Molecular phylogeny of *Panaspis* and *Afroablepharus* skinks (Squamata: Scincidae) in the savannas of sub-Saharan Africa

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ARTICLE INFO

ABSTRACT

African snake-eyed skinks are relatively small lizards of the genera *Panaspis* and *Afroablepharus*. Species allocation of these genera frequently changed during the 20th century based on morphology, ecology, and biogeography. Members of these genera occur primarily in savanna habitats throughout sub-Saharan Africa and include species whose highly conserved morphology poses challenges for taxonomic studies. We sequenced two mitochondrial (*16S* and *cyt b*) and two nuclear genes (*PDC* and *RAG1*) from 76 *Panaspis* and *Afroablepharus* samples from across eastern, central, and southern Africa. Concatenated gene-tree and divergence-dating analyses were conducted to infer phylogenies and biogeographic patterns. Molecular data sets revealed several cryptic lineages, with most radiations occurring during the mid-Miocene to Pliocene. We infer that rifting processes (including the formation of the East African Rift System) and climatic oscillations contributed to the expansion and contraction of savannas, and caused cladogenesis in snake-eyed skinks. Species in *Panaspis* and *Afroablepharus* used in this study, including type species for both genera, formed a monophyletic group. As a result, the latter genus should be synonymized with the former, which has priority. Conservatively, we continue to include the West African species *P. breviceps* and *P. togoensis* within an expanded *Panaspis*, but note that they occur in relatively divergent clades, and their taxonomic status may change with improved taxon sampling. Divergence estimates and cryptic speciation patterns of snake-eyed skinks were consistent with previous studies of other savanna vertebrate lineages from the same areas examined in this study.

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1. Introduction

There are currently 154 genera and 1602 species assigned to the Family Scincidae (Uetz and Hošek, 2015, but see Hedges, 2014 for an alternative arrangement). Several studies have revealed concealed genetic divergence in multiple lineages of skinks from different regions of the world (Daniels et al., 2009; Engelbrecht et al., 2013; Heideman et al., 2011; Portik et al., 2011; Siler et al., 2011). The family exhibits a wide variety of ecomorphs, but the fossorial/semi-fossorial forms typically have reduced vagility that can facilitate population fragmentation and divergence by historical climatic and geographic processes.

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The semi-fossorial, African snake-eyed skink genus *Panaspis* currently includes eight savanna and lowland rainforest species distributed throughout sub-Saharan Africa (Uetz and Hošek, 2015). In the 20th century, the taxonomic composition of the genus *Panaspis* was based on morphological characters, including skull morphology, head scalation, and distinctive characters in the lower eyelid (Broadley, 1989; Fuhn, 1969, 1972; Greer, 1974; Perret, 1973, 1975, 1982). As a result, some African and Eurasian skink species were moved back and forth between different scincid taxa, including *Ablepharus*, *Afroablepharus*, *Lacertaspis*, *Leptosiaphos*, and *Panaspis* (Fuhn, 1969, 1970; Greer, 1974; Perret, 1973, 1975).

The recurrent allocation of African savanna scincid species among these closely related genera in the 20th century resulted from the disparate morphological work of several herpetologists. After the ablepharine (lower eyelid fused with the supercilium) and pre-ablepharine (lower eyelid not completely fused, forming a palpebral slit) eye conditions were discovered (Boulenger, 1887), and Fuhn (1969) noted that skull morphology could be used to delimit scincid taxa, the genus *Panaspis* was restricted to African species. Continued use of skull morphology also supported the separation of the family Scincidae into four subfamilies: Acontinae, Feylininae, Lygosominae, and Scincinae (Greer, 1970). Recent molecular and morphological evidence (Hedges, 2014; Hedges and Conn, 2012; Skinner et al., 2011) suggested skinks could be divided into as many as nine families. Although considered controversial, ignored, or rejected by subsequent authors (e.g., Lambert et al., 2015; Linkem et al., in press; Pyron et al., 2013), this new subdivision continues to support skinks as a monophyletic group (Hedges, 2014). Under a modified version of this classification, the genera *Afroablepharus*, *Lacertaspis*, *Leptosiaphos*, and *Panaspis* are allocated to the Subfamily Eugongylinae (Hedges, 2014; Uetz and Hošek, 2015).

Relying on osteological patterns rather than eye anatomy, Fuhn (1970, 1972) added more skink species with movable lower eyelids and a transparent disc to *Panaspis*. Morphological work by Perret (1973, 1975) divided *Panaspis* species into three groups according to general morphology (mabuiform, lacertiform, and sepsinoid). Greer (1974) erected the genus *Afroablepharus* to accommodate African skinks with an ablepharine eye, and moved all species with movable lower eyelids and pre-ablepharine eyes to other genera, including semiaquatic species to the genus *Cophoscincopus* and terrestrial species to the genus *Panaspis*. As a result, *Leptosiaphos* was synonymized with *Panaspis* based on the movable lower eyelid character, and the only taxon with the pre-ablepharine eye condition was *P. cabindae*, the type species of *Panaspis*. Perret (1975) reduced *Afroablepharus* to a subgenus and described the new subgenus *Lacertaspis* to accommodate two species (*P. reichenowi* and *P. rohdei*) that fitted his lacertiform description from two years earlier. Broadley (1989) revised the genera in question and restricted *Panaspis* to species residing in African savannas and having ablepharine or pre-ablepharine eyes. He then restored *Leptosiaphos* to full genus rank for forest and montane grassland species that had a movable lower eyelid. Lastly, he erected a new subgenus, *Perretia*, to accommodate a newly described species, *Leptosiaphos* (*Perretia*) *rhomboidalis*, which had distinctive cephalic lepidosis. A recent revision by Schmitz et al. (2005) recognized *Afroablepharus*, *Lacertaspis*, and *Leptosiaphos* as distinct genera.

Although Schmitz et al. (2005) gave *Afroablepharus* full-genus rank, insufficient sampling did not fully resolve the genus-level boundaries between *Afroablepharus* and *Panaspis*, as only two species each of *Afroablepharus* and *Panaspis* were assessed, and samples of the type species of the latter genus (*Panaspis cabindae*) were not available at that time. The included species of *Panaspis* (*P. breviceps* and *P. togoensis*) were also not ideal representatives, because they have unique morphological characters and habitat preferences that differ from most remaining members of the genus. This taxonomic arrangement is currently recognized in a recent reptile atlas of South Africa (Bates et al., 2014) and the Reptile Database (Uetz and Hošek, 2015). In this study, our objective is to investigate the monophyly of *Afroablepharus* and *Panaspis*, and clarify their relationship to closely related African genera, including *Lacertaspis* and *Leptosiaphos*. *Afroablepharus wahlbergi* is the most common and widespread snake-eyed skink in sub-Saharan Africa, but its distribution is disjunct and poorly known (Branch, 1998; Fuhn, 1970; Spawls et al., 2002). Greer (1974) designated *A. wahlbergi* as the type species of *Afroablepharus*. The type locality was vaguely defined by Smith (1849)—as "country to the eastward of the Cape Colony," but it is likely to be in the southeastern part of KwaZulu-Natal (Broadley and Howell, 1991). The species has been reported from mainly southern and eastern African countries from South Africa to Kenya, and even Namibia (Fuhn, 1970; Jacobsen and Broadley, 2000; Spawls et al., 2002). Other sub-Saharan African endemics are known to have a similar widespread distribution over savanna and/or woodland habitats, including birds (Voelker et al., 2012), anurans (Evans et al., 2015; Zimkus et al., 2010), mammals (Gaubert et al., 2005), insects (Simard et al., 2009), and other skinks (Portik and Bauer, 2012).

Herein, we examine evolutionary relationships of skinks in the genera *Panaspis* and *Afroablepharus*. We follow the General Lineage Concept (de Queiroz, 1998, 2007; de Queiroz and Gauthier, 1990), which recognizes species as separately evolving lineages. With this species concept, we reject the use of subspecies as natural groups and use molecular data sets to identify separately evolving species. Our concatenated analyses are used to address the following questions: (1) Are *Afroablepharus* and *Panaspis* distinct, reciprocally monophyletic lineages? (2) What is the extent of cryptic speciation within the *Afroablepharus wahlbergi* complex? (3) When did *Afroablepharus*/*Panaspis* species diversify? and (4) Can diversification of *Afroablepharus*/*Panaspis* species be linked to climatic and biogeographic events?

2. Materials and methods

2.1. Taxon sampling

Specimens of the genera *Panaspis* and *Afroablepharus* were collected from multiple localities in sub-Saharan Africa, and 76 samples were sequenced (Table 1, Fig. 1). Additional comparative material was obtained from collections listed by Sabaj Pérez (2013). We generated 75 sequences of *16S*, 70 of *cyt b*, 65 of *PDC*, and 41 of *RAG1*. Two species of *Trachylepis*, one species of *Typhlosaurus* (Scincidae) and *Cordylus marunguensis* (Cordylidae) were used as outgroups to root the trees. Additional sequences of closely related genera (*Lacertaspis*, *Leptosiaphos*, and *Mochlus*) were also sequenced or included from GenBank (Table 1).

2.2. PCR amplification and sequencing

The DNA of alcohol-preserved muscle or liver tissue samples was extracted using the Qiagen DNeasy Blood and Tissue Kit (Valencia, CA), or the IBI DNA Extraction Kit (Shelton Scientific, Peosta, IA). Two mitochondrial (*16S* and *cyt b*) and two nuclear (*PDC* and *RAG1*) genes were amplified (Table 2) in 25 μL PCRs, with an initial denaturing temperature of 95 °C for 2 min, followed by denaturation at 95 °C for 35 seconds (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 or 34 cycles (for mitochondrial or nuclear genes, respectively). The PCR amplicons were visualized with a 1.5% agarose gel with SYBRsafe gel stain (Invitrogen, Carlsbad, CA), and these products were purified with Agencourt AMPure XP magnetic bead

Table 1
Field numbers and localities for specimens used in genetic analyses. DRC = Democratic Republic of Congo, E = east, Moz = Mozambique, N = north, NW = northwest, SW = southwest, S = south, SA = South Africa.

Species	Field number	Collection number	Locality	16S	cyt *b*	PDC	RAG1
Cordylus marunguensis	EBG 2993	UTEP 20374	Pepa, Katanga, DRC	JQ389803	KU298723	KU298803	KU298675
Trachylepis megalura	EBG 1409	UTEP 21195	Lwiro, South Kivu, DRC	KU236715	KU298724	KU298804	–
Trachylepis striata	EBG 1407	UTEP 21172	Lwiro, South Kivu, DRC	KU236716	KU298725	KU298805	–
Typhlosaurus braini	AMB 6338	CAS 214579	Rooibank, Erongo Region, Namibia	HQ180128	–	–	HQ180137
Typhlosaurus braini	AMB 6340	CAS 214581	Rooibank, Erongo Region, Namibia	HQ180025	–	–	HQ180106
Lacertaspis chriswildi	–	ZFMK 75735	Tchabal Mbabo, Cameroon	KU236797	KU298801	KU298874	–
Lacertaspis gemmiventris	RCD 13251	CAS 207854	Bioko Island, Equatorial Guinea	KU236793	KU298797	KU298870	KU298720
Lacertaspis gemmiventris	RCD 13255	CAS 207858	Bioko Island, Equatorial Guinea	KU236792	KU298796	–	KU298719
Lacertaspis reichenowi	E56.12	–	–	AY308235	–	–	KU298717
Lacertaspis rohdei	–	ZFMK 75382	Mt. Nlonako, Cameroon	KU236790	KU298795	–	KU298717
Leptosiaphos blochmanni	EBG 1610	UTEP 21177	Bichaka, South Kivu, DRC	KU236798	KU298802	KU298875	KU298722
Leptosiaphos koutoui	–	MNHN 2001.0697	Meiganga, Adamaoua Plateau, Cameroon	KU236789	KU298794	KU298868	KU298716
Leptosiaphos meleagris	ELI 2844	UTEP 21178	Rwenzori Mountains National Park, Uganda	KU236799	–	–	–
Leptosiaphos sp.	–	ZFMK 69552	Mt. Nlonako, Cameroon	KU236794	KU298798	KU298871	KU298721
Leptosiaphos sp.	–	ZFMK 75381	Mt. Nlonako, Cameroon	KU236791	–	KU298869	KU298718
Mochlus afer	E56.17	ZFMK 54317	Kiyawetanga, Kenya	KU705386	–	KU764776	KU841442
Afroablepharus africanus	–	Uncatalogued	Príncipe, Gulf of Guinea	KU705385	–	KU764775	–
Afroablepharus africanus	Pm3	–	Montalegre, Príncipe, Gulf of Guinea	EU164477	–	–	–
Afroablepharus africanus	E62.17	BMNH, Uncatalogued	Príncipe, Gulf of Guinea	AY308286	–	–	–
Afroablepharus annobonensis	An15	–	Annobon, Gulf of Guinea	EU164494	–	–	–
Afroablepharus annobonensis	An9	–	Annobon, Gulf of Guinea	EU164488	–	–	–
Panaspis breviceps	ELI 558	UTEP 21176	Byonga, South Kivu, DRC	KU236717	–	–	–
Panaspis breviceps	MM 106	ZFMK 87663	Mawne, Cameroon	KU236787	KU298792	KU298866	KU298715
Panaspis breviceps	MM 105	ZFMK 87662	Mawne, Cameroon	KU236786	KU298791	–	KU298714
Panaspis breviceps	–	ZFMK 75380	Mt. Nlonako, Cameroon	KU236796	KU298800	KU298873	–
Panaspis cabindae	WRB 804	PEM R20256	Soyo, NW Angola	KU236768	KU298775	KU298851	KU298708
Panaspis cabindae	PM 050	Uncatalogued	Luango-Nzambi, Bas-Congo, DRC	KU236751	KU298758	KU298834	KU298698
Panaspis cabindae	PM 049	Uncatalogued	Luango-Nzambi, Bas-Congo, DRC	KU236750	KU298757	KU298833	KU298697
Panaspis cabindae	WRB 810	PEM R21594	Riverine Forest, Bengo, Angola	KU236765	KU298772	KU298848	KU298705
Panaspis cabindae	ANG 21	PEM R19467	Lagoa Carumbo, Angola	KU236741	KU298749	KU298826	KU298690
Panaspis cabindae	ELI 1722	UTEP 21173	Bombo-Lumene Reserve, Kinshasa, DRC	KU236753	KU298760	KU298836	–
Panaspis cabindae	ANL 52	MTD 48612	Kimpa Vita Uni Campus, Uíge, N Angola	KU236771	–	KU298854	–
Panaspis cabindae	MBUR 2128	Uncatalogued	S Leba Pass, Huila District, SW Angola	KU236740	KU298748	KU298825	–
Afroablepharus maculicollis	ANG 421	PEM R20475	Benero Campsite, near Jamba, Angola	KU236770	KU298778	KU298853	KU298711
Afroablepharus maculicollis	MBUR 02843	Uncatalogued	Phalaborwa, Limpopo, SA	KU236748	KU298755	KU298831	KU298695
Afroablepharus maculicollis	MBUR 02848	Uncatalogued	Phalaborwa, Limpopo, SA	KU236749	KU298756	KU298832	KU298696
Afroablepharus maculicollis	MCZF 38848	CAS 234188	Farm Nooitgedacht, Limpopo Province, SA	KU236728	KU298736	KU298816	KU298694
Afroablepharus maculicollis	MCZF 38790	CAS 234135	Farm Vrienden, Limpopo Province, SA	KU236747	KU298754	KU298830	KU298694
Afroablepharus maculicollis	MCZF 38733	CAS 234099	Farm Vrienden, Limpopo Province, SA	KU236720	KU298728	KU298808	KU298678
Afroablepharus sp. Ethiopia	TJC 264	–	Oromia, western Ethiopia	KU236752	KU298759	KU298835	–
Afroablepharus sp. Katanga 1	ELI 294	UTEP 21174	Mulongo, Katanga, DRC	KU236730	KU298738	KU298818	KU298686
Afroablepharus sp. Katanga 1	ELI 295	UTEP 21175	Mulongo, Katanga, DRC	KU236729	KU298737	KU298817	KU298685
Afroablepharus sp. Katanga 2	WRB 575	PEM R17454	Kalakundi Copper Mine, S Katanga, DRC	KU236736	KU298744	KU298822	KU298689
Afroablepharus sp. Katanga 2	WRB 576	PEM R17455	Kalakundi Copper Mine, S Katanga, DRC	KU236737	KU298745	KU298823	–
Afroablepharus sp. Katanga 2	JHK 26	Uncatalogued	Kisanfu Camp, Katanga, DRC	KU236726	KU298734	KU298814	KU298682
Afroablepharus sp. Katanga 2	WRB 0047	PEM R20327	Fungurume Camp, Katanga, DRC	KU236745	KU298752	KU298829	–
Afroablepharus sp. Katanga 2	WRBNimb083	–	NW Zambia	KU236742	KU298750	KU298827	KU298691
Afroablepharus sp. Limpopo	MCZ-A 27176	–	Hoedspruit, Limpopo, SA	KU236743	KU298751	KU298828	KU298692
Afroablepharus sp. Limpopo	MCZ-A 27177	CAS 248791	Hoedspruit, Limpopo, SA	KU236744	–	–	–
Afroablepharus sp. Malawi	WRB 568	PEM R20247	Sombani Trail, Mt. Mulanje, Malawi	KU236732	KU298740	KU298819	KU298687
Afroablepharus sp. Malawi	WRB 570	PEM R20800	Likabula Station, Mt. Mulanje, Malawi	KU236733	KU298741	–	–
Afroablepharus sp. Mozambique 1	WC 1251	PEM R20561	Ecofarm, Chemba, Moz	KU236764	KU298771	KU298847	KU298704
Afroablepharus sp. Mozambique 1	WC 1249	No voucher	Ecofarm, Chemba, Moz	KU236763	KU298770	KU298846	–
Afroablepharus sp. Mozambique 1	WC 1169	PEM R20565	Boabab Ore Mine, Masamba, Moz	HQ761761	KU298768	KU298844	–
Afroablepharus sp. Mozambique 1	WC 1186	PEM R20566	Boabab Ore Mine, Masamba, Moz	KU236762	KU298769	KU298845	–
Afroablepharus sp. Mozambique 1	SVN 693	–	Gorongosa National Park, Moz	KU236754	KU298761	KU298837	KU298699
Afroablepharus sp. Mozambique 1	WRB 886	PEM R20591	Ruoni Hill S, Tete Province, Moz	KU236769	KU298777	–	KU298710
Afroablepharus sp. Mozambique 2	WC 1358	Uncatalogued	Quiterajo, Cabo Delgado, Moz	KU236776	–	KU298859	–
Afroablepharus sp. Mozambique 2	ENI 038	Uncatalogued	Mocimboa da Praia, Cabo Delgado, Moz	KU236780	–	–	–
Afroablepharus sp. Mozambique 3	WC 1051	No voucher	NW of Rapale, Nampula, Moz	KU236772	KU298779	KU298855	–
Afroablepharus sp. Mozambique 3	WC 1067	PEM R20557	E of Ribuae, Nampula, Moz	KU236773	KU298780	KU298856	–
Afroablepharus sp. Mozambique 3	WC 1133	No voucher	NW of Mecuburi, Nampula, Moz	KU236774	KU298781	KU298857	–
Afroablepharus sp. Mozambique 3	WC 1161	PEM R20558	Rapale, Nampula, Moz	KU236778	KU298784	KU298861	–
Afroablepharus sp. Mozambique 4	WRB 855	PEM R20569	Syran graphite mine, Balama, Moz	KU236766	KU298773	KU298849	KU298706
Afroablepharus sp. Mozambique 4	WRB 856	PEM R20576	Syran graphite mine, Balama, Moz	KU236767	KU298774	KU298850	KU298707
Afroablepharus sp. Mozambique 4	WC 1317	Uncatalogued	Pemba, Cabo Delgado, Moz	KU236775	KU298782	KU298858	–
Afroablepharus sp. Mozambique 4	WC 1404	Uncatalogued	Pemba, Cabo Delgado, Moz	KU236777	KU298783	KU298860	–
Afroablepharus sp. Mozambique 4	ENI 037	Uncatalogued	Quirimbas National Park, Moz	KU236779	KU298785	KU298862	–
Afroablepharus sp. Mozambique 5	DMP 187	MVZ 266148	Serra Jeci, Moz	KU236739	KU298747	–	–
Afroablepharus sp. Namibia	AMB 7634	MCZ R183767	Sesfontein, Namibia	KU236727	KU298735	KU298815	KU298683
Afroablepharus sp. Namibia	WRB 567	–	Otavi, Namibia	KU236731	KU298739	–	–
Afroablepharus sp. Tanzania 1	WRB 0021	–	Arusha, Tanzania	KU236719	KU298727	KU298807	KU298677
Afroablepharus sp. Tanzania 1	WRB 0026	–	Arusha, Tanzania	KU236718	KU298726	KU298806	KU298676

Table 1 (*continued*)

Species	Field number	Collection number	Locality	16S	cyt *b*	PDC	RAG1
Afroablepharus sp. Tanzania 2	WRB 572	PEM R16769	Klein's Camp, Serengeti, Tanzania	KU236734	KU298742	KU298820	–
Afroablepharus sp. Tanzania 2	WRB 573	PEM R20799	Klein's Camp, Serengeti, Tanzania	KU236735	KU298743	KU298821	KU298688
Panaspis togoensis	–	ZFMK 42212	–	KU236788	KU298793	KU298867	–
Panaspis togoensis	2426	MVZ 249793	Kyabobo National Park, Ghana	KU236795	KU298799	KU298872	–
Panaspis togoensis	DCB 34707	–	Gashaka Gumti National Park, Nigeria	KU236725	KU298733	KU298813	–
Panaspis togoensis	TJH 2561	TCWC 94519	W National Park, Alibori, Benin	KU236756	KU298763	KU298839	–
Panaspis togoensis	TJH 2629	TCWC 94557	Dogo Forest, Benin	KU236758	KU298765	KU298841	KU298701
Panaspis togoensis	TJH 2600	TCWC 94544	W National Park, Alibori, Benin	KU236757	KU298764	KU298840	–
Afroablepharus wahlbergi	SVN 742	NMB R10286	Beira, Mozambique	KU236755	KU298762	KU298838	KU298700
Afroablepharus wahlbergi	WRB 745	PEM R16455	Bluff, Durban, KwaZulu-Natal, SA	–	KU298776	KU298852	KU298709
Afroablepharus wahlbergi	WC 2723	PEM R21297	Doornkop Reserve, Mpumalanga, SA	KU236782	KU298787	–	KU298713
Afroablepharus wahlbergi	WC 2721	PEM R21298	Doornkop Reserve, Mpumalanga, SA	KU236781	KU298786	–	KU298712
Afroablepharus wahlbergi	DMP 127	MVZ 266147	Inhambane, Mozambique	KU236738	KU298746	KU298824	–
Afroablepharus wahlbergi	–	TM 84299	Groblersdal, Limpopo, SA	KU236746	KU298753	–	KU298693
Afroablepharus wahlbergi	MCZF 38852	CAS 234194	Limpopo Province, SA	KU236724	KU298732	KU298812	KU298681
Afroablepharus wahlbergi	AMB 8279	MCZR 184432	Limpopo Province, SA	KU236723	KU298731	KU298811	–
Afroablepharus wahlbergi	AMB 8293	MCZR 184443	Limpopo Province, SA	KU236722	KU298730	KU298810	KU298680
Afroablepharus wahlbergi	MCZF 38868	CAS 234209	Limpopo Province, SA	KU236721	KU298729	KU298809	KU298679
Afroablepharus wahlbergi	TJH 3253	TCWC 95588	Kimberley, Northern Cape, SA	KU236760	KU298767	KU298843	KU298703
Afroablepharus wahlbergi	TJH 3213	TCWC 95563	Kimberley, Northern Cape, SA	KU236759	KU298766	KU298842	KU298702
Afroablepharus wahlbergi	WRB inh18	PEM R21757	Inhambane, Mozambique	KU236783	KU298788	KU298863	–
Afroablepharus wahlbergi	WRB inh19	PEM R21758	Inhambane, Mozambique	KU236784	KU298789	KU298864	–
Afroablepharus wahlbergi	WRB inh30	PEM R21759	Inhambane, Mozambique	KU236785	KU298790	KU298865	–
Broadleysaurus major	–	–	–	AJ416922	DQ090881	–	HM161157
Xantusia vigilis	–	–	–	DQ249035	DQ249101	HQ426258	–
Plestiodon inexpectatus	–	–	–	AY217990	AY217837	HQ426253	AY662632
Plestiodon japonicus	–	–	–	–	EU203045	–	HM161196
Tiliqua rugosa	–	–	–	AY308319	–	EF534856	–

solution (Beckman Coulter, Danvers, MA) with the manufacturer's protocols. Forward and reverse strands of PCR products were sequenced on an ABI 3700xl capillary DNA sequencer at the University of Texas at El Paso (UTEP) Border Biomedical Research Center (BBRC) Genomic Analysis Core Facility.

2.3. Phylogenetic analyses

We conducted phylogenetic analyses of single-gene and concatenated data sets, consisting of 2278 characters from the mitochondrial genes *16S* (518 bp) and *cyt b* (619 bp), and nuclear genes *PDC* (442 bp) and *RAG1* (699 bp). Hypervariable regions in the 16S ribosomal gene, totaling 50 base pairs, were removed from the final analysis. The program SeqMan (Swindell and Plasterer, 1997) was used to interpret chromatograph data. Sequences were aligned using the ClustalW algorithm in the program MEGALIGN (DNASTAR, Madison, WI) and adjusted in MacClade v4.08 (Maddison and Maddison, 2000). A maximum-likelihood tree (ML) was estimated with the GTRGAMMA model in RAxML v7.2.6 (Stamatakis, 2006). All parameters were estimated and a random starting tree was used. Node support was assessed with 1000 nonparametric bootstrap replicates (Stamatakis et al., 2008). Bayesian inference (BI) was conducted with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Our model included ten data partitions: a single one for 16S and independent partitions for each codon position of the protein-coding genes *cyt b*, *PDC*, and *RAG1*. Concatenated data sets were partitioned identically for ML and BI analyses. The Akaike information criterion implemented in jModelTest 2 (Darriba et al., 2012) was used to identify the best-fit model of evolution given our data for subsequent BI analyses. Bayesian analyses were conducted with random starting trees, run for 20,000,000 generations, and Markov chains were sampled every 1000 generations. Are we there yet? (AWTY) (Nylander et al., 2008) was used to verify that multiple runs converged, and the first 25% of the trees were discarded as burn-in. Phylogenies were visualized using FigTree v1.4.2 (Rambaut, 2012).

2.4. Divergence time estimation

Divergence dates were estimated using BEAST v1.8.1 (Drummond et al., 2012). There are no fossil calibrations available for the genera *Panaspis* or *Afroablepharus*, and therefore, two external calibrations were incorporated from Mulcahy et al. (2012). We used the fossil cordyliform *Konkasaurus* from the Maastrichtian (Upper Cretaceous) of Madagascar (Krause et al., 2003) as the minimum age estimate for the most recent common ancestor (MRCA) of Cordyliformes (i.e., Cordylidae and Gerrhosauridae) + xantusiids, because Mulcahy et al. (2012) noted that the earliest stem group xantusiid fossil is *Paleoxantusia* from the Torrejonian of the early Paleocene. This date was implemented using a lognormal distribution with a real space mean of 10, log(stdev) of 0.7, and offset of 58, yielding a 95% interval of 60.4–82.7 mya (million years ago). The second calibration incorporated the crown-group scincid fossils *Contogenys* and *Sauriscus* from multiple formations between the Late Cretaceous and Early Paleocene (Bryant, 1989; Carroll, 1988; Estes, 1969; Mulcahy et al., 2012) to provide a minimum-age estimate for the Family Scincidae. This calibration was enforced using a lognormal distribution with a real space mean of 10, log(stdev) of 0.7, and offset of 63, yielding a 95% interval of 65.4–87.7 mya. Dating analyses incorporated all four genes, partitioned by mtDNA (*cyt b*, *16S*) and nucDNA (*RAG1*, *PDC*) markers. Relevant outgroups were selected from GenBank (Table 1). Ingroup sampling was limited to one or two representative lineages with complete data sets, and inclusion of ingroup and outgroup samples required at least one locus per partition. Including members with missing sequences could yield potentially problematic results (Blankers et al., 2013).

Dating analyses were run for 5×10^7 generations with sampling every 5000 generations. The Yule model of speciation was used as the tree prior, uncorrelated relaxed lognormal clock models were applied, and both clock and substitution models were unlinked across partitions. The underlying lognormal distribution for the clock model (ucld.mean) was given a broad exponential prior (mean = 10, offset = 0, initial = 1). Runs were assessed using Tracer v1.6 to examine convergence and confirm that ESS values were
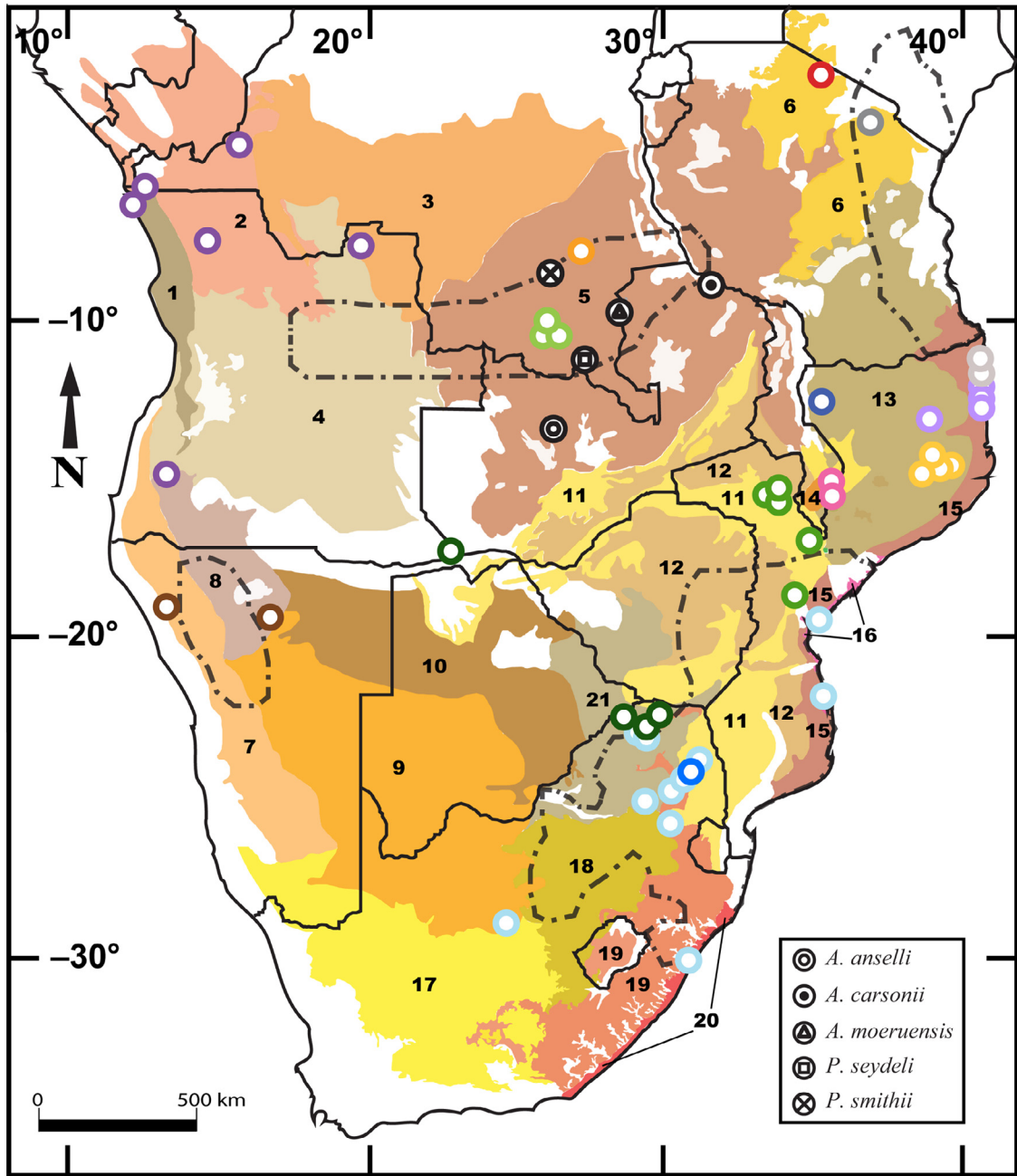
Fig. 1. Map of central, eastern and southern Africa showing the historical, disjunct distribution of *Afroablepharus wahlbergi* (in dotted lines). Ecoregions containing genetic samples for this study are colored and assigned with numbers from 1 to 21. Sampled locality colors correspond to the clades in Fig. 2. Map was modified from Branch (1998), Burgess et al. (2004), and Spawls et al. (2002). Black circles indicate type localities of *Ablepharus anselli* (Kasempa, Zambia), *Ablepharus moeruensis* (Kilwa Island, Lake Mweru between Zambia and Katanga Province, DRC), *Panaspis seydeli* (Lubumbashi, southeastern Katanga Province, DRC) and *P. smithii* (Nyonga, central Katanga, DRC), which are currently considered to be synonyms of *Afroablepharus seydeli* (Broadley and Cotterill, 2004; Uetz and Hošek, 2015). A fifth black circle indicates the type locality for *Ablepharus carsonii* (Fwambo [aka, Fwamba], northeastern Zambia).

Table 2
Primer sequences used in this study.

Primer	Gene	Reference	Sequence
16L9	16S	Pramuk et al. (2008)	5′-CGCCTGTTTACCAAAAACAT-3′
16H13	16S	Pramuk et al. (2008)	5′-CCGGTCTGAACTCAGATCACGTA-3′
CytbCBJ10933	cyt b	Vences et al. (2003)	5′-TATGTTCTACCATGAGGACAAATATC-3′
CytbC	cyt b	Vences et al. (2003)	5′-CTACTGGTTGTCCTCCGATTCATGT-3′
PHOF2	PDC	Bauer et al. (2007)	5′-AGATGAGCATGCAGGAGTATGA-3′
PHOR1	PDC	Bauer et al. (2007)	5′-TCCACATCCACAGCAAAAAACTCCT-3′
RAG1 G396	RAG1	Groth and Barrowclough (1999)	5′-TCTGAATGGAAATTCAAGCTGTT-3′
RAG1 G397	RAG1	Groth and Barrowclough (1999)	5′-GATGCTGCCTCGGTCGGCCACCTTT-3′
RAG1f700	RAG1	Bauer et al. (2007)	5′-GGAGACATGGACACAATCCATCCTAC-3′
RAG1r700	RAG1	Bauer et al. (2007)	5′-TTTGTACTGAGATGGATCTTTTTGCA-3′

acceptable (>200) (Rambaut and Drummond, 2009). A burn-in of 25% was set and maximum-clade credibility trees were created with median date estimates from 7500 trees for each analysis with TreeAnnotator v1.8.1 (Drummond et al., 2012).

3. Results

3.1. Phylogenetic analyses

One sample failed to amplify for the *16S* gene, 6 for *cyt b*, 11 for *PDC*, and 35 for *RAG1* (Table 1). This could be attributed to several factors, including tissue degradation, poor extraction quality, and/ or reagent deterioration. Other studies have shown that phylogenetic analyses with missing data can still be accurately inferred if they have an appropriate amount of informative characters. Support actually improves when taxa with missing data are included, as opposed to excluding these taxa altogether (Jiang et al., 2014; Mulcahy et al., 2012; Wiens and Morrill, 2011). For the BI analyses, the models of nucleotide substitution selected by jModelTest 2 are listed in Table 3. When a relatively complex model selected by jModelTest 2 was not available in MrBayes, the least restrictive model (GTR) was implemented. The concatenated topologies for the ML and BI analyses were identical, and strong support values were similar for most clades (Fig. 2). These concatenated ML and BI analyses resulted in the same topologies as our single-gene mtDNA analyses (not shown). Separate topologies of our nuclear genes *PDC* (41 parsimony-informative sites) and *RAG1* (102 parsimony-informative sites) are provided in the Supplementary materials (Supplementary Figs. 1 and 2). The ML analysis (concatenated data set) likelihood score was −18445.331817.

The ML and BI analyses of the concatenated data (Fig. 2) demonstrated that neither *Panaspis* nor *Afroablepharus* are monophyletic. However, both analyses recovered strong support (>70% ML bootstrap values and >0.95 BI posterior probabilities) for a clade including all *Panaspis* and *Afroablepharus* samples, which is sister to a well-supported clade including *Lacertaspis* and *Leptosiaphos*. Two clades corresponding to *P. togoensis* and *P. breviceps* (known from forest/savanna mosaic habitats and forests, respectively) were recovered in basal and sister positions, respectively, to the remaining samples from savanna habitats, which formed a well-supported clade. Within the latter group, a western clade including *A. africanus*, *A. annobonensis*, and *P. cabindae* was recovered as sister to other samples from central, eastern, and southern Africa, which formed a well-supported clade.

We recovered a high level of geographic structuring within the latter clade. Herein, we label geographically distinct populations to allow easy reference throughout the text and to designate these populations as candidates for further taxonomic investigation. We recovered the following lineages: (1) *A.* sp. Limpopo in northern South Africa, (2) *A.* sp. Namibia, (3) *A. maculicollis* from northern South Africa and southeastern Angola, (4) *A.* sp. Mozambique 1, in Gorongosa National Park and provinces in the northwestern side

of the country, (5) *A.* sp. Mozambique 2, located near the northeastern coast of the country, (6) *A.* sp. southern Malawi, (7) *A.* sp. Mozambique 3 in Nampula Province, in northeastern Mozambique about 170 km south of the following lineage, (8) *A.* sp. Mozambique 4 from Cabo Delgado Province in the northeastern side of the country, (9) *A. wahlbergi*, including presumably topotypic samples, from multiple localities in eastern South Africa and adjacent Mozambique, (10) *A.* sp. Tanzania 1 in the suburbs of the city of Arusha, on the eastern side of the Great Rift Valley, (11) *A.* sp. Tanzania 2, from "Klein's Camp" at the northeastern tip of Serengeti National Park, (12) *A.* sp. Katanga 1 in eastern Katanga Province, DRC, and (13) *A.* sp. Katanga 2 at the southernmost side of the latter province. Unique, divergent samples included *A.* sp. Ethiopia from western Ethiopia and *A.* sp. Mozambique 5 from Serra Jeci, Niassa Province, northwestern Mozambique.

3.2. Divergence time estimation

Our BEAST analysis indicates the time to the most recent common ancestor of *Panaspis*/*Afroablepharus* clade as in the Eocene, approximately 51.6 mya (42.7–62.4 mya, 95% highest posterior densities [HPD]). Whilst the analysis indicated the origin of the entire clade in the Eocene, a majority of *Panaspis*/*Afroablepharus* lineages diversified during the Miocene (Fig. 3, Table 4). The topology of the BEAST tree differs only slightly from that of the ML and BI analyses (Fig. 2) by the following well-supported, monophyletic clades: (1) *Lacertaspis*, (2) *Panaspis breviceps* and *P. togoensis*, and (3) *Afroablepharus* sp. Ethiopia, *A.* sp. Limpopo, *A.* sp. Namibia, *A.* sp. Mozambique 1, and *A. maculicollis*.

3.3. Taxonomic ramifications

Because the type species of both *Panaspis* (*P. cabindae*) and *Afroablepharus* (*A. wahlbergi*) were recovered in a well-supported clade along with all available congeners (Fig. 2), we transfer *Afroablepharus* Greer, 1974 to the synonymy of *Panaspis* Cope, 1868, which has taxonomic priority. To avoid further nomenclatural confusion in the following text we thus adopt this new arrangement in all further discussion.

4. Discussion

4.1. Biogeography

Our analyses recovered strongly supported lineages that are mainly distributed in non-forested areas reaching elevations up to 1884 m. The clades found at the eastern side of sub-Saharan Africa are situated around the Afromontane Archipelago, which consists of a series of discontinuous mountain formations along eastern Africa, ranging from the southernmost tip of South Africa to the Arabian Peninsula (Grimshaw, 2001). Although most of our recovered lineages are not considered to be Afromontane, their

Table 3
Models of nucleotide substitution selected by jModelTest 2 for the Bayesian Inference analyses.

Gene	Position	Model
16S	–	TIM2 + I + G
cyt b	Codon 1	TPM3uf + I + G
	Codon 2	TPM2uf + G
	Codon 3	TIM2 + I + G
PDC	Codon 1	TIM3 + I
	Codon 2	TPM3uf + I
	Codon 3	TPM1uf + G
RAG1	Codon 1	TPM1uf + G
	Codon 2	HKY + G
	Codon 3	TPM1 + G

divergences might be explained by the irregular physiography seen along the areas where these populations occur. This pattern of micro-endemism has been documented in other skinks (Parham and Papenfuss, 2009), geckos (Travers et al., 2014), chameleons (Glaw et al., 2012), chelonians (Daniels et al., 2007; Petzold et al., 2014), birds (Husemann et al., 2013), and mammals (Stoffberg et al., 2012; Taylor et al., 2011). For example, Tanzania has two populations that are separated by the Great Rift Valley: *P.* sp. Tanzania 1, located in Arusha at 1400 m elevation, and *P.* sp. Tanzania 2, located at "Klein's Camp" in Serengeti National Park at approximately 1884 m elevation. Both populations are located in the disjunct Southern *Acacia-Commiphora* Bushlands and Thickets Ecoregion (note the genus *Acacia* in Africa is now either *Vachellia* or *Senegalia*, *sensu* Miller et al., 2014), which consists of tropical and subtropical grasslands and savanna (Burgess et al., 2004). Similar patterns of diversification are seen in savanna-adapted snakes (Broadley, 2001b).

Mozambique harbors the greatest genetic diversity of snake-eyed skinks found in our study (Figs. 1 and 2). The country is dominated by tropical and subtropical grasslands, savannas and shrublands, and contains a variety of hills, low plateaus, and highlands (Burgess et al., 2004). The *P.* sp. Mozambique 1 clade resides within the Southern Miombo Woodlands, a lowland ecoregion with mainly tropical and subtropical savannas. This ecoregion is disjunct, covering the northwestern tip and central area of Mozambique. A few samples also fall inside the subhumid Zambezian and Mopane Woodlands Ecoregion (Burgess et al., 2004), which occupies most of western Mozambique and is located between the latter ecoregion's disjunct areas. Though the Zambezian and Mopane Woodlands have scant vertebrate endemics, reptile endemism is represented by Lang's worm lizard (*Chirindia langi*) and the Sabi quill-snouted snake (*Xenocalamus sabiensis*) (Burgess et al., 2004).

The distinct clades at the northeastern tip of Mozambique (*P.* sp. Mozambique 2–4) are located in areas with different types of habitats. Cabo Delgado Province harbors the neighboring populations of *P.* sp. Mozambique 2 and 4 along the northeastern coastline. The corresponding ecoregion is called the Southern Zanzibar-Inhambane Coastal Forest Mosaic, and one sample of *P.* sp. Mozambique 4 falls within the Eastern Miombo Woodlands Ecoregion. Although both ecoregions contain mosaics of tropical and subtropical grasslands and savannas, the coastal mosaic forests have been described as "biologically valuable" (Burgess et al., 2003), harboring a great variety of plant and vertebrate endemics. The *P.* sp. Mozambique 3 clade is located in Nampula Province, south of Cabo Delgado. This population falls within the Eastern Miombo Woodlands Ecoregion, with elevations ranging from 300 to 500 m. The specimen that corresponds to *P.* sp. Mozambique 5 was collected in mid-elevation grassland on Serra Jeci (1358 m), a massif in northwestern Mozambique. Given the large number of endemic reptiles described from Mozambique in recent years (Branch and

Bayliss, 2009; Branch and Tolley, 2010; Branch et al., 2014; Broadley, 1990, 1992; Portik et al., 2013b), it should not be surprising that the country harbors a large number of cryptic species of snake-eyed skinks.

The only population sampled from Malawi (Fig. 2) was found on the lower slopes of the Mt. Mulanje Massif, which rises up to 3000 m elevation above the Phalombe Plain at the border with Mozambique. The massif includes many herpetofaunal endemics (Branch and Cunningham, 2006; Broadley, 2001a; Günther, 1893; Loveridge, 1953), and represents an important center of endemism in the Afromontane Archipelago (Burgess et al., 2004), as well as a site of important conservation concern for amphibians (Conradie et al., 2011). Similar studies suggest this region harbors cryptic species of other taxa, including bats (Curran et al., 2012), insects (Dijkstra and Clausnitzer, 2006), and birds (Voelker et al., 2010), which resulted from formation of sky islands. The taxonomic status of this population and of other snake-eyed skinks recorded from Malawi (e.g., Cholo and Nchisi Mountains, Nyika Plateau), and their relationship to *Ablepharus carsonii* Boulenger, 1894, described from Fwambo, Zambia, and also recorded from the Nyika Plateau (Boulenger, 1897), requires further study.

Although specimens in Namibia were collected in localities that are distant from each other (one from the Northern Namibian Escarpment [NNE] at Sesfontein and another from the Otavi Highlands on the Namibian central plateau), they formed a well-supported clade with minimal genetic divergence from each other. Much of Namibia comprises xeric savanna and represents a center of high reptile diversity and endemism, but many areas remain understudied (Herrmann and Branch, 2013). However, Bauer (2010) explained that even though the NNE and Otavi Highlands are known for having substantial biodiversity, long-term isolation and thus endemism decrease owing to the "low relief" and accessibility to surrounding areas. This might explain the close relationship between the two Namibian samples. There is also a Namibian population of mole rats with a widespread distribution (Faulkes et al., 2004), which is also attributed to low relief and high accessibility in the landscape.

According to Jacobsen and Broadley (2000), *P. wahlbergi* can be found in a variety of habitats, from rocky outcrops to highveld grassland at altitudes ranging from sea level to 2000 m. Our sampling suggests that *P. wahlbergi* occupies mostly montane shrublands and grasslands in eastern South Africa with an elevation ranging from 1000 to 1300 m. Included in the *P. wahlbergi* clade are our Mozambique samples, which are genetically slightly divergent from the South African samples. Although the Indian Ocean coastal ecoregion extends from KwaZulu-Natal to Mozambique, the Limpopo River valley might limit gene flow between the latter populations.

Knowledge of the distribution of *P. wahlbergi* has changed over time as field guides were updated through fieldwork efforts in the late 20th century (Branch, 1998; Spawls et al., 2002). Although the species was reported from Saudi Arabia (Al-Jumaily, 1984), this population was certainly misidentified because of the disparate locality and habitat, and its morphological resemblance to the Asian skink *Ablepharus pannonicus* (Schätti and Gasperetti, 1994). Another clade (*P.* sp. Limpopo, Figs. 1 and 2) is sympatric with populations of *P. maculicollis* and *P. wahlbergi*. Located in an area dominated by diverse habitats and high endemism (Burgess et al., 2004), this clade likely represents a new species, because it is morphologically distinct from both *P. maculicollis* and *P. wahlbergi* (MFM, unpubl. data).

We recovered two clades residing within the Central Zambezian Miombo Woodlands Ecoregion (*P.* sp. Katanga 1 and 2) in Katanga Province (DRC) and northern Zambia, which contains high physiographic diversity. Southeastern Katanga is dominated by various high relief areas, which contain numerous ravines, depressions,
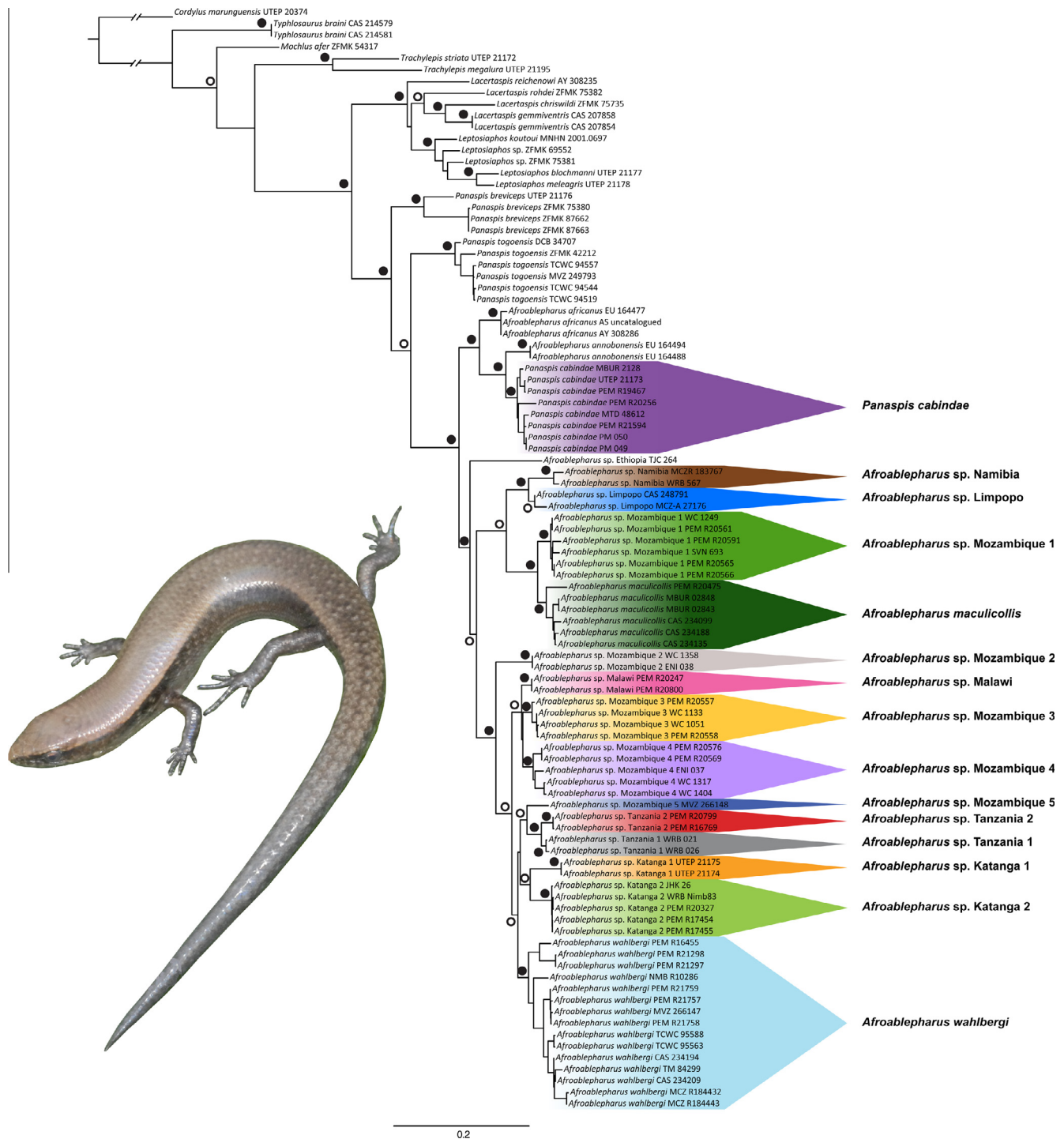
Fig. 2. Maximum-likelihood phylogenetic tree derived from *16S, cyt b, PDC,* and *RAG1* DNA sequences. Tree topology was identical in both BI and ML analyses. Nodes supported by Bayesian posterior probability of ⩾0.95 and maximum likelihood bootstrap support of ⩾70 are indicated by black circles. Nodes supported by maximum likelihood values of ⩾70 only are indicated by open circles. Photo (UTEP 21174) shows *Afroablepharus* sp. Katanga 1.

and drainage systems. This region is dominated by miombo/woodland savanna (Burgess et al., 2004) and harbors various hotspots for plant and reptile endemism (Broadley and Cotterill, 2004). Plateaus in southeastern Katanga are believed to have formed from sands in the Plio-Pleistocene that coincided with extensive aridification processes. In an area with such geological and vegetation complexity, it was not surprising to recover unknown skink lineages in our phylogeny (Fig. 2). The extremely close morphological resemblance between several taxa known from Katanga and

Zambia justifies the actions of earlier herpetologists, who merged *Panaspis anseli*, *P. moeruensis*, and *P. seydeli* into a single currently recognized species, *P. seydeli* (Broadley and Cotterill, 2004). The only unsampled snake-eyed skink species known from Katanga, *P. smithii*, has at least three distinct morphological traits, including white dorsolateral stripes that are lacking in *P. seydeli* (Broadley and Cotterill, 2004). Considering the extensive habitat diversity in Katanga and the large number of lineages recovered in our phylogeny, all four taxa may prove to be specifically distinct species
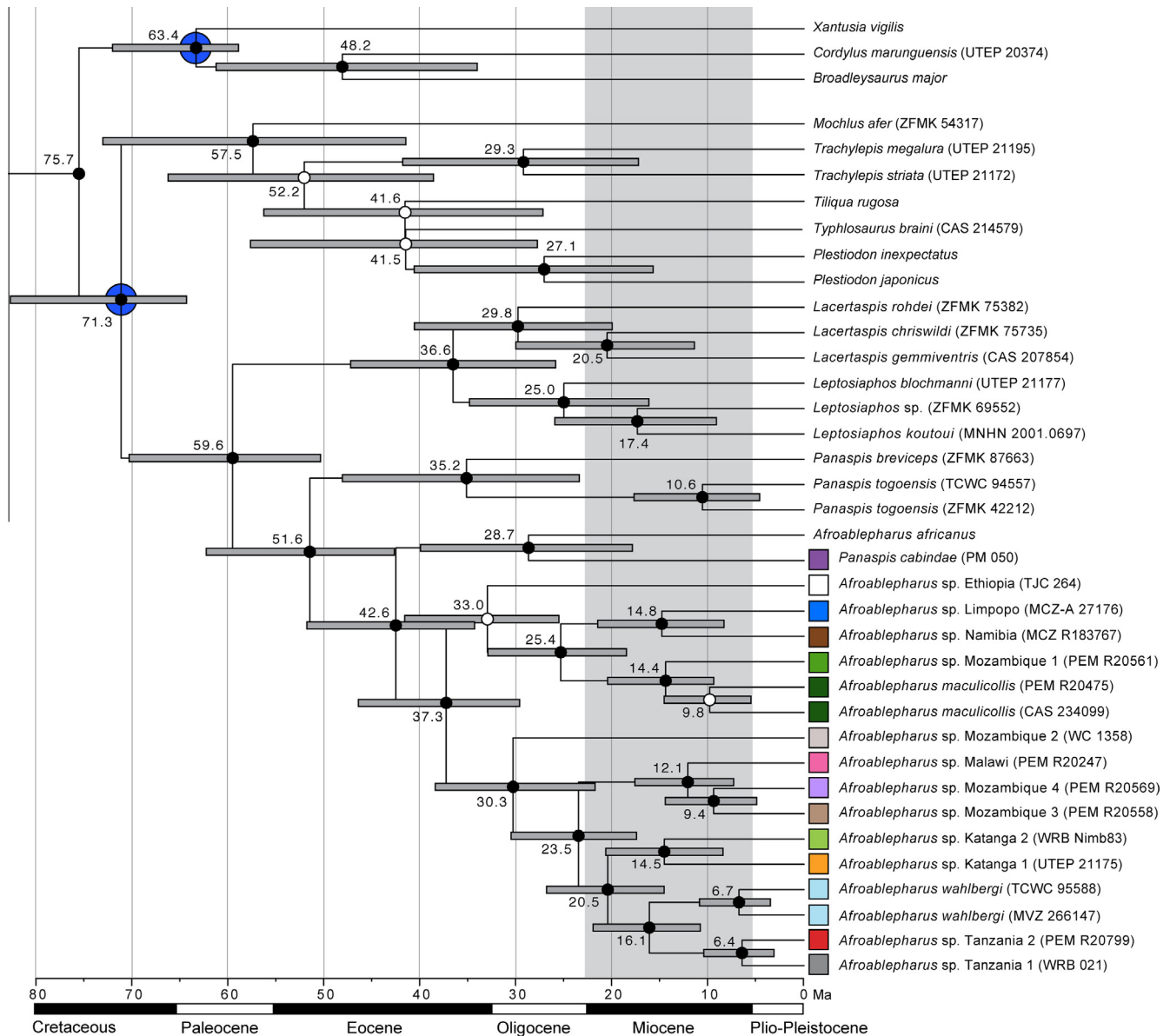
Fig. 3. Chronogram resulting from BEAST, based on two fossil calibration points. Nodes with high support (posterior probability >0.9) are black; those with lower support (posterior probability <0.9) are white. Median age estimates are provided along with error bars representing the 95% highest posterior densities (HPD). Blue circles around nodes indicate fossil calibrations. Colored boxes correspond to the clade color scheme used in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with additional sampling and morphological evidence. A fifth species described from northeastern Zambia in the same ecoregion (Fig. 1), *Ablepharus carsonii* Boulenger, 1894, has been overlooked in recent revisions and may prove to be a distinct species as well.

4.2. Divergence dating

According to our dating analysis, most of the *Panaspis* lineages emerged in the Miocene (Fig. 3). Our dating analyses suggest that the most recent common ancestor of the *Panaspis* clade first emerged in the Eocene, when savanna and grassland habitats began to expand, following global cooling and the fragmentation of the pan-African forest (Couvreur et al., 2008; Zachos et al., 2001). Diversification continued from the early Miocene to the Plio-Pleistocene as cooling conditions progressed, causing the expansion of ideal habitats for *Panaspis* in sub-Saharan Africa. The presence of wind-pollinated taxa and grazing vertebrates in

the fossil record helped determine that main savanna development in southern Africa took place from the early Miocene to the Holocene (Jacobs, 2004). This timeframe coincides with the transition from C_3 to C_4 vegetation, which altered the diets of many mammalian grazers and caused shifts in their distribution (Sepulchre et al., 2006). Northern and southern savanna areas increased in East Africa during the mid-Miocene, encouraging colonization by various vertebrate lineages to "open" habitats (Voelker et al., 2012). Transition from woodlands to grasslands in the Miocene is also attributed to alterations in the concentration of atmospheric CO_2 caused by cooling of the Indian Ocean and glacial cycles (Sepulchre et al., 2006). Further climate changes were also caused by rifting processes such as the formation of the East African Rift System in the early Oligocene and its completion in the mid-Miocene (Roberts et al., 2012). Global temperature changes during the Pliocene caused the Afrotropical forest to expand eastward to coastal Kenya, and resulted in the division of the northern and

Table 4
Estimated median dates and highest posterior densities (HPD) for nodes of interest from our BEAST analysis.

Node	Median Age (mya)	Epoch	95% HPD (mya)
Konkasaurus (Krause et al., 2003)	63.4	Late Cretaceous	59.0–72.2
Contogenys and *Sauriscus* (Bryant, 1989; Estes, 1969)	71.3	Late Cretaceous	64.5–82.9
Panaspis/Afroablepharus	51.6	Eocene	42.8–62.4
P. togoensis	10.6	Miocene	4.6–17.7
Afroablepharus africanus, *Panaspis cabindae*	28.7	Late Oligocene	17.9–40.0
A. sp. Ethiopia	33.0	Oligocene	25.6–41.7
A. sp. Limpopo, *A.* sp. Namibia, *A. maculicollis*, *A.* sp. Mozambique 1	25.4	Late Oligocene	18.5–33.0
A. sp. Limpopo, *A.* sp. Namibia	14.8	Miocene	8.3–21.5
A. maculicollis, *A.* sp. Mozambique 1	14.4	Miocene	9.4–20.5
A. sp. Mozambique 2	30.3	Late Oligocene	21.8–38.5
A. sp. Malawi, *A.* sp. Mozambique 3, *A.* sp. Mozambique 4	12.1	Miocene	7.3–17.6
A. sp. Katanga 1, *A.* sp. Katanga 2, *A. wahlbergi*, *A.* sp. Tanzania 1, *A.* sp. Tanzania 2	20.5	Miocene	14.6–26.8
A. sp. Katanga 1, *A.* sp. Katanga 2	14.5	Miocene	8.4–20.7
A. wahlbergi	6.7	Late Miocene	3.1–10.5
A. sp. Tanzania 1, *A.* sp. Tanzania 2	6.4	Late Miocene	3.5–10.9

southern savanna regions (Voelker et al., 2012). This loss of savanna habitat connectivity triggered diversification of major arid-adapted vertebrate lineages, which might explain the emergence of divergent *Panaspis* populations from Katanga, Mozambique, South Africa, and Tanzania (Fig. 3).

We found congruence between the ages of diversification of our clades and climatic and geologic events in sub-Saharan Africa. The western branch of the East African Rift System, covering northern Mozambique, formed in the late Oligocene around 25–26 mya (Roberts et al., 2012), and its completion thereafter (~20 mya) coincides with the radiations in our Mozambique clades (Fig. 3). Tiercelin and Lezzar (2002) suggested that the Eastern Arc Mountains and Southern Highlands of Tanzania arose during the late Miocene. Climatic shifts also took place during that time and encouraged the development of forest refugia in the region (Menegon et al., 2014). The sister clades from Arusha and Serengeti shared a common ancestor during this period (Fig. 3), and these climatic and orogenic changes likely promoted their allopatric speciation. Fossil records suggest rich reptile faunas during the Miocene in Namibia (Rage, 2003). There is congruence between our speciation patterns and aridification processes in that epoch, and the dates in our BEAST trees concur (Fig. 3). Tolley et al. (2008) described southwestern Africa as a "cradle of diversity" for species that survived the transition from C_3 to C_4 plant habitats. The extinction of C_3-dependent species implies that while not all species survived this transition, the remaining ones had the opportunity to diversify, thus creating a biodiversity hotspot. The presence of three sympatric populations in South Africa (*P. maculicollis*, *P. wahlbergi*, and *P.* sp. Limpopo) is supported by this hypothesis.

Similar patterns and timing of diversification have been demonstrated in other vertebrate groups with non-forest distributions, including African clawed frogs (Furman et al., 2015), cobras (Trape et al., 2009), and lizards (Diedericks and Daniels, 2014; Dowell et al., 2016; Makokha et al., 2007), which have all been shown to form complexes of divergent populations correlated with the expansion of C_4 grasslands during the Miocene. Subsequent aridification in the Pliocene and Pleistocene likely explain the more recent cladogenic events in our analyses (Fig. 3), which are similar to patterns in lions (Barnett et al., 2014; Bertola et al., 2011), mole rats (Faulkes et al., 2004), and ungulates (Lorenzen et al., 2012).

Recent divergence between the sister clades *P. maculicollis* and *P.* sp. Mozambique 1, and that of *P. wahlbergi* and numerous cryptic taxa in northern Mozambique (*P.* sp. Mozambique 2–5) and southern Malawi (*P.* sp. Malawi), may also be influenced by contemporaneous effects of the southwest extensions of the East African Rift System. Moore and Larkin (2001) suggested that flexure along the Kalahari-Zimbabwe (Rhodesia) axis severed the links between the Limpopo and the Okavango, Cuando and Zambezi Rivers, with

the formation of lakes in the depression northwest of the axis. The development of the Okavango, Linyanti and Zambezi Rivers, and their associated swamps and palaeolakes, as well as the concomitant decline of the influence of the Limpopo drainage, are all relatively recent (3 mya to present) events, and have been strongly affected by the tectonic history of the region (McCarthy, 2013; Moore and Larkin, 2001). The influence of these events on the biodiversity and biogeography of aquatic organisms have been studied (Cotterill, 2003, 2004; Goodier et al., 2011), however, the barrier effects of these changing patterns of inundation and drainage on fossorial and semifossorial species remain in their infancy.

4.3. Taxonomy and species boundaries

Greer (1974) erected *Afroablepharus* based on discrete morphological differences—the frontal scale being in contact with one supraocular, and the ablepharine eye condition. He restricted *Panaspis* to skinks with smooth body scales and terrestrial or fossorial habits. Greer's (1974) only specific characteristics for diagnosing *Panaspis* were having the frontal scale in contact with two supraoculars, and either a pre-ablepharine eye or lower mobile eyelids, the latter only applicable to *P. breviceps* and *P. togoensis*. All examined vouchers from our study that are formerly attributed to the genus *Afroablepharus* are consistent with Greer's (1974) explicit characteristics reserved for the genus. Schmitz et al. (2005) used mitochondrial data and broad sampling from *Panaspis sensu lato* to support the recognition of *Afroablepharus* as a full genus. However, they suggested an in-depth assessment of *Panaspis sensu stricto* because differences existed in the ecology of some of its species (e.g., *P. breviceps* is a lowland rainforest species). Furthermore, DNA sequences of *P. cabindae* (the type species) were not included, thus restricting taxonomic conclusions of the latter study. Based on our results as noted above, *Afroablepharus* (Greer, 1974) is transferred to the synonymy of *Panaspis* (Cope, 1868), which has taxonomic priority.

The species *P. breviceps* and *P. togoensis* formed reciprocally monophyletic clades with relatively long branch lengths (Fig. 2), thus refuting previous ideas that *P. togoensis* was a subspecies of *P. breviceps* (Hoogmoed, 1980; Loveridge, 1952). Because these two species are morphologically and genetically distinct from *Panaspis sensu stricto* (Fig. 2), their generic allocation, and that of *P. tristaoi* (a senior synonym of *P. nimbaensis*, Trape and Ineich, 2012), should be reassessed in the future. Excluding these species, our molecular and morphological analyses confirm that *Panaspis* should accommodate savanna skinks with pre-ablepharine and ablepharine eyes, as Broadley (1989) suggested.

We recovered strong support for the reciprocal monophyly of at least 13 lineages of *Panaspis*, most of which are likely to be new

species. Prior to this study, the prevailing belief was that *Panaspis wahlbergi* inhabited an enormous geographic area in southern and eastern Africa (Fig. 1, Branch, 1998; Spawls et al., 2002), and that this disjunct distribution could be explained by gaps in sampling. However, our phylogeny of samples initially identified as *P. wahlbergi* demonstrated that the species is included in a complex of at least 13 cryptic lineages that are genetically distinct (Fig. 2). Unpublished morphological data (MFM and EG, unpubl. data) also suggests the lineages are candidate species.

According to Spawls et al. (2002), *P. wahlbergi* is presumed to occupy a large area along the eastern coast of Tanzania and a small, disjunct population occurs at the southwestern tip of Lake Tanganyika (Fig. 1). However, it is likely that *P.* sp. Tanzania 1 corresponds to *Panaspis megalurus*, known from "the mid-altitude central plains of Tanzania, north and northwest of Dodoma" (Spawls et al., 2002). The suggested range for *P. megalurus* extends throughout the ecoregion it is found in, from Arusha southward to Dodoma. This range coincides with our samples from Arusha (Fig. 2). The type locality Kinjanganja in "Turu," as written by Nieden (1913), could not be pinpointed with accuracy (only latitude coordinates were provided in the original description), but it is believed to be located in central Tanzania, close to Dodoma (Uetz and Hošek, 2015), within the presumed range of this species.

Exploration of northwestern Mozambique has resulted in the description of new species and identification of reptile and amphibian taxa with unresolved taxonomic statuses (Branch et al., 2005; Portik et al., 2013a). Large areas of Mozambique remain unexplored because of inaccessibility in the Lichinga Plateau where Serra Jeci is situated, but the Niassa Game Reserve (NGR), located to the east of the plateau, is known to have the highest reptile diversity in Mozambique, including *Panaspis* (Branch et al., 2005). Our data suggest high levels of genetic diversity within *Panaspis* occurring in Mozambique, which requires additional population-level sampling for proper taxonomic assessment. To date, there are thorough vertebrate biodiversity assessments from very few areas of Mozambique and most lie south of the Zambezi River (Schneider et al., 2005). Political turmoil and loss of infrastructure have, until recently, curtailed exploration of northern Mozambique (Branch et al., 2005; Branch and Bayliss, 2009; Portik et al., 2013a). Peace, a burgeoning human population, and a surge in development are placing increasing environmental pressure on the region. Further herpetofaunal surveys in the region are urgently required to improve understanding of its biodiversity, endemism, and conservation priorities.

Based on distinctive morphology and proximity to type localities, we matched three lineages of our phylogeny to known species: *P. wahlbergi* (Smith, 1849), *P. maculicollis* Jacobsen and Broadley, 2000, and *P. cabindae* Bocage, 1866. *Panaspis wahlbergi* was described from the "country to the eastward of the Cape Colony" (Smith, 1849). The type locality of *P. wahlbergi* could not be pinpointed with accuracy because Smith (1849) gave ambiguous locality descriptions for most of his specimens, including this species (AMB, pers. comm.). Broadley and Howell (1991) restricted the type locality to Durban, KwaZulu-Natal to best fit Smith's (1849) description (i.e., likely the southeastern part of KwaZulu-Natal in South Africa). A problem with morphology also exists because, as stated by FitzSimons (1937), Smith collected various specimens, but the surviving types he chose to represent *P. wahlbergi* were not congruent in morphology with the dimensions he described. Given the problematic type localities from Smith for other species (AMB, pers. comm.), we recognize the type locality is most likely from eastern South Africa (Broadley and Howell, 1991; Smith, 1849). The type locality for *P. maculicollis* is from Klein Tshipise, in northeastern Limpopo Province, South Africa, and morphometric data for our *P. maculicollis* vouchers were nearly identical to the type description from Jacobsen and Broadley

(2000). The type species *P. cabindae* was described from the Cabinda Enclave in the northwestern, disjunct tip of Angola, and our vouchers are again consistent with the original description (Bocage, 1866).

Genetic samples from the *P. wahlbergi* clade in South Africa were collected from the putative restricted type locality (the Bluff, Durban per Broadley and Howell, 1991), and also from widely distributed localities within the country and its greater presumed range (light blue[1] samples in Fig. 1). Morphometric and color pattern data were used to match the examined types of *P. wahlbergi* (BMNH 1946.8.18.49 and 1946.8.18.50; MFM and EG, unpubl. data) to our vouchers from this clade. Based on our phylogenetic analyses, *P. wahlbergi* has a potentially large distribution that has yet to be thoroughly explored (Fig. 1), and broader sampling in eastern South Africa and Mozambique is needed to improve understanding of the distribution of the species.

The species *P. maculicollis* and *P. wahlbergi* were previously reported from Namibia (Bauer et al., 1993; Branch, 1998; Herrmann and Branch, 2013). However, it is unlikely that these Namibian populations are conspecific with either *P. wahlbergi* or *P. maculicollis*, because the ecoregions they inhabit are completely different, and our samples from Namibia are genetically distinct (Fig. 2). Namibia is mainly dominated by arid ecoregions, whereas South Africa contains mostly tropical and subtropical savannas. Nonetheless, both areas share a portion of the Kalahari Desert. Further sampling is required to document the full distribution of *P.* sp. Namibia and describe it as a new species. A sample (ANG 421) with a distinctive branch length from the southeastern corner of Angola, adjacent to the Namibian Zambezia Province (Caprivi Strip), was nested in our *P. maculicollis* clade, and additional sampling is needed to understand the distribution of this lineage as well.

The distribution of our *P. maculicollis* samples suggests the species is sympatric with *P. wahlbergi*, because they were collected in nearby localities (Fig. 1). There is an unknown lineage of *Panaspis* (*P.* sp. Limpopo) located in the vicinity of *P. maculicollis* and *P. wahlbergi*. Further research on this lineage is needed, as it is sister to the clade from Namibia, where *P. wahlbergi* and *P. maculicollis* had been previously reported. A similar case of this disjunct Namib-Limpopo distribution in skinks involves the species complex *Trachylepis punctulata* (Portik and Bauer, 2012). Additional sampling in and around the Kalahari may help clarify both cases of this biogeographic pattern. Specimens from various locations in Angola and western DRC were nested in a well-supported clade belonging to *P. cabindae*, demonstrating the species is more widespread in south-central Africa than previously assumed.

Several snake-eyed skink species from sub-Saharan Africa lack molecular sampling and are poorly known in general. Two of these species were described from the Rwenzori Massif between Uganda and DRC more than half a century ago: *Panaspis helleri* (Loveridge, 1932) at 2895 m (DRC) and *Panaspis burgeoni* (de Witte, 1933) at 2073 m (DRC). The Ethiopian species *Panaspis tancredi* should retain its full species status, but extensive sampling is required to confirm its distribution, because few specimens have been found (Boulenger, 1909; Largen and Spawls, 2006). Our only sample from western Ethiopia (TJC 264) is genetically distinct, and although it is morphologically similar to *P. wahlbergi*, the locality is outside the distribution of *P. tancredi* based on Largen and Spawls (2006), and therefore, we suspect it is a new species. The availability of *Ablepharus carsonii* for snake-eyed skinks from Zambia and Malawi (and possibly from Katanga, DRC), overlooked since being synonymized with *P. wahlbergi* by Loveridge (1953), also requires further study. The West African members of *Panaspis*,

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[1] For interpretation of color in Fig. 1, the reader is referred to the web version of this article.

P. breviceps (Peters, 1873), *P. togoensis* (Werner, 1902), and *P. tristaoi* (Monard, 1940), need to be examined in greater detail, because they all have lower mobile eyelids. Considering remaining taxa that were formerly members of the synonymized genus *Afroable-pharus*, *P. wilsoni* is only known from Sudan and *P. duruarum* resides in Cameroon, whereas *P. africanus* and *P. annobonensis* are located on volcanic islands of the Gulf of Guinea (Uetz and Hošek, 2015). The morphologically distinct species *P. smithii* (de Witte, 1936) is known from southeastern Katanga Province (DRC) and should be included in future studies.

We briefly explored the relationships between the closely related genera *Lacertaspis* and *Leptosiaphos*. Our phylogeny includes the respective type species of each genus and adopted the taxonomic nomenclature of Schmitz et al. (2005) (Fig. 2). All samples in the genus *Leptosiaphos* were recovered in a well-supported, distinct clade, but *Lacertaspis* was not reciprocally monophyletic (Fig. 2). However, these genera were recovered in reciprocally monophyletic clades in the BEAST analysis (Fig. 3). A more extensive phylogenetic analysis with deeper sampling of these genera is underway (EG, MFM, AS, unpubl. data) to tackle taxonomic discrepancies between these genera.

4.4. Conservation

African savannas cover large parts of the central and southern parts of the continent (Sodhi et al., 2007). They harbor the world's greatest diversity of ungulates and therefore a variety of predators. Termites are also abundant and contribute to soil fertility and serve as a principal food source for many semi-fossorial reptiles. About two fifths of land in Africa is covered by savannas, and most of that land is currently used for livestock farming to sustain local populations (Hassler et al., 2010; Sodhi et al., 2007). Savannas are constantly exposed to degradation because of poor farming management, uncontrolled fires, and mining, all of which threaten biodiversity in many unique areas of Africa, including the Niassa Game Reserve, Mt. Mulanje Biosphere Reserve, Quirimbas National Park of coastal northeastern Mozambique, the xeric savannas of Namibia, and the largely unprotected Katanga miombo savannas (Herrmann and Branch, 2013; Sodhi et al., 2007). However, many species of lizards in savannas are resilient after fires (Andersen et al., 2012; Costa et al., 2013; Gorissen et al., 2015) and other anthropogenic disturbances (Smart et al., 2005). Indeed, several specimens in our study were found in disturbed areas, including mining concessions (Table 1), agricultural plots (DMP, pers. comm), and even adjacent to an outhouse in the Bombo-Lumene Game Reserve (EG, pers. comm.). While it is likely that some of these species occur in relatively small populations, future studies are needed to determine whether the *Panaspis* included in this study should be assessed as threatened species.

Acknowledgments

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.2016.04.026.

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