape Province, Hluleka	–	MG775922	MG746806	MG775812	MG775726
Amblyodipsas concolor	PEM R19795	WC 483	SA: Eastern Cape Province, Dwesa Point	–	MG775923	MG746807	MG775813	MG775727
Amblyodipsas concolor	PEM R20284	WC 975	SA: Eastern Cape Province, Mazeppa Bay	–	MG775921	MG746805	MG775811	MG775725
Amblyodipsas dimidiata	–	CMRK 311	Tanzania	–	DQ486322	DQ486346	DQ486170	–
Amblyodipsas dimidiata	PEM R15626	–	–	–	–	AY612027	AY611936	–
Amblyodipsas microphthalma	–	SP3	SA: Limpopo Province, Soutpansberg	MG746914	MG775927	MG746808	MG775818	MG775729
Amblyodipsas polylepis	–	AMB 6114	SA: Limpopo Province, Farm Guernsey	–	MG775932	–	MG775823	MG775734
Amblyodipsas polylepis	MCZ-R 190174	AMB 7960	Namibia: East Caprivi	–	MG775931	MG746812	MG775822	MG775733
Amblyodipsas polylepis	–	UP 052	DRC: Katanga Province, Kiubo	–	MG775929	MG746810	MG775820	MG775731
Amblyodipsas polylepis	PEM R22492	MBUR 00353	SA: Limpopo Province	MG746921	MG775928	MG746809	MG775819	MG775730
Amblyodipsas polylepis	PEM R18986	632	SA: Limpopo Province, Phalaborwa	–	MG775930	MG746811	MG775821	MG775732
Amblyodipsas polylepis	–	PVP9 WRB	Angola	MG746922	MG775933	MG746813	–	–
Amblyodipsas polylepis	–	MTSN 7571	Tanzania: Ruaha	MG746923	–	MG746814	–	–
Amblyodipsas polylepis	–	3128WW	–	MG746924	–	–	–	–
Amblyodipsas polylepis	PEM R23535	WC 4651	Angola: Moxico	MG746925	–	–	–	–
Amblyodipsas unicolor	–	PB-11-502	Guinea: Kankan	MG746917	MG775924	MG746815	MG775814	MG775728
Amblyodipsas unicolor	–	PGL-15-116	Ivory Coast: Yamassoukro	–	–	MG746816	MG775815	–
Amblyodipsas unicolor	–	2209N	Chad: Baibokoum	MG746918	MG775925	MG746817	MG775816	–
Amblyodipsas unicolor	–	2286N	Chad: Baibokoum	–	MG775926	MG746818	MG775817	–

(*continued on next page*)

Table 1 (*continued*)

Species	Collection no.	Field no.	Locality	*16S*	*ND4*	*cyt b*	*c-mos*	*RAG1*
Amblyodipsas ventrimaculata	PEM R23320	WC 3920	Angola: Moxico Province, Cuito River Source	MG746919	–	MG746819	–	–
Amblyodipsas ventrimaculata	–	R-SA	SA: Lephalale	MG746920	–	–	–	–
Aparallactus capensis	MCZ-R 184403	AMB 8180	SA: Eastern Cape Province, Farm Newstead	MG746971	MG776002	MG746888	MG775885	–
Aparallactus capensis	MCZ-R 184404	AMB 8181	SA: Eastern Cape Province, Farm Newstead	–	MG776003	MG746889	MG775886	–
Aparallactus capensis	MCZ-R 184501	AMB 8365	SA: Limpopo Province	–	MG776004	MG746890	MG775887	–
Aparallactus capensis	–	GPN 134	Mozambique: Gorongosa National Park	MG746988	MG776000	MG746886	MG775883	MG775781
Aparallactus capensis	ZMB 83259	GPN 310	Mozambique: Gorongosa National Park	MG746983	–	–	–	–
Aparallactus capensis	ZMB 83260	GPN 333	Mozambique: Gorongosa National Park	MG746979	–	–	–	–
Aparallactus capensis	–	GPN 351	Mozambique: Gorongosa National Park	MG746977	–	–	–	–
Aparallactus capensis	–	GPN 352	Mozambique: Gorongosa National Park	MG746978	–	–	–	–
Aparallactus capensis	ZMB 83342	GPN 359	Mozambique: Gorongosa National Park	MG746976	–	–	–	–
Aparallactus capensis	ZMB 83343	GPN 394	Mozambique: Gorongosa National Park	MG746981	–	–	–	–
Aparallactus capensis	ZMB 83261	GPN 429	Mozambique: Gorongosa National Park	MG746975	–	–	–	–
Aparallactus capensis	–	KB 2	Rwanda: Akagera National Park	–	MG775996	MG746882	MG775879	–
Aparallactus capensis	–	KB 5	Rwanda: Akagera National Park	MG746987	MG775995	MG746881	MG775878	MG775777
Aparallactus capensis	–	KB 8	Tanzania: Kigoma	–	MG775998	MG746884	MG775881	MG775779
Aparallactus capensis	–	KB 23	Rwanda: Akagera National Park	–	MG775997	MG746883	MG775880	MG775778
Aparallactus capensis	PEM R17909	648	Malawi: Mt. Mulanje	–	MG775984	MG746870	MG775867	MG775765
Aparallactus capensis	–	655	SA: Eastern Cape Province, Middleton	–	MG775987	–	MG775870	MG775768
Aparallactus capensis	PEM R17453	657	DRC: Katanga Province, Kalakundi	MG746970	MG775986	–	MG775869	MG775767
Aparallactus capensis	–	659	Tanzania	–	MG775985	MG746871	MG775868	MG775766
Aparallactus capensis	HLMD J156	–	SA	AY188045	–	AY188006	AY187967	–
Aparallactus capensis	NMB R10885	MBUR 01229	SA: KwaZulu-Natal	MG746985	–	MG746878	MG775876	–
Aparallactus capensis	NMB R11380	MBUR 01592	SA: Limpopo Province	–	MG775992	MG746876	MG775875	MG775773
Aparallactus capensis	NMB R11381	MBUR 01593	SA: Limpopo Province	–	MG775991	MG746875	MG775874	MG775772
Aparallactus capensis	NMB R11382	MBUR 01609	SA: Limpopo Province	–	–	MG746873	MG775872	MG775770
Aparallactus capensis	NMB R11383	MBUR 01642	SA: Limpopo Province	MG746984	MG775993	MG746877	–	MG775774
Aparallactus capensis	–	WC 1352	Mozambique: Cabo Delgado Province, Pemba region	–	MG775999	MG746885	MG775882	MG775780
Aparallactus capensis	PEM R20693	WC 2612	SA: Eastern Cape Province, Tsolwana	–	MG775994	MG746880	MG775877	MG775776
Aparallactus capensis	MCZ-R 27164	–	SA: Limpopo Province	MG746973	–	MG746892	–	–
Aparallactus cf. capensis	PEM R18438	677	SA: Limpopo Province	–	MG775988	MG746872	MG775871	MG775769
Aparallactus cf. capensis	NMB R10997	MBUR 00871	SA: Limpopo Province	MG746986	–	MG746879	–	MG775775
Aparallactus cf. capensis	NMB R11379	MBUR 01554	SA: Limpopo Province	–	–	MG746874	MG775873	MG775771
Aparallactus cf. capensis	MCZ-R 27805	–	SA: Limpopo Province	MG746972	MG776005	MG746891	–	–
Aparallactus cf. capensis	–	GPN 242	Mozambique: Gorongosa National Park	MG746989	MG776001	MG746887	MG775884	MG775782
Aparallactus cf. capensis	–	GPN 357	Mozambique: Gorongosa National Park	MG746982	–	–	–	–
Aparallactus cf. capensis	ZMB 83344	GPN 403	Mozambique: Gorongosa National Park	MG746980	–	–	–	–
Aparallactus cf. capensis	–	2118 WW	SA: Limpopo Province, Bela Bela	MG746969	–	–	–	–
Aparallactus cf. capensis	–	2119 WW	SA: Limpopo Province, Bela Bela	MG746968	–	–	–	–
Aparallactus cf. guentheri	–	MTSN 8341	Tanzania: Nguru Mts	MG746974	–	MG746899	–	–
Aparallactus cf. guentheri	PEM R5678	–	Tanzania: Usambara Mts	–	–	AY235730	–	–
Aparallactus jacksonii	–	649	Tanzania: Mt. Kilimanjaro	MG746960	MG775980	MG746866	–	–
Aparallactus jacksonii	–	650	Tanzania: Oldonyo Sambu	MG746962	MG775983	MG746869	MG775866	MG775764
Aparallactus jacksonii	–	651	Tanzania: Oldonyo Sambu	MG746961	MG775981	MG746867	MG775864	MG775762
Aparallactus jacksonii	–	654	Tanzania: Ndukusiki	–	MG775982	MG746868	MG775865	MG775763
Aparallactus jacksonii	–	MTSN 8301	Tanzania: Nguru Mts	MG746963	–	–	–	–
Aparallactus jacksonii	–	MTSN 8303	Tanzania: Nguru Mts	MG746967	–	–	–	–
Aparallactus jacksonii	–	MTSN 8323	Tanzania: Nguru Mts	MG746964	–	–	–	–
Aparallactus jacksonii	–	MTSN 8352	Tanzania: Nguru Mts	MG746965	–	–	–	–
Aparallactus jacksonii	–	MTSN 8353	Tanzania: Nguru Mts	MG746966	–	–	–	–
Aparallactus lunulatus	–	653	Tanzania: Nguru Mts	MG746991	MG776006	–	MG775891	MG775784
Aparallactus lunulatus	–	2158N	Chad: Baibokoum	–	MG776009	MG746896	MG775888	–
Aparallactus lunulatus	–	2178N	Chad: Baibokoum	MG746993	MG776010	MG746897	MG775889	–
Aparallactus lunulatus	TMHC 2013-09-315	–	Ethiopia: Borana	MG746992	MG776008	MG746895	–	–
Aparallactus lunulatus	TMHC 2013-09-316	–	Ethiopia: Simien Mts.	–	MG776007	MG746894	–	–
Aparallactus lunulatus	–	WBR 957	NE of Lake Albert	MG746990	–	MG746893	MG775890	MG775783
Aparallactus modestus	IPMB J284	–	Gabon: Ogooué-Maritime Province: Rabi	AY611824	FJ404332	AY612007	AY611916	–
Aparallactus modestus	MCZ-R 182624	–	Republic of Congo: Bomassa	–	–	MG746863	MG775862	–
Aparallactus modestus	MCZ-R 182625	–	Republic of Congo: Bomassa	–	MG775977	MG746864	MG775863	–
Aparallactus modestus	MVZ 252411	–	Ghana: Ajenjua Bepo	MG746957	MG775978	MG746865	–	–
Aparallactus modestus	USNM 584365	–	Republic of Congo: Impongui	MG746949	MG775958	MG746844	MG775844	MG775747
Aparallactus modestus	ZFMK 87627	–	–	MG746959	–	–	–	–
Aparallactus modestus	–	5009G	Guinea: Kissidougou	MG746958	MG775979	–	–	–
Aparallactus modestus	–	CRT 4045	DRC: Orientale Province, Bomane	–	MG775964	MG746850	MG775850	–
Aparallactus modestus	–	CRT 4181	DRC: Orientale Province, Lieki	–	MG775966	MG746852	–	MG775752
Aparallactus modestus	–	CRT 4256	DRC: Orientale Province, Lieki	–	MG775967	–	–	MG775753
Aparallactus modestus	UTEP 21609	EBG 2609	DRC: Orientale Province, Bazinga	MG746950	MG775959	MG746845	MG775845	–
Aparallactus modestus	UTEP 21605	ELI 1379	DRC: South Kivu Province, Kihungwe	MG746951	MG775960	MG746846	MG775846	MG775748
Aparallactus modestus	UTEP 21606	ELI 1419	DRC: South Kivu Province, Kihungwe	MG746952	MG775961	MG746847	MG775847	MG775749
Aparallactus modestus	No voucher	ELI 2138	DRC: Equateur Province, Npenda Village	MG746948	MG775957	MG746843	–	–

(*continued on next page*)

Table 1 (*continued*)

Species	Collection no.	Field no.	Locality	*16S*	*ND4*	*cyt b*	*c-mos*	*RAG1*
Aparallactus modestus	UTEP 21601	ELI 2221	DRC: Equateur Province, Npenda Village	MG746953	MG775962	MG746848	MG775848	–
Aparallactus modestus	UTEP 21602	ELI 2222	DRC: Equateur Province, Npenda Village	MG746954	MG775963	MG746849	MG775849	MG775750
Aparallactus modestus	UTEP 21608	ELI 2914	DRC: Orientale Province, Kisangani	MG746955	MG775968	MG746853	MG775852	–
Aparallactus modestus	–	KG 457	DRC: Orientale Province, Bagwase	–	MG775970	MG746855	MG775855	MG775755
Aparallactus modestus	–	KG 467	DRC: Orientale Province, Bagwase	–	MG775972	MG746858	MG775858	MG775758
Aparallactus modestus	–	KG 499	DRC: Orientale Province, Bagwase	–	MG775973	–	MG775859	MG775759
Aparallactus modestus	–	KG 501	DRC: Orientale Province, Bagwase	–	MG775971	MG746857	MG775857	MG775757
Aparallactus modestus	–	KG 503	DRC: Orientale Province, Bagwase	–	MG775969	MG746854	MG775854	MG775754
Aparallactus modestus	–	KG 511	DRC: Orientale Province, Bagwase	–	MG775975	MG746860	MG775861	MG775761
Aparallactus modestus	–	KG 528	DRC: Orientale Province, Bagwase	–	–	MG746856	MG775856	MG775756
Aparallactus modestus	–	KG 572	DRC: Orientale Province, Bagwase	–	MG775974	MG746859	MG775860	MG775760
Aparallactus modestus	–	MSNS REPT 34	Gabon: Ogooué-Lolo Province: Mt. Iboundji	–	–	MG746862	–	–
Aparallactus modestus	–	PB 11-733	Guinea: Nzerekore	–	MG775976	MG746861	MG775853	–
Aparallactus modestus	–	UAC 038	DRC: Bas-Congo Province, Yoko	–	MG775965	MG746851	MG775851	MG775751
Aparallactus modestus	PEM R22331	MBUR 03449	Republic of Congo: Niari Province	MG746956	–	–	–	–
Aparallactus niger	–	8075X	Guinea: Nzerekore	MG746994	MG776011	MG746898	MG775892	–
Aparallactus werneri	FMNH 2504400	–	Tanzania: Tanga	–	U49315	AF471035	–	–
Chilorhinophis gerardi	–	635	Zambia: Trident Mine	MG746995	MG776012	MG746900	MG775893	MG775785
Macrelaps microlepidotus	PEM R20944	–	SA: KwaZulu-Natal, Hillcrest	MG746927	MG775938	–	–	–
Macrelaps microlepidotus	–	28666	–	–	MG775935	MG746821	MG775824	–
Macrelaps microlepidotus	PEM R19791	WC DNA 511	SA: Eastern Cape Province, Dwessa Nature Reserve	MG746926	MG775934	MG746820	–	–
Macrelaps microlepidotus	PEM R20167	WC DNA 928	SA: Eastern Cape Province, Hogsback	–	MG775937	MG746823	–	–
Macrelaps microlepidotus	PEM R20295	WC DNA 973	SA: Eastern Cape Province, Mazeppa Bay	–	MG775936	MG746822	–	–
Micrelaps bicoloratus	–	CMRK 330	–	–	–	DQ486349	DQ486173	–
Micrelaps muelleri	R 15654	–	Israel: Salti	–	–	MG746781	–	–
Micrelaps muelleri	R 16469	–	Israel: Malkishua	–	–	MG746782	MG775895	–
Micrelaps muelleri	R 16738	–	Israel: Bet Nehemya	–	–	MG746783	MG775896	–
Micrelaps muelleri	R 16944	–	Israel: Ein Hod	–	MG776013	MG746784	MG775897	–
Micrelaps cf. muelleri	R 16426	–	Israel: Afiq	–	–	MG746780	MG775894	–
Polemon acanthias	PEM R1479	–	Ivory Coast: Haute Dodo	AY611848	FJ404341	AY612031	AY611940	–
Polemon acanthias	–	PLI-12-053	Liberia: Nimba County	–	MG775954	MG746841	MG775841	MG775745
Polemon acanthias	–	PLI-12-208	Liberia: Nimba County	MG746946	MG775955	MG746842	MG775842	MG775746
Polemon acanthias	–	T266	Togo: Mt. Agou	MG746947	MG775956	–	MG775843	–
Polemon christyi	UTEP 21618	DFH 535	Uganda: Road to Budongo Central Forest Reserve	MG746945	MG775953	MG746840	–	–
Polemon cf. christyi	PEM R17452	–	DRC: Katanga Province, Kalakundi	MG746943	MG775951	MG746838	MG775839	MG775743
Polemon cf. christyi	PEM R20734	–	DRC: Katanga Province, Fungurume	MG746944	MG775952	MG746839	MG775840	MG775744
Polemon collaris	PEM R19893	TB 28	Angola: North-west region	MG746931	MG775943	MG746827	MG775829	–
Polemon collaris	UTEP 21612	ELI 561	DRC: South Kivu Province, vicinity of Byonga	MG746928	MG775939	MG746824	MG775825	MG775735
Polemon collaris	UTEP 21613	ELI 1317	DRC: South Kivu Province, Fizi	MG746930	MG775941	MG746826	MG775827	MG775737
Polemon collaris	UTEP 21614	ELI 2464	DRC: Equatuer Province, Watsi Kengo, Salonga River	MG746929	MG775940	MG746825	MG775826	MG775736
Polemon collaris	–	KG 523	DRC: Orientale Province, Bagwase	–	MG775944	MG746828	MG775830	–
Polemon collaris	–	MSNS REPT 110	Gabon: Ogooué-Lolo Province: Mt. Iboundji	MG746934	–	MG746829	–	–
Polemon collaris	–	UAC 062	DRC: Bas-Congo Province, Yoko	MG746933	MG775942	–	MG775828	–
Polemon collaris	PEM R22747	MBUR 03862	Republic of Congo: Niari Province	MG746932	–	–	–	–
Polemon fulvicollis	PEM R5388		Gabon: Ogooué-Maritime Province: Rabi	AY611846	FJ404342	AY612029	AY611938	–
Polemon fulvicollis laurenti	UTEP 21615	ELI 3046	DRC: Orientale Province, Bombole Village	MG746942	MG775949	MG746837	MG775837	–
Polemon graueri	–	CRT 4007	DRC: Orientale Province, Bomane	–	MG775947	MG746833	MG775834	MG775740
Polemon graueri	UTEP 21610	EBG 1376	DRC: South Kivu Province, Irangi	MG746940	–	MG746835	MG775836	MG775742
Polemon graueri	No voucher	EBG 2294	DRC: Orientale Province, between Beni and Bunia	MG746938	–	MG746832	MG775833	–
Polemon graueri	UTEP 21611	ELI 2842	Uganda: Rwenzori Mts National Park	MG746939	MG775948	MG746834	MG775835	MG775741
Polemon graueri	–	MTSN 7378	Rwanda: Nyungwe National Park	MG746941	–	MG746836	–	–
Polemon notatus	–	29395	Gabon	MG746935	MG775950	–	MG775838	–
Polemon notatus	PEM R5404	–	Gabon: Ogooué-Maritime Province: Rabi	AY611847	FJ404343	AY612030	AY611939	–
Polemon cf. robustus	UTEP 21617	ELI 2594	DRC: Equateur Province, Salonga River	MG746936	MG775945	MG746830	MG775831	MG775738
Polemon robustus	UTEP 21616	ELI 2069	DRC: Bandundu Province, Isongo, Lake Mai-Ndombe	MG746937	MG775946	MG746831	MG775832	MG775739
Xenocalamus bicolor	MCZ-R 27160	–	SA: Limpopo Province	–	MG775911	MG746794	MG775800	–
Xenocalamus bicolor	MCZ-R 27161	–	SA: Limpopo Province	MG746905	MG775912	MG746795	MG775801	–
Xenocalamus bicolor	PEM R17377	615	SA: Northern Cape Province, Kimberly	–	MG775903	–	MG775795	MG775710
Xenocalamus bicolor	–	616	SA: KwaZulu-Natal	–	–	MG746787	–	–
Xenocalamus bicolor	PEM R17438	647	SA: Northern Cape Province, Kimberly, Rooipoort	–	MG775902	MG746786	MG775794	MG775709
Xenocalamus bicolor	NMB R10851	MBUR 00925	SA: Limpopo Province	MG746904	MG775910	MG746793	MG775799	MG775716
Xenocalamus bicolor	NMB R11418	MBUR 01553	SA: Limpopo Province	–	MG775907	MG746790	MG775797	MG775714
Xenocalamus bicolor	–	TGE T3 28	SA: Northern Cape Province	–	MG775905	MG746788	MG775796	MG775712
Xenocalamus bicolor	–	TGE T3 29	SA: Northern Cape Province	–	MG775908	MG746791	MG775798	MG775715
Xenocalamus bicolor	–	TGE T3 32	SA: Northern Cape Province	–	MG775909	MG746792	–	–
Xenocalamus bicolor	–	TGE T4 14	SA: Free State Province	–	MG775906	MG746789	–	MG775713

(*continued on next page*)

Table 1 (*continued*)

Species	Collection no.	Field no.	Locality	*16S*	*ND4*	*cyt b*	*c-mos*	*RAG1*
Xenocalamus bicolor australis	PEM R22083	–	SA: Northern Cape Province, Kimberly	MG746906	MG775913	MG746796	MG775802	–
Xenocalamus bicolor lineatus	–	13321	–	–	–	MG746797	MG775803	–
Xenocalamus bicolor machadoi	–	666	Angola: Moxico	MG746903	MG775904	–	–	MG775711
Xenocalamus mechowii	PEM R23533	WC 4654	Angola: Moxico	MG746908	–	–	–	–
Xenocalamus mechowii	PEM R23463	WC 4695	Angola: Cuando Cubango	MG746907	–	–	–	–
Xenocalamus michelli	UTEP 21619	ELI 209	DRC: Katanga Province, Kyolo	MG746909	MG775914	MG746798	MG775804	MG775718
Xenocalamus michelli	UTEP 21620	ELI 355	DRC: Katanga Province, Manono	MG746910	MG775915	MG746799	MG775805	MG775719
Xenocalamus transvaalensis	NMB R10888	MBUR 01107	SA: KwaZulu-Natal, Ndumo Game Reserve	MG746913	–	MG746800	–	MG775717
Xenocalamus transvaalensis	–	FO57-51-51	SA: KwaZulu-Natal, Maputaland	MG746911	–	–	–	–
Xenocalamus transvaalensis	PEM R:TBA	–	SA: KwaZulu-Natal, Hluhluwe	MG746912	–	–	–	–
Xenocalamus transvaalensis	PEM R12103	–	SA: KwaZulu-Natal, Maputaland	AY611842	FJ404344	AY612025	AY61193	–

Aparallactinae?

Herein, we follow the biogeographic classification of Linder et al. (2012), who partitioned Africa into several biogeographic regions, including: Congolian, (sub-regions: Congolian, Shaba, and Guinean) (central African tropical and relict forests); Sudanian, Zambesian, and Somalian (savannas, woodlands and forests of eastern, central, and southern Africa); South Africa (sub-regions: Cape Region, Natal, and Kalahari—desert, forest, and savanna habitats of southern Africa).

2. Materials and methods

2.1. Taxon sampling

Specimens from the subfamily Aparallactinae were collected from multiple localities in sub-Saharan Africa (Fig. 1). We generated sequences of three mitochondrial genes (*16S*, *ND4*, and *cyt b*) and two nuclear genes (*c-mos* and *RAG1*) for 165 aparallactine individuals (Tables 1 and 2). Our study included sequences from six of the eight (not including *Brachyophis* and *Hypoptophis*) aparallactine genera (6/9 species of *Amblyodipsas*; 7/11 species of *Aparallactus*; 1/2 species of *Chilorhinophis*; 1/1 species of *Macrelaps*; 6/12 species of *Polemon*; 4/5 species of *Xenocalamus*) (Wallach et al., 2014; Uetz et al., 2017). Sequences from some of these individuals have been published previously (Kelly et al., 2009, 2011); new sequences were deposited in GenBank (Table 1). Concatenated trees were rooted with *Acrochordus granulatus* (Acrochordidae; Caenophidia) (not shown on Fig. 2). Three species of Viperidae (*Agkistrodon piscivorus*, *Atheris nitschei*, and *Crotalus viridis*) (not shown on Fig. 2), three species of Elapidae (*Dendroaspis polylepis, Naja annulata,* and *N. kaouthia*), eight species of Lamprophiinae (*Boaedon fuliginosus*, *B. upembae*, *Bothrophthalmus lineatus*, *Bothrolycus ater*, *Gonionotophis brussauxi*, *Lycodonomorphus laevissimus*, *L. rufulus,* and *Lycophidion capense*), one species of Psammophiinae (*Psammophylax variabilis*), and seven species of Atractaspidinae (*Atractaspis bibronii, A. boulengeri, A. corpulenta, A. irregularis, A. micropholis, A. microlepidota,* and *Homoroselaps lacteus*) were used as outgroups for the concatenated analyses (Table 1, Fig. 2). Additionally, 2/4 species of *Micrelaps* (previously in Aparallactinae) were included in all analyses. For divergence dating analyses, additional samples from squamate outgroup taxa included Scincidae, Leptotyphlopidae, Viperidae, Colubrinae, and Dipsadinae (Table 1). When possible, voucher specimens were compared to type specimens to verify identifications.

2.2. Laboratory protocols

Genomic DNA was isolated from alcohol-preserved muscle or liver tissue samples with the Qiagen DNeasy tissue kit (Qiagen Inc., Valencia, CA, USA). Primers used herein are shown in Table 2. We used 25 μL PCR reactions with gene-specific primers with an initial denaturation step of 95 °C for 2 min, followed by denaturation at 95 °C for 35 s (s), annealing at 50 °C for 35 s, and extension at 72 °C for 95 s with 4 s added to the extension per cycle for 32 (mitochondrial genes) or 34 (nuclear gene) cycles. Amplification products were visualized on a 1.5% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen Corporation, Carlsbad, CA, USA). Sequencing reactions were purified with CleanSeq magnetic bead solution (Agencourt Bioscience, La Jolla, CA) and sequenced with an ABI 3130xl automated sequencer at the University of Texas at El Paso (UTEP) Border Biomedical Research Center Genomic Analysis Core Facility.

2.3. Sequence alignment and phylogenetic analyses

Phylogenetic analyses were conducted for single-gene mitochondrial and nuclear data sets, and a five-gene concatenated data set. Chromatograph data were interpreted using the program SeqMan (Swindell and Plasterer, 1997). An initial alignment for each gene was produced in MUSCLE (Edgar, 2004) in the program Mesquite v3.10

Table 2
Primers used for sequencing mitochondrial and nuclear genes.

Gene name	Primer name	Primer sequence (5′ to 3′)	Primer source
16S	L2510	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
	H3059	CCGGTCTGAACTCAGATCACGT	
	L2510mod/16Sar	CCG ACT GTT TAM CAA AAA CA	Zaher et al. (2009)
	H3056mod/16Sbr	CTC CGG TCT GAA CTC AGA TCA CGT RGG	
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994) and Pook et al. (2009)
	HIS1276	TTCTATCACTTGGATTTGCACCA	
cyt b	L14910	GAC CTG TGATMTGAAAAACCAYCGTTGT	Burbrink et al. (2000)
	H16064	CTTTGG TTTACAAGAACAATGCTTTA	
c-mos	S77	CATGGACTGGGATCAGTTATG	Slowinski and Lawson (2002)
	S78	CCTTGGGTGTGATTTTCTCACCT	
RAG1	G396 (R13)	TCTGAATGGAAATTCAAGCTGTT	Groth and Barrowclough (1999)
	G397 (R18)	GATGCTGCCTCGGTCGGCCACCTTT	

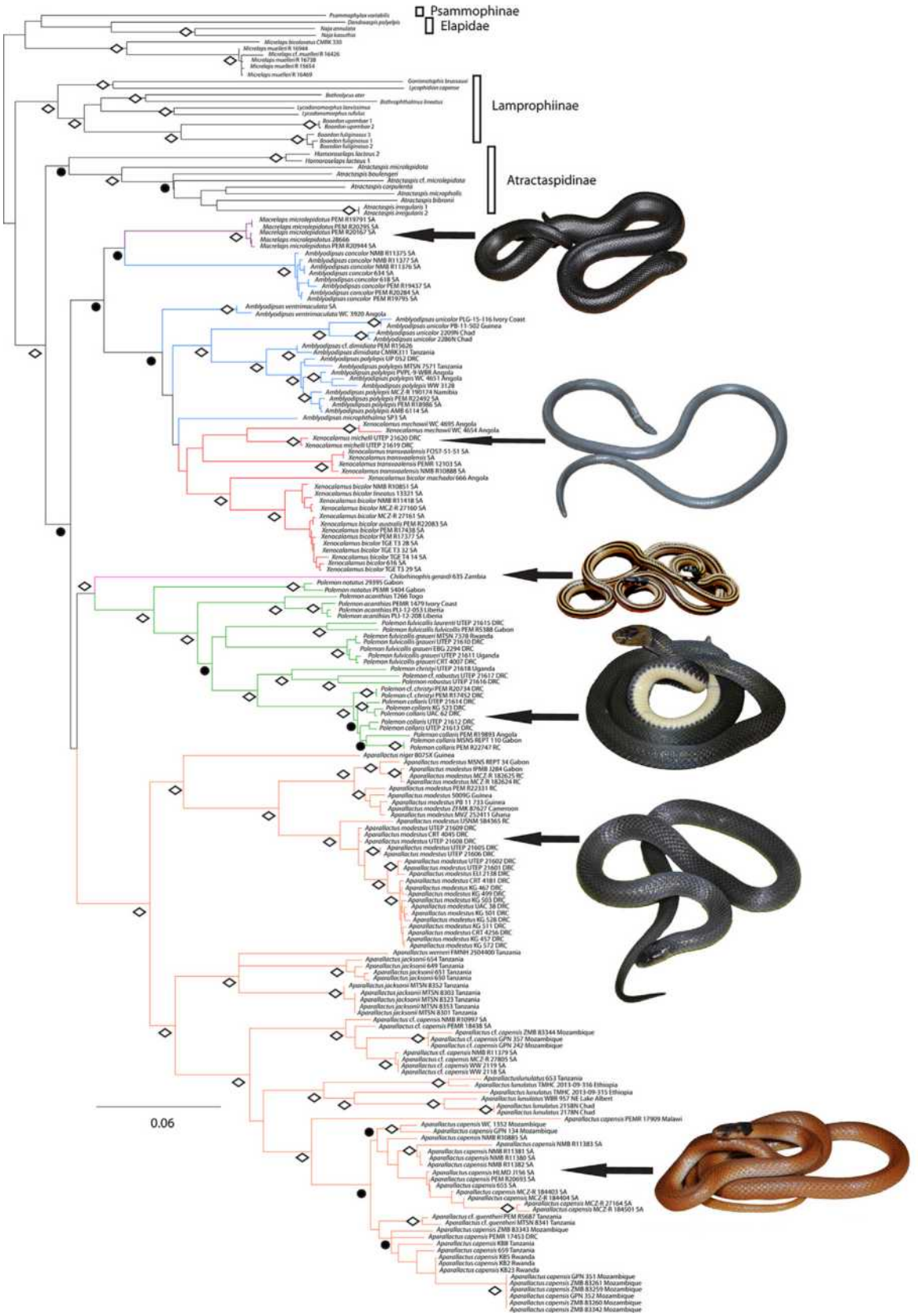

Fig. 2. Maximum-likelihood phylogeny of Aparallactinae with combined *16S*, *ND4, cyt b*, *c-mos* and *RAG1* data sets. Closed circles denote clades with Bayesian posterior probability values ≥ 0.95. Diamonds denote clades with strong support in both maximum likelihood (values ≥ 70) and Bayesian analyses (posterior probability values ≥ 0.95). DRC = Democratic Republic of the Congo, RC = Republic of Congo, SA = South Africa.

(Maddison and Maddison, 2017), and manual adjustments were made in MacClade v4.08 (Maddison and Maddison, 2005). Maximum Likelihood (ML) analyses of single gene and concatenated data sets were conducted using the GTRGAMMA model in RAxML v8.2.9 via the Cipres Science Gateway (Miller et al., 2017). All parameters were estimated and a random starting tree was used. Support values for clades inferred by ML analyses were assessed with the rapid-bootstrap algorithm with 1,000 replicates (Stamatakis, 2006; Stamatakis et al., 2008). Bayesian inference (BI) analyses were conducted with MrBayes v3.2.6 via the Cipres Science Gateway (Miller et al., 2017). The model included 13 data partitions: independent partitions for each codon position of the protein-coding genes *ND4*, *cyt b*, *c-mos* and *RAG1*, and a single partition for the mitochondrial gene *16S*. Phylogenies were constructed based on concatenated data, which included the latter five genes. Concatenated data sets were partitioned identically for ML and BI analyses. The program PartitionFinder v1.1.1 (Lanfear et al., 2012, 2014) was used to find the model of evolution that was most consistent with our data for BI analyses. Bayesian analyses were conducted with random starting trees, run for 20 million generations, and sampled every 1,000 generations. Phylogenies were visualized using FigTree v1.2.3 (Rambaut and Drummond, 2010). A second type of analysis was run using only nuclear data with the same parameters as above.

2.4. Biogeographic analyses

The program BEAST v1.8.3 via Cipres Science Gateway (Miller et al., 2017) was used to estimate divergence times across aparallactine phylogeny with the five-gene data set. Substitution and clock models were unlinked for all partitions; trees were unlinked across the nuclear loci, but were linked for the mitochondrial partitions because these evolve as a single unit. We implemented an uncorrelated log-normal relaxed clock model with a Yule tree prior. Two independent analyses were run for 100 million generations, sampling every 10,000 generations. Primary calibration points were obtained from Head et al. (2016) and a secondary calibration point was obtained from Kelly et al. (2009), including: the split between Scolecophidia and all other snakes (97 Ma [120–92]); split between Caenophidia and its nearest sister taxon, Booidea (72.1–66 Ma); split between Colubroidea and its nearest sister taxon, (*Acrochordus* + Xenodermatidae) (72.1–50.5 Ma); the divergence of Colubridae + Elapoidea (30.9 ± 0.1 Ma); and the split between Crotalinae and Viperinae (23.8–20.0 Ma). All calibrations were constrained with a log-normal mean of 0.01, a normal standard deviation of 2.0 (first calibration point), and 1.0 (the last four calibration points). Parameter values of the samples from the posterior probabilities on the maximum clade credibility tree were summarized using the program TreeAnnotator v.1.8.3 via Cipres Science Gateway (Miller et al., 2017). We used DEC (dispersal-extinction-cladogenesis) analyses implemented in RASP (Yu et al., 2015) to reconstruct the possible ancestral ranges of aparallactines. The DEC analysis allows distributions to span multiple regions and allows for reduced probability of dispersal between distant regions (Yu et al., 2015). To account for uncertainties in the phylogeny, we used 10,000 trees selected from the BEAST output after burnin. The number of maximum areas was set to two. Outgroups were removed from the trees and only the aparallactine ingroup was included. The total number of geographic areas for analyses was set at nine African biogeographic regions *sensu* Linder et al. (2012) (sub-regions were included): A = Cape Region; B = Kalahari; C = Natal; D = Zambezian; E = Congolian; F = Shaba; G = Guinean; H = Sudanian; and I = Somalian (Fig. 5). We allowed dispersal to and from all regions because several species possess wide ranges and we treated all potential dispersal events as equal (Uetz et al., 2017).

3. Results

3.1. Concatenated gene-tree analyses

Our data set consisted of 3933 base pairs (*16S* [546 bp], *ND4* [679 bp], *cyt b* [1094 bp], *c-mos* [605 bp], and *RAG1* [1009 bp]). Individuals with missing data were included in the concatenated sequence analyses, because placement of individuals that are missing a significant amount of sequence data can be inferred in a phylogeny, given an appropriate amount of informative characters (Wiens, 2003; Wiens and Morrill, 2011; Pyron et al., 2011; Anderson and Greenbaum, 2012). Additionally, Jiang et al. (2014) demonstrated that excluding genes with missing data often decreases accuracy relative to including those same genes, and they found no evidence that missing data consistently bias branch length estimates.

The following models of nucleotide substitution were selected by PartitionFinder for BI analyses: *16S* (GTR + G), *ND4* 1st codon position (GTR + G), *ND4* 2nd codon position (TVM + G), and *ND4* 3rd codon position (HKY + I + G); *cyt b* 1st codon position (TVM + G), *cyt b* 2nd codon position (HKY + I + G) and *cyt b* 3rd codon position (GTR + G); *c-mos* and *RAG1* 1st, 2nd and 3rd codon positions (HKY + I). The best ML analysis likelihood score was – 60211.067400. The concatenated ML and BI analyses recovered similar topologies (Fig. 2), as did single-gene mtDNA analyses (not shown). The relationships of Elapidae, Lamprophiinae, *Micrelaps*, and *Psammophylax* with respect to the ingroup Aparallactinae, were not strongly supported in either ML or BI analyses. There was strong support for a monophyletic group including the subfamilies Aparallactinae and Atractaspidinae, and the monophyly of Aparallactinae was strongly supported in BI analyses (Fig. 2). Three well-supported clades within Aparallactinae were recovered: (1) *Aparallactus*, (2) *Polemon/Chilorhinophis*, and (3) *Amblyodipsas/Macrelaps/Xenocalamus* (BI), but relationships among these clades were weakly supported. Within the *Macrelaps/Amblyodipsas/Xenocalamus* clade, BI analyses recovered *Macrelaps microlepidotus* as sister to *Amblyodipsas concolor,* but the two lineages were deeply divergent (Fig. 2). There was strong support for a clade containing all *Xenocalamus* and all remaining *Amblyodipsas* species (excluding *Amblyodipsas concolor*). In BI analyses, *Xenocalamus bicolor, X. michelli*, *X. mechowii*, and *X. transvaalensis* were all strongly supported, but relationships among *Xenocalamus* species were weakly supported. The western African samples of *Amblyodipsas unicolor* were strongly supported as sister to *A. polylepis* and *A. dimidiata. Amblyodipsas microphthalma* and *A. ventrimaculata* were both nested within the strongly supported *Amblyodipsas* + *Xenocalamus* clade, rendering both genera paraphyletic. *Chilorhinophis* was strongly supported as the sister to *Polemon*, but these lineages were deeply divergent from each other. Within the well-supported *Polemon* clade, most clades were well supported (Fig. 2). *Aparallactus* was divided into two deeply divergent groups, both strongly supported: the western and central African *A. niger/A. modestus* clade, and the southern, eastern, and western African *Aparallactus capensis*, *A.* cf. *guentheri*, *A. jacksonii*, *A. lunulatus*, and *A. werneri* clade. Several lineages within *A. modestus* were deeply divergent, giving the *A. modestus* clade a highly structured appearance, including distinct western and central African lineages. *Aparallactus werneri* was recovered within a strongly supported clade containing two divergent *A. jacksonii* lineages. Among our samples of *A. capensis*, several distinct and strongly supported lineages were recovered, but the inclusion of *A. lunulatus* rendered *A. capensis* paraphyletic. These clades of *A. capensis* did not seem to be partitioned geographically, because at least two clades contained samples from similar localities (Table 1 and Fig. 2). Two samples of *A.* cf. *guentheri* were nested within a large and well-supported *A. capensis* clade.

Both nuclear phylogenetic analyses (*c-mos* and *RAG1*) recovered a monophyletic group including samples of Aparallactinae and Atractaspidinae (Fig. 3). The phylogeny based on *c-mos* data (A) recovered a well-supported Aparallactinae + Atractaspidinae clade (Fig. 3), but a well-supported Atractaspidinae clade was strongly

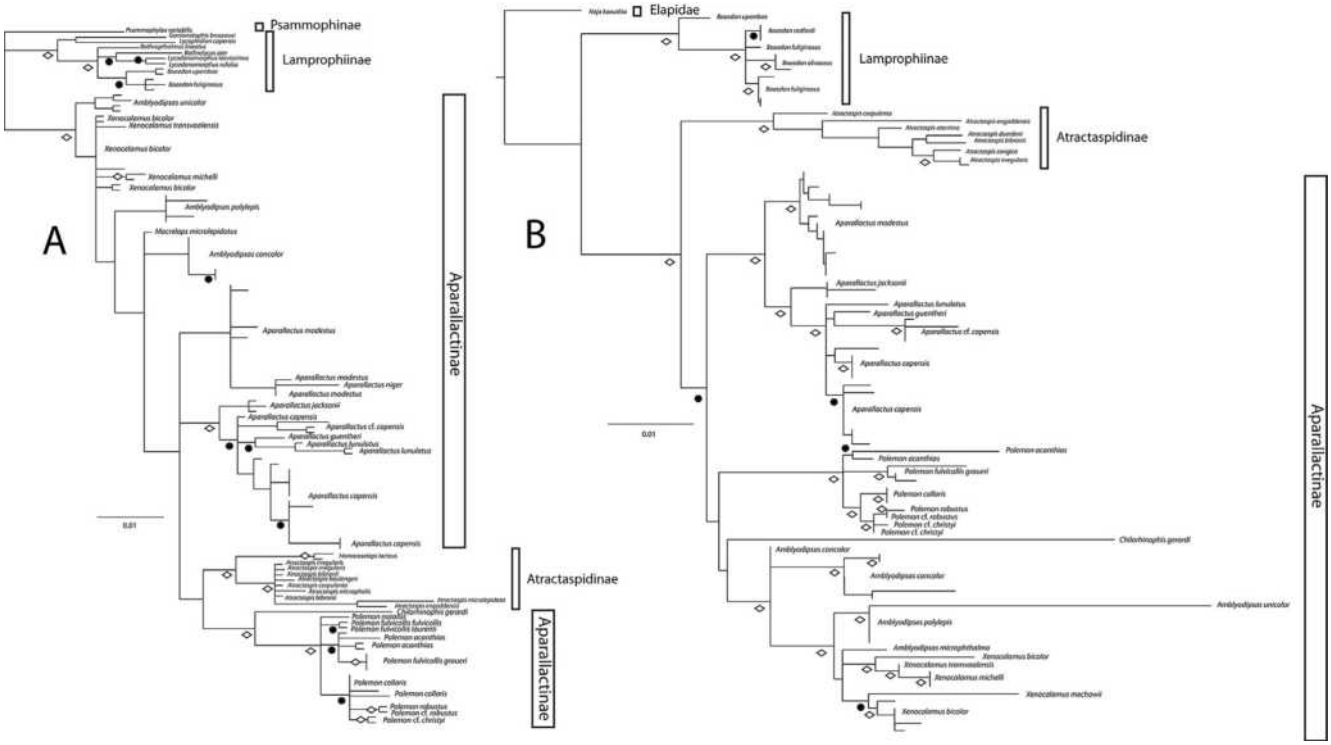

Fig. 3. Maximum-likelihood phylogeny of Aparallactinae including *c-mos* (A) and *RAG1* (B) data. Closed circles denote clades with Bayesian posterior probability values ≥ 0.95. Diamonds denote clades with strong support in both maximum likelihood (values ≥ 70) and Bayesian analyses (posterior probability values ≥ 0.95).

supported as sister to a well-supported (BI) Aparallactinae clade based on *RAG1* data (B) (Fig. 3). The *RAG1* phylogeny was fairly consistent with the concatenated tree results. Several major aparallactine clades/genera (*Amblyodipsas/Xenocalamus*, *Aparallactus*, and *Polemon*) were recovered with strong support in the *RAG1* phylogeny. *Polemon* was recovered in a monophyletic group with strong support in the *c-mos* phylogeny, but other aparallactine genera were not (Fig. 3).

3.2. Divergence dating

Topologies from the BEAST analyses were consistent with results from RAxML and MrBayes analyses (Fig. 4). Dating estimates suggested aparallactines split from atractaspidines during the Oligocene around 29.7 mya (25.8–31.8 mya, 95% highest posterior densities [HPD]) (Fig. 4 and Table 3). Subsequent diversification of Aparallactinae occurred in the late-Oligocene. Divergences within each of the major aparallactine genera occurred during the early Miocene, with subsequent radiations during the mid- to late Miocene. No currently recognized species included in our analyses originated after the Pliocene.

3.3. Ancestral area reconstruction

For the subfamily Aparallactinae (node 2), DEC analyses suggested four possible ancestral ranges: DG (Zambezian + Guinean), DE (Zambezian + Congolian), D (Zambezian), and CD (Natal + Zambezian) (Fig. 5). The most likely ancestral range at node 23 (*Aparallactus*) was DG (Zambezian + Guinean) (60.7%). The most likely ancestral range at node 26 was D (100%); the most likely ancestral range at node 24 (*Aparallactus niger/A. modestus*) was less clear (G, 48.8%) (Fig. 5). Results suggested DE (Zambezian + Congolian) (54.8%) as the likely ancestral range for node 4 (*Chilorhinophis/Polemon*). Ancestral range E (Congolian) (51.5%) was recovered with the highest likelihood for node 5 (*Polemon*). For node 14 (*Amblyodipsas/Macrelaps/Xenocalamus*), analyses suggested CD (Natal + Zambezian) (100%) as the likely ancestral range. For node 15 (*Amblyodipsas concolor/Macrelaps microlepidotus*), a C (Natal) origin was recovered (100%). For node 16 (remainder of *Amblyodipsas* and *Xenocalamus*), a CD (Natal + Zambezian) ancestral range was recovered with the highest likelihood (60.1%).

4. Discussion

4.1. Biogeography of Aparallactinae

Results from our phylogenetic trees clearly separated taxa that are from central, southern, western, and eastern Africa. This is congruent with the biogeographic region partitioning of sub-Saharan Africa proposed by Linder et al. (2012). Results suggested that aparallactines began to diversify during the late Oligocene (Fig. 4) around 29 mya, and originated in sub-Saharan Africa (Fig. 5). During this time period, icehouse earth conditions (when glaciers reached their maximum extent and sea levels were low) were widespread (Zachos et al., 2001; Feakins and Demenocal, 2010). Aside from several periodic climatic optima, the climate was relatively cool, which induced aridification and forest fragmentation (Couvreur et al., 2008; Feakins and Demenocal, 2010; Tolley et al., 2011). Based on our data, many major radiations within aparallactine genera occurred during the Miocene, which was characterized by an early climatic optimum (warm and tropical), subsequently followed by increasingly cooler and xeric conditions, which led to the expansion of savannas globally (Bouchenak-Khelladi et al., 2010; Schnitzler et al., 2011). Several lineages of aparallactines (e.g., *Xenocalamus*) are from xeric habitats and the expansion of dry savannas could have facilitated their diversification. This trend has also been shown in xeric adapted plants, which experienced expansion and diversification events during the Miocene (Bouchenak-Khelladi et al., 2010). Other squamate groups such as typhlopid snakes and skinks of the genus *Panaspis*, which are prey items of aparallactines, also experienced diversification events from the late Oligocene to the late Miocene (Kornilios et al., 2013; Medina et al., 2016). Results from Morales-Castilla et al. (2011) showed that African alethinophidian (all

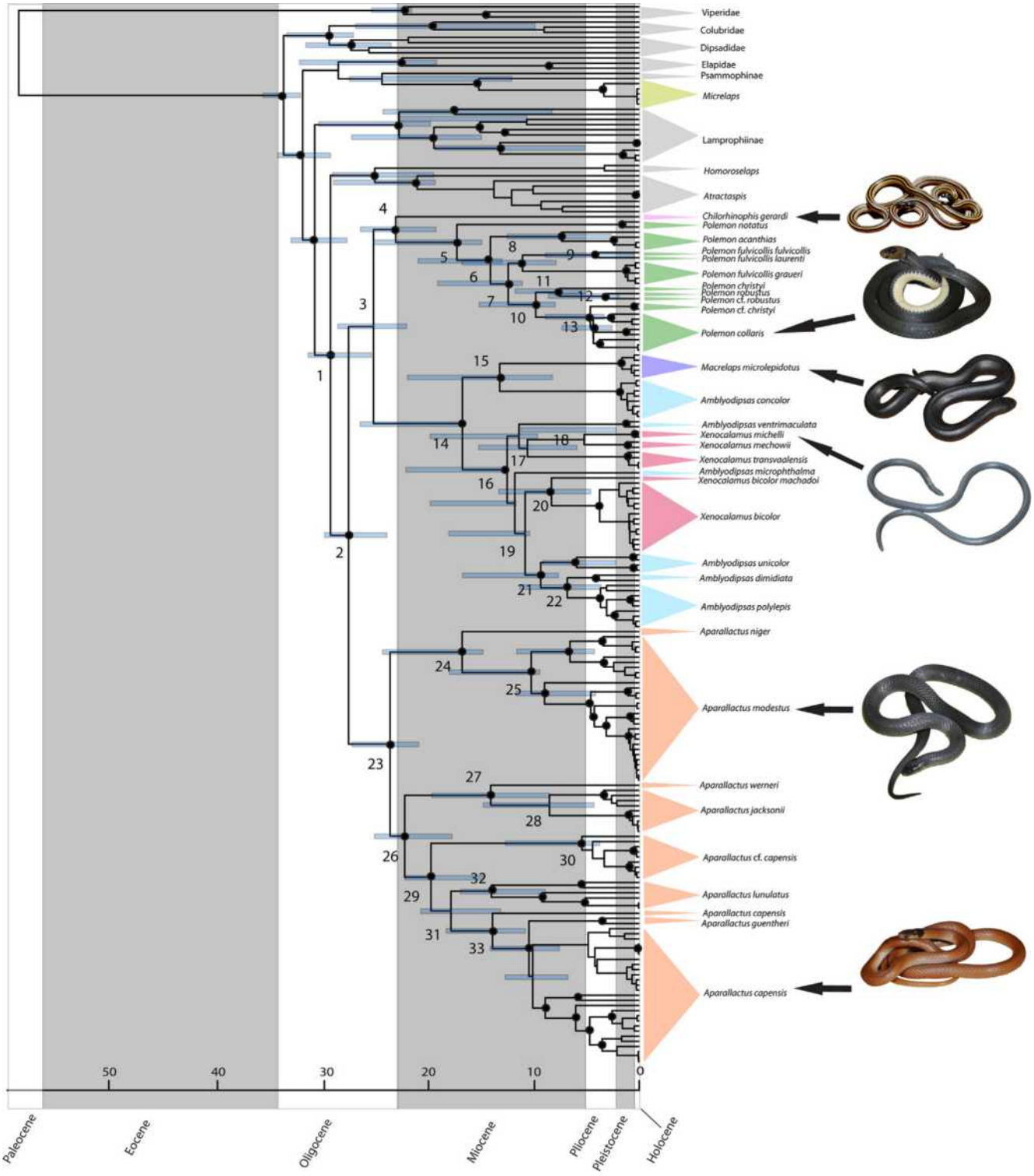

Fig. 4. Phylogeny resulting from BEAST, based on five calibration points. Nodes with high support (posterior probability > 0.95) are denoted by black circles. Median age estimates are provided along with error bars representing the 95% highest posterior densities (HPD) (Table 3).

snakes excluding Typhlopidae and Leptotyphlopidae) snake diversity is higher in tropical habitats than xeric habitats, and these modern snake lineages experienced major radiation events during a climatic optimum in the early to mid-Miocene. Our results reflect this trend, because several aparallactine forest groups experienced initial diversification events during the mid- to late Miocene (Fig. 4) (e.g., *Aparallactus modestus*, *A. niger*, *Polemon* spp., *Macrelaps microlepidotus*, *Amblyodipsas concolor*, *A. polylepis,* and *A. unicolor*).

A major split within *Aparallactus* occurred around the early Miocene, separating the central and western African *A. modestus/A. niger* group from the eastern/southern/western African *A. capensis/A.* cf. *guentheri/A. jacksonii/A. lunulatus/A. werneri* group. Aside from relatively cool and dry conditions during the late Oligocene, rift-related volcanism and lake formation in central and eastern Africa began around 25 mya (Feakins and Demenocal, 2010; Roberts et al., 2012). Additionally, continental erosion was associated with uplift in southern

Table 3
Estimated date and 95% highest posterior densities (HPD) of main nodes. The node labels correspond to those in Fig. 4.

Node	Event	Estimated age in mya (95% HPD)
1	Split between Aparallactinae and Atractaspidinae	29.7 (25.8–31.8)
2	Basal divergence of Aparallactinae	28.0 (24.1–30.3)
3	Split between *Polemon/Chilorhinophis* and *Amblyodipsas/Macrelaps/Xenocalamus*	25.6 (22.5–29.0)
4	Split between *Chilorhinophis* and *Polemon*	23.4 (19.5–26.8)
5	Basal divergence of *Polemon*	17.5 (15.2–26.8)
6	Split between *Polemon acanthias* and *P. fulvicollis/P. robustus/P. bocourti/P. graueri/P. christyi/P. collaris*	14.3 (13.1–25.5)
7	Split between *Polemon fulvicollis* lineages and *P. christyi/P. robustus/P.* cf. *robustus/P.* cf. *christyi/P. collaris*	12.6 (11.2–19.4)
8	Split between *Polemon f. fulvicollis/P. f. laurenti* and *P. f. graueri*	11.2 (8.0–17.1)
9	Split between *Polemon fulvicollis fulvicollis* and *P. f. laurenti*	4.2 (1.3–8.9)
10	Split between *Polemon christyi/P. robustus/P.* cf. *robustus* and *P.* cf. *christyi/P. collaris*	9.9 (8.2–15.3)
11	Split between *Polemon christyi* and *P. robustus/P.* cf. *robustus*	7.6 (5.2–11.9)
12	Split between *Polemon robustus* and *P.* cf. *robustus*	3.2 (1.6–8.3)
13	Split between *Polemon* cf. *christyi* and *P. collaris*	5.0 (3.3–9.0)
14	Basal divergence of the *Amblyodipsas/Macrelaps/Xenocalamus* clade	17.0 (17.1–26.8)
15	Split between *Macrelaps microlepidotus* and *Amblyodipsas concolor*	13.4 (8.3–22.3)
16	Basal divergence of *Amblyodipsas/Xenocalamus*	12.9 (13.1–22.6)
17	Split between *Xenocalamus mechowii/X. michelli* and *X. transvaalensis*	10.7 (5.9–15.5)
18	Split between *Xenocalamus mechowii* and *X. michelli*	5.2 (2.2–11.2)
19	Split between *Amblyodipsas dimidiata/A. polylepis/A. unicolor* and *Xenocalamus bicolor*	10.9 (10.5–18.3)
20	Split between *Xenocalamus bicolor* and *X. b. machadoi*	8.4 (4.6–13.5)
21	Split between *Amblyodipsas polylepis/A. dimidiata* and *A. unicolor*	9.4 (7.7–16.8)
22	Split between *Amblyodipsas dimidiata* and *A. polylepis*	6.8 (3.7–11.8)
23	Basal divergence of *Aparallactus*	24.0 (21.2–27.5)
24	Split between *Aparallactus niger* and *A. modestus*	17.0 (15.0–24.8)
25	Basal divergence of the *Aparallactus modestus* complex	10.4 (9.5–18.3)
26	Split between *Aparallactus capensis/A.* cf. *guentheri* /*A. lunulatus* and *A. werneri/A. jacksonii* group	22.5 (17.9–25.5)
27	Split between *Aparallactus werneri* and *A. jacksonii*	14.2 (8.5–19.9)
28	Basal divergence of *Aparallactus jacksonii*	8.6 (4.3–15.0)
29	Split between *Aparallactus* cf. *capensis* and *A. capensis/A.* cf. *guentheri/A. lunulatus*	20.0 (14.8–22.5)
30	Basal divergence of *A.* cf. *capensis*	5.4 (3.7–12.8)
31	Split between *A. lunulatus* and *A. capensis/A.* cf. *guentheri*	18.1 (13.2–20.9)
32	Basal divergence of *Aparallactus lunulatus*	14.2 (9.0–17.1)
33	Basal divergence of *A. capensis/A.* cf. *guentheri* complex	14.1 (10.9–18.6)

Africa (Feakins and Demenocal, 2010). This coincides with and could have played a role in the partitioning of major clades in *Aparallactus*. Subsequent radiations between *Aparallactus* species took place during the mid- to late Miocene (Fig. 4), which coincides with several habitat shifts that occurred within *Aparallactus* (e.g., Zambezian to Congolian/Guinean) (Fig. 5). Lineages of *Naja nigricollis*, which inhabit open habitats throughout sub-Saharan Africa, began diversifying around 12–15 mya (Wüster et al., 2007; Pook et al. 2009). These dates correspond with the evidence of increasing grassland coverage of parts of Africa in the early to mid-Miocene and occured during similar dates for radiations within *Aparallactus capensis, A. lunulatus*, and *Amblyodipsas/Xenocalamus* (Table 3) (Cerling, 1992; Meadows and Linder, 1993; Wüster et al., 2007). The late Miocene also corresponds with biogeographic shifts in *Xenocalamus* from a Zambezian region to Cape and or Natal regions (Fig. 5). Several *Xenocalamus* species inhabit open habitats (Broadley, 1990; Marais, 2004) and aridification events of the late Miocene could have driven diversification within *Xenocalamus* and other morphologically similar species (e.g., *Amblyodipsas microphthalma*), allowing species from drier, open habitats to expand their ranges. Recent paleontological evidence shows that forest habitats were replaced by open habitats associated with C_3 grasses in the early to mid-Miocene (Edwards et al. 2010; Barlow et al., 2013). These habitats were subsequently replaced by C_4 grassland habitats around the late Pliocene to early Pleistocene (Edwards et al. 2010; Barlow et al., 2013). Results from Pook et al. (2009), Barlow et al. (2013), and this study suggest that many snake lineages associated with open habitats throughout sub-Saharan Africa may have existed in habitats preceding C_3 habitats, which contrasts with savanna mammals and some grassland snake species (e.g., *Naja haje* and *Bitis arietans*) whose origins and diversifications were closely associated with the shift to C_4 grasslands during the Pliocene and Pleistocene (Hewitt 2004; Ségalen et al. 2007; Pook et al. 2009; Barlow et al., 2013). Inland regions of South Africa and southern Namibia seem to have been inhospitable during glacial maxima due to colder winter temperatures and less precipitation (Barlow et al., 2013). These harsher inland conditions could have influenced fragmentation of southern African populations of *Aparallactus*.

Several unique *Aparallactus* lineages in our study are from the Eastern Arc Mountains in Tanzania. One of the major processes that likely influenced radiation events of organisms in these mountains is the isolation of each Eastern Arc Mountain fragment, including the Nguru, Udzungwa, and Usambara mountains (Sepulchre et al., 2006; Loader et al., 2013, 2014). *Aparallactus jacksonii* is composed of two deeply divergent clades that are represented by far northern Tanzanian populations, and a population from the Nguru Mountains (Figs. 1 and 2). Climatic changes could have caused episodic contacts and separations between montane forests, explaining the complex phylogeographic structure of our samples of *A. jacksonii* (Fig. 2). Similarly, results from Loader et al. (2013, 2014) demonstrated that anuran taxa such as brevicipitids and *Arthroleptides* (Barej et al., 2014) experienced long-term isolation in the Eastern Arc Mountains. Other taxa that have shown high population structure in the Eastern Arc Mountains include *Arthroleptis* (Blackburn and Measey, 2009), *Hyperolius* (Lawson, 2013), caecilians (Loader et al., 2007), and birds (Fjeldså and Bowie, 2008). Like *A. jacksonii*, the spotted reed frog (*Hyperolius substriatus*) and avian taxa from montane forests such as the green barbet (*Stactolaema olivacea*) and streaky canary (*Serinus striolatus*), have shown substantial genetic divergence between populations found in extreme northern Tanzania (Usambara, Taita, and Pare mountains) and those to the south (Uluguru, Ukaguru, Nguru, and Malundwe mountains) (Fjeldså and Bowie, 2008; Lawson, 2010). In contrast, *Aparallactus* cf. *guentheri* showed little genetic differentiation between samples found in the Usambara Mountains and Nguru Mountains (Fig. 2). The phylogenetic pattern of *Aparallactus* cf. *guentheri* was more similar to the one seen in the treefrog *Hyperolius spinigularis*, which doesn't show clear genetic partitioning in the Eastern Arc Mountains. These results further demonstrate that evolutionary history of taxa in the Eastern Arc

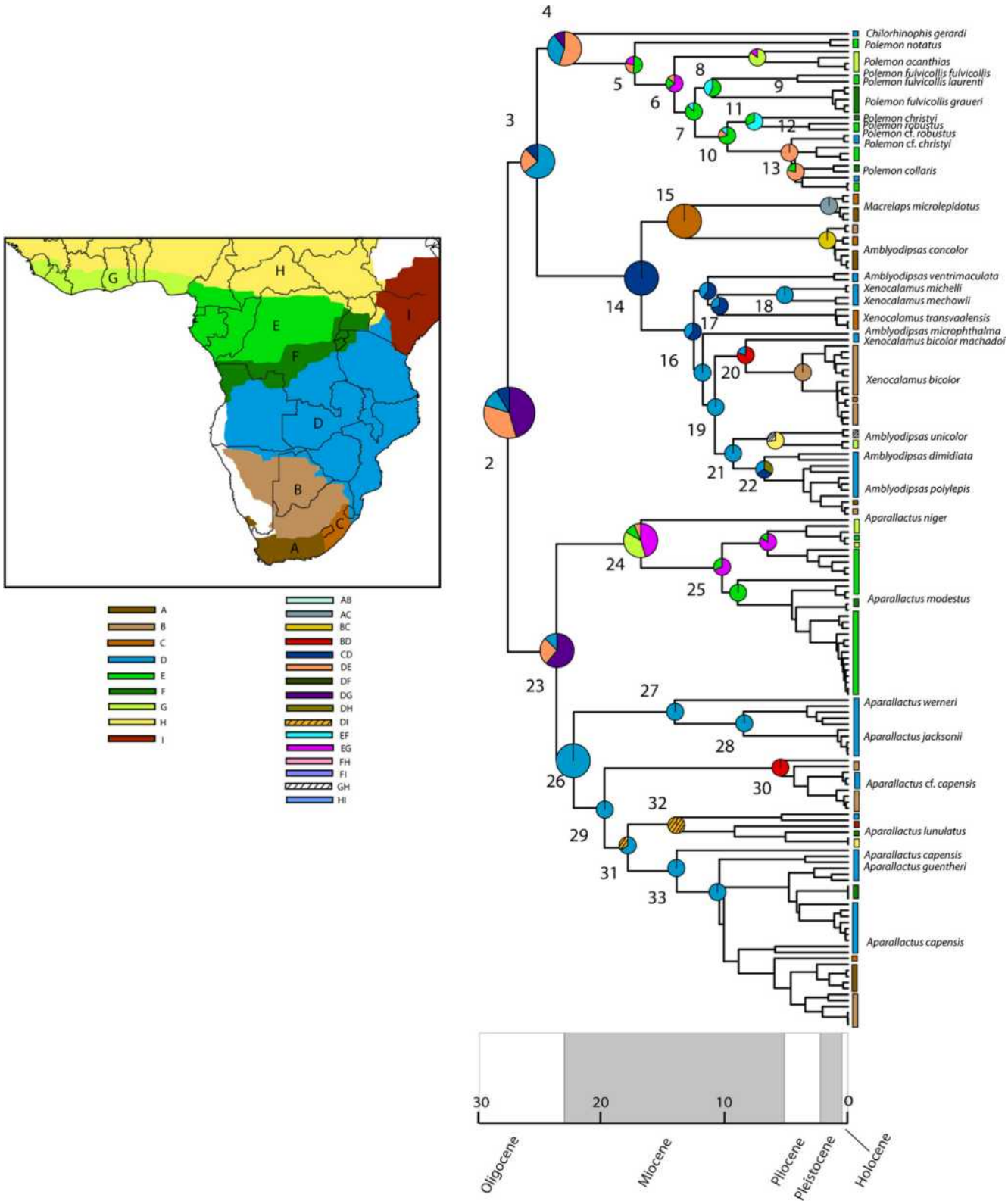

Fig. 5. Graphical output from DEC (RASP). Numbers at the base of the nodes correspond to Table 3. Biogeographical regions: A, Cape Region; B, Kalahari; C, Natal; D, Zambezian; E, Congolian; F, Shaba; G, Guinean; H, Sudanian; I, Somalian.

Mountains is lineage specific (Lawson, 2010). Certain Eastern Arc *Aparallactus* (e.g., *A. jacksonii*) may be restricted to montane habitats, which may explain the deeply divergent lineages in the phylogenies. We speculate that forest habitats occurred at lower elevations in the past, which formed corridors for dispersal between mountains (Feakins and Demenocal, 2010). With increased aridity during the late Miocene (when our populations of *A. jacksonii* last shared a common ancestor, Fig. 4), montane forests would have shifted to higher elevations, thus limiting or eliminating gene flow between populations from different mountains of the Eastern Arc.

Polemon spp., *Aparallactus modestus*, and *A. niger* are mainly central and western African groups (de Witte and Laurent, 1947), with most species inhabiting tropical forests. Results indicated that *Polemon* began to diversify during the early Miocene. Our phylogenetic estimates suggest that these groups experienced radiations during the climatic optimum and subsequent drier and cooler conditions of the late Miocene. Subsequently, most *Polemon* species included in our analyses radiated during the mid- to late Miocene as global cooling transformed Africa's equatorial forests (Fig. 4) (Couvreur et al., 2008). Western and central African *Aparallactus* also experienced diversification events that coincided with those of *Polemon*. Forest habitats (where the above taxa occur) were likely fragmented during the expansion of open habitats in the mid- to late Miocene, which seems to explain radiations within these groups. Trends of climate cooling with concomitant drier conditions may also explain the partitioning of western and central African *Aparallactus modestus* (Fig. 4). Other studies of central African forest herpetofauna (*Atheris*, *Amietia*, *Boaedon*, *Kinyongia*, *Leptopelis,* and *Ptychadena*) recovered similar results of Miocene diversification events (Menegon et al., 2014; Greenbaum et al., 2015; Portillo et al., 2015; Larson et al., 2016; Zimkus et al., 2017; Hughes et al., 2017).

Another possible explanation for some of the aparallactine species complexes, specifically central African species, could be rivers. A few aparallactine taxa in Central Africa (e.g., *Aparallactus modestus* and *Polemon collaris*) experienced some diversification within their clades in the late Miocene and early Pliocene. The diversion of the northeastern section of the Congolian Aruwimi River is attributed to the early to mid-Miocene (Flügel et al., 2015). Age estimates from primates, fish, and understory birds suggested some of the rivers of the Congo Basin influenced their diversification during the Pliocene and Pleistocene across the Congolian lowland rainforests (Harcourt and Wood, 2012; Flügel et al., 2015; Huntley and Voelker, 2016). The Cross River has been shown to structure amphibian distribution between western and central African populations (Penner et al., 2011). However, not all rivers are considered to be effective barriers to dispersal, and they seem to be less important as biogeographic barriers for plants and reptiles (Lowe et al., 2010; Bell et al., 2015; Greenbaum et al., 2015; Kindler et al., 2016). Wider and faster flowing rivers present more effective barriers compared to narrower, smaller tributaries (Harcourt and Wood, 2012; Flügel et al., 2015). Harcourt and Wood (2012) noted that the lower section of the Congo River seems to be a more effective barrier than its smaller tributaries and headwaters. Indeed, *Aparallactus modestus* and *Polemon collaris* can be found on either side of the Congo River in DRC (Fig. 6).

4.2. Phylogeny and taxonomy of Aparallactinae

Results from this study support the monophyly of aparallactines and its sister relationship with atractaspidines, a finding that is consistent with previous phylogenetic assessments of these snakes (Pyron et al., 2013). Figueroa et al. (2016) recovered a monophyletic Aparallactinae but not a monophyletic Atractaspidinae or *Atractaspis*.

Within aparallactines, we verified three major clades: *Aparallactus*, *Polemon/Chilorhinophis,* and *Amblyodipsas/Macrelaps/Xenocalamus* (Figs. 2–4). Although aparallactine samples were limited, Pyron et al. (2013) already noted the non-monophyly of *Amblyodipsas*, a result confirmed herein (Fig. 2). Neither *Amblyodipsas* nor *Xenocalamus* were recovered as monophyletic in our results. Results inferred that *Amblyodipsas concolor* is sister to the semi-fossorial, monotypic *Macrelaps microlepidotus.* There are no clear synapomorphies available to define these genera because there is a large amount of morphological variation within *Amblyodipsas*, as currently understood. It is likely that lineages including *Amblyodipsas microphthalma, A. ventrimaculata, A. katangensis,* and *Xenocalamus* evolved longer snouts as an adaptation for burrowing. It should be noted that *Amblyodipsas microphthalma*, *A. ventrimaculata*, and *Xenocalamus* species inhabit drier, more open habitats than other *Amblyodipsas* species and *Macrelaps,* which have broader snouts and bodies (de Witte and Laurent, 1947; Broadley, 1990; Marais, 2004). *Amblyodipsas katangensis* (not included in this study) is likely closely related to *Xenocalamus*, with which it shares several morphological characters (e.g., prefrontal and internasal scales are fused) (de Witte and Laurent, 1947; FP pers. obs). Figueroa et al. (2016) synonymized *Xenocalamus* with *Amblyodipsas*, but the placement of *A. concolor* in our study would render the genus *Amblyodipsas* paraphyletic. Despite the complicated phylogenetic patterns we recovered for *Amblyodipsas*, *Macrelaps*, and *Xenocalamus*, we currently withhold taxonomic changes due to the ramifications of various options. Additional sampling and morphological analyses are in progress that will help clarify the correct taxonomy for these groups.

Aparallactus jacksonii was represented in this study by two highly divergent Tanzanian populations (extreme northern Tanzania [Oldonyo Sambu and Mt. Kilimanjaro], and Nguru Mountains), both related to *A. werneri*. A possible explanation for this result is that one clade of *A. jacksonii* was represented by only a single gene (*16S*) of sequence data. However, there were several other samples that had one gene of sequence data available and their placement did not seem ambiguous (Fig. 2 and Table 1). Another possible explanation is that gene flow from these two lineages was cut off with the isolation of different

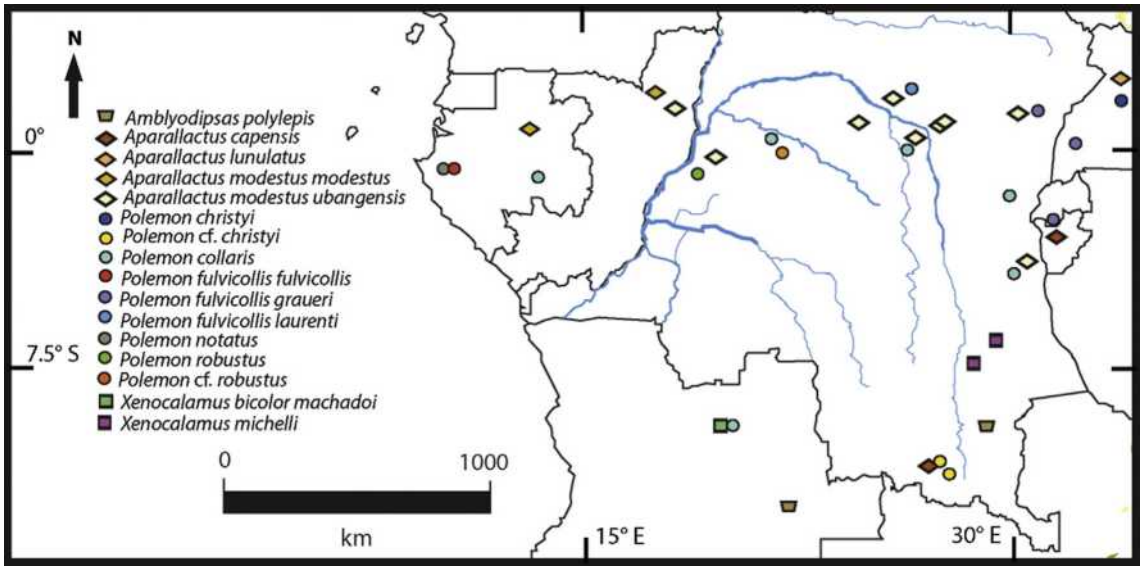

Fig. 6. Map of the Democratic Republic of Congo illustrating rivers and localities of aparallactine samples used in this study.

mountains in the Eastern Arc Mountains. Our results indicated that *Aparallactus capensis* is a non-monophyletic species complex (Figs. 2 and 3). *Aparallactus capensis* are similar to *A. jacksonii*, but the former taxon usually lacks a dorsal stripe (de Witte and Laurent, 1947). Samples of *A.* cf. *guentheri* were nested within a large *A. capensis* clade. Additionally, *A. lunulatus* represents several highly divergent lineages. There are several synonyms associated with these species and further taxonomic studies should be conducted on *A. capensis*, *A.* cf. *guentheri*, and *A. lunulatus* to fully resolve their taxonomy. *Aparallactus modestus* also represents a species complex, with at least two major lineages partitioned by western and central African samples. It is likely that the major partitioning of *A. modestus* represents the two-recognized subspecies: *A. m. ubangensis* (type locality: "Zongo, Ubangi Rapids, DRC") and *A. m. modestus* (type locality: "West Africa") (Uetz et al., 2017).

Chilorhinophis and *Polemon* were strongly supported as sister taxa. Only a single sample of *Chilorhinophis* was available for this study, and its relationship with respect to other aparallactines cannot be fully resolved until taxon sampling increases. The genus *Polemon* is composed of 13 species of ophiophagous snakes, some of which are known to prey on snakes equal to their own size (Kusamba et al., 2013). *Polemon* species are distributed throughout western, central, and eastern Africa (de Witte and Laurent, 1947). *Polemon fulvicollis fulvicollis* comprises three deeply divergent lineages, which geographically and morphologically (F. Portillo unpublished data) correspond to three subspecies: *P. f. fulvicollis*, *P. f. graueri*, and *P. f. laurenti*. Given that the sister relationship of *P. f. graueri* and *P. f. fulvicollis* + *P. f. laurenti* isn't always supported (Fig. 4), the deep divergences between these lineages, and comparison of our vouchers to type specimens (F. Portillo unpublished data), we elevate *P. graueri* to full species status. *Polemon christyi* was not monophyletic—two samples from southern DRC (Katanga Province) were recovered as the sister lineage to *P. collaris* and the description of this new taxon has been submitted for publication. Topotypic *Polemon christyi* from Uganda was recovered in a clade with *P. bocourti* and *P. robustus*, both from DRC (Fig. 2). These results are unsurprising considering that southeastern Katanga Province (DRC) is dominated by miombo/woodland savanna (Burgess et al., 2004) in contrast to the primary or relic forest habitats that *P. christyi* inhabits in Uganda (type locality) (Pitman, 1974). Additionally, Katanga Province is known for containing areas of high plant and reptile endemism (Broadley and Cotterill, 2004; Medina et al., 2016).

Multiple studies (Kelly et al., 2011; Pyron et al, 2011, 2013) have shown that the genus *Micrelaps* (included as an outgroup in our study) is unrelated to both aparallactines and atractaspidines. *Micrelaps* is composed of four species ranging from eastern Africa to the Middle East in Asia (*Micrelaps muelleri* is the type species of the genus, described from near Jerusalem, Israel). Because studies have lacked multiple samples of *Micrelaps*, and the placement of *Micrelaps* changes among colubroidean snakes depending on the study, we are reluctant to assign this enigmatic genus to another family. Therefore, we continue to recognize *Micrelaps* as *incertae sedis* (Figueroa et al., 2016). Further work should include efforts to obtain samples of *Brachyophis* (Somalia and Kenya) and *Hypoptophis* (southern DRC and Zambia) to determine their phylogenetic positions with respect to other aparallactines and even *Micrelaps*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.03.019.

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