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Out-of-Africa again: A phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions

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ABSTRACT

Despite the long popularity of *Charaxes* among collectors and researchers, their evolutionary history is largely unknown. The current and accepted species groupings and relationships within the genus are based exclusively on adult morphology and life histories. Here, we examine the monophyly and evolutionary affinities of the species-groups within the genus *Charaxes* and explore how they relate to members of their closest genera (*Euxanthe*, *Polyura* and *Palla*) using 4167 bp of sequence data from five (1 mitochondrial and 4 nuclear) gene regions. Within the proposed phylogenetic framework, we estimate ages of divergence within the genus and also reconstruct their historical biogeography. We included representatives of all known species-groups in Africa and Asia, all known species of *Euxanthe* and *Palla* and two exemplar species of *Polyura*. We found the genus *Charaxes* to be a paraphyletic group with regard to the genera *Polyura* and *Euxanthe*, contrary to the earlier assumption of monophyly. We found that 13 out of 16 morphologically defined species-groups with more than one species were strongly supported monophyletic clades. *Charaxes nichetes* is the sister group to all the other *Charaxes*. *Polyura* grouped with the Zoolina and Pleione species-groups as a well-supported clade, and *Euxanthe* grouped with the Lycurgus species-group. Our results indicated that the common ancestor of *Charaxes* diverged from the common ancestor of *Palla* in the mid Eocene (45 million years ago) in (Central) Africa and began diversifying to its extant members 15 million years later. Most of the major diversifications within the genus occurred between the late Oligocene and Miocene when the global climates were putatively undergoing drastic fluctuations. A considerable number of extant species diverged from sister species during the Pliocene. A dispersal–vicariance analysis suggests that many dispersal rather than vicariance events resulted in the distribution of the extant species. The genus *Polyura* and the Indo-Australian *Charaxes* are most likely the results of three independent colonizations of Asia by African *Charaxes* in the Miocene. We synonymize the genera *Polyura* (syn. nov.) and *Euxanthe* (syn. nov.) with *Charaxes*, with the currently circumscribed *Charaxes* subdivided into five subgenera to reflect its phylogeny.

1. Introduction

The genus *Charaxes* Ochsenheimer, 1816 (Lepidoptera, Nymphalidae, Charaxinae) comprises about 250 species distributed mainly in the African continent with a few (~30) occurring in tropical Asia and Australia, as well as one species (*Charaxes jasius*) which extends its range to the Palaearctic. The genus *Charaxes* is the most speciose group of butterflies in Africa apart from *Acraea* Fabricius 1807 (Larsen, 2005). They are generally medium to large sized and robust in structure, strong and powerful in flight, ubiquitous in distribution, colorful and showy in appearance and behavior. They are also versatile in feeding; their food sources range from fruits, through dung to carrion, with the last being the most preferred by the males. *Charaxes* are perhaps the most fascinating and admirable group of butterflies in Africa (if not the world). As Ackery et al. (1999) recount, no group of butterflies in Africa evokes so much passion and emotion as *Charaxes*. For this reason they have long been very popular with collectors. Testament to the extensive fondness for this group of butterflies among collectors is the enormous and readily available ecological information on the group and the existence of a relatively well-known alpha taxonomy.

Due to the high species richness of *Charaxes*, taxonomists often prefer to summarize and study them under subgroups. Consequently, species of *Charaxes* are at the moment placed into 19 putative species-groups in Africa, based almost exclusively on the morphology of the adult (hind)wings (Van Someren, 1963, 1964, 1966, 1967, 1969, 1970, 1971, 1972, 1974, 1975; Henning, 1989).

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Although Turlin (2005, 2007) proposes 22 species-groups, his review, also based on adult morphology and life history, is still under way and incomplete. Turlin's species-group categorization is not as widely accepted and operational as Van Someren's (1969–1975) and Henning's (1989) species-groups hypotheses. We therefore adopt van Someren (and Henning's) *Charaxes* species-group categorization for this study. Using this putative species-group categorization, the number of members in a species-group ranges from one (for four subgroups – Hadrianus, Zingha, Jahlusa, and Nichetes) to over 50 (in Etheocles). In the absence of a robust phylogenetic hypothesis, these traditional morphological hypotheses represent tentative phenetic relationships of *Charaxes* species in Africa. However, the lack of discretionary power of phenetic analyses to distinguish between phylogenetically uninformative traits inherited from an ancestor (plesiomorphies) and traits that evolved anew after divergence (synapomorphies) makes them liable to mislead.

The field of molecular systematics has grown significantly within the last decade with an advanced battery of molecular markers. Characteristic of this development is an increase in confidence, precision and accuracy of hypotheses used for testing the monophyly or otherwise of putative species-groups (Brooks et al., 2007). Using these improved technologies and advancements in molecular systematics, *Charaxes* and its putative sister taxa are being recovered and resolved as a distinctive clade (Charaxinae) in various higher level butterfly systematic studies (e.g. Brower, 2000; Wahlberg et al., 2003; Freitas and Brown, 2004; Peña et al., 2006; Peña and Wahlberg, 2008). Charaxinae consist of ~350 species and 20 genera worldwide. *Charaxes* alone makes up over 70% of the species in the subfamily Charaxinae. Two genera (*Palla* Hübner, 1819 and *Euxanthe* Hübner, 1819) also placed in Charaxinae are found exclusively in the Afrotropics. The remaining 17 genera (comprising ~125 species) occur mainly in the Neotropical region, with a few genera being found in the Oriental and Australasian regions. The relationships of the Charaxinae genera have not been the focus of any major study, although Peña and Wahlberg (2008) sampled single exemplar species of almost all of the genera in their study on the evolutionary history of Satyrinae butterflies. They found that *Charaxes*, *Euxanthe* and *Polyura* Billberg, 1820 form a monophyletic clade, with *Euxanthe* being the most immediate sister group to *Charaxes*. On the other hand, in taxonomic reviews (e.g. Smiles, 1982; Larsen, 2005), the closest group of butterflies to *Charaxes* is considered to be the genus *Polyura*, which is restricted to the Oriental region in distributional range. However, the evolutionary relatedness of this group with *Charaxes* has never been explored in detail.

As the evolutionary history of *Charaxes* is poorly known, the origin of the group and the reason for their success in Africa is unknown. To fully understand and appreciate the biogeographic and evolutionary patterns among these groups of butterflies in different continents, a molecular systematic probe into when and where important divergence events happened has recently been advocated (Wahlberg, 2006). The investigation of origin and times of diversification of species-groups is gaining place in modern systematics (Avise, 2000; Rutschman, 2006). Linked to an existing robust phylogenetic hypothesis, they provide useful information of the plausible drivers of the speciation process and/or events of the taxa group in study. A recent study (Peña and Wahlberg, 2008) postulates that the major Charaxinae lineages began diversifying between the Paleocene and Eocene era (35–52 million years ago or Mya), but it was not until between late Oligocene and early Miocene era (25–20 Mya) that the ancestor of *Charaxes* diverged from its immediate sister candidates and presumably started rapidly diversifying. Forces and reasons for this presumed rapid radiation of *Charaxes* over evolutionary time and their current wide distribution will be best studied within a robust phylogenetic framework.

Against this background, the aim of the study was to test the monophyly of *Charaxes* and its putative subgroups within a phylogenetic hypothesis reconstructed from molecular data of five gene regions. We also investigated the evolutionary relatedness of the *Charaxes* species-groups in Africa and how they naturally relate to the species on other continents. The position of *Charaxes* among its two Africa sister candidates (*Palla* and *Euxanthe*) and its closest morphological sister groups (*Polyura*) were examined in this study. Using the proposed phylogenetic hypothesis, we also estimated the times of the major splits in *Charaxes* and related these divergence times with external factors that might have contributed to the diversification of the genus. Finally a zoogeographic hypothesis and probable events that might have led to the wide colonization and/or dispersal of the *Charaxes* in other continents were investigated using a dispersal–vicariance analysis (Ronquist, 1997).

2. Materials and methods

2.1. Laboratory protocols

Selection of taxa for the study was based on available taxonomic information on the *Charaxes* species-group (Ackery et al., 1995; Larsen, 2005; Williams, 2008). As ingroups, the exemplar species were selected such that they represented all known 'informal' species-groups of *Charaxes* in Africa (a total of 125 specimens of 83 species). We also included as ingroups all known species of the two Charaxinae genera (*Euxanthe* and *Palla*) in Africa, three of ca. 30 Oriental *Charaxes* and two exemplar species of *Polyura.* Outgroups were selected to include other members of Charaxinae which are putatively closely related to *Charaxes*. The trees were rooted with two species of Satyrinae (*Bicyclus anynana* and *Morpho helenor*) and one species of Calinaginae (*Calinaga buddha*). Individuals of the selected taxa were collected from the field either by the authors or through collaborative effort with other collectors and researchers. Legs of sampled individuals were removed and either preserved dried or conserved in 96% ethanol. Detailed information of the sampled specimens is given in Table 1. Voucher specimens are deposited at the following centers: Eric Vingerhoedt collections, Belgium; African Butterfly Research Institute (ABRI), Kenya; Kwame Nkrumah University of Science & Technology, Ghana; Nymphalidae Systematics Group, Finland; and can be viewed at http://nymphalidae.utu.fi/db.php.

We extracted DNA from one or two leg(s) of individuals using QIAgen's DNEasy extraction kit. Samples stored in ethanol were first air dried at least two hours before extraction. We then amplified the following five gene regions of each extracted DNA sample; 1487 base pairs (bp) region of the cytochrome oxidase subunit I gene (COI) from the mitochondrial genome and four gene regions from the nuclear genome: 1240 bp of the Elongation Factor-1α (EF-1α) gene, 400 bp of the *wingless* (wg) gene, 617 bp of ribosomal protein subunit 5 (RpS5) gene and 411 bp of ribosomal protein subunit 2 (RpS2) gene. Primer-pairs for amplifying each specific gene region using Polymerase Chain Reaction (PCR) technique were taken from Wahlberg and Wheat (2008), and included the universal forward/reverse tail, which facilitated sequencing. The first three gene regions are considered to be standard in butterfly molecular systematics (Wahlberg et al., 2005), RpS5 has been used successfully in recent studies of nymphalids (Peña and Wahlberg, 2008; Wahlberg et al., 2009), and RpS2 was chosen as it appeared to be phylogenetically informative (Wahlberg and Wheat, 2008) and it amplified well from most *Charaxes* samples. GAPDH, which has also been successfully used in recent studies (Peña and Wahlberg, 2008; Wahlberg et al., 2009) does not amplify from *Charaxes* samples with the existing primers.

Table 1
Sampled species for the study, along with GenBank accession numbers and their current distribution. Percentages after the first mention of a species-group name give the coverage of all species sampled in this study. For the gene regions, – = PCR amplification failed. For the distribution NA, not applicable; C, Central Africa; E, Eastern Africa; S, Southern Africa; W, Western Africa; M, Malagasy; P, Palaearctic; and A, Asia.

Species-group	Species	Voucher code	Source of specimen	COI	EF-1α	Wingless	RpS5	RpS2	Distribution
Outgroup	*Calinaga buddha*	NW64-3	Stratford Butterfly Farm, UK	AY090208	AY090174	AY090141	EU141406	EU141685	NA
Outgroup	*Bicyclus anynana*	EW10-5	Zimbabwe	AY218238	AY218258	AY218276	EU141374	EU141660	NA
Outgroup	*Morpho helenor*	NW66-5	London Pupae Supplies, UK	AY090210	AY090176	AY090143	EU141407	EU141686	NA
Outgroup	*Agatasa calydonia*	NW111-8	Malaysia, Cameron Highlands	EU528310	EU528288	EU528266	EU528420	–	NA
Outgroup	*Agrias hewitsonius*	CP-M264	Peru	EU528311	EU528289	EU528267	EU528421	–	NA
Outgroup	*Anaea troglodyta*	NW92-2	USA, Florida	DQ338573	DQ338881	DQ338599	EU141428	EU141705	NA
Outgroup	*Anaea troglodyta*	NW152-18	Dominician Republic	GQ256760	GQ256896	GQ256650	–	–	NA
Outgroup	*Anaeomorpha splendida*	CP05-41	Peru	EU528313	–	EU528269	EU528423	–	NA
Outgroup	*Archaeoprepona demophon*	NW81-9	Stratford Butterfly Farm	AY090220	AY090186	AY090153	EU141424	–	NA
Outgroup	*Coenophlebia archidona*	CP-M269	Peru	EU528316	EU528293	EU528272	EU528429	–	NA
Outgroup	*Consul fabius*	NW109-16	Costa Rica	EU528317	EU528294	EU528273	EU528430	GQ257088	NA
Outgroup	*Fountainea ryphea*	NW106-1	Brazil	GQ256890	GQ257004	GQ256758	GQ257210	GQ257092	NA
Outgroup	*Hypna clytemnestra*	NW127-11	Brazil	DQ338574	DQ338882	DQ338600	EU528439	GQ257093	NA
Outgroup	*Memphis appias*	NW127-6	Brazil	DQ338575	DQ338883	DQ338601	EU528445	GQ257094	NA
Outgroup	*Polygrapha tyrianthina*	CP06-88	Peru	EU528324	EU528301	EU528281	EU528458	–	NA
Outgroup	*Prepona* sp.	CP-CI142	Peru	EU528326	EU528303	EU528283	EU528460	–	NA
Outgroup	*Prothoe franck*	NW103-5	Indonesia, Java	EU528327	EU528304	EU528284	EU528462	GQ257097	NA
Outgroup	*Siderone galanthis*	NW124-6	Costa Rica	EU528329	EU528306	EU528285	EU528464	–	NA
Outgroup	*Zaretis* sp.	CP05-05	Peru	EU528332	EU528309	–	EU528470	–	NA
Palla 4 (100%)	*Palla decius*	NW124-7	Ghana	DQ338576	DQ338884	–	EU141389	EU141674	WC
Palla	*Palla publius*	NW123-24	Ghana	GQ256891	GQ257005	–	GQ257211	–	WC
Palla	*Palla ussheri*	NW123-22	Ghana	GQ256892	GQ257006	–	GQ257212	–	WCE
Palla	*Palla violinitens*	KAP132	Ghana	GQ256893	GQ257007	–	GQ257213	–	WC
Palla	*Palla violinitens*	NW123-19	Ghana	GQ256894	GQ257008	–	GQ257214	–	WC
Nichetes 1 (100%)	*Charaxes nichetes*	ABRI-004	Zambia	GQ256840	GQ256964	GQ256716	GQ257168	GQ257062	WCE
Nichetes	*Charaxes nichetes*	ABRI-034	Zambia	GQ256841	GQ256965	GQ256717	GQ257169	GQ257063	WCE
Nichetes	*Charaxes nichetes*	NW114-14	Zambia	GQ256842	GQ256966	GQ256718	GQ257170	GQ257064	WCE
Lycurgus 5 (80%)	*Charaxes lycurgus*	KAP506	Ghana	GQ256833	GQ256957	GQ256709	GQ257160	GQ257056	WCE
Lycurgus	*Charaxes mycerina*	EV-0062	DR Congo	GQ256839	GQ256963	GQ256715	GQ257167	GQ257060	WC
Lycurgus	*Charaxes porthos*	NW118-11	Uganda	GQ256858	GQ256979	GQ256733	GQ257183	GQ257073	WC
Lycurgus	*Charaxes zelica*	KAP228	Ghana	GQ256876	GQ256995	GQ256749	GQ257200	–	WCE
Euxanthe 6 (100%)	*Euxanthe crossleyi*	NW103-15	Uganda	GQ256885	GQ257001	GQ256755	–	–	CE
Euxanthe	*Euxanthe eurinome*	NW131-10	Ghana	EU141357	EU136664	EU141238	EU141390	EU141675	WCE
Euxanthe	*Euxanthe madagascariensis*	EV-0066	Madagascar	GQ256886	GQ257002	GQ256756	GQ257207	GQ257089	M
Euxanthe	*Euxanthe tiberius*	EV-0064	Tanzania	GQ256887	–	–	–	GQ257090	E
Euxanthe	*Euxanthe trajanus*	FM-15	Cameroon	GQ256888	GQ257003	GQ256757	GQ257208	GQ257091	C
Euxanthe	*Euxanthe wakefieldi*	EV-0065	Tanzania	GQ256889	–	–	GQ257209	–	ES
Eupale 4 (100%)	*Charaxes dilutus*	UN0509	Zambia	GQ256795	GQ256925	GQ256679	GQ257127	–	CE
Eupale	*Charaxes eupale*	NW164-3	Uganda	GQ256807	GQ256935	GQ256690	–	GQ257039	WCE
Eupale	*Charaxes montis*	EV-0044	Rwanda	GQ256838	GQ256962	GQ256714	GQ257166	GQ257059	C
Eupale	*Charaxes subornatus*	EV-0043	Gabon	GQ256867	–	GQ256740	GQ257191	GQ257078	WCE
Polyura 21 (10%)	*Polyura moori*	NW121-24	Indonesia	EU528325	EU528302	EU528282	EU528459	GQ257095	A
Polyura	*Polyura schreiberi*	NW114-19	Indonesia	GQ256895	GQ257009	GQ256759	GQ257215	GQ257096	A
Pleione 2 (100%)	*Charaxes paphianus*	KAP108	Ghana	GQ256849	GQ256972	GQ256725	GQ257176	GQ257068	WCE
Pleione	*Charaxes pleione*	KAP100	Ghana	GQ256856	GQ256977	GQ256731	GQ257181	GQ257071	WCE
Zoolina 2 (100%)	*Charaxes kahldeni*	ABRI-023	DR Congo	GQ256825	GQ256951	GQ256704	GQ257153	GQ257053	C
Zoolina	*Charaxes kahldeni*	EV-0009	Cameroon	GQ256826	GQ256952	GQ256705	GQ257154	GQ257054	C
Zoolina	*Charaxes zoolina*	ABRI-024	Ethiopia	GQ256879	GQ256998	GQ256752	GQ257203	GQ257085	CESM
Zoolina	*Charaxes zoolina*	ABRI-026	Ethiopia	GQ256880	GQ256999	GQ256753	GQ257204	GQ257086	CESM
Zoolina	*Charaxes zoolina*	EV-0010	Rwanda	GQ256882	GQ257000	GQ256754	GQ257206	GQ257087	CESM
Bernardus 30 (7%)	*Charaxes bernardus*	NW134-10	Bangladesh	GQ256777	GQ256910	GQ256664	GQ257112	GQ257022	A
Bernardus	*Charaxes bernardus*	NW134-11	Bangladesh	GQ256778	GQ256911	GQ256665	–	GQ257023	A
Bernardus	*Charaxes bernardus*	NW134-12	Vietnam	GQ256779	–	GQ256666	GQ257113	GQ257024	A
Bernardus	*Charaxes marmax*	UN0479	Vietnam	GQ256836	GQ256960	GQ256712	GQ257164	GQ257057	A
Solon 1 (100%)	*Charaxes solon*	NW134-13	Bangladesh	GQ256866	–	GQ256739	GQ257190	GQ257077	A
Jahlusa 2 (100%)	*Charaxes jahlusa*	ABRI-022	Kenya	GQ256818	GQ256946	GQ256700	GQ257148	GQ257048	CES
Jahlusa	*Charaxes jahlusa*	ABRI-025	Kenya	GQ256819	GQ256947	GQ256701	GQ257149	GQ257049	CES
Etesipe 6 (83%)	*Charaxes achaemenes*	ABRI-018	Zambia	GQ256761	GQ256897	GQ256651	GQ257098	GQ257010	WCES
Etesipe	*Charaxes achaemenes*	KAP505	Ghana	GQ256762	GQ256898	GQ256652	GQ257099	GQ257011	WCES
Etesipe	*Charaxes cacuthis*	EV-0042	Madagascar	GQ256788	GQ256919	–	GQ257121	–	M
Etesipe	*Charaxes etesipe*	KAP149	Ghana	GQ256799	GQ256929	GQ256683	GQ257131	GQ257037	WCES
Etesipe	*Charaxes etesipe*	NW164-2	Uganda	GQ256800	–	GQ256684	GQ257132	GQ257038	WCES

(*continued on next page*)

Table 1 (*continued*)

Species-group	Species	Voucher code	Source of specimen	COI	EF-1α	Wingless	RpS5	RpS2	Distribution
Etesipe	*Charaxes penricei*	EV-0041	DR Congo	GQ256851	GQ256974	GQ256727	GQ257178	GQ257070	CE
Etesipe	*Charaxes tavetensis*	ABRI-003	Kenya	GQ256869	GQ256988	GQ256742	GQ257193	GQ257080	ES
Anticlea 7 (71%)	*Charaxes anticlea*	KAP292	Ghana	GQ256772	GQ256907	GQ256661	GQ257109	GQ257020	WCE
Anticlea	*Charaxes baumanni*	ABRI-008	Kenya	GQ256774	GQ256908	GQ256662	GQ257110	GQ257021	E
Anticlea	*Charaxes blanda*	ABRI-013	Kenya	GQ256782	GQ256914	GQ256669	GQ257116	GQ257027	E
Anticlea	*Charaxes hildebrandti*	KAP113	Ghana	GQ256815	GQ256943	GQ256697	GQ257145	GQ257046	WC
Anticlea	*Charaxes opinatus*	ABRI-015	Rwanda	GQ256847	GQ256971	GQ256723	GQ257175	GQ257067	E
Etheocles 56 (32%)	*Charaxes aubyni*	EV-0051	Kenya	GQ256773	–	–	–	–	NA
Etheocles	*Charaxes berkeleyi*	EV-0052	Kenya	GQ256776	GQ256909	GQ256663	GQ257111	–	E
Etheocles	*Charaxes congdoni*	EV-0053	Tanzania	GQ256792	GQ256923	GQ256677	–	–	E
Etheocles	*Charaxes ethalion*	ABRI-001	Tanzania	GQ256801	GQ256930	GQ256685	GQ257133	–	ES
Etheocles	*Charaxes ethalion*	EV-0054	Kenya	GQ256802	GQ256931	GQ256686	GQ257134	–	ES
Etheocles	*Charaxes etheocles*	KAP147	Ghana	GQ256803	GQ256932	GQ256687	GQ257135	–	WCE
Etheocles	*Charaxes etheocles*	KAP296	Ghana	GQ256804	GQ256933	GQ256688	GQ257136	–	WCE
Etheocles	*Charaxes galawadiwosi*	EV-0058	Ethiopia	GQ256812	GQ256940	GQ256695	GQ257143	–	E
Etheocles	*Charaxes guderiana*	EV-0057	Rep. Democratic of Congo	GQ256813	GQ256941	GQ256696	GQ257144	GQ257044	ES
Etheocles	*Charaxes howarthi*	EV-0059	Rep. Democratic of Congo	GQ256816	GQ256944	GQ256698	GQ257146	–	E
Etheocles	*Charaxes kirki*	EV-0056	Ethiopia	GQ256827	GQ256953	GQ256706	GQ257155	–	E
Etheocles	*Charaxes maccleeryi*	EV-0048	Tanzania	GQ256834	GQ256958	GQ256710	GQ257161	–	E
Etheocles	*Charaxes mafuga*	ABRI-012	Rwanda	GQ256835	GQ256959	GQ256711	GQ257162	–	C
Etheocles	*Charaxes northcotti*	EV-0068	Cameroon	GQ256845	GQ256969	GQ256721	GQ257173	–	W
Etheocles	*Charaxes pembanus*	EV-0067	Pemba island	GQ256850	GQ256973	GQ256726	GQ257177	GQ257069	E
Etheocles	*Charaxes petersi*	EV-0049	Guinea	GQ256852	GQ256975	GQ256728	GQ257179	–	W
Etheocles	*Charaxes plantroui*	KAP507	Ghana	GQ256855	GQ256976	GQ256730	GQ257180	–	W
Etheocles	*Charaxes sidamo*	EV-0050	Ethiopia	GQ256863	GQ256984	–	–	–	E
Etheocles	*Charaxes turlini*	ABRI-016	Uganda	GQ256871	GQ256990	GQ256744	GQ257195	–	E
Etheocles	*Charaxes virilis*	KAP071	Ghana	GQ256873	GQ256992	GQ256746	GQ257197	–	WC
Etheocles	*Charaxes virilis*	KAP508	Ghana	GQ256874	GQ256993	GQ256747	GQ257198	–	WC
Zingha 1 (100%)	*Charaxes zingha*	KAP165	Ghana	GQ256877	GQ256996	GQ256750	GQ257201	GQ257084	WC
Zingha	*Charaxes zingha*	NW133-1	Ghana	GQ256878	GQ256997	GQ256751	GQ257202	–	WC
Hadrianus 1(100%)	*Charaxes hadrianus*	EV-0039	Guinea	GQ256814	GQ256942	–	–	GQ257045	WC
Cynthia	*Charaxes boueti*	KAP050	Ghana	GQ256786	GQ256917	GQ256672	GQ257119	GQ257030	WCE
Cynthia	*Charaxes cynthia*	NW107-11	Uganda	GQ256794	GQ256924	GQ256678	GQ257126	GQ257035	WCE
Cynthia	*Charaxes lasti*	EV-0020	Tanzania	GQ256830	–	–	GQ257157	–	E
Cynthia	*Charaxes protoclea*	KAP251	Ghana	GQ256860	GQ256981	GQ256735	GQ257185	–	WCES
Cynthia	*Charaxes protoclea*	KAP163	Ghana	GQ256859	GQ256980	GQ256734	GQ257184	–	WCES
Varanes 8 (38%)	*Charaxes acuminatus*	ABRI-010	Tanzania	GQ256765	GQ256901	GQ256655	GQ257102	GQ257014	E
Varanes	*Charaxes fulvescens*	KAP299	Ghana	GQ256811	GQ256939	GQ256694	GQ257142	GQ257043	WCE
Varanes	*Charaxes varanes*	KAP503	Ghana	GQ256872	GQ256991	GQ256745	GQ257196	GQ257082	WCESP
Candiope 5 (60%)	*Charaxes antamboulou*	EV-0018	Madagascar	GQ256771	GQ256906	GQ256660	GQ257108	GQ257019	M
Candiope	*Charaxes candiope*	KAP504	Ghana	GQ256790	GQ256921	GQ256675	GQ257123	GQ257033	WCES
Candiope	*Charaxes candiope*	KAP273	Ghana	GQ256789	GQ256920	GQ256674	GQ257122	GQ257032	WCES
Candiope	*Charaxes cowani*	ABRI-020	Madagascar	GQ256793	–	–	GQ257125	–	M
Jasius 21 (48%)	*Charaxes ansorgei*	ABRI-002	Rwanda	GQ256769	GQ256904	GQ256657	GQ257105	GQ257016	CE
Jasius	*Charaxes ansorgei*	EV-0029	Rwanda	GQ256770	GQ256905	GQ256658	GQ257106	GQ257017	CE
Jasius	*Charaxes brutus*	KAP081	Ghana	GQ256787	GQ256918	GQ256673	GQ257120	GQ257031	WCES
Jasius	*Charaxes castor*	NW78-3	Stratford Pupae Farm	AY090219	AY090185	AY090152	EU141422	EU141700	WCES
Jasius	*Charaxes druceanus*	ABRI-032	South Africa	GQ256796	GQ256926	GQ256680	GQ257128	–	CES
Jasius	*Charaxes druceanus*	EV-0028	Kenya	GQ256797	GQ256927	GQ256681	GQ257129	–	CES
Jasius	*Charaxes ducarmei*	EV-0034	DR Congo	GQ256798	GQ256928	GQ256682	GQ257130	GQ257036	C
Jasius	*Charaxes eudoxus*	ABRI-019	Rwanda	GQ256805	GQ256934	GQ256689	GQ257137	–	WCE
Jasius	*Charaxes jasius*	EV-0022	Kenya	GQ256822	GQ256948	GQ256702	GQ257150	GQ257050	WCESP
Jasius	*Charaxes jasius*	EV-0030	Ethiopia	GQ256823	GQ256949	–	GQ257151	GQ257051	WCESP
Jasius	*Charaxes jasius*	NW147-3	Italy	GQ256824	GQ256950	GQ256703	GQ257152	GQ257052	WCESP
Jasius	*Charaxes legeri*	EV-0023	Nigeria	GQ256831	GQ256955	GQ256707	GQ257158	GQ257055	W
Jasius	*Charaxes pollux*	KAP501	Ghana	GQ256857	GQ256978	GQ256732	GQ257182	GQ257072	WCE
Jasius	*Charaxes richelmani*	EV-0035	DR Congo	GQ256862	GQ256983	–	GQ257187	–	C
Lucretius 4 (50%)	*Charaxes lactetinctus*	EV-0025	Guinea	GQ256828	GQ256954	–	GQ257156	–	WC
Lucretius	*Charaxes lucretius*	KAP069	Ghana	GQ256832	GQ256956	GQ256708	GQ257159	–	WCE
Nobilis 3 (67%)	*Charaxes nobilis*	EV-0002	DR Congo	GQ256843	GQ256967	GQ256719	GQ257171	GQ257065	WC
Nobilis	*Charaxes nobilis*	EV-0003	Guinea	GQ256844	GQ256968	GQ256720	GQ257172	–	WC
Nobilis	*Charaxes superbus*	EV-0001	Gabon	GQ256868	GQ256987	GQ256741	GQ257192	GQ257079	C
Acraeoides 2 (100%)	*Charaxes acraeoides*	EV-0007	Gabon	GQ256764	GQ256900	GQ256654	GQ257101	GQ257013	C
Acraeoides	*Charaxes acraeoides*	ABRI-028	Gabon	GQ256763	GQ256899	GQ256653	GQ257100	GQ257012	C
Acraeoides	*Charaxes fournierae*	EV-0004	Rép. of Center Africa (RCA)	GQ256808	GQ256936	GQ256691	GQ257139	GQ257040	WC
Acraeoides	*Charaxes fournierae*	EV-0005	Rwanda	GQ256809	GQ256937	GQ256692	GQ257140	GQ257041	WC
Acraeoides	*Charaxes fournierae*	EV-0006	Guinea	GQ256810	GQ256938	GQ256693	GQ257141	GQ257042	WC
Tiridates 17 (71%)	*Charaxes ameliae*	KAP280	Ghana	GQ256767	GQ256903	GQ256656	GQ257104	GQ257015	WCE

Table 1 (*continued*)

Species-group	Species	Voucher code	Source of specimen	COI	EF-1α	Wingless	RpS5	RpS2	Distribution
Tiridates	*Charaxes bipunctatus*	KAP222	Ghana	GQ256780	GQ256912	GQ256667	GQ257114	GQ257025	WCE
Tiridates	*Charaxes bipunctatus*	KAP290	Ghana	GQ256781	GQ256913	GQ256668	GQ257115	GQ257026	WCE
Tiridates	*Charaxes bohemani*	ABRI-031	Zambia	GQ256784	GQ256915	GQ256670	GQ257117	GQ257028	CES
Tiridates	*Charaxes bohemani*	UN0504	Zambia	GQ256785	GQ256916	GQ256671	GQ257118	GQ257029	CES
Tiridates	*Charaxes cithaeron*	EV-0032	Kenya	GQ256791	GQ256922	GQ256676	GQ257124	GQ257034	ES
Tiridates	*Charaxes imperialis*	EV-0038	Guinea	GQ256817	GQ256945	GQ256699	GQ257147	GQ257047	WCE
Tiridates	*Charaxes mixtus*	EV-0036	Gabon	GQ256837	GQ256961	GQ256713	GQ257165	GQ257058	C
Tiridates	*Charaxes numenes*	KAP509	Ghana	GQ256846	GQ256970	GQ256722	GQ257174	GQ257066	WCE
Tiridates	*Charaxes phenix*	ABRI-005	Tanzania	GQ256853	–	–	–	–	NA
Tiridates	*Charaxes pythodoris*	EV-0037	Kenya	GQ256861	GQ256982	GQ256736	GQ257186	GQ257074	WCE
Tiridates	*Charaxes smaragdalis*	KAP502	Ghana	GQ256864	GQ256985	GQ256737	GQ257188	GQ257075	WCE
Tiridates	*Charaxes smaragdalis*	UN0798	Uganda	GQ256865	GQ256986	GQ256738	GQ257189	GQ257076	WCE
Tiridates	*Charaxes tiridates*	KAP098	Ghana	GQ256870	GQ256989	GQ256743	GQ257194	GQ257081	WCE
Tiridates	*Charaxes xiphares*	EV-0033	Rwanda	GQ256875	GQ256994	GQ256748	GQ257199	GQ257083	ES

All PCRs were performed in a 20 μL reaction volume. The thermal cycling profile for COI, Wingless and the second half of EF-1α (Al-EfrcM4) primer-pairs was 95 °C for 7 min, 40 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min followed by a final extension period of 72 °C for 10 min. The thermal cycling profile for RpS5, RpS2 and the first half of EF-1α (Starsky-Monica) differed only in an elevated annealing temperature of 55 °C, compared to 50 °C in the previous thermal cycling profile. All successful PCR products were cleaned of singled stranded DNA and unused primers using exonuclease I and calf intestine alkaline phosphatase enzymes. We then sequenced all cleaned PCR products using the universal primers (Wahlberg and Wheat, 2008). All DNA sequencing was done with an ABI PRISMR 3130xl capillary sequencer using dye terminator kits and following the recommendations by the manufacturers.

2.2. Phylogenetic analysis

The resultant DNA sequences of targeted gene regions were aligned by eye using the program BioEdit (Hall, 1999). Some of the sequences used in the study were taken from previous studies (Peña and Wahlberg, 2008). Phylogenetic and molecular evolutionary analyses were done separately for each gene and for all five genes combined. We assessed individual sequence properties using MEGA v. 4 (Tamura et al., 2007). For parsimony analyses, we treated all character states as equal and unordered, and employed the four New Technology Search algorithms (sectorial search, ratchet, drift and tree fusing) in combination with the traditional search algorithms in the program TNT (Goloboff et al., 2004) to heuristically search for the most parsimonious trees using 1000 random addition replicates. A strict consensus tree of all equally most parsimonious trees was subsequently produced. To appraise the stability and/or confidence of the resultant topology, we performed 1000 iterations of non-parametric re-sampling with replacement (bootstrapping) in TNT to generate support values (bootstrap percentages) for the individual nodes of our hypothesized most parsimonious phylogenetic tree. Bremer Support (BS) values were also calculated within the same TNT program for each internal node of the tree. For convenience, we refer to weak support for bootstrap values 50–64% (Bremer Support values 1–2), moderate support for bootstrap values 65–75% (Bremer Support values 3–5), good support for bootstrap values 76–88% (Bremer Support values 6–8) and strong support for bootstrap values 89–100% (Bremer Support values >10) (as in Peña et al., 2006) in the results and discussion sections. The contribution of each of the five gene partitions to the BS values was assessed using Partitioned Bremer Support (PBS) (Baker et al., 1998). We computed the PBS values for all nodes recovered in the strict consensus tree from the maximum parsimony analysis using a script written for TNT (see Peña et al., 2006). The degree of congruence between the five separate datasets was summarized using the Partition Congruence Index (PCI, Brower, 2006). This index is equal to the Bremer Support value when there is no conflict between datasets and has negative values when there is strong conflict between datasets (Brower, 2006). These analyses were intended to evaluate which nodes would be robust and stable to addition of new data.

2.3. Molecular dating

We also performed a Bayesian analysis using the program BEAST (Drummond and Rambaut, 2007). An advantage of BEAST compared to other software packages like MrBayes is its ability to estimate the topology and dates of divergence simultaneously, based on sequence data and specified model parameters. For this analysis, we first partitioned the data into two, based on genome. One partition consisted of combined sequence of the four nuclear genes, with the mitochondrial (COI) gene being the other partition. Although we assigned both partitions with the GTR+G model, the parameters were estimated separately for each partition. This model was preferred to the GTR+G+I, which chosen for both partitions based on AIC values in ModelTest (Posada and Crandall, 1998), because the parameters I (proportion of Invariant positions) and G (Gamma distribution) are strongly correlated and deeply intertwined such that it is impossible to tease them apart (Ren et al., 2005), making it likely that it leads to complications in estimating values for these parameters. The gamma function is enough for correcting for the rate variations among sites, including sites which do not change at all in the dataset.

There are no known fossils of *Charaxes*. However, a recent study based on fossil records estimates the age of the crown group of Charaxinae as 51.7 ± 5.7 Mya (Peña and Wahlberg, 2008). We therefore used this age as the calibration point for the crown group Charaxinae node in the analysis of times of divergence. The estimation of divergence times was performed within the Bayesian phylogenetic analysis, using the above model specifications. The relaxed molecular clock technique was used for the molecular dating, allowing branch lengths to vary according to an uncorrelated Lognormal distribution. The tree prior was set to the Yule process, and the "treeModel.RootHeight" prior (i.e. the age at the root of the tree) was set to 51.7 million years (with a standard deviation of 5.7 million years), in accordance with results from Peña and Wahlberg (2008). All other priors were left to the defaults in BEAST.

We then ran the analysis twice for 10,000,000 generations of MCMC analyses in BEAST and the chains were sampled at every 1000 generations, yielding a total of 10,000 samples for each run. We determined whether our parameter estimates and tree topol-

Fig. 1. The biogeographical areas used in this study.

ogy were at equilibrium using the program Tracer (Drummond and Rambaut, 2007). The first 1,000,000 generations (or 1000 trees) were discarded as burn-in. Posterior probabilities and error estimates (Posterior probability for the nodes, standard deviation and Bayesian credibility interval for the age estimates) were computed for each internal node estimate.

2.4. Biogeographical analysis

We constructed the historical biogeography of *Charaxes* using a dispersal–vicariance optimization model implemented in the DIVA program (Ronquist, 1997). The model, unlike the classic vicariance single pattern model, acknowledges the need for some level of dispersal in explaining the occurrence of widespread ancestors. DIVA therefore assigns a cost of one for assumed dispersal and extinction events and a zero cost for vicariance and within-area speciation. The optimal ancestral reconstruction of the DIVA model is the one with the least cost, i.e. the most parsimonious. DIVA requires that the phylogenetic relationships among species are fully resolved; we thus used the Bayesian topology for this analysis.

Based on earlier attempts to study butterfly zoogeography in Africa (Carcasson, 1981; Larsen, 2005), we categorized the distribution of African *Charaxes* into the following: Western African (W), Central Africa (C), Eastern Africa (E), Southern Africa (S) and Malagasy region (M) (Fig. 1). These delineations did not necessarily follow the subregional political boundaries. In this paper, Western Africa is bordered by the Sahara in the north, the Atlantic Ocean in the west and south and Western Nigeria and Niger in the east (as in Larsen, 2005). Central Africa stretches from eastern Nigeria to the western border of Uganda, down to the upper portions of Angola and Zambia. Eastern Africa covers areas from main Uganda to the Indian Ocean on the east and from Sudan and Eritrea in the north to northern portions of Mozambique. Stretching from lower Angola and Zambia to the Indian Ocean in the east and Atlantic in the west is the Southern Africa. The Malagasy region includes the main island Madagascar and all surrounding minor islands. Sampled taxa with geographical distribution outside the African continent were also included in the biogeographical analysis. These included the Oriental and Palaearctic regions.

3. Results

3.1. General properties of sequences

The final dataset consisted of 144 taxa, including 19 outgroups. For certain groups of taxa, we were unable to amplify all the five target genes (Table 1). For instance, we could not amplify and sequence the *wingless* gene of all our *Palla* exemplar samples. Similarly, generating RpS2 gene sequences of almost all the black Etheocles-group (except for *Charaxes blanda* and *C. guderiana*) was futile. Missing genes were coded as missing data in the combined analyses. In all, the complete combined sequence data contained 4167 nucleotides of which 1712 sites were variable. Approximately 80% (1328) of the variable sites were parsimony informative. At the individual gene level, *wingless* had the highest proportion of parsimony informative sites at 38%, followed closely by COI with 36%. The nuclear ribosomal genes (RpS2 and RpS5) and EF-1α on the other hand showed the highest proportions of conserved sites with each gene partition having about 62% of their sites being invariable (Table 2). On the whole, base frequencies were fairly even in the four nuclear genes but were strongly A–T biased in the mitochondrial COI gene (A = 0.308, T = 0.408, G = 0.138, C = 0.148).

3.2. Congruence of genes

An assessment of the relative contribution of each gene to the combined tree revealed that most of the conflicts in the combined tree were coming from the two ribosomal protein nuclear gene (RpS5 and RpS2) partitions. Out of the 122 nodes recovered in our strict consensus tree, RpS2 and RpS5 datasets conflicted in 34 and 32 nodes, respectively. The COI partition on the other hand, contained the least nodal conflict; lending support to the combined tree at 98 of its 122 nodes (Table 3). Interestingly there were very few cases of strong conflict between gene partitions (as suggested by PCI values in Table 3), with most conflict ranging between PBS values of −0.3 and −3. We observed that the COI gene partition carried most of the phylogenetic signal, sometimes overcoming the nodal conflicts emanating from the nuclear genes datasets. It carried on average 8 units of Bremer Support per node compared to next highest of 2.6 in RpS5 and 1.8 in EF-1α gene partitions. The COI gene resolved recent (shallow and terminal taxa) divergences with good support but deeper nodes were weakly supported in general. The opposite was true of EF-1α which had 13 and 9 of its 25 total conflicts occurring at the terminal and deep

Table 2
Basic statistics for the five sequenced genes.

	COI	EF-1α	Wgl	RpS5	RpS2
Taxa amplified	144	133	126	134	92
Base pairs	1487	1240	400	617	411
Conserved	805 (54.1%)	771 (62.2%)	224 (56%)	384 (62.2%)	256 (62.3%)
Parsimony informative	535 (36.0%)	321 (25.9%)	152 (38%)	193 (31.3%)	127 (30.9%)
Variable sites	682	469	176	230	155

Table 3
Support values for each branch node in Fig. 2. Bremer Support indices and bootstrap values from Maximum Parsimony analyses. PCI, Partition Congruence Index; PBS, Partitioned Bremer Support. PP (posterior probability) from Bayesian analysis. – = node has less than 50% bootstrap or PP.

Node		Bremer	PCI	PBS values					Bootstrap	PP
				COI	EF-1α	wgl	RpS5	RpS2		
1	Charaxinae	25	24.8	2.9	12.5	−2.6	13.1	−0.8	99	1.00
2	Outgroup	44	44.0	13.5	12.5	5	14	−1	100	
3	Outgroup	9	7.1	−5.5	7.5	6	4	−3	88	
4	Outgroup	30	29.9	19.5	7.5	4	−2	1	99	
5	Outgroup	13	12.8	−0.5	2.5	−1	8	4	92	
6	Outgroup	9	8.3	5.5	2.5	1	−3	3	59	
7	Outgroup	35	34.8	15.5	3.5	15	4	−3	99	
8	Outgroup	2	−3.0	6.5	0.5	−1	−1	−3	–	
9	Outgroup	2	−3.0	6.5	0.5	−1	−1	−3	–	
10	Outgroup	2	−3.0	6.5	0.5	−1	−1	−3	–	
11	Outgroup	2	−3.0	6.5	0.5	−1	−1	−3	–	
12	Outgroup	28	27.9	9	12	8	−1	0	100	
13	Outgroup	23	22.6	8.1	3.6	1.7	13.2	−3.7	100	
14	Palla	24	23.9	21.8	1.2	0	2	−1	100	0.99
15	Palla – Palla violinitens spp.	2	0.0	2.5	0.5	0	−2	1	92	0.99
16	Palla internal node (decius + violinitens)	3	1.7	2.5	1.5	0	−2	1	81	0.87
17	Palla internal node (ussheri + decius + violinitens)	12	11.7	10.5	2.5	0	−2	1	100	0.99
18	*Charaxes*	23	22.7	19.9	0.7	−3.5	5.5	0.4	100	0.99
19	Nichetes – *Charaxes* nichetes spp.	81	81.0	40.5	0.5	0	41	−1	100	1.00
20	*Charaxes* without Nichetes	8	6.6	6	−2.5	−3	4.5	3	100	0.62
21	Lycurgus internal node (mycerian + lycurgus)	3	0.8	4.4	−2	0	−1	1.5	51	0.85
22	Lycurgus internal node (porthos + zelica)	2	1.6	1.8	0.2	0.3	−0.4	0.1	–	0.86
23	Lycurgus subgroup	43	43.0	15.5	4.5	6	11	6	100	1.00
24	Euxanthe	20	19.8	20.8	0.2	0	−2	1	100	1.00
25	Euxanthe (eurinome) madagacariensis – wakefieldi	20	20.0	16.9	−0.2	1.7	1.3	0.3	100	1.00
26	Euxanthe (tiberius) tiberius + trajanus	24	23.6	20.5	−1.5	−3.5	2.5	6	100	1.00
27	Euxanthe internal node (crossleyi + wakefieldi)	1	0.0	1.4	−0.3	0	−0.2	0.1	–	–
28	Euxanthe internal node (eurinome – wakefieldi)	3	3.0	2.4	0.6	0	0	0	50	–
29	Euxanthe + Lycurgus-group	1	−5.0	0.5	0.5	3	−2	−1	–	0.49
30	Eupale internal node (dilutus + eupale)	21	20.9	19.5	0.5	0	2	−1	100	1.00
31	Eupale internal node (subornatus + dilutus + eupale)	6	5.1	3	0.8	−2.3	4.9	−0.4	56	1.00
32	Euplae subgroup	48	48.0	28.5	6.5	−1	9	5	100	1.00
33	Eupale + pleione + polyura + zoolina)	1	−4.8	−2.1	1.5	2.4	0	−0.8	–	–
34	Polyura	19	19.0	1.5	5.5	7	3	2	99	0.99
35	Polyura + Pleione group	3	1.5	2.4	1.4	0.5	−2.2	0.9	27	0.50
36	Polyura + zoolina + paphianus subgroup	15	14.6	−3.1	4.1	4.3	4.7	5	78	0.99
37	Pleione subgroup	22	21.6	21.8	−0.8	−3.5	2.5	2	100	0.99
38	Zoolina – *Charaxes* zoolina spp.	17	17.1	6.6	5.2	1.6	2.2	1.5	100	1.00
39	Zoolina – khaldeniEV-009 + zoolinaEV-0010	1	1.0	0	1	0	0	0	?36	1.00
40	Zoolina internal node (khaldeniEV-009 + zoolinaEV-0010 + khaldeniABRI023)	19	19.0	13	4	0	0	2	100	1.00
41	Zoolina subgroup	64	64.0	40.9	7.1	3	6.3	6.7	100	1.00
42	Etesipe – *Charaxes* achaemenes	45	45.0	26.4	8.7	0	2.8	7.1	100	1.00
43	Etesipe internal node (etesipe + penricei + taventensis)	26	26.0	21.7	−0.4	2.4	−0.1	2.4	100	1.00
44	Etesipe internal node (penricei + taventensis)	3	3.0	1.8	0.1	0	1.3	−0.1	100	1.00
45	Etesipe subgroup	28	28.0	11.5	−0.5	3	8	6	100	1.00
46	Etesipe + Hildebrandti subgroup	1	−5.0	0.5	0.5	3	−2	−1	–	–
47	Etesipe + Hildebrandti + Anticlea + Etheocles	1	−5.0	0.5	0.5	3	−2	−1	–	–
48	Etesipe + Hildebrandti + Anticlea + Etheocles + Jahlusa + Solon	5	3.0	−5.1	1.5	1	3.3	4.3	92	1.00
49	Anticlea + Etheocles (+ blanda + guderiana) subgroup	2	0.5	0.5	−1.4	0.7	2.3	−0.1	–	0.87
50	Anticlea internal node (opinatus + anticlea)	2	0.0	2.9	1.1	−0.6	−0.9	−0.5	–	0.99
51	Anticlea subgroup	34	34.0	18.3	−0.3	5	5	6	100	1.00
52	Etheocles – *Charaxes* ethalion spp.	2	1.2	1.7	0.7	0	−0.8	0.4	90	1.00
53	Etheocles – *Charaxes* etheocles spp.	4	4.0	2	2	0	0	0	98	1.00
54	Etheocles – *Charaxes* virilis	5	4.9	5.3	−0.3	0	0	0	99	1.00
55	Etheocles internal (blanda + guderiana)	1	−10.0	4.5	−3.5	−1	−1	2	–	–
56	Etheocles internal node (aubyni + ethalion)	2	0.8	3.2	−1.2	0	0	0	–	–
57	Etheocles internal node (berkeleyi – virilis)	3	2.8	3.3	−0.3	0	0	0	51	1.00
58	Etheocles internal node (cacuthis – virilis)	4	3.3	5.4	−1.3	0	−0.1	0	56	1.00
59	Etheocles internal node (howarthi – virilis)	1	−4.6	−2.7	3.5	0	0.3	−0.1	30	1.00
60	Etheocles internal node (maccleeryi + congdoni + cacuthis)	9	9.0	7	2	0	0	0	99	1.00
61	Etheocles internal node (pembanus – cacuthis)	2	1.0	0.3	1.5	1.2	−0.9	−0.1	–	1.00
62	Etheocles internal node (petersi + etheocles)	5	1.9	11.2	−4.7	−0.5	−2.5	1.5	91	1.00
63	Etheocles internal node (sidamo + galawadiwosi)	6	5.3	8.2	−2.2	0	0	0	96	1.00
64	Etheocles internal node (turilini – mafuga)	3	3.0	1.7	1.3	0	0	0	83	0.99
65	Etheocles subgroup (excluding blanda + guderiana)	9	8.6	9.8	0.2	0	−2	1	98	1.00
66	Etheocles subgroup (including blanda + guderiana)	15	15.1	14.6	0.6	0	−0.3	0.2	99	1.00
67	Zingha – *Charaxes* zingha spp.	56	56.0	45	4.5	0	1.5	5	100	1.00
68	Cynthia – *Charaxes* protoclea spp.	47	47.0	33.5	12.5	0	2	−1	100	0.99
69	Cynthia + Hadrianus subgroup	15	14.8	4.1	3.6	−0.6	0.4	7.4	96	1.00
70	Cynthia internal node (cynthia + lasti)	4	4.0	2	0	0	2	0	69	1.00
71	Cynthia internal node boueti – lasti)	29	29.0	22	0	0	7	0	100	1.00

(*continued on next page*)

Table 3 (*continued*)

Node		Bremer	PCI	PBS values					Bootstrap	PP
				COI	EF-1α	wgl	RpS5	RpS2		
72	Cynthia subgroup	9	8.4	−1.5	9.5	0	2	−1	87	1.00
73	Varanes internal node (acuminatus + varanes)	7	6.4	9	−2	0	0	0	90	0.97
74	Varanes subgroup	26	26.0	25.3	0.7	0	0	0	100	1.00
75	Asian clade (bernadus)	29	29.0	18	0	0	11	0	100	1.00
76	Asian clade (bernadus) internal node (bernadusNW134 – marmax)	1	−1.0	2	0	0	−1	0	–	–
77	Asian clade (bernadus) internal node (bernadusNW134 + marmax)	3	2.3	4	0	0	−1	0	73	1.00
78	Candiope – *Charaxes* candiope spp.	11	11.0	10.5	0	0	0	0.5	99	1.00
79	Candiope + Asian (bernardus) subgroup	21	20.8	−1	4.4	6.5	8.4	2.6	100	1.00
80	Candiope internal node (antambolou + candiope)	8	8.0	8	0	0	0	0	96	1.00
81	Candiope subgroup	11	11.0	4	0	0	7	0	99	1.00
82	Jasius – *CharaxesCharaxes* ansorgei spp.	5	4.9	−0.1	2.8	−0.1	2.4	0	98	1.00
83	Jasius internal node (castor + jasiusEV-0022)	2	1.5	0.2	0.8	1.3	0	−0.4	73	1.00
84	Jasius internal node (druceanus – lactetinctus)	1	−0.5	−0.7	1.5	−0.2	0.5	0	–	–
85	Jasius internal node (druceanus + ansorgei)	13	13.0	5	6	2	0	0	99	1.00
86	Jasius internal node (euxodus – lactetinctus)	5	3.0	−5	8.9	0	1.2	−0.1	81	1.00
87	Jasius internal node (jasiusEV-0023 – castor)	5	4.3	6.7	0	−0.7	0	−1	88	1.00
88	Jasius internal node (jasiusNW147-3 – castor)	48	48.0	31	3	3	5	6	100	1.00
89	Jasius internal node (lactetinctus + ducarmei + druceanus)	1	−0.4	0.9	0.5	0	−0.7	0.3	–	0.97
90	Jasius internal node (lucretius – lactetinctus)	1	−5.0	−2.5	3	−0.5	1	0	–	–
91	Jasius internal node (pollux – druceanus)	1	1.0	1	0	0	0	0	55	0.49
92	Jasius internal node (richelmani – druceanus)	5	3.5	8.4	0	−1	−1	−1.5	96	0.99
93	Jasius subgroup – Clade 1	13	13.0	4.5	0.5	1	6	1	99	1.00
94	Jasius subgroup – Clade 1 + 2	7	6.0	−2.5	3.5	1	6	−1	66	1.00
95	Nobilis – *Charaxes* nobilis	26	26.0	21.4	2.6	1.5	−0.5	1	100	1.00
96	Nobilis subgroup	40	39.9	17.4	0	0	22	0.5	100	1.00
97	Acraeiodes – *Charaxes* acraeiodes spp.	19	19.0	13.4	−0.3	3	0.8	2.1	100	1.00
98	Acraeiodes + Nobilis + tiridates subgroup	12	10.8	−7	10	1	4	4	94	0.99
99	Acraeiodes + Nobilis subgroup	6	5.9	1	−0.4	3.6	0.8	1	70	1.00
100	Acraeiodes subgroup	49	49.0	20.5	0.5	0	29	−1	100	1.00
101	Acraeoides – *Charaxes* fournierae spp.	21	20.9	20	2	0	0	−1	100	1.00
102	Acraeoides – *Charaxes* fournierae spp. (3 taxa)	22	22.0	17	1	2	2	0	100	1.00
103	Jahlusa subgroup	66	66.0	39.5	1.5	5	13	7	100	1.00
104	Tiridates – *Charaxes* bipunctatus spp.	21	21.0	17	1	1	1	1	100	1.00
105	Tiridates – *Charaxes* smaragdalis spp.	4	4.0	2	0	0	1	1	98	1.00
106	Tiridates internal node (ameliae + numenes)	1	−9.0	−5	3	1	1	1	–	1.00
107	Tiridates internal node (ameliae – smaragdalis)	2	−1.5	−3.3	2.5	−0.2	0.9	2.1	–	0.99
108	Tiridates internal node (bipunctatus – smaragdalis)	18	18.0	11.9	2.2	0	0.4	3.5	100	1.00
109	Tiridates internal node (bohemani ABRI_31 – smaragdalis)	6	5.7	4	−1	0	2	1	88	0.96
110	Tiridates internal node (bohemani_UN0504 – smaragdalis)	1	−6.0	−3.5	2	0.5	1	1	–	–
111	Tiridates internal node (bohemani_UN0504 + phenix)	3	2.7	1	−0.4	0	1.7	0.7	79	–
112	Tiridates internal node (cithaeron + tiridates)	3	2.3	1	−1	0	1	2	52	1.00
113	Tiridates internal node (mixtus + smaragdalis)	17	16.9	16	−1	1	1	0	100	1.00
114	Tiridates internal node (pythodoris – smaragdalis)	5	3.4	−4.1	2.9	0.2	2.2	3.8	–	1.00
115	Tiridates internal node (xiphares – smaragdalis)	1	−3.0	−2	1	0.3	1	0.7	–	–
116	Tiridates internal node (xiphares + tiridates + cithaeron)	2	−2.0	−3	1	−1	4.5	0.5	–	1.00
117	Tiridates subgroup	12	12.0	4	2	0	5	1	98	1.00
118	Tiridates + acraeoides + nobilis + jasius	3	−0.1	−3.7	3.2	−0.2	4.8	−1	–	–
119	Tiridates + acraeoides + nobilis + jasius + Asian + candiope	5	4.6	4	0.5	0	1.5	−1	58	0.99
120	Tiridates + acraeoides + nobilis + jasius + Asian + candiope + varanes	4	2.3	2.2	−0.4	−3	0.3	4.9	–	0.89
121	Tiridates + acraeoides + nobilis + jasius + Asian + candiope + varanes + cynthia + hadrianus	6	3.0	−8.6	3.2	4.2	5.9	1.2	–	0.99
122	Tiridates + acraeoides + nobilis + jasius + Asian + candiope + varanes + cynthia + hadrianus + zingha	1	−4.7	−0.6	0.7	2.1	0.6	−1.9	–	0.69

nodes, respectively (Table 3). They were however useful at the deeper splits, often overcoming the conflicts of the COI gene partition at those nodes.

3.3. Phylogenetic analyses

The maximum parsimony (MP) analysis of the combined data resulted in 36 equally parsimonious trees, of which the strict consensus is shown in Fig. 2. The Bayesian analysis produced a topology which was largely congruent with the strict consensus tree produced in the maximum parsimony analysis (Fig. 3). The Bayesian topology however was more resolved compared to the strict consensus tree of the most parsimonious trees. Also significant in this topology, is the position of the genus *Palla* as the sister group to *Charaxes*. The estimated parameter values of the models used in the Bayesian phylogenetic analysis are listed in Table 4.

Based on a comparison of the two topologies (Figs. 2 and 3), there appear to be several well-supported, distinct lineages within the *Charaxes* clade. According to this phylogenetic hypothesis, the genus *Charaxes* is not a monophyletic group with regard to *Euxanthe* and *Polyura*. The clade including all *Charaxes*, *Polyura* and *Euxanthe* species is however strongly supported. The genus *Euxanthe* is deeply nested inside *Charaxes* and appears to be sister to the Lycurgus-group of *Charaxes*, although this position has little support. The low support for the Lycurgus + *Euxanthe* node is due to some conflict from the ribosomal protein (RpS5, RpS2) genes. The

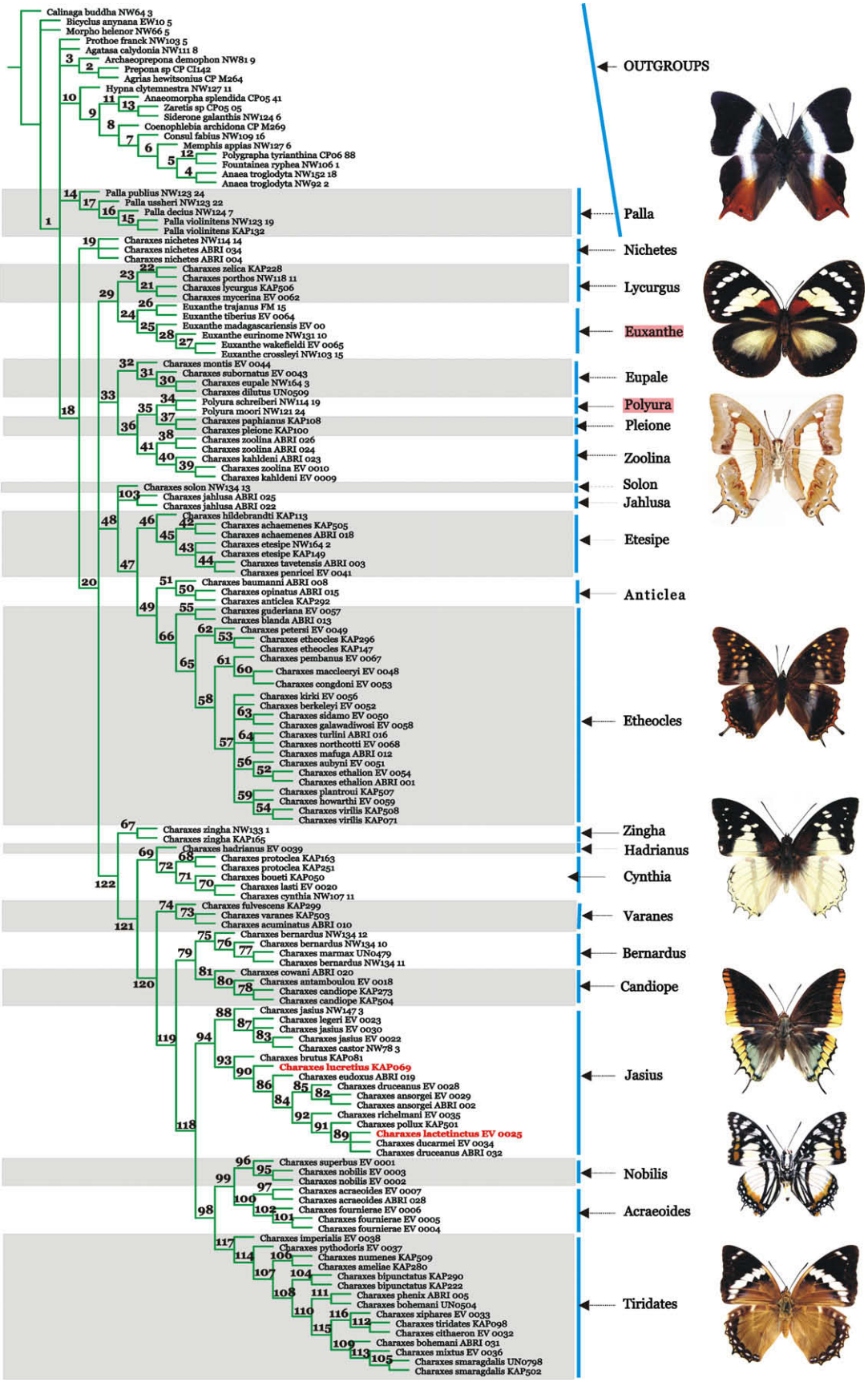

Fig. 2. Strict consensus of 36 most parsimonious trees found for the 5-gene combined dataset. Length = 10,250 steps, CI = 0.265, RI = 0.665. Clade numbers are indicated above branches. Corresponding bootstrap values, Bremer Support values, Partitioned Bremer Support values and Partition Congruence Indices are given in Table 3. Figured species are, from top to bottom, *Palla decius*, *Euxanthe trajanus*, *Polyura moori*, *Charaxes etheocles*, *Charaxes hadrianus*, *Charaxes epijasius*, *Charaxes superbus* and *Charaxes numenes*.

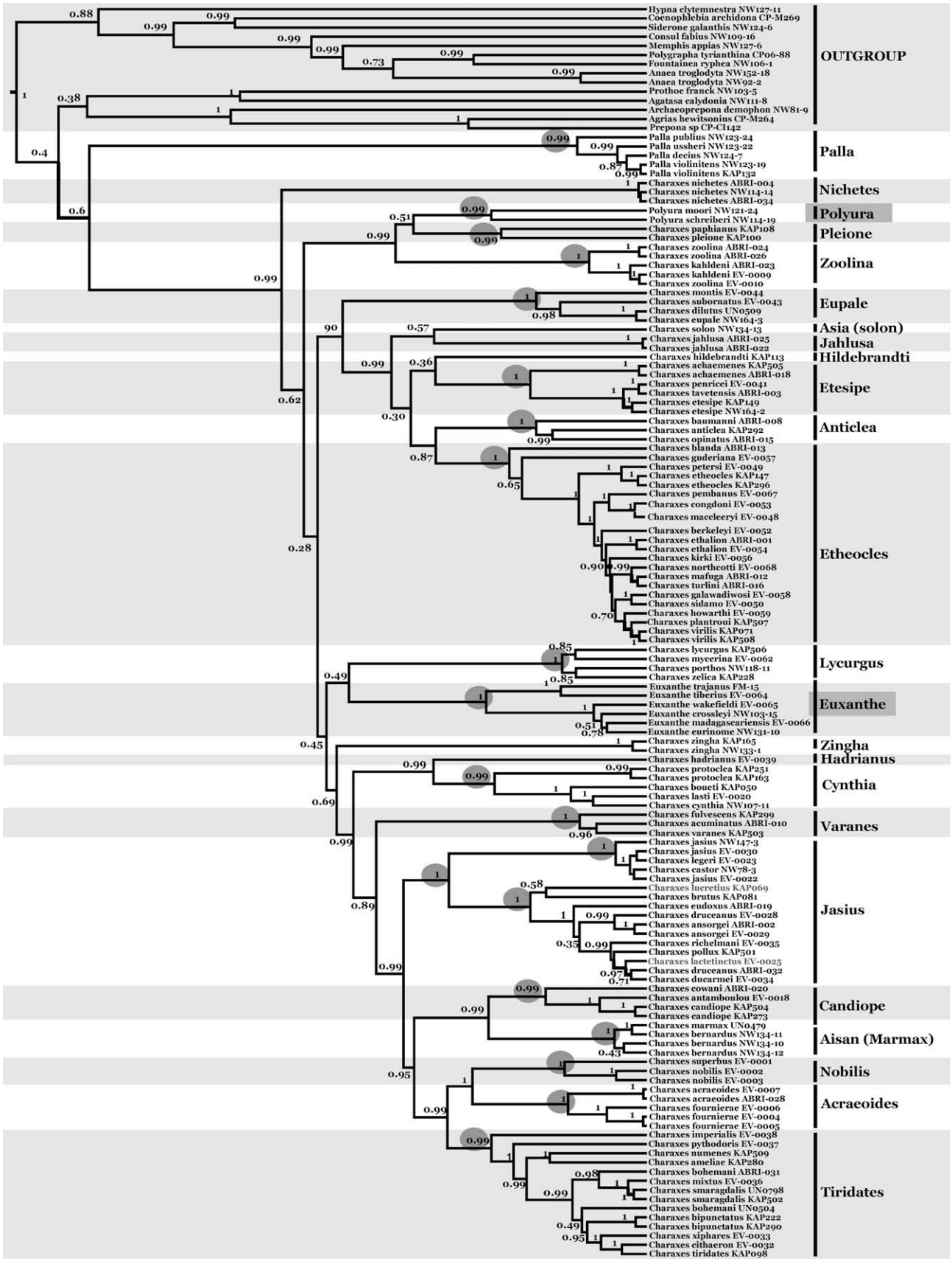

Fig. 3. Bayesian topology from the BEAST analyses. Numbers to the left of each node are the posterior probabilities of those nodes. Posterior probabilities of species-group clades are highlighted.

Table 4
Parameter values and their standard deviations in the Bayesian analysis using the program BEAST.

Parameter	Mean value	SD
likelihood	−49900	0.537
meanRate	0.003258	0.00000899
treeModel.rootHeight	50.437	0.07795
yule.birthRate	0.1	0.0002791
gtr.ac mtDNA	0.09547	0.000122
gtr.ag mtDNA	0.281	0.0002219
gtr.at mtDNA	0.178	0.0001044
gtr.cg mtDNA	0.01117	0.00006697
gtr.gt mtDNA	0.02195	0.00003888
siteModel.alpha mtDNA	0.195	0.00008895
gtr.ac nDNA	0.14	0.0001481
gtr.ag nDNA	0.503	0.0006957
gtr.at nDNA	0.266	0.0002735
gtr.cg nDNA	0.06388	0.00008375
gtr.gt nDNA	0.113	0.0001308
siteModel.alpha nDNA	0.278	0.00009175
ucld.mean	0.003322	0.00001156
ucld.stdev	0.216	0.00255
coefficientOfVariation	0.213	0.002546
covariance	0.001851E−3	0.001382
treeLikelihood mtDNA	−23960	0.622
treeLikelihood nDNA	−25940	0.657
speciation	−448.304	0.349

genus *Polyura* clustered with the Pleione- and Zoolina-groups with strong Bremer Support. In both topologies the Nichetes-group is the sister group to the rest of *Charaxes* including *Polyura* and *Euxanthe*, although the position of the Nichetes-group is not very strongly supported. Both analyses recover 13 of the 16 putative species-groups of African *Charaxes* with more than one species as monophyletic and with appreciable support values. The Anticlea-, Jasius- and Lucretius-groups were not recovered as monophyletic, with the Lucretius-group being polyphyletic within the Jasius-group. The Oriental *Charaxes* came out in two separate clades. The first monophyletic group consisted of *Charaxes bernardus* and *C. marmax* which appeared to share a common ancestor with the Candiope-group of *Charaxes* in Africa. The other Oriental clade was a monotypic group of *Charaxes solon*. Of the species with more than one specimen sampled, all were monophyletic except for *Charaxes bohemani*, *C. jasius*, *C. bernardus* and the two Zoolina-group species (*C. zoolina* and *C. kahldeni*). Most of the deeper nodes in the topologies were either unresolved or weakly supported obscuring the natural relationships among some subgroups.

3.4. Estimation of times of major divergence

Our times of divergence analysis revealed that the most recent common ancestor of *Charaxes* diverged from the common ancestor of the genus *Palla* in the mid Eocene (45 Mya) (Fig. 4). This geological period is characterized by the cooling of the early Eocene warm global climate and the reduction of global tropical forest dominance. Within *Charaxes*, we observed that the Nichetes-group is the oldest extant lineage of *Charaxes*, appearing to have diverged from the common ancestor of the rest of *Charaxes* in the Oligocene era (~30 Mya); 15 million years after the major split between *Palla* and *Charaxes*. The next group of *Charaxes* to have diverged after Nichetes was the common ancestor of the *Polyura* + Pleione + Zoolina clade. This occurred in the mid Oligocene (27 Mya). The Oligocene–Miocene boundary marked the beginning of major *Charaxes* diversification (Fig. 4). However, the peak of the evolutionary radiations, which subsequently gave rise to the current species-groups, appeared to have happened during the Miocene (24–10 Mya). The putative genera *Polyura* and *Euxanthe* are estimated to have branched off from their concomitant sister groups about 24 and 19 Mya, respectively. The estimated times of divergence between the African and the Asian (Solon and Bernardus) *Charaxes* species-groups are between 17 and 13 Mya.

3.5. DIVA inference of biogeographical patterns

Based on the dispersal–vicariance model, the resultant optimal ancestral state reconstruction suggested that the ancestor of *Charaxes* diverged from the ancestor of *Palla* in Africa, implying that *Charaxes* is of African origin. Where exactly in Africa this split occurred is uncertain. Although, as our DIVA analysis tells us, the ancestors of *Charaxes* might have been widely distributed in forests in Central and Eastern Africa with slight possibility of having been in Western Africa as well (Fig. 4). Many dispersal rather than vicariance events are responsible for the current *Charaxes* geographic distribution in and out of the Africa continent. It appears that Central Africa has been a very important area for the diversification of the older lineages of the genus. The ancestors of all the five identified old lineages of *Charaxes* traced back to the Central African region as their place of origin in the late Oligocene (Fig. 4). Our results suggest that there were several independent colonizations of species from Central Africa to the other parts of mainland Africa during this period of global forest expansion. Similar independent colonization events from Central Africa are observed to have occurred also in the Miocene era resulting in the common ancestors of the extant putative species-groups like Eupale-, Nobilis-, Acraeoides-, Lycurgus-, Tiridates- and Jasius-groups. Eastern Africa was also instrumental in the diversification of certain species-groups. Etheocles (and Anticlea) are clearly of East African origin. The distribution of the *Polyura* + Pleione + Zoolina clade is inferred from our DIVA analysis to be in forests in Central Africa, suggesting that the origin of the genus *Polyura* is Central African. The genus *Euxanthe* is believed to have diverged and started diversifying in forest refugia in Central and Eastern Africa. It also appears that Asia has been colonized independently three times, once by the ancestor of *Polyura*, once by the ancestor of *C. solon* and once by the ancestor of the rest of the Asian *Charaxes*.

4. Discussion

4.1. Phylogeny and systematic relationships

Many nodes in our phylogenetic hypotheses were resolved with moderate to strong support values and were stable to method used. The few unresolved or not well-supported nodes had relatively short branches, indicating low signal owing to possible rapid radiations rather than conflicting signals from the different gene partitions (Table 3). One factor likely to have contributed to the strong phylogenetic signal is our extensive taxon sampling coverage (Zwickl and Hillis, 2002). In most cases, we had sampled not less than 75% of all known species from a *Charaxes* species-group (Table 1). Because this study was primarily focused on African *Charaxes*, we only included few exemplar species of non-African *Charaxes* and *Polyura*. However, lack of adequate sampling for these groups did not seem to considerably affect the resolution of our trees.

We have clearly shown in our results that the genus *Charaxes* is a paraphyletic group, contrary to the earlier monophyletic assumption (Figs. 2 and 3). We recovered as part of *Charaxes* the genera *Polyura* and *Euxanthe*. The MP and Bayesian analyses produced a similar topology and with a well-supported node for these relationships. The recovery of *Euxanthe* as part of the *Charaxes* clade is unexpected and rather surprising. Morphologically, they look quite different to *Charaxes*, their strongly rounded forewings, as opposed to the falcate wings in *Charaxes*, and the complete lack

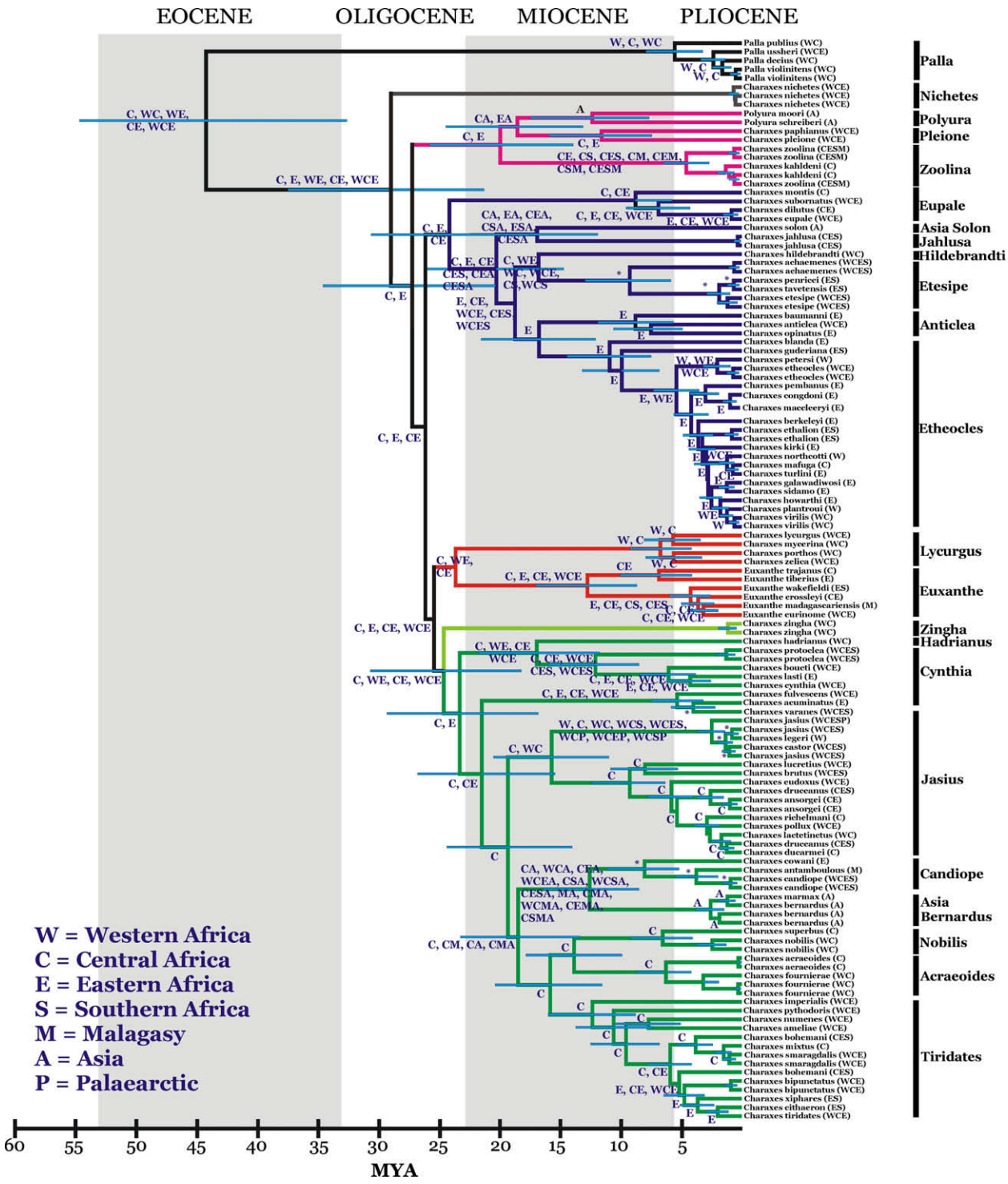

Fig. 4. Chronogram from the BEAST analyses with associated posterior credibility limits. Results of a dispersal–vicariance analysis, with maxareas set to 4 ancestral areas, are shown for each node. For nodes marked with asterisks there were too many possible ancestral distributions to fit on the figure. Colored clades reflect suggested subgenus divisions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of tails on the hindwing have even won them a separate tribe status among taxonomists. However, they share with *Charaxes* the serrated forewing costa. Interestingly, in common with our proposed phylogeny and earlier cladistic studies and revisions (Van Someren, 1975; Smiles, 1985; Larsen, 2005; Williams, 2008) of *Euxanthe*, is the splitting of the members into two groups with similar wing shape (often placed in two subgenera *Euxanthe* Hübner, 1819 and *Hypomelaena* Aurivillius, 1899). Although the wing shape of *Euxanthe* and *Charaxes* differ considerably, examination of their early stages also suggests they are closely related (Rydon, 1971; Van Someren, 1975). The relationship or position of *Euxanthe* with other *Charaxes* groups is not stable although it paired with the Lycurgus-group in both the MP and Bayesian topologies, but with weak Bremer Support (1) and very low posterior probability. From our phylogenetic analyses, the two groups diverged early and have undergone long independent evolution and that might well explain

the obscured or low phylogenetic signal. There are however some morphological traits shared by both *Euxanthe* and Lycurgus-groups. The absence or near lack of tails of members of these group is one such shared trait.

The recovery of *Polyura* within *Charaxes* was also unexpected. Originally planned in the study to be an outgroup, they clustered well inside the *Charaxes* clade with a more or less well-defined position and affinity with other *Charaxes* groups. On the other hand, species of *Polyura* in general look and behave very much like species of *Charaxes*, despite being given the status of a separate genus by earlier taxonomists (Smiles, 1982). Perhaps the only important morphological difference between these two closely related taxa is the venation of the hindwing cell, which is open in *Polyura*, but is closed in all *Charaxes* (Smiles, 1982). Aside from this trivial difference (known to vary considerably in Nymphalidae, e.g. Freitas and Brown, 2004), they share almost all the important synapomorphic characters used to define *Charaxes* (Smiles, 1982). There is even superficial resemblance in the underside pattern of some members of *Polyura* and Zoolina-groups. We suspect that the lack of a stable position of *Polyura* within the Zoolina + Pleione clade is due largely to inadequate taxon sampling of the former. We sampled only two of ~21 known *Polyura* species. However, we must add that we believe an increase in the taxon sampling of the group will not challenge our position of *Polyura* being part the *Charaxes* clade.

Our hypothesized topologies indicate strong evolutionary relatedness within the *Charaxes* species-groups. Most of the species-groups cluster as clades with moderate to strongly supported nodes. With the exception of the Anticlea, Jasius and Lucretius species-groups, our proposed phylogenetic hypotheses recovered the putative *Charaxes* species-groups in Africa as well-supported monophyletic groups. The two sampled members of the Lucretius-group (*Charaxes lucretius* and *C. lactetinctus*) were recovered in different positions within the Jasius-group. This is congruent with the recent revision of the genus by Turlin (2007) which does not recognize the putative Lucretius species-group proposed by other authors (Van Someren, 1963, 1964, 1966, 1967, 1969, 1970, 1971, 1972, 1974, 1975; Henning, 1989) as a natural group. In his revision, Turlin splits the widely accepted and used Lucretius-group into two separate species-groups (Lucretius and Lactetinctus) which our phylogenetic hypothesis corroborates, although at the cost of making the Jasius-group paraphyletic.

Our results therefore, to a large extent, support earlier *Charaxes* species-group hypotheses which were based almost exclusively on morphological similarities (Van Someren, 1963, 1964, 1966, 1967, 1969, 1970, 1971, 1972, 1974, 1975; Rydon, 1971; Henning, 1989). There were however a few but important inconsistencies. One such differing view is the grouping of *Charaxes hildebrandti* with the Anticlea-group in earlier species-group hypotheses. Our proposed hypothesis suggests *C. hildebrandti* deserves a discrete monospecies-group status. Quite surprisingly, it appears to be the sister species of the Etesipe-group, although this relation has weak Bremer Support (BS value of 1). Nevertheless, *C. hildebrandti* is definitely not within the Anticlea-group as earlier circumscribed.

Again, our hypothesis advocates a split of the Jasius-group into at least two subgroups to reflect the two clearly defined monophyletic units recovered within the Jasius clade. Turlin (2005) even suggests four subgroups, although our results suggest that his Pollux-, Euxodus- and Brutus-groups are not monophyletic and together form a clade distinct to his Jasius-group. We recovered Turlin's Lactetinctus monospecific group and two of his subgroups (Pollux and Euxodus) as a well-supported monophyletic group with 0.99 posterior probability and Bremer Support value of 5. Similarly we recovered Turlin's Lucretius-group with one of his subgroups (Brutus) as a clade but with low support. However, the putative Lucretius- and Lactetinctus-groups, together with Turlin's Pollux-, Euxodus-, Brutus-groups, constitutes one of the two strongly supported monophyletic groups within the putative Jasius clade recovered in our analyses. Perhaps it is more useful to redefine these species-groups as a single species-group to reflect this clade. These two monophyletic groups within the Jasius clade appear to have diverged about 16 Mya.

Further examination within the Jasius-group seems to lend support to an earlier position held by some taxonomists (Torben B. Larsen, pers. communication) that the only Mediterranean *Charaxes* species (*C. jasius*) is a distinct lineage and hence a separate species to the taxon called *C. jasius* found in tropical Africa. We sampled three individuals (and subspecies) of *C. jasius* from Italy, Kenya and Ethiopia. However, these three putatively conspecific individuals could not be recovered as a monophyletic group in our phylogenetic analysis. The Mediterranean sample (nominate subspecies, voucher code NW147-3) was observed to differ considerably from the samples of mainland Africa, which also did not cluster as expected of conspecific individuals. Rather, the Kenyan (*saturnus* ssp., voucher code EV-022) and Ethiopian (*epijasius* ssp., voucher code EV-020) *C. jasius* specimens grouped with *C. castor* and *C. legeri*, respectively. We think a detailed study of this complex from the Cape of South Africa through to the Mediterranean would yield insights that will further elucidate our understanding of this species-group.

Our study presents the first attempt to establish the internal evolutionary relationships within the genus *Charaxes* using molecular data. If the systematic order by which *Charaxes* subgroups (and species) appear in literature (e.g. Larsen, 2005; Williams, 2008) are taken to mean some kind of phylogenetic relatedness, then our study calls for a systematic revolt within the genus. The order by which these species-group appear hints of an informal acceptance of Henning's (1989) cladistic analysis of morphological characters, which puts the Varanes-group as a separate subgenus and Candiope-group as the sister group of all other *Charaxes* species-groups. Henning's (1989) proposed relationships are clearly and largely at variance with our proposed hypothesis. The position of the Varanes-group in our hypothesis suggests that the use of a subgenus (*Stonehamia* Cowan, 1968) for the subgroup is unnecessary. The Candiope-group, according our hypothesis, is not the sister to most of the species-groups, but rather part of a clade that includes the Jasius-group, to which the type species of *Charaxes* belongs.

Our results suggest that *Polyura* and *Euxanthe* should be synonymized with *Charaxes*, a taxonomic act which is bound to cause consternation among lepidopterists, since both genera have a long history of use. The alternative would be to split the currently circumscribed *Charaxes* into new genera, which in practice would mean that each of the well-supported species-groups should receive a genus-level name. We do not advocate such excessive splitting and thus recommend that *Polyura* Billberg, 1820 (syn. nov.) and *Euxanthe* Hübner, 1819 (syn. nov.) should be synonymized with *Charaxes* Ochsenheimer, 1816. The names remain available for use as subgenera, which we feel is the least disruptive way to classify species in the genus *Charaxes*.

There have been at least three separate connections of the African *Charaxes* with Asia. This is evidenced in the strong affinities our sampled Asian *Charaxes* have with some subgroups in Africa. For instance the Asian *Charaxes solon* was recovered by the Bayesian analysis as the immediate sister to the monospecific *Charaxes* subgroup Jahlusa. An even stronger affinity was observed between the Asian Bernardus-group and the Candiope-group in Africa. The last connection with Asia is evidenced in relation between *Polyura* and Zoolina + Pleione species-groups. An ongoing study is showing that the Asian *Charaxes* form a monophyletic group corresponding to our Bernardus-group, to the exclusion of *C. solon* (C. Muller, pers. communication), thus we believe the three Asian groups we have found represent all the connections between Africa and Asia in the *Charaxes* clade.

4.2. Historical biogeography

Africa is clearly shown in our study as the origin of the genus *Charaxes*. We suspect that the evolution of *Charaxes* and many of the divergence events were most likely climate-mediated. The genus is estimated to have evolved during the mid Eocene (45 Mya) when the world's climate and ecosystems began undergoing significant transformation. It is generally assumed that the beginning of the Eocene saw almost the entire earth covered by forests owing to a moist, conducive environment created by high temperatures and warm oceans. For instance we know that large portions Northern Africa, that are currently desert, were covered by rainforest (Jacobs, 2004). It is plausible that the ancestral populations of *Charaxes* at this period were distributed in large ranges of forests throughout Africa. However, most of these populations are suspected to have suffered from the mass global extinction that characterized the late Eocene and early Oligocene (40–33 Mya). Flora and fauna which could neither cope nor adapt to the drastic global cooling which sharply transformed the warm and humid climates to a relatively harsh dry one were forced into extinction. Perhaps the reason for the survival of the common ancestors of the Nichetes-group and other *Charaxes* could be inferred from habitats of the extant *C. nichetes*, which is a resilient species and able to thrive in varying environmental conditions. Their present geographical distribution spans across most parts of Africa with vicariant subspecies specializing in different forest and savannah habitats (Williams, 2008). It is likely they had the physiological capacity to adapt to the cooler and drier Eocene–Oligocene boundary environments.

Notwithstanding the strong resilience of the Nichetes-group, we also believe some refugial forests may have provided them with some level of protection from the harsh late Eocene and early Oligocene climate. Many of these postulated forest refugia that provided relatively stable forest environments are in Central and Eastern Africa (Couvreur et al., 2008). We suspect that ancestors of *Charaxes* were 'trapped' in some of the refugia until conditions were favorable (warmer and wetter) for them to expand their ranges. This perhaps explains why extant *Charaxes* only started diversifying 15 Mya after their split from the common ancestor with *Palla*. *Charaxes* diversification began in the Oligocene–Miocene boundary when the climate was relatively stable and saw concomitant expansion of rainforests in Africa. It appears that all the well-supported species-group lineages diverged fairly quickly during the mid to late Oligocene (30–23 Mya). The Oligocene–Miocene boundary is known to have marked the beginning of major diversification in many other Africa taxa, including African *Hyperolius* frog (Wieczorek et al., 2000), birds (Roy et al., 2001), Africa genets (Mayaux et al., 2004), mammals (Moritz et al., 2000) and trees in Annonaceae (Couvreur et al., 2008). The ancestors of *Charaxes* presumably expanded their ranges during this time through dispersal to new forest habitats.

However, the closure of the Tethys Sea in the mid Miocene caused drastic cooling of global temperature, reducing the ability of the atmosphere to absorb moisture (Zachos et al., 2001). Africa became drier and the condition gradually forced most forested lands to give way to grassland. The western and eastern forests were eventually separated during this period. The widespread aridification continued into the late Miocene, resulting in isolated refugia forests separated by savannah. For instance in Western Africa, the Guinea forests were separated by the Dahomey Gap (Lovett et al., 2005). Large tracts of Southern African subtropical woodlands were replaced by Fynbos (Scott et al., 1997). The rifting and uplifts of the Central African plateau and Eastern Mountain Arc are also believed to have further shrunk the refugial tropical rainforests in East Africa and thereby increasing the separation in lowland taxa. By the late Miocene, rainforests in Africa were limited to small patches in upland and possibly lowland river systems. This resulted in many major distributional disjunctions in populations of African taxa, most likely also leading to isolated populations of surviving *Charaxes* ancestors in fragmented landscapes, allowing for speciation by genetic drift. Adaptations of different species to particular forest fragments also set conditions for local speciation. As evidenced in our data, many of the present-day *Charaxes* lineages evolved during this period of rainforest retractions.

There are at least three separate links with Asia, giving rise to *Polyura*, the Bernardus-group and *C. solon*. The monophyly of *Polyura* has not been tested, but based on morphology it is quite likely to be a monophyletic group (Smiles, 1982). The monophyly of the Bernardus-group has been studied previously (C. Muller, personal communication), and the 30 species were found to form a strongly supported monophyletic group to the exclusion of *C. solon*, which appears as an independent Asian *Charaxes* lineage just as we found in our study. At the continental scale, the likelihood that vicariance played a significant role in this diversification process is rather low given that the break-up of Gondwana is known to have occurred about 100 Mya (Jokat et al., 2003). The three colonization events into Asia are dated between 19 and 14 Mya. Interestingly, land connection between the Africa and Asia is believed to have formed at this time (Willis and McElwain, 2002). It is therefore most likely that some descendants of the African *Charaxes* colonized Asia across the Arabian Peninsula, much as has been found for the nymphalid genus *Junonia* (Kodandaramaiah and Wahlberg, 2007). Contraction of tropical forest into isolated fragments following the intense cooler and drier climate in the mid and late Miocene perhaps caused permanent isolation of the populations in Africa and Asia.

The presence of *Charaxes* on Madagascar requires explanation, as its separation from Africa in the early Cretaceous (Rabinowitz et al., 1983) is much older than the age of the butterflies. There are nine Charaxinae members on Madagascar (8 *Charaxes* and an *Euxanthe*), which are all endemic. We sampled four of nine Madagascar Charaxinae members (*Euxanthe madagascariensis*, *Charaxes cowani*, *C. antamboulou*, *C. zoolina*). Our age estimates analysis suggest that at least three independent dispersal events from mainland Africa to Madagascar occurred between 20 and 13 Mya. Mainland African *Charaxes* dispersal to Madagascar is expected to be more than the observed because the unsampled Madagascar extant species fall into three other separate putative *Charaxes* species-groups, which intuitively suggests at least three additional colonization events.

Another significant period of *Charaxes* diversification is the Pliocene. The early Pliocene (5–3.5 Mya) was characterized by moist climate and rainforest expansion. Perhaps the role of Pleistocene climate oscillations in the diversification of taxa in African tropical rainforests was more significant for *Charaxes* than earlier supposed (Larsen, 2005). The oscillations resulted in repeated expansion and retraction of forests. Depending on the time lapse between the oscillations, new species could arise by adaptation and genetic drift as the evolutionary forces. During glacial maxima, species of *Charaxes* were perhaps limited to areas of high degrees of humidity and shade like galley forests in lowland and montane regions, which means large numbers of local extinctions were also likely. The Great Rift Valley and Congo Basin were both developed during the Pliocene and early Pleistocene (Plana, 2004), increasing the range of environment habitat options available to *Charaxes*. Most of the extant *Charaxes* species were defined during this period. One group of *Charaxes* that benefited immensely from these cyclic climatic changes is the Etheocles-group which appears still to be radiating. Three of the four extant *Palla* species only diverged recently (2.5–0.5 Mya), and even the fourth species *P. publius* diverged from the common ancestor of all extant *Palla* species only about 5 Mya.

5. Conclusion

We have shown that the genus *Charaxes* is a paraphyletic group with regard to *Euxanthe* and *Polyura*, contrary to the earlier assumptions of monophyly. The ancestors of *Charaxes* diverged from *Palla* in the mid Eocene (~45 Mya) and started diversifying 15 million years later. Past climatic events have been very instrumental in shaping the history of this species rich group. The estimated dates of major divergence and patterns of *Charaxes* diversifications are quite similar to the ones put forth for the nymphalid genus *Junonia* (Kodandaramaiah and Wahlberg, 2007) and *Bicyclus* (Monteiro and Pierce, 2001) in Africa. It is most probable that similar evolutionary signatures could be found in other African dominated nymphalid taxa like *Bebearia*, *Acraea*, *Euphaedra*, *Euriphene*, *Henotesia*, *Cymothoe*, *Neptis* and a number of others whose phylogeny has never been studied. We recommend future phylogenetic work on these African dominated nymphalid taxa. Our study furthers our understanding of the evolutionary processes that generate and sustain biodiversity in tropical faunas, and it is apparent that both Miocene and Pliocene climatic fluctuations shaped the current biodiversity distribution and composition.

The phylogenetic and biogeographic hypotheses now provide a framework within which we can implement studies of the possible reasons behind the success of *Charaxes* in Africa, where they occur abundantly. Further studies should investigate whether or not evolution of host plant use has had any effect on speciation rates. Finally, our results also demonstrate that the current systematics of the genus *Charaxes* does not reflect the phylogeny of the group. Based exclusively on molecular evidence provided in this study, we propose the following classification within the genus *Charaxes* (species-groups as defined by Henning, 1989):

New subgenus
- Species-group: Nichetes

Subgenus *Polyura* Billberg, 1820
- Species-group: Pyrrhus
- Species-group: Pleione
- Species-group: Zoolina

Subgenus *Eriboea* Hübner 1819
- Species-group: Eupale
- Species-group: Solon
- Species-group: Jahlusa
- Species-group: Hildebrandti
- Species-group: Etesipe
- Species-group: Anticlea
- Species-group: Etheocles

Subgenus *Euxanthe* Hübner, 1819
- Species-group: Euxanthe
- Species-group: Lycurgus

Subgenus *Charaxes* Ochsenheimer, 1816
- Species-group: Zingha
- Species-group: Hadrianus
- Species-group: Cynthia
- Species-group: Varanes
- Species-group: Jasius
- Species-group: Lucretius
- Species-group: Candiope
- Species-group: Bernardus
- Species-group: Tiridates
- Species-group: Nobilis
- Species-group: Acraeoides

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