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Savel Regan Daniels PhD
Stellenbosch University, South Africa, srd@sun.ac.za

E E. Phiri

Sebastian Klaus PhD
J. W. Goethe-University, Frankfurt, Germany

C Albrecht

Neil Cumberlidge
Northern Michigan University, ncumberl@nmu.edu

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Multilocus Phylogeny of the Afrotropical Freshwater Crab Fauna Reveals Historical Drainage Connectivity and Transoceanic Dispersal Since the Eocene

Savel R. Daniels[1,*], Ethel E. Phiri[1], Sebastian Klaus[2,3], Christian Albrecht[4], and Neil Cumberlidge[5]

[1]*Department of Botany and Zoology, Private Bag X1, University of Stellenbosch, Matieland 7602, South Africa;* [2]*Department of Ecology and Evolution, J. W. Goethe-University, Biologicum, Frankfurt am Main 60438, Germany;* [3]*Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Peoples Republic of China;* [4]*Department of Animal Ecology and Systematics, Justus Liebig University, Giessen 35392, Germany; and* [5]*Department of Biology, Northern Michigan University, Marquette, MI 49855-5376, USA*
Correspondence to be sent to: Department of Botany and Zoology, Private Bag X1, University of Stellenbosch, Matieland 7602, South Africa; E-mail: mailto:srd@sun.ac.za

Abstract.—Phylogenetic reconstruction, divergence time estimations and ancestral range estimation were undertaken for 66% of the Afrotropical freshwater crab fauna (Potamonautidae) based on four partial DNA loci (12S rRNA, 16S rRNA, cytochrome oxidase one [COI], and histone 3). The present study represents the most comprehensive taxonomic sampling of any freshwater crab family globally, and explores the impact of paleodrainage interconnectivity on cladogenesis among freshwater crabs. Phylogenetic analyses of the total evidence data using maximum-likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) produced a robust statistically well-supported tree topology that reaffirmed the monophyly of the Afrotropical freshwater crab fauna. The estimated divergence times suggest that the Afrotropical Potamonautidae diverged during the Eocene. Cladogenesis within and among several genera occurred predominantly during the Miocene, which was associated with major tectonic and climatic ameliorations throughout the region. Paleodrainage connectivity was observed with specimens from the Nilo-Sudan and East African coast proving to be sister to specimens from the Upper Guinea Forests in West Africa. In addition, we observed strong sister taxon affinity between specimens from East Africa and the Congo basin, including specimens from Lake Tanganyika, while the southern African fauna was retrieved as sister to the Angolan taxa. Within the East African clade we observed two independent transoceanic dispersal events, one to the Seychelles Archipelago and a second to Madagascar, while we observe a single transoceanic dispersal event from West Africa to São Tomé. The ancestral area estimation suggested a West African/East African ancestral range for the family with multiple dispersal events between southern Africa and East Africa, and between East Africa and Central Africa The taxonomic implications of our results are discussed in light of the widespread paraphyly evident among a number of genera. [Eocene; paleodrainage; Potamonautidae; sub-Saharan Africa and associated islands; zoogeography.]

Africa is the second largest continent and comprises approximately 20% of the total land surface of the planet. The terrestrial Afrotropical biota exhibits significant biodiversity richness and hosts 5 of the 25 global hotspots of biodiversity, including the Western Indian Ocean Islands (e.g., Madagascar and the Seychelles Archipelago), the Cape Floristic Region, the Succulent and Nama Karoo, the Guinean Forests, and the Eastern Afromontane hotspot (Myers et al. 2000). These regions exhibit remarkable levels of alpha and beta diversity, species richness, and endemism (Myers et al. 2000). Nevertheless, despite Africa's high biodiversity richness the evolutionary affinities of its fauna and flora have been poorly studied in comparison to those from Europe and North America. The Afrotropical region includes continental Africa south of the Sahara, plus the southern part of the Arabian Peninsula and a number of offshore islands including Socotra, the Seychelles Archipelago, and Madagascar (Fig. 1). The region has undergone significant paleoclimatic oscillations and geotectonic ameliorations that have impacted the rates of diversification and extinction and created unique biogeographic patterning among the continental terrestrial biota (Zachos et al. 2001; deMenocal 2004; Huber and Caballero 2011). Physiographically, the African continent can be divided into High Africa and Low Africa by a NE to SW imaginary diagonal line. High Africa, south of this line, includes the

Ethiopian Highlands, the East African Rift Valley, and the higher lands of southern Africa, while Low Africa, north of this line, includes the Sahara, the Sahel, and the lowland rainforests of the Congo drainage basin. The continent exhibits a complex spatial and temporal geohydrological network that includes several large river basins (e.g., the Congo, Nile, Niger, and Zambezi) and a series of large ancient lakes in the East African Rift Valley (e.g., Albert, Edward, Kivu, Tanganyika, Malawi, and Victoria). Structurally two major features are associated with the African hydrological network: the Central African Shear Zone and the East African Rift Valleys. The Central African Shear Zone produced by the general uplift of the African continent resulted in the modern drainage division between the Congo and the Nilo-Sudan drainage systems (Goudie 2005; Runge 2008). Some of these drainages date back to the Cretaceous, while others are remarkably young (Goudie 2005; Stankiewicz and de Wit 2006). The Congo basin is Africa's most ancient drainage system and possesses the highest diversity of endemic freshwater taxa on the continent (Cumberlidge 1999, 2011a, 2011b; Skelton 2001). During the late Cretaceous/Paleogene, the paleo-Congo drained east into the Indian Ocean (Stankiewicz and de Wit 2006) and was joined to the middle Zambezi-Luanga (Goudie 2005). Following the uplift of East Africa during the Oligocene or Eocene (30–40 Ma), the flow direction of the paleo-Congo reversed, and the
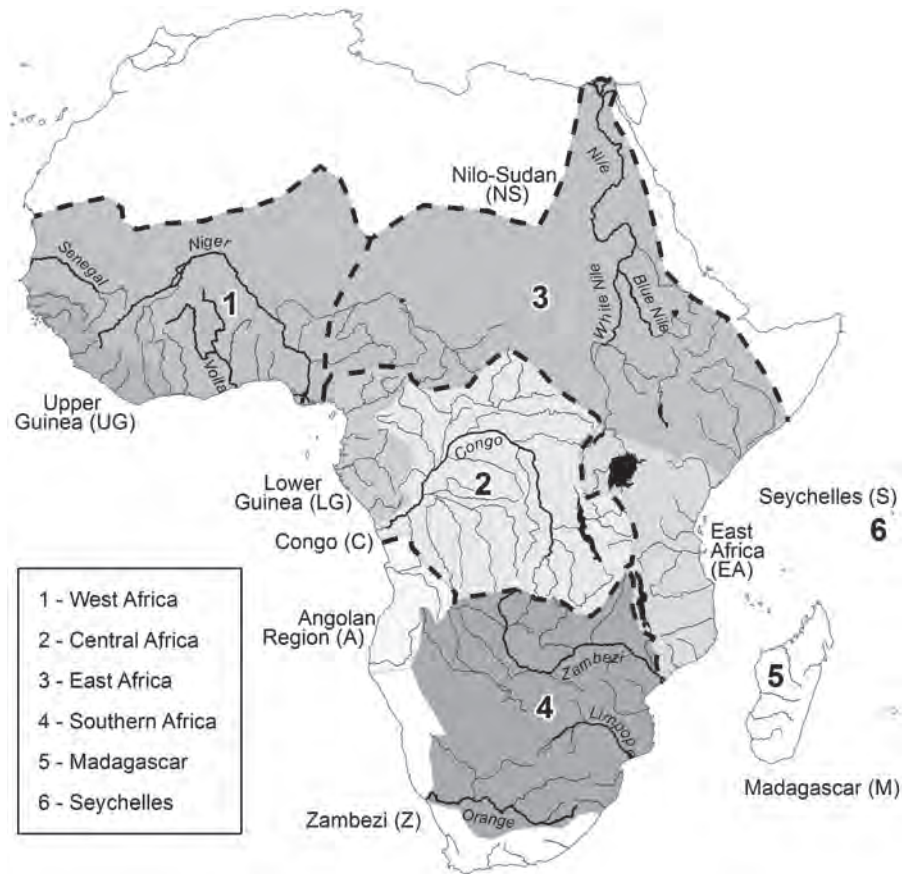
FIGURE 1. Modification of the main ichthyological provinces of the Afrotropical region, after Lévêque (1997) with major extant drainages on the African continent. In brackets are the major regions relevan1t to our study on the Afrotropical freshwater crab fauna in the family Potamonautidae. The Nile basin lies outside of the Afrotropical region. The shading on the map indicates the boundaries of freshwater regions. For our biogeographic inference we recognized the six areas shown on the figure legend.

river drained west (Goudie 2005). Initially the uplift formed a landlocked basin that was captured during the Miocene causing the modern-day outlet into the Atlantic Ocean. The western fringes of the Congo drainage can be linked to the Lower Guinea systems in Central Africa as well as with Lake Tanganyika in the East African Rift (Giresse 2005; Goudie 2005; Stankiewicz and de Wit 2006; Genner et al. 2007; Wagner et al. 2012). In West Africa, the Niger is a dominant and old river (Goudie 2005). During the Tertiary, the Niger was fed by a major trans-African paleodrainage that arose in the Egypt-Sudan region (McCauley et al. 1982) and was captured by the growth of the Nile system via a series of lake basins. However, the latter hypothesis is disputed (Goudie 2005). The evolution of the Nile is complex and generally comprised five distinct phases summarized in Issawi and McCauley (1992). At the source of the Nile, the river owes it origin to the uplift of the Ethiopian Highlands, initiated by volcanic activity during the Oligocene, 30 Ma (Goudie 2005; Stankiewicz and de Wit 2006). The modern flow of the Nile River is the result of several drops in the Mediterranean sea level during the Miocene and Pleistocene. Rifting in the

East African system was initiated during the Miocene 30 Ma. Lake Tanganyika began to form between 9 and 12 Ma with the northern and southern portions of the basins being younger (7–8 Ma and 2–4 Ma, respectively) (Cohen et al. 1993), while the Lake Malawi basin has been actively subsiding since the Miocene (Contreras et al. 2000). In southern Africa, the upper and lower portions of the Zambezi River were formerly separate systems, with the upper system joined to the Limpopo and the middle Zambezi to the Shire systems (Moore and Larkin 2001; Goudie 2005; Moore et al. 2009). These links are thought to have been maintained until as recently as the Pliocene or mid-Pleistocene (Thomas and Shaw 1991), facilitating faunal exchanges (Skelton 2001). The Orange River is thought to have existed since the Cretaceous, and achieved its present-day drainage pattern following capture by the Kao River during the Miocene. The Orange River is thought to have drained a large part of the Kalahari via the Trans-Tswana River, and some authors suggest that the catchment extended into Central Africa. However, this is a contested hypothesis (McCarthy 1983). De Wit (1999) argues that two rivers, the Karoo and Kalahari Rivers, drained southern Africa

during the Cretaceous. Uplift, subsidence and faulting have all impacted paleodrainage connectivity on the African continent, resulting in a complex network of historical faunal boundaries. While an excellent body of literature on paleodrainage evolution exists for the Afrotropical region, considerable debate and conjecture prevail about the accuracy, timing, and cessation of drainage links, making a coherent synthesis of hydrographic evolution elusive. Molecular phylogenetic studies, coupled with divergence time estimations of the continental Afrotropical ichthyological fauna, have proved useful in elucidating paleodrainage connectivity including some of the most classic and spectacular examples of adaptive radiations, such as the cichlid fauna of the East African Rift Valley Lakes (Kocher 2004; Salzburger and Meyer 2004; Salzburger et al. 2005, Seehausen 2006; Genner et al. 2007; Wagner et al. 2012; Day et al. 2013). Several recent studies on Asian and African riverine freshwater fishes have demonstrated a strong correlation between paleodrainage chronology and biogeographic patterning (Kocher 2004; Goodier et al. 2011; Wagner et al. 2012; Schwarzer et al. 2012; Day et al. 2013; De Bruyn et al. 2013; Pinton et al. 2013). For example, Salzburger et al. (2005) reported that the haplochromine cichlids of the Congo drainage are ancestral to the southern African taxa. Similarly, Schwarzer et al. (2012) reported shared haplotypes between haplochromine fishes from the upper Kwanza-Okavango and the Congo-Zambezi watersheds. Goodier et al. (2011) report that West African drainages systems shared species with the Nile system, suggesting a recent faunal exchange, while Day et al. (2013) observed a sister taxon phylogenetic relationship between catfishes from West Africa and the Nilo-Sudan region, with some West African taxa being sister to the Congo basin. It would be reasonable to assume that evolutionary studies of other obligatory freshwater taxa could also act as an evolutionary archive of the impact of paleodrainage rearrangements. However, the utilization of African freshwater invertebrates to infer paleodrainage evolution is limited and remains largely neglected. No single broad scale evolutionary study has yet been conducted on any Afrotropical invertebrate taxon that inhabits both lotic and lacustrine environments despite the potential novel insights into paleodrainage patterning this could yield.

Freshwater decapods such as crabs and crayfish have proven to be excellent taxa to correlate phylogenetic and biogeographic patterning (Crandall et al. 2000; Daniels et al. 2006a, 2006b). Freshwater crabs (Potamoidea) are distributed circumtropically, exhibit high levels of species diversity and endemism, exhibit direct development, lack a dispersive larval phase and complete their entire life cycle in freshwater (Daniels et al. 2006a; Marijnissen et al. 2006; Cumberlidge and Ng 2009; Klaus et al. 2013). Freshwater crabs comprise in excess of 1320 extant species (Ng et al. 2008; Ng P.K.L., personal communication) and represent the largest group within Brachyura (Ng et al. 2008). Primary freshwater crabs (Yeo et al. 2008, 2014) are assigned to five families, four of which are probably monophyletic (Gecarcinucidae, Potamidae, Potamonautidae, and Pseudothelphusidae), while the fifth family, the Neotropical Trichodactylidae, probably represents a separate invasion of freshwaters (Tsang et al. 2014).

In the Afrotropics, freshwater crabs are the largest inland aquatic invertebrates and are critical to ecosystem function and structure (Dobson 2004). Freshwater crabs in Africa are medically important because they are vectors in the transmission of river blindness and paragonimiasis in humans (Cumberlidge 1999). Most species of Afrotropical freshwater crabs are riverine, but there are a significant number of species that have adapted to live in nearly all types of inland freshwater systems ranging from high altitude first and second order mountain streams to lakes, wetlands, swamp forests, and ephemeral pans. Some species are highly terrestrial and live in phytotelmic freshwater pools that form in holes in forest trees (Cumberlidge 1999; Cumberlidge et al. 2005; Cumberlidge 2011b). Afrotropical freshwater crabs belong to the family Potamonautidae and comprise two subfamilies; Deckeniinae contain 11 genera, 3 on the African continent (*Afrithelphusa*, *Deckenia*, and *Globonautes*) and 1 in the Seychelles Archipelago (*Seychellum*) that are thought to be sister to the 7 genera from Madagascar (*Boreathelphusa*, *Foza*, *Hydrothelphusa*, *Madagapotamon*, *Malagasya*, *Marojejy*, and *Skelosophusa*), while Potamonautinae contain seven continental genera (*Erimetopus*, *Liberonautes*, *Louisea*, *Platythelphusa*, *Potamonautes*, *Potamonemus*, and *Sudanonautes*) (Daniels et al. 2006a; Cumberlidge et al. 2008). Notable taxonomic incongruence exists in the generic division among the two subfamilies based on the classification proposed by Cumberlidge et al. (2008) in comparison to that of De Grave et al. (2009). Potamonautidae contains an estimated 145 described species, however, this is likely to be a conservative estimation of true diversity (Cumberlidge 2014a,b). Recent alpha taxonomic endeavours on Afrotropical freshwater crabs have resulted in the description of a plethora of novel species (e.g., Meyer and Cumberlidge 2011; Cumberlidge and Clark 2012; Daniels and Bayliss 2012; Phiri and Daniels 2013, 2014; Cumberlidge and Daniels 2014; Daniels et al. 2014). *Potamonautes* is the most species-rich genus containing an estimated 90 species, and the genus is the most widely distributed in sub-Saharan Africa (including the Nile basin as far north as Cairo), with the Congo basin and East Africa representing the areas where endemism and diversity are highest (Cumberlidge 2009a, 2014a). West Africa is the most diverse region for freshwater crabs and contains seven genera, five of which are endemic to the region (Cumberlidge 1999). Africa's continental offshore islands (e.g., Bioko, Zanzibar, and Pemba) and some of its oceanic islands (e.g., São Tomé and Príncipe) harbor additional species, some of which are endemic (Cumberlidge 2008).

Phylogenetic studies of freshwater crab relationships globally have largely concentrated on regionally discrete geographic areas (e.g., southern Africa, Indo-China, the Aegean, the Seychelles Archipelago and Borneo) and have generally focused on a specific ingroup (Daniels et al. 2002; Shih et al. 2009; Jesse et al. 2010; Daniels 2011; Klaus et al. 2013) However, most freshwater crab phylogenetic studies have suffered from limited taxon sampling, coupled with a low number of sequence loci and a near exclusive reliance on mtDNA. Comprehensive taxon sampling for any biogeographic region has hitherto remained unattainable and has precluded strong inferences of divergence time and hampered our ability to test evolutionary hypotheses (Daniels et al. 2002, 2006a; Klaus et al. 2006; Shih et al. 2009). The most complete study of the Afrotropical freshwater crab fauna to date (Daniels et al. 2006a) lacked several key genera (*Afrithelphusa*, *Boreathelphusa*, *Erimetopus*, *Globonautes*, and *Louisea*) and included only a fraction of the described species (Daniels et al. 2006a). Daniels et al. (2006a) demonstrated the monophyly of the Afrotropical fauna, with island species from the Seychelles and Madagascar sister to the East African *Deckenia*, while those from Socotra belonging to a separate lineage (the Potamidae). That study also revealed considerable taxonomic inaccuracies and did not support a Gondwanan origin of the Potamonautidae. Two overarching hypotheses have been advanced to explain the contemporary distribution patterns of the Potamonautidae. Kensley's (1981) hypothesized that African freshwater crabs originated in East Africa and migrated southwards using the large river systems which led to the evolution of species and species groups that are characteristic of a particular river or river system. An alternative scenario was proposed by Cumberlidge (1999); hypothesizing a Central/West African origin of the Potamonautidae, with the current biogeographic patterns in West and Central Africa arising from past range contractions and expansions associated with climate oscillations during the Pleistocene glacial periods. When glacial maxima in the high latitudes produced dry climates in Africa the humid forest species survived only in Pleistocene forest glacial refugia in West and Central Africa, which were places where there was still sufficient rainfall to support rain forest growth. When the glaciers retreated in the northern latitudes, the climate in Africa became wetter, rainforests expanded, and the forest species of freshwater crabs subsequently expanded their range. Neither of these hypotheses has been tested using a phylogenetic framework; hence, the potential role of paleodrainage rearrangements on the cladogenesis of freshwater crab lineages within the Afrotropical region remains equally obscure in the absence of a near complete phylogeny.

Considering the vast geographic area under study, a complex evolutionary patterning of dispersal and vicariance is likely to exist among the Afrotropical freshwater crabs. The robust generic sampling within the Potamonautidae in the present work is to the best of our knowledge the most comprehensive of any biogeographic freshwater crab study undertaken thus far. The present study offers the opportunity to employ biogeographic patterning to aid conservation management decisions. Specifically, we ask (1) when and where did potamonautid freshwater crabs originate? Was it in East Africa (hypothesis of Kensley's 1981) or in Central/West Africa (hypothesis of Cumberlidge 1999)? (2) When and where were the present drainage basins in Africa connected in the past in ways that would have allowed freshwater crabs to cross between major watersheds (paleodrainage connectivity)? (3) Can we see effects that can be attributed to Pleistocene rain forest contraction and the existence of refuges and connectivity? and (4) How do the distribution patterns observed among freshwater crabs correspond to what has been reported for freshwater fishes?

Materials and Method

Taxon Sampling

The present study includes a total of 96 species of Afrotropical freshwater crabs in the Potamonautidae, representing 17 of the 18 genera and about 66% of the entire known species diversity (Table 1). *Louisea* is the only genus absent from the present study, and neither of the two species in this genus has been collected since 1910 (Cumberlidge 1999). A recent molecular phylogenetic study of Brachyura suggests that the Potamidae and the Gecarcinucidae are sister to the Potamonautidae, hence six species from these two families were selected as outgroups (Tsang et al. 2014). During the present study we adopted a modified version of the ichthyological bioregions of Africa proposed by Lévêque (1997) as a template to demarcate aquatic regions to reflect the paleodrainage links that have been corroborated by biogeographic patterning observed among freshwater fishes. The areas we defined differ in that we merged Upper Guinea and western Nilo-Sudan (including the entire Niger drainage) into a West African region (1); Lower Guinea and Congo into a Central African region (2); East Africa and Eastern Nilo-Sudan into an East African region (3), and the Angolan region and Zambezi into a southern African region, (4). The Gondwanic island Madagascar and the Seychelles Archipelago were coded as two distinct regions 5 and 6, respectively (Fig. 1).

Molecular Techniques and Sequencing

DNA was extracted from ethanol preserved specimens from pereiopod muscle or gill tissue provided by various museum collections For formalinpreserved museum specimens we explicitly targeted gravid females for DNA extraction since these rapidly dividing cells contain a large number of mtDNA copies. The formalin-preserved specimens were extracted in a separate laboratory with equipment dedicated exclusively for the use of museum material, in order to limit possible contamination. DNA

TABLE 1. A list of the Afrotropical freshwater crab species, and outgroups used during the present phylogenetic study

Species	Reference Study	Country	Museum accession number	12S rRNA	16S rRNA	COI	Histone 3
Afrithelphusa monodosa	Present	Guinea	NMU 25.IV.2005.C	KP640386	KP640430	KP640469	KP640507
Boreathelphusa uglowi	Present	Madagascar	FMNH 5732	KP640387	KP640431	KP640470	KP640508
Deckenia imitatrix	2	Kenya	NMU 1998.1	AY803503	AY803544	AY803576	AY803698
Deckenia mitis	Present	Tanzania	Unaccessioned	KP640388	KP640432	KP640471	KP640509
Erimetopus brazzae	Present	D.R.Congo	MCZ 4255	KP640389	KP640433	—	KP640510
Foza raimundi	Present	Madagascar	FMNH 7438	KP640390	KP640434	KP640472	KP640511
Foza goudoti	2	Madagascar	FMNH 4652	AY803507	AY803548	AY803579	AY803702
Globonautes macropus	Present	Guinea	NMU VII. 1988	KP640391	KP640435	—	—
Hydrothephusa agilis	2	Madagascar	FMNH 5729	AY803505	AY803546	AY803578	AY803700
Hydrothelphusa bombetokensis	2	Madagascar	FMNH 6878	AY803506	AY803546	—	AY803701
Hydrothelphusa madagascariensis	2	Madagascar	FMNH 7438	AY803508	AY803549	AY803580	AY803703
Hydrothelphusa vencesi	Present	Madagascar	FMNH 13940	KP640392	KP640436	KP640473	
Liberonautes chaperi	Present	Liberia	NMU VII.1988a.1	KP640393	KP640441	—	KP640512
Liberonautes latidactylus	Present	Liberia	SAM A78435	KP640396	KP640439	KP640474	KP640513
Liberonautes lugbe	Present	Guinea	Unaccessioned	KP640398	KP640442	KP640475	KP640514
Liberonautes nanoides	Present	Liberia	NMU 14.XII.1988	KP640397	KP640440	—	KP640515
Liberonautes nimba	Present	Liberia	SAM A48202	KP640395	KP640438	KP640477	KP640516
Liberonautes rubrigimanus	Present	Liberia	SAM A48201	KP640394	KP640437	KP640476	KP640517
Liberonautes sp.	Present	Sierra Leone	SAM A78436	KP640399	KP640443	KP640478	KP640518
Platythelphusa armata	2	Tanzania	ZMA Crust.De.204685	AY803491	AY803531	KP640479	KP640519
Platythelphusa conculcata	4	Tanzania	Unaccessioned	DQ203190	DQ203218	—	—
Platythelphusa denticulate	4	Tanzania	Unaccessioned	DQ203194	DQ203220	—	—
Platythelphusa echinata	4	Tanzania	Unaccessioned	DQ203196	DQ203222	—	—
Platythelphusa immaculate	4	Tanzania	Unaccessioned	DQ203198	DQ203224	—	—
Platythelphusa maculate	4	Tanzania	Unaccessioned	DQ203203	DQ203228	—	—
Platythelphusa praelongata	4	Tanzania	Unaccessioned	DQ203206	DQ203232	—	—
Platythelphusa tuberculata	4	Tanzania	Unaccessioned	DQ203204	DQ203230	—	—
Potamonautes anchietae	Present	Angola	Unaccessioned	KP640400	KP640444	KP640480	—
Potamonautes bayonianus	1 + Present	Botswana	Unaccessioned	AY042321	AY042243	AF510868	KP640520
Potamonautes brincki	1	South Africa	Unaccessioned	AY042322	AY042244	AF510875	AY803674
Potamonautes calcaratus	1	South Africa	Unaccessioned	AY042323	AY042242	AF510867	AY803675
Potamonautes choloensis	6+Present	Mozambique	SAM A46802	JF799164	JF799214	JF799210	KP640521
Potamonautes clarus	1	South Africa	Unaccessioned	AY042320	AY042241	AF510872	AY803676
Potamonautes dentatus	1	South Africa	Unaccessioned	AY042324	AY042246	AF510878	AY803677
Potamonautes depressus	1	South Africa	Unaccessioned	AY042325	AY042247	AF510877	AY803678
Potamonautes ecorssei	2	Mali	NMU 07.01.2003.1	AY803492	AY803532	—	AY803679
Potamonautes emini	2	Tanzania	ZMA Crust. De. 204680	AY803493	AY803533	—	AY803680
Potamonautes sp 12	Present	Zambia	Unaccessioned	KP640401	KP640445		KP640522
Potamonautes granularis	1	South Africa	Unaccessioned	AY042326	AY042254	AF510876	AY803681
Potamonautes kundudo	present	Ethiopia	SAM A48200	KP640403	KP640447	KP640482	KP640523
Potamonautes idjuwiensis	present	D.R.Congo	SAM A78437	KP640402	KP640446	KP640481	KP640524
Potamonautes lirrangensis	2	Malawi	ZMA Crust. De. 204681	AY803494	AY803534	AY803568	AY803682
Potamonautes montivagus	present	Mozambique	Unaccessioned	KP640404	KP640448	KP640483	KP640525
Potamonautes lividus	1	South Africa	Unaccessioned	AY 042327	AY042248	AF 510879	AY 803683
Madagapotamon humberti	2	Madagascar	FMNH 11049	AY803509	AY803550	—	AY803704
Malagasya antongilensis	2+Present	Madagascar	NMU 29.4.11.2001B	AY803511	AY803551	KP640493	AY803706
Malagasya goodmani	2+Present	Madagascar	FMNH unaccessioned	AY803512	—	KP640494	AY803707
Marojejy longimerus	2	Madagascar	FMNH 4656	AY803513	AY803552	AY803582	AY803708
Skelosphusa eumeces	2	Madagascar	FMNH 11059	AY803514	AY803553	AY803583	AY803709
Skelosphusa prolixa	Present	Madagascar	FMNH 7596	KP640417	KP640457	KP640495	KP640539
Potamonautes sp. 10	Present	D.R.Congo	SAM A78444	KP640428	KP640467	KP640497	KP640548
Potamonautes sp. 8	Present	D.R.Congo	SAM A78443	KP640426	KP640466	KP640498	KP640547
Potamonautes sp. 11	Present	Kenya	SAM A78442	KP640421	KP640460	KP640496	KP640540
Potamonautes flavusjo	8+Present	South Africa	SAM A48207	KJ713427	KJ713479	KJ713530	KP640542
Potamonautes bellarussus	8+Present	Mozambique	SAM A48218	KJ713445	KJ713497	KJ713549	KP640541
Potamonautes sp. 2	Present	Mozambique	NMU-PN3	KP640424	KP640459	KP640500	KP640544
Potamonautes sp. 1	Present	São Tomé	Cal. Acad. No. 796	KP640423	KP640458	KP640499	KP640543
Potamonautes stanleyensis	Present	D.R.Congo	SAM A78442	KP640418	KP640463	KP640501	KP640550
Potamonautes langi	Present	D.R.Congo	SAM A78438	KP640419	KP640464	KP640502	KP640551
Potamonautes ballayi	Present	D.R.Congo	SAM A78439	KP640420	KP640465	KP640503	KP640552
Potamonautes sp. 7	Present	D.R.Congo	SAM A78440	KP640429	KP640468	—	KP640549
Potamonautes sp. 9	Present	Uganda	SAM A78441	KP640422	KP640462	KP640504	KP640546
Potamonautes sp. 6	Present	D.R.Congo	Unaccessioned	KP640427	KP640461	—	KP640545
Geothelphusa sp.	2	Japan	Unaccessioned	AY803519	AY803558	KP640505	AY803714
Parathelphusa sp.	2	Malaysia	NMU 16.08.2003.1	AY803523	AY803561	AY803588	AY803718

TABLE 1. Continued

Species	Reference Study	Country	Museum accession number	Gen Bank accession numbers			
				12S rRNA	16S rRNA	COI	Histone 3
Rouxana sp. 1	2	P.N.Guinea	NMU A21.09.2000.1	AY803524	AY803562	—	AY803719
Rouxana sp. 2	2	P.N.Guinea	NMU B21.09.2000.1	AY803525	AY803563	AY803589	AY803720
Socotra pseudocardisoma	2	Socotra	NMU 10.1998.1	AY803516	AY803555	AY803585	AY803711
Johora tiomanensis	2+Present	Malaysia	ZRC 1999.0899	AY803517	AY803556	KP640506	AY803712
Potamonautes loveni	Present	Kenya	NHM 2004.1-6	KP640405	KP640449	KP640484	KP640526
Potamonautes mutariensis	7+Present	Zimbabwe	SAM A45934	KC768246	KC768273	KC768299	KP640527
Potamonautes mulanjeensis	6	Malawi	SAM A46801	JF799175	JF799139	JF799201	—
Potamonautes namuleensis	6	Mozambique	SAM A46797	JF799170	JF799140	JF799196	KP640528
Potamonautes niloticus	2+Present	Tanzania	ZMA Crust. De. 204683	AY803469	AY803536	KP640485	AY803685
Potamonautes obesus	2	Tanzania	Unaccessioned	AY803497	AY803537	AY803647	AY803686
Potamonautes odhneri	2	Tanzania	NMU 14.07.2004	AY803498	AY803538	AY803571	AY803687
Potamonautes parvicorpus	1	South Africa	Unaccessioned	AY042328	AY042252	AF510869	AY803688
Potamonautes parvispina	1	South Africa	Unaccessioned	AY042329	AY042253	AF510873	AY803689
Potamonautes perlatus	1	South Africa	Unaccessioned	AY042330	AY042249	AF510874	AY803690
Potamonautes barnardi	Present+3	South Africa	SAM A41012	KP640406	DQ028709	KP640486	KP640529
Potamonautes barbarai	Present+3	South Africa	SAM A41060	KP640407	DQ028686	KP640487	KP640530
Potamonautes platynotus	2	Tanzania	NMU 23.04.2003.6	AY803499	AY803539	AY803572	AY803691
Potamonautes raybouldi	2	Tanzania	ZMA Crust. De. 204684	AY803540	AY803500	AY803573	AY803692
Potamonautes rukwanzi	Present	Uganda	NMU 16.VII.1993b.1	KP640408	KP640450	—	KP640531
Potamonautes sidneyi	1	South Africa	Unaccessioned	AY042331	AY042245	AF510871	AY803693
Potamonautes subukia	2	Kenya	NMU 18.10.2003	AY803495	AY803535	AY803569	AY803684
Potamonautes suprasulcatus	Present	Tanzania	NHM unaccessioned	KP640425	KP640451	KP640488	KP640532
Potamonautes unispinus	1	South Africa	Unaccessioned	AY042332	AY042250	AF510870	AY803694
Potamonautes warren	1	South Africa	Unaccessioned	AY042333	AY042251	AF510880	AY803695
Potamonemus sachsi	2	Cameroon	NMU 09.04.1983	AY803490	AY803530	—	—
Potamonemus mambilorum	Present	Cameroon	NHM 1991.183	KP640409	KP640452	—	KP640533
Potamonemus asylos	Present	Cameroon	NHM 1994.588-591	KP640410	KP640453	KP640489	KP640534
Sudanonautes aubryi	Present	Nigeria	NMU 23.04.1984A	AY803502	AY803542	AY803575	—
Sudanonautes floweri	Present	Gabon	Unaccessioned	AY803501	AY803541	AY803574	AY803696
Potamonautes sp. 5	Present	Angola	Unaccessioned	KP640413	KP640456	KP640490	KP640535
Potamonautes sp. 4	Present	Angola	Unaccessioned	KP640412	KP640455	KP640491	—
Potamonautes sp. 3	Present	Angola	Unaccessioned	KP640411	KP640454	KP640492	—
Seychellum alluaudi	5+Present	Seychelles	SAM A48220	KP640414	JF799294	JF799368	KP640536
Seychellum mahefregate	5+Present	Seychelles	SAM A48235	KP640415	JF799260	JF799319	KP640537
Seychellum silhouette	5+Present	Seychelles	SAM A48226	KP640416	JF799277	JF799351	KP640538

Notes: All the DNA sequences generated during the present study were deposited in GenBank and combined with those from (1–3) Daniels et al. (2002, 2006a, 2006b), (4) Marijnissen et al. (2006), (5) Daniels (2011), (6) Daniels and Bayliss (2012), (7) Phiri and Daniels (2013), and (8) Daniels et al. (2014). The reference study refers to one of the eight studies listed above. Specimens from the following museum collections were used; the British Natural History Museum, London, UK (NHM); the Field Museum, Chicago, USA (FMNM); the Zoological University Museum, Amsterdam, the Netherlands (ZMA); the South African Natural History-Iziko Museums of Cape Town, South Africa (SAM); the Zoological Reference Collection, Raffles Museum, National University of Singapore, Singapore (ZRC); the Museum of Comparative Zoology, Harvard University, USA (MCZ); the Northern Michigan University Museum, USA (NMU) and the California Academy of Science (Cal Acad.) A dash (—) indicates a gene region that was not sequenced.

extraction of museum specimens was always conducted using a blank sample (containing only reagents) as a control. Tissues sampled were washed repeatedly in sterilized water and then subjected to standard DNA extraction using a Machery & Nagel DNA extraction kit following the manufacturer's protocol. However, instead of digesting tissue for 3 h as for ethanol preserved specimens, we digested the formalin museum tissue samples for 2–3 days adding 20 µl of proteinase K daily and vortexed frequently for 5 min. DNA from museum specimens was eluted in 20 µl of elution buffer due to the lower quality nucleic acids. DNA was then stored at −20°C until required for polymerase chain reaction (PCR) (Daniels et al. 2006a, 2006b).

Prior to use in PCR, a 1 µl:20 µl dilution was made (DNA: millipore water). We amplified three mitochondrial partial gene fragments (mtDNA). For 12S rRNA, the primer pair 12Sai (5′-AAA CTA GGA TTA GAT ACC CTA TTA T-3′) and 12Smb (5′-GAG AGT GAC GGG CGA TGT GT-3′) (Kocher et al. 1989) was used. For the 16S rRNA locus two primer pairs were used, the first set of primers were used on recently collected taxa 16Sa (5′-ACT TGA TAT ATA ATT AAA GGG CCG-3′) and 16Sb (5′-CTG GCG CCG CTC TGA ACT CAA ATC-3′) (Palumbi et al. 1991), while the primer pair 16L29 (5′-YGCCTGTTTATCAAAAACAT-3′) and 16H37 (5′-CCGGTYTGAACTCAAATCATGT-3′) was used for the museum specimens and amplified a shorter fragment of the 16S rRNA locus. The latter fragment was 100 bp shorter in comparison to the longer 16S rRNA fragment. For cytochrome oxidase I (COI) the primer pairs LCOI-1490 (5′-GGT CAA CAAA TCA TAAA GAT ATTG-3′) and HCOI-2198 (5′-TAAA CTT CAG GGT GAC CAAA AAA TCA-3′) (Folmer et al. 1994) was used. Histone three (H3) was

amplified using the primer pairs H3AF (5′-ATG GCT CGT ACC AAG CAG ACVGC-3′) and H3AR (5′-ATA TCC TTR GGC ATR ATR GTG AC-3′) (Colgan et al. 1998). Daniels et al. (2006a) used these four loci in their study on the evolution of Afrotropical freshwater crabs allowing for the results generated during the present study to be combined with the earlier data set. We included 16S rRNA and 12S rRNA DNA sequences for seven *Platythelphusa* species from GenBanks (see Table 1; Marijnissen et al. 2006). The eighth *Platythelphusa* species, *Platythelphusa armata* was sequenced for all four-marker loci in the earlier study by Daniels et al. (2006a). Representatives of all the mtDNA sequences for recently described species generated subsequent to the Daniels et al. (2006a) publication on Afrotropical freshwater crabs, were downloaded from GenBank and included in the present study (Daniels 2011; Daniels and Bayliss 2012; Phiri and Daniels 2013, 2014; Daniels et al. 2014; Cumberlidge and Daniels 2014). The present study includes 13 undescribed freshwater crab species (12 within *Potamonautes*, and one in *Liberonautes*). For certain specimens we were unable to obtain all the sequence data, specifically for the two protein coding loci (see Table 1 for details). The total evidence data set included the following number of missing sequences, one sequence for both 16S rRNA and 12S rRNA, 21 COI sequences, and 15 histone 3 sequences. These sequences were coded as absent during the phylogenetic analyses.

For PCR, a 25-μl reaction was used that contained, millipore water, 2.5 μl $MgCl_2$, $10 \times Mg^{2+}$ free buffer, 10 mM dNTP solution, 10 mM forward and reverse primers, 0.1 μl of Taq polymerase, and 2.5 μl of the 1:19 μl template DNA dilution. For 12S rRNA and 16S rRNA the following PCR conditions were used: 95°C (5 min), (95°C [30 s], 50°C [40 s], 72°C [1 min]) for 36 cycles; for COI: 94°C (4 min), (94°C [30 s], 42°C [40 s], 72°C [45 s]) for 36 cycles; and for H3: 95°C (5 min), 95°C (30 s), 48–46°C (40 s), 72°C (1 min). The final extension for all PCR conditions was 72°C (10 min). PCR products were electrophoresed (3 h in a 1.5% agarose gel containing ethidium bromide) and the PCR products were then gel purified with the BioFlux purification kit (Bioer Technology Co., Ltd), after which they were sent to Macrogen for sequencing (Macrogen Inc., Amsterdam, The Netherlands). Sequences were checked for ambiguities and aligned with MUSCLE (Edgar 2004). Multiple alignment as executed in MEGA6 (Tamura et al. 2013). Both the COI and H3 (the two protein-coding genetic loci) sequences were translated to amino acids and checked for stop codons using EMBOSS-Transeq (http://www.ebi.ac.uk/emboss/transeq/). All new sequences were deposited in GenBank (see Table 1 for GenBank accession numbers).

Phylogenetic Reconstructions

We reconstructed the Afrotropical freshwater crab phylogeny using MP, ML, and BI on the total evidence data set including all four loci. Analyses were conducted

separately to confirm topology congruence. MP analyses were executed in PAUP*4 v. beta 10 (Swofford 2002). For the MP analyses, trees were generated using the heuristic search option with TBR branch swapping using 100 random taxon additions, with gaps treated either as fifth character states or as missing data in the 16S rRNA and 12S rRNA partitions. Phylogenetic confidence in the nodes recovered from MP was estimated by bootstrapping (Felsenstein 1985) analyzing 1000 replicates of the data set. For the ML and BI analyses the best-fit partitioning scheme as well as the best substitution models for the respective partitions were found with PartitionFinder v.1.1.1 (Lanfear et al. 2012) using the Bayesian Information Criterion and considering GTR, TrN, HKY, and JC models with and without gamma distributed substitution frequencies. This resulted in two partitions, one comprising the mitochondrial rRNA genes and the second codon position of COI, and one with histone H3 and first and third COI codon positions. The best-fit model of sequence evolution was assigned to both partitions (GTR model with gamma distributed substitution frequencies). ML analysis was conducted in RAxML v.7.2.7 (Stamatakis 2006). The robustness of branches of the best ML tree was assessed with 1000 bootstrap replicates using the CAT algorithm for fast bootstrapping, while the final tree search was conducted under the GTR+Γ model for both partitions (see above). The Bayesian phylogenetic reconstruction was executed in MrBayes v. 3.2.2 (Ronquist et al. 2012). The partitioned analysis was conducted with two parallel runs, each with four Metropolis coupled Markov Chain Monte Carlo (MC3) chains of 10×10^6 generations, each chain starting from a random tree. Sampling from both runs was conducted every 5×10^3 generations. Convergence of sampled parameters and potential autocorrelation (effective sampling size/ESS for all parameters > 100) was investigated in Tracer 1.6 (Rambaut et al. 2013). Additionally, the average standard deviation of split frequencies between both runs was checked (< 0.01; Ronquist et al. 2012). The maximum credibility tree was found and annotated in Tree Annotater v. 1.7.5 (part of the BEAST package) using the trees of the first run after removal of 500 trees as burn-in. The best tree topology from each of the phylogenetic reconstruction methods was chosen and drawn to scale. Nodes were considered to be statistically well supported when bootstrap values were ⩾ 75% and Bayesian posterior probabilities (BPPs) ⩾ 0.95.

Divergence Time Estimation

Estimation of divergence times was performed using a Bayesian approach in BEAST MC3 v. 1.7.5 (Drummond et al. 2012a, 2012b) with three chains for 50×10^6 iterations sampling every 10,000 iterations. Convergence of sampled parameters and potential autocorrelation (ESS for all parameters > 100) was investigated in Tracer v. 1.6 (Rambaut et al. 2013). We discarded the

first 2700 trees as burn-in, keeping 1300 trees. The maximum credibility tree was found and parameter values annotated with TreeAnnotator (part of the BEAST package). As we applied external substitution rates for specific genes, we had to modify the previously used partitioning scheme, recognizing three partitions (mitochondrial rRNAs, COI, and H3) with unlinked molecular clock rates and substitution models. We applied the substitution models as suggested by jModelTest v. 2.1.3 (Darriba et al. 2012), considering models with equal/unequal base frequencies and with/without rate variation among sites (base tree for likelihood calculations = ML tree; tree topology search operation = NNI; the best model was inferred by the Bayesian Information Criterion): HKY + Γ for the COI gene, GTR + Γ for the H3 gene, and for the combined 16S and 12S rRNA genes. We applied a Yule tree prior and adjusted the exchange rates for the mitochondrial gene partition after initial test runs (normal distributed priors with mean values as inferred by jModelTest and 10% SD). We have chosen an uncorrelated lognormal relaxed molecular clock using published brachyuran substitution rates as priors and one additional fossil calibration. In detail, these are:

(1) A mean rate of 1.09% per Ma (normal distribution) for the mitochondrial rRNAs (SD = 0.239% per Ma; 5–95% quantile = 0.63–1.4% per Ma), that resulted from a phylogeny of Old World freshwater crabs dated with three fossil calibration points (Klaus et al. 2010). These are the most recent common ancestor (MRCA) of the genus *Potamon* (divergence *Potamon fluviatile* and *Potamon persicum*) calibrated with Early Miocene fossil *Potamon quenstedti*; the MRCA of the gecarcinucid genus *Sartoriana* based on Pliocene fossil claws from the South Asian Siwalik formation; and the MRCA of *Potamonautes niloticus* and *P. armata* based on Late Miocene *P.* aff. *niloticus*. The taxonomy and chronostratigraphy of the potamid and gecarcinucid fossils was recently assessed (Klaus and Gross 2010), and associated uncertainty was modeled conservatively in the study of Klaus et al. (2010). Each of these calibration points is placed in one of the three Old World freshwater crab families (Potamonautidae, Potamidae, and Gecarcinucidae), that form a monophyletic group (Tsang et al. 2014). Therefore, these rates are highly applicable for a potamonautid phylogeny.

(2) 0.19% per Ma for the H3 gene (SD = 0.04% per Ma; 5–95% quantile = 0.12–0.26% per Ma). This rate is also derived from the study of Klaus et al. (2010), see above.

(3) Estimates for the arthropod substitution rate of the COI locus show a large variance with most published values being between 0.7% and 2.0% per Ma (Schubart et al. 1998; Farrell 2001; Quek

et al. 2004; Papadopoulou et al. 2010). There are, however, much larger rates available for marine crustaceans (11.89% per Ma, Crandall et al. 2012). Instead of fixing the COI rate to a specific value, we therefore modeled this uncertainty by using a broad normally distributed prior for the substitution rate with a 5–95% quantile of 0.77–3.9% per Ma (mean 2.33% per Ma, SD = 1.00% per Ma). Similar values have been used in previous studies on freshwater crabs (Daniels 2011; Klaus et al. 2013).

(4) As described above, Klaus et al. (2010) calibrated the split between *P. niloticus* and *P. armata* based on Late Miocene *P.* aff. *niloticus* (see Carriol and Secrétan 1992). As we could show with the more thorough taxon sampling that both species are not direct sister taxa this most likely resulted in a bias for younger divergence times. Therefore, to adjust this error, we additionally constrained the age of the divergence between *P. niloticus* and (*P. stanleyensis* + *Potamonautes* sp. 6) with a gamma distributed calibration density (offset 6.0 Ma, shape parameter 2.0, scale factor 1.0, 5–95% quantile = 6.4–10.7 Ma; following Klaus et al. 2010).

To investigate the influence of the fossil adjustment and the various external rates on divergence times and to select the calibration scheme that fits best to the data, we have run different analyses under different schemes. The partitions that have not been set to a specific rate were subject to a uniform rate of sequence evolution (interval of $0–1^{100}$% per Ma) during these test runs. We compared the Bayes factor between these models to identify the best-fit substitution scheme, with marginal likelihoods estimated using the path sampling algorithm (Baele et al. 2013). This resulted in two models that were equally superior to the others with strong support (2× logBayes factor > 7, criteria of Kaas and Raftery 1995). A model with the additional fossil calibration and external rates for all genes, and one model with external rates for the mitochondrial partitions only (Supplementary Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.85569). Resulting credibility intervals of divergence times were highly congruent for both models, with the full model showing slightly narrower credibility intervals (Supplementary Fig. S1, available on Dryad at http://dx.doi.org/10.5061/dryad.85569). We chose the latter model for subsequent range estimation and diversification analysis, the corresponding tree is given in Figure 3. The resulting substitution rates for the fully calibrated model were 0.81% per Ma for the rRNA genes (95% CI 0.59–1.01%), for the COI gene 2.85% per Ma (95% CI 2.09–3.66%), and 0.25% per Ma (95% CI 0.19–0.32%) for H3 locus, thus showing only a slightly slower rate for the rRNA genes and a slightly faster rate for H3 compared with the study of Klaus et al. (2010).

Ancestral Range Estimation

For the biogeographic analyses each specimen was coded to one of six areas according to its sampling locality (Table 1; Fig. 1). To initially test if these regions correlate with our phylogenetic estimation, that is, if more closely related taxa are more likely to share the same area than expected by chance, we conducted phylogeny trait correlations (Association index, Fitch parsimony score, and monophyletic clade [MC] size statistic) as implemented in BaTS beta v.2 (Parker et al. 2008) using the 1300 post burn-in trees of the Bayesian divergence time estimation and using 1000 randomizations to test for significance. The results indicate a significant correlation of occurrence data and phylogenetic position for all statistics ($P < 0.05$; with exception of the MC statistic for the Central African area) (see Supplementary Table S3, available on Dryad at http://dx.doi.org/10.5061/dryad.85569, for detailed results). Therefore, we conclude that our area assignment does reflect paleogeographic entities, and allows us to subsequently infer number and timing of past freshwater crab dispersal between drainages.

To infer the geographical origin of potamonautid lineages and investigate the number of transitions between drainage systems we conducted likelihood analyses of ancestral ranges based on a dispersal–extinction–cladogenesis model allowing for founder speciation (DEC+J; Matzke 2014) after comparing with a simple DEC model (deltaAIC = 80). Analyses were conducted with the R-package BioGeoBEARS (Matzke 2013). The specimens were coded for the regions from which they were sampled (Table 1). The areas we defined are similar to the ichthyological bioregions but differ in that we merged the Congo Basin and Lower Guinea into a Central region; East Africa and Nilo-Sudan into an East African Nilo-Sudan region while the Angolan region and Zambezi is merged into a southern African region. Thus, we incorporate larger drainage systems a priori as biogeographical entities, without hampering biogeographical inference by too fine-scaled range assignment (i.e., considering each non-connected river system as an independent range). The maximum credibility tree with removed outgroups was used as phylogenetic information. West Africa was coded as non-adjacent to southern Africa, the Seychelles, and Madagascar. We allowed ancestral ranges to comprise a maximum of three areas. Connectivity of areas was modeled with dispersal multipliers such that adjacent areas receive a dispersal multiplier of 1.00, decreasing for every additional dispersal step to another area by 0.25. To test which area is the most likely ancestral one for all Potamonautidae, we conducted a set of analysis, restricting in each run dispersal into one of the continental areas (dispersal set to 1.0E−5 in the dispersal multiplier matrix) and compared the AIC of the different models. It was not possible to constrain dispersal into South Africa as the initial likelihood of this constraint was too low to perform this analysis. Also, the null hypothesis without any constraint (shown in

Fig. 3) showed to be the best model (deltaAIC = 15–18), restricting a more rigorous testing of the potamonautid origin (Supplementary Table S2, available on Dryad at http://dx.doi.org/10.5061/dryad.85569).

RESULTS

Phylogenetic Reconstruction

For the 12S rRNA, 16S rRNA, COI, and histone 3 partial DNA fragments the length of the four loci was 370, 474 600, and 284 bp, respectively, yielding a total evidence data set of 1728 bp. The substitution models for the four loci are listed in Supplementary Material S1, available on Dryad at http://dx.doi.org/10.5061/dryad.85569. For MP when gaps were treated as missing characters (for the two rRNA markers, 12S rRNA and 16S rRNA), a total of 24 trees were retrieved, with a tree length of 7615 steps, a CI of 0.23, and a RI of 0.55, when gaps were treated as fifth character states we retrieved 108 trees, with a tree length of 7898 steps, a CI of 0.21, and a RI of 0.55 The tree topologies produced by ML/MP and the BI were very similar hence only the ML tree is shown and discussed (Fig. 2). The Potamonautidae, and each of the two subfamilies (Deckeniinae and Potamonautinae) were retrieved as monophyletic with strong statistical support ($> 75\%/> 0.95$ BPP) (Fig. 2). Within the Deckeniinae, the tribe Deckeniini was monophyletic with the two West African genera *Globonautes* and *Afrithelphusa* being retrieved as sister taxa with strong nodal support ($> 75\%/> 0.95$ BPP). The latter clade was sister to a clade comprising the two species of *Deckenia* from East Africa and the three species of *Seychellum* from the Seychelles Archipelago ($> 75\%/> 0.95$ BPP). The tribe Deckeniini was sister to the monophyletic tribe Hydrothelphusini $> 75\%/> 0.95$ BPP) from Madagascar. Among the continental Potamonautinae, the genus *Liberonautes* was monophyletic ($> 75\%/> 0.95$ BPP), however, *Sudanonautes* was nested within *Potamonemus* while both *Platythelphusa* and *Erimetopus* were nested within the genus *Potamonautes* suggesting widespread paraphyly. Deeper nodal relationships within *Potamonautes* were poorly supported ($<75\%/<0.95$ BPP) and four clades were evident in the genus (Fig. 2). Some of the East African *Potamonautes* species (clade 1) represent the earliest branching with *Potamonautes obesus* basal to *Potamonautes calcaratus*, which was in turn sister to *Potamonautes namuleensis* and *Potamonautes choloensis*. The second potamonautid clade (clade 2) comprised West African taxa, with *Potamonautes* sp. 1 from São Tomé Island (in the Atlantic Ocean) sister to a clade comprising *Potamonautes ecorssei* from Mali sister to two species from the Congo drainage (*Potamonautes ballayi* and *Erimetopus brazzae*). Clade 3 comprised southern African species sister to Angolan species. Clade 4 comprised exclusively East African and species from the Congo basin. The two mountain-living species *Potamonautes bellarussus* and *Potamonautes kundudo* were basal, albeit with poor statistical support. All
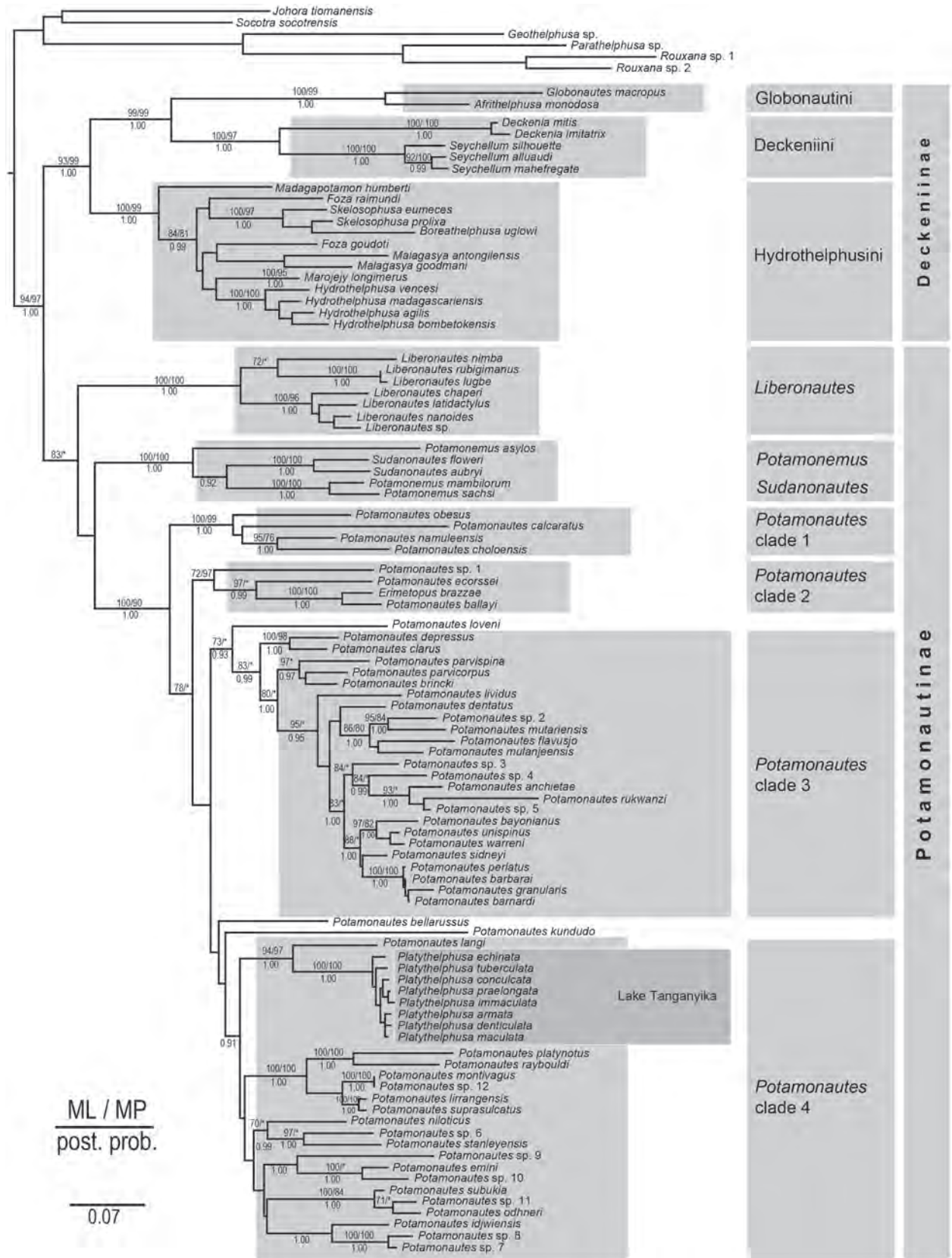
FIGURE 2.   ML tree topology derived from four partial loci (12S rRNA, 16S rRNA, COI and histone 3 genes) for the Afrotropical Potamonautidae. Statistical values above the nodes indicate bootstrap support (%) while values below the nodes are BPPs of the Bayesian analysis (MrBayes). Only bootstrap values > 75% and BPP values > 0.095 BPP are shown. An asterisk indicates values that were below the support threshold.

eight species of the monophyletic *Platythelphusa* that is endemic to Lake Tanganyika (> 75%/ > 0.95 BPP) were deeply nested within the East African *Potamonautes* and sister to *Potamonautes langi* from the Congo drainage (> 75%/ > 0.95 BPP). Within the genus *Platythelphusa,* sister species relationships were poorly supported and characterized by short internal branches (a result supported by the estimated recent divergence time estimation for these species, Fig. 3). Furthermore, clade 4 comprised several large-bodied freshwater crab species sister to a clade comprising Congo species and East African mountain species. Within the East African clade, the mountain-living species *Potamonautes subukia, Potamonautes odhneri,* and an undescribed species (*Potamonautes* sp. 11), together with several small-bodied freshwater crab species from the Congo drainage formed a clade (> 75%/ > 0.95 BPP).

Divergence Time Estimation

The Potamonautidae originated between 63 and 34 Ma (Paleocene to Eocene; 95% highest posterior density, HPD) (Fig. 3). The West African Deckeniinae (*Globonautes* and *Afrithelphusa*) and the East African Deckeniinae (*Deckenia* and *Seychellum*) diverged from Eocene to Early Miocene (95% HPD 42–19 Ma) while these two sister clades diverged from the Malagasy Hydrothelphusini during the Palaeocene to Oligocene (95% HPD 53–27 Ma). The MRCA of the Hydrothelphusini lived during the Eocene to Miocene (95% HPD 37–16 Ma). The divergence of the genus *Liberonautes* occurred during the Oligocene and Miocene (95% HPD 56–30 Ma). The divergence of the genera *Sudanonautes* and *Potamonemus* clade from *Potamonautes* occurred during the Eocene to Miocene (95% HPD 53–29 Ma); and the MRCA of the genus *Potamonautes* also originated during the Eocene to Miocene (95% HPD 42–23 Ma). Mountain-living potamonautid species were constantly branching early in both of the major East and southern African clades, and most likely represent relictual lineages characterized by prolonged isolation and absence of diversification. The divergence of the southern African *Potamonautes* species occurred during the Late Eocene to Miocene (95% HPD 35–19 Ma), while divergence among the East African *Potamonautes* clade occurred during the Oligocene to Miocene (95% HPD 33–18 Ma). The diversification within the endemic Lake Tanganyika genus *Platythelphusa* started most likely during the Late Miocene to Pliocene (95% HPD 8–2 Ma), while the divergence of the genus *Platythelphusa* from *P. langi* (of the Congo drainage) occurred during the Miocene (95% HPD 19–7 Ma). These divergence time estimation should be regarded as our working hypothesis.

Ancestral Range Estimation

The ancestral range of the MRCA of the Deckeniinae was estimated to be wide with a disjunct distributional area that included West, Central, and East Africa (with subsequent dispersal to the Seychelles Archipelago and Madagascar). The separation of the Madagascan lineage occurred only once (53–16 Ma). Range estimates at deeper nodes within the Deckeniinae are, however, arbitrary (see Supplementary Material, available on Dryad at http://dx.doi.org/10.5061/dryad.85569, for alternative, less likely, scenarios). Dispersal from West Africa to East Africa within the Deckeniinae is reconstructed at the root of the Potamonautidae, however, with some uncertainty, and dispersal to the Seychelles Archipelago at 42–11 Ma. Within the Potamonautinae the range of the MRCA is estimated to be West Africa. The initial diversification occurred in West Africa, and this was subsequently followed by dispersal to East Africa. Although not estimated by the biogeographical model, it is likely that this dispersal took place over a long time period (given the slow dispersal capabilities of freshwater crabs) that resulted in a widespread range that included West, Central, and East Africa. The present-day disjunct distribution of this well-supported clade (Deckeniinae) in West and East Africa but not in Central Africa could be explained by an extinction event in Central Africa. However, the latter observation is speculative, although Cumberlidge et al. (2008) also advanced this hypothesis in an attempt to explain the contemporary distribution of the Deckeniinae. Potamonautids reached southern Africa from East Africa independently three times (at 30–16, 29–13, and 28–11 Ma). Dispersal from East Africa to Central Africa occurred independently five times (at 33–21, 20–7, 15–6, 16–5, and 10–2 Ma).

DISCUSSION

Phylogenetic and Biogeographic Implications

The multilocus estimations of phylogenetic relationships and divergence times together with the ancestral area estimates presented during the present study provide overwhelming evidence for the historical signature of paleodrainage rearrangement on the present-day biogeographic patterning of the Afrotropical freshwater crab fauna. The observed phylogeny is congruent with results for similar studies of riverine and lacustrine freshwater fishes of the Afrotropical region (Salzburger et al. 2002, 2005; Kocher 2004; Seehausen 2006; Wagner et al. 2012; Day et al. 2013). An Eocene origin for the monophyletic Afrotropical Potamonautidae was retrieved here, a result congruent with a period of intensive brachyuran radiation evident from the paleontological record (Brösing 2009). Although climatic data for this period are sparse for the African continent, the Eocene was characterized by a significant increase in global temperatures that resulted in sea level rises and the flooding of coastal plains of the continents as well as increases in mean precipitation that led to the expansion of forests and woodland biomes in general (Parrish et al. 1982; Zachos et al. 2001; Huber and Caballero 2011). A Late Cretaceous origin for
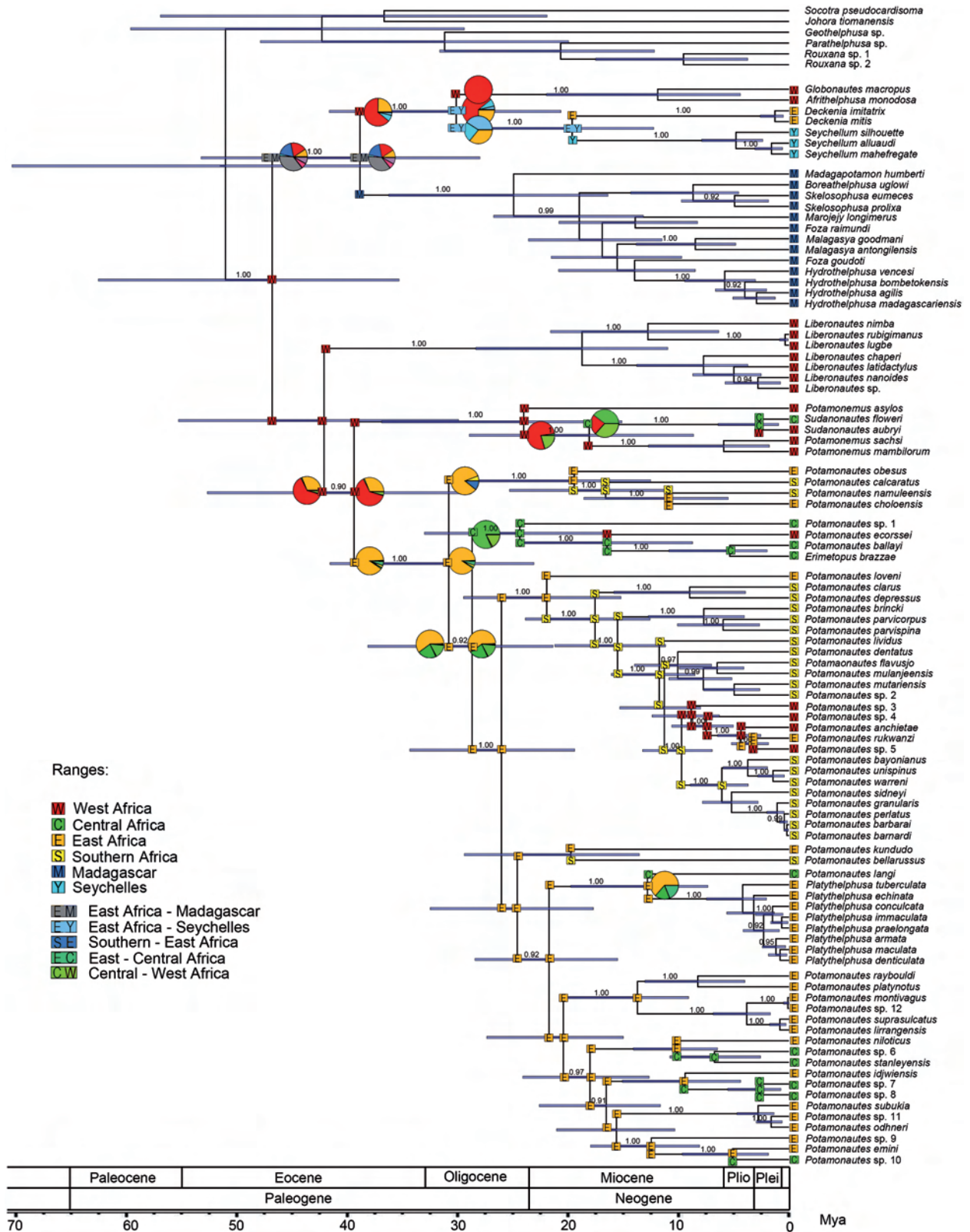
FIGURE 3.   Chronogram of the Afrotropical Potamonautidae inferred from the Bayesian relaxed molecular clock dating (BEAST 1.7.5). The biogeographic range inheritance is estimated using BioGeoBEARS under a DEC+J model for the six major biogeographic regions. The boxes represent the estimated ranges. In cases where ranges covered several areas the boxes contain several letters. The pie charts are the relative probabilities for the states.

the Potamonautidae was reported by Tsang et al. (2014) (divergence from Potamidae 100–74 Ma). Given that our results suggest the Potamonautidae started to diversify 63–34 Ma, the divergence from the Potamidae might be closer to the younger limit within the credibility interval of Tsang et al. (2014). We are cognisant of the fact that only a limited number of freshwater crab fossils exist and this potentially hampers more accurate divergence time estimations, an observation that extends to the young age of the Afrotropical freshwater crab fossil fauna in general. However, using older fossils for calibration that have a higher uncertainty regarding their correct phylogenetic placement (Tsang et al. 2014) may introduce significant error. The dense taxonomic sampling in the present study, representing 66% of the described potamonautid freshwater crab fauna, together with our use of both locus-specific substitution rates and fossil calibration, adds confidence that our results reflect a reasonably accurate estimation for the divergence time of the Potamonautidae. We observed a clear historical faunistic link between freshwater drainage basins, as evidenced by sister taxon relationships between crabs from the Congo and the Zambezi drainages, or the Zambezi/Cunene/Kwanza and Limpopo Orange drainages and the Congo and East African Rift Valley systems, suggesting that evolutionary partitioning at a continental scale is different from that seen on islands. This contrasts with freshwater crab diversification on the Greater Sunda Islands in Southeast Asia, where every island hosts a unique species radiation despite recent Pleistocene drainage connectivity (Klaus et al. 2013). The biogeographical reconstruction and the sister taxon relationship between the Deckeniini of East Africa and the Seychelles Archipelago (the genera *Deckenia* and *Seychellum*) and of West Africa (the genera *Afrithelphusa* and *Globonautes*) implies that the Deckeniini had a widespread range during the Oligocene/Miocene (42–19 Ma) and points to a past paleohydrological connection between West Africa (Upper Guinea), Central Africa (Nilo-Sudan), and East Africa (despite the fact that there is currently no evidence for the presence of deckeniine crabs in central Africa). The latter results support the controversial hypothesis of a paleodrainage link between East and West Africa (McCauley et al. 1982). Marine fossil evidence indicates that the proto-Congo basin was submerged by a shallow marine incursion during the Cretaceous–Oligocene when major rivers drained east toward the Indian Ocean (Stankiewicz and de Wit 2006). The subsequent uplift of the East African Rift System during the Miocene resulted in the Congo River system draining westwards toward the Atlantic Ocean (Stankiewicz and de Wit 2006). Corroborative evidence for an Oligocene connection between East Africa and the Upper Guinean basins is well documented for several vertebrate taxa. For example, phylogenetic analyses of Afrotropical freshwater catfish species of the genus *Synodontis* also demonstrated an Oligocene divergence between West and East African clades (Pinton et al. 2013; Day et al.

2013). A more recent Miocene East-West Africa split has been documented among fossil freshwater fishes of the genera *Semlikiichthys* and *Calarius* (Otero et al. 2009) as well as among sister species of potamophilous mammalian taxa (Boisserie et al. 2005).

Paleodrainage rearrangement is however not the exclusive mechanism responsible for biogeographic patterning, because there is also evidence for transoceanic dispersal. Within the East African Deckeniinae we observe two single transoceanic dispersal events, one from East Africa to the Seychelles Archipelago and one from East Africa to Madagascar as evident from the monophyletic relationship of the freshwater crabs on each of the two Gondwanan islands (Daniels et al. 2006a; Daniels 2011). Transoceanic dispersal from East Africa to the latter two Indian Ocean islands is well established for several terrestrial vertebrate and invertebrate groups (Vences et al. 2003, 2004; Yoder et al. 2003, Yoder and Nowak 2006; Daniels 2011; Townsend et al. 2011; Maddock et al. 2014; Pyron 2014). Paleo-oceanographic modeling suggests that the prevailing oceanic currents flowed eastwards from the East African coast toward these two Gondwanic islands during the Paleogene and the Miocene, which would favor transoceanic dispersal by freshwater crab ancestors from East Africa (Ali and Huber 2010; Esser and Cumberlidge 2011). The Malagasy freshwater crab fauna have undergone a spectacular Miocene radiation, suggesting that several vacant niches possibly existed on the island during the initial colonization period. Similarly, an Eocene/Miocene transoceanic dispersal has also recently been advocated for Malagasy hyperoliid frogs (*Tachycnemis* and *Heterixalus*) (Pyron 2014; Maddock et al. 2014). Within *Potamonautes*, there is a probable transoceanic dispersal events to the volcanic islands of São Tomé in the Atlantic Ocean off the coast of West Africa (Cumberlidge 2008). A West African origin (specifically from the Congo drainage) was recently postulated for the cinnamon-bellied reed frog *Hyperolius cinnamomeoventris* from São Tomé and Principe, with an estimated late Miocene/early Pliocene divergence (Bell et al. 2014). These transoceanic dispersal events were generally restricted to the Miocene epochs suggesting that they are likely the result of stochastic events during mesic periods. Our results underscore that successful transoceanic dispersal events and colonization are rare. Mechanisms that have facilitated transoceanic dispersal remain dubious; however, rafting on vegetation during severe monsoon storms and the survival of species in freshwater plumes are some of the methods postulated to explain transoceanic dispersal across salt barriers (Esser and Cumberlidge 2011).

Within the continental Potamonautinae the West African genus *Liberonautes*, and a clade consisting of [*Sudanonautes* + *Potamonemus*], were consistently retrieved as early branching lineages, suggesting a West African origin of the Potamonautinae at 63–34 Ma, as corroborated by the ancestral area estimation. These three genera cover a range that is considered to include

Pleistocene rain forest refuges (Cumberlidge 1999). However, the old age of the MRCAs of the two clades (*Liberonautes*: 29–12 Ma; [*Potamonemus+Sudanonautes*]: 35–14 Ma) makes it unlikely that these are the result of a recent, post-Pleistocene, range expansion and diversification. This does not support the hypothesis of Cumberlidge (1999) that the species diversity patterns in West Africa reflect dispersal out of Pleistocene refugia. At the species level, however, the potential existence of glacial refuges of rain forest adapted freshwater crabs still has to be tested with population genetic tools.

Our phylogeny and biogeographic inference does not support the hypothesis advocated by Kensley's (1981) that the southern African species of the genus *Potamonautes* are simply derived from a recently migrated ancestral stock from East Africa. Our results suggest that southern Africa underwent at least three independent colonization events as evident from the ancestral area reconstruction and the non-monophyly of the geographic region. This result suggests that Kensley's (1981) hypothesis, derived from Barnard's (1950) observation that several drainages share species complexes, rather reflects the historically poor taxonomic status of the group and emphasizes the need for an accurate taxonomy to underscore biogeographic relationships.

In South Africa the mountain-living species of freshwater crabs are early branches in the clade and are characterized by a Miocene divergence ranging from 6 to 8 Ma for the Drakensberg and Cape Fold Mountains respectively. Here we report on the existence of a large clade of semi-terrestrial and riverine freshwater taxa with a strong tropical affinity that is mostly confined to the north-eastern parts of southern Africa (Mozambique and Malawi). The remaining species in this clade comprise large-bodied riverine species from South Africa that are sister to species from Angola and diverged 13–7 Ma during the Miocene. The latter result reflects recent drainage interconnectivity between the Zambezi, the Okavango delta, and the Limpopo/Orange drainage systems corroborated by the recent divergence time estimations among these species. For example, *Potamonaute bayonianus* occurs in the Okavango delta, while *Potamonaute unispinus* occurs in the Limpopo, Zambezi, and Congo drainages and *P. warreni* occurs in the Orange drainage. The divergence between these three species occurred during the Plio/Pleistocene (95% HDP 6–2 Ma). Within *P. unispinus*—the sole species shared between these large drainages, populations in both drainages show a sequence divergence of <1% for COI, indicating very recent divergence and/or continuous gene flow between these drainages (Daniels S.R., unpublished data). Evidence for recent drainage admixture between the Zambezi/Cunene/Limpopo and Orange rivers is well documented for the ichthyological fauna, particularly among the riverine haplochromine cichlids of southern Africa (Skelton 2001; Salzburger et al. 2005). Goodier et al. (2011) reported a similar phylogeographic pattern, with evidence of shared haplotypes among several

African tigerfish species of the genus *Hydrocynus*. It is interesting to note that despite the geographic proximity of the Angola region to the Congo drainage basin (Fig. 1) no phylogenetic links were shared among freshwater crabs from these two regions in the present study. In contrast, freshwater fishes of the Angola region are generally phylogenetically associated with the Congo drainage, as demonstrated for cichlids between the Kwanza/Okavango and Congo/Zambezi watersheds (Schwarzer et al. 2012). However, this result might reflect a sampling bias toward the southern Angolan drainages during the present study, denser taxonomic sampling from north-eastern Angola is required to explore possible links with the Congo drainage.

The East African potamonautid fauna represents the second largest clade within the Potamonautidae. Within this clade we observed a strong sister taxon relationship between East African species and species from the Congo drainage. A similar pattern was found in viviparid freshwater snails (*Bellamya* and *Neothauma*) (Schultheiß et al. 2014). Some of the small-bodied mountain-living freshwater crabs to the south (*P. bellarussus* from northern Mozambique) and north of the East African Rift System (*P. kundudo* from Ethiopia) are early branches of the large East African clade. These species are characterized by Oligocene to Early Miocene divergence and long branches with poor phylogenetic support, suggesting prolonged mountainous isolation and an absence of further diversification. In contrast, toward the southern Eastern Arc Mountains a more recent Pliocene divergence can be observed, and this is congruent with widespread geotectonic uplift in the region that most likely has promoted allopatric fragmentation and cladogenesis (Trauth et al. 2005). The Eastern Arc Mountains of Tanzania are characterized by high diversity and endemism in a suite of taxonomic groups, including freshwater crabs (Reed and Cumberlidge 2006). We have a relatively poor taxonomic coverage of the *Potamonautes* species for this region, which limits our phylogenetic and biogeographic inferences.

We observed two independent invasions of Lake Tanganyika. In the first instance, the lacustrine *Potamonaute, platynotus* is endemic to Lake Tanganyika and is sister to *Potamonaute, raybouldi* from Kenya and Tanzania, with divergence between these two species dated to the Late Miocene (13–4 Ma), corroborating an East African hydrological connection with the proto Lake Tanganyika. A second invasion of Lake Tanganyika is represented by the endemic genus *Platythelphusa* that is sister to *P. langi* from the Congo basin, indicating a shared paleodrainage history between the proto Lake Tanganyika and the Congo drainage basin roughly at the same time, or slightly earlier (Early to Middle Late Miocene; 19–7 Ma). Similarly, a close phylogenetic relationship between the riverine freshwater fish fauna of the Congo basin and Lake Tanganyika is well documented (Sturmbauer et al. 2001). The initial stages of the formation of proto Lake Tanganyika interrupted the flow of the ancient proto Malagarasi–Congo River, and lake formation progressed

in step with the formation of the East African Rift Valley. Variations in Lake Tanganyika were driven primarily by tectonism and climatic oscillations. The proto Lake Tanganyika basin is thought to have formed 20 Ma, but only reached deep-lacustrine conditions 12–6 Ma, with occasional fluctuations in water levels (Cohen et al. 1993; Sturmbauer et al. 2001; Koblmüller et al. 2008). This is in line with the colonization of freshwater crabs as inferred in the present study. A recent Late Miocene to Pleistocene (8–2 Ma) *in situ* rapid adaptive radiation of *Platythelphusa* is supported by the short internal branches and the low statistical support among species (Fig. 2). Marijnissen et al. (2008) demonstrated some degree of ecological differentiation in habitat and diet exists among *Platythelphusa* species. The dates obtained for the divergence time estimation for *Platythelphusa* are broadly congruent with what has been documented for the cichlid radiations in Lake Tanganyika (Salzburger et al. 2005; Genner et al. 2007; Day et al. 2008). During this period, cichlids are thought to have accumulated species richness and morphological diversification (Genner et al. 2007). While our divergence estimations fall at the lower end of the divergence scale for cichlids, the ancestral riverine origin of Lake Tanganyika's freshwater crab fauna is in broad agreement with the reported divergence dates and riverine origin of its cichlid fish fauna (Genner et al. 2007). Genetic markers with accelerated mutation rates such as non-coding mtDNA sequences and rapidly evolving nuclear markers such as microsatellites, amplified fragment length polymorphism (AFLP), and next-generation sequencing data would benefit the delineation of *Platythelphusa* species boundaries and enhance insight into the evolutionary mechanics of speciation and rapid cladogenesis in a lacustrine environment. Sexual selection is thought to be one of the major drivers for the high cichlid diversity in the East African Rift lake systems but sexual selection is poorly studied in freshwater crabs and its role in cladogenesis is unexplored.

The Miocene is a major epoch for lineage diversification among Afrotropical freshwater crabs as evident from the divergence time estimations. During the early Miocene the climate was wetter than at present with higher levels of precipitation resulting in larger scale paleovegetation changes on the African continent (Zachos et al. 2001). During the early Miocene forested areas were widespread but climatic amelioration in the late Miocene resulted in a decrease in forest cover and an increase in mixed C_3 grasslands. Marked geotectonic uplift along the East African Rift valley during the Miocene reduced precipitation cycles and increased aridity, impacting faunal drainage basin connections (Zachos et al. 2001). In East Africa, forests survived in isolated patches with mixed savannah, while toward the late Miocene C_4 grasses became widely established in association with declining pCO_2-levels (Zachos et al. 2001). During the middle Miocene the proto Benguela oceanic current developed along the southern African west coast

which resulted in decreased rainfall and marked xeric climatic conditions together with the development of highly seasonal rainfall regimes (Siesser 1980). The drier climatic conditions continued throughout the Pliocene/Pleistocene with periodical oscillations between mesic and xeric cycles resulting in significant biome shifts that coincided with the intensification of high-latitude glacial cycles (De Menocal 2004). Based on our divergence time estimations, Miocene cladogenesis appeared to be particularly marked among freshwater crabs, freshwater fishes and frogs, and likely reflects a response to mesic climatic shifts that have resulted in marked allopatric differentiation among lineages (Pinton et al. 2013; Day et al. 2013; Loader et al. 2014).

Taxonomic Considerations and Conclusion

Considerable taxonomic rearrangement is required based on the results of the present study. Our phylogenetic results disagree with the taxonomy proposed by De Grave et al. (2009) and corroborate the taxonomic classification advanced by Cumberlidge et al. (2008). De Grave et al. (2009) included the Malagasy genus *Foza* in the Potamonautinae. However, our phylogenetic results indicate that the seven Malagasy genera (*Boreathelphusa*, *Foza*, *Hydrothelphusa*, *Madagapotamon*, *Malagasya*, *Marojejy*, and *Skelosophusa*) exclusively comprise the monophyletic tribe Hydrothelphusini hence we restrict the latter tribe to Madagascar. Within the Hydrothelphusini, the genera *Foza* and *Skelosophusa* are paraphyletic and in need of revision (Fig. 2). In addition, De Grave et al. (2009) placed the two West African genera *Afrithelphusa* and *Globonautes* within the Malagasy Hydrothelphusini, however, our phylogenetic result does not support this conclusion, hence we transfer *Afrithelphusa* and *Globonautes* to the tribe Globonautini as they are sister to the tribe Deckeniini (*Seychellum* and *Deckenia*) within the subfamily Deckeniinae. The phylogenetic position of the West African genus *Louisea* remains uncertain. Unpublished data suggest that the classification particularly within *Hydrothelphusa* has been problematic, and a study is currently under way to demarcate species boundaries within that genus (Daniels S.R., unpublished data). We restrict the Potamonautinae to continental Africa and its associated inshore islands. The evolutionary relationships for several genera within Potamonautinae are in need of taxonomic revision. For example, *Potamonemus* and *Sudanonautes* were paraphyletic and require further taxonomic investigation. Similarly, *Potamonautes* was retrieved as paraphyletic with respect to *Platythelphusa* and *Erimetopus* and comprised four clades, indicating that a complete revision is required to stabilize the taxonomy. Several undescribed lineages appear to exist within *Potamonautes* (we detected at least 11 novel lineages in the present study) suggesting that species diversity in this widespread genus has likely been underestimated. A complete cladistics analysis of morphological data should be undertaken when a larger

diversity of *Potamonautes* species has been sequenced and phylogenetically analyzed to identify useful morphological characters that can help disentangle the four clades discovered within the genus during the present study. Consequently, we propose the increased use of molecular data in alpha taxonomic studies of freshwater crabs (Cumberlidge and Daniels 2014; Daniels et al. 2014).

The present Afrotropical freshwater crab phylogeny revealed a close link between paleodrainge patterning underscoring the role of both dispersal and vicariance in the biogeographic pattern. A large-scale multitaxon study of other obligatory freshwater taxa in the Afrotropical region would be useful to reconstruct and test historical drainage connectivity patterns and designate biogeographic provinces. This process will also aid in conservation management of the continent's inland fauna, because aquatic biodiversity patterns are likely to reveal unique historical biogeographic areas and evolutionary patterns and processes. Comparative studies with the Neotropical and Oriental faunas would be useful to explore the impact of paleodrainage basin changes on cladogenesis in other obligatory freshwater taxa.

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