

TALL TALES FROM SMALL ANIMALS:
DIVERSITY, PHYLOGENY AND BIOGEOGRAPHY OF
NEGLECTED SOUTHERN AFRICAN ARTHROPODS

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ABOUT THE AUTHOR

Professor Savel Daniels matriculated from Diazville Senior Secondary School in Saldanha in 1991. Subsequently, he graduated with a BSc degree in 1995, a BSc (Hons) in 1996 and an MSc (*cum laude*) in 1998 from the University of the Western Cape. In 1999 he was awarded a freestanding merit bursary from the National Research Foundation (NRF) and enrolled for a PhD at Stellenbosch University (SU). During this period, he also received a SU merit bursary and a Harry Crossly bursary.

Upon completion of his PhD in 2002, he was appointed as junior lecturer and promoted to lecturer the following year in the SU Department of Botany and Zoology. He was awarded a Claude Leon Foundation postdoctoral fellowship that would have permitted him to undertake postdoctoral research in South Africa. However, he declined this fellowship in favour of a two-year (2004–2005) NRF-funded postdoctoral fellowship in the United States of America to work under the supervision of Prof K. Crandall.

Prof. Daniels returned to the Department of Botany and Zoology at SU and became a senior lecturer in 2006 and an associate professor in 2009. His field of expertise is molecular systematics, phylogeography and the conservation of invertebrates. The main objective of his work is to better understand and document the spectacular biological diversity of South Africa to aid the conservation of these groups.

He was a recipient of the Rector's award in 2010, 2011 and 2012 and received a Fulbright Research Scholarship and an Oppenheimer Memorial Trust Research Grant for his sabbatical at Harvard University during 2012. He was awarded a C1 NRF rating in 2012 and was promoted to full professor at SU as from 1 January 2014.

To date, Prof. Daniels has authored and/or coauthored 63 peer-reviewed manuscripts in top-tier international journals. His *h* factor is currently 17 on Scopus and 21 on Google Scholar. In addition, he has presented the results of his research at several local and international conferences. He has successfully supervised and/or cosupervised two PhD, seven MSc and five honours students.

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INTRODUCTION

Invertebrates comprise a staggering 95% of eumetazoan diversity. The term 'invertebrates' constitutes an artificial taxonomic grouping since several invertebrate lineages are phylogenetically more closely related to vertebrates than to other non-vertebrates. True protostome invertebrates comprise several lineages that have fascinated evolutionary biologists; hence, they have provided critical insight into key innovations in the development of eumetazoa. Nevertheless, despite their ecological and evolutionary importance, several invertebrate phyla have remained recalcitrant to modern systematic endeavours using DNA sequence and phylogenomic data in comparison to vertebrates. The clear bias toward vertebrates has resulted in a taxonomic picture that is highly skewed in favour of vertebrate diversity. Consequently, the taxonomic diversity among invertebrates remains largely underestimated and significant debate exists in the literature on the true diversity among invertebrate lineages. Estimates of biodiversity range from three to five million species, with only 1.5 million described species. The number of undiscovered eumetazoan species remains debateable; however, unknown taxonomic diversity is most prevalent among the invertebrates. The underestimated taxonomic diversity among invertebrates is particularly prevalent in temperate, tropical and subtropical regions and generally in areas outside the Northern Hemisphere where systematic expertise is limited, such as, for example, in the Southern Hemisphere regions where several large biodiversity hotspots are present. They provide critical ecological services and are functionally important in several spheres, ranging from agriculture to vectors for several diseases that impact human health, such as for example bilharzia and malaria.

VELVET WORMS

The phylum Arthropoda, the largest invertebrate phylum (also sometimes referred to as a superphylum), is exceptionally diverse taxonomically and contains a spectacularly diverse assembly of faunal

groups. Velvet worms, also called Onychophora, represent one of the most basal Arthropod lineages, and their phylogenetic placement has historically been the subject of vigorous scientific debate. Similarly, the group has also been at the centre of some controversial 'scientific' ideas. The Lobopodia, a now extinct marine group present in the Burgess shale, are widely regarded to be ancestral to velvet worms and date back to 550 million years ago. Extant velvet worms are terrestrial and confined to humid environments such as saproxylic environments in forested environments. Two families occur globally; the Peripatidae are distributed circumtropically in the Neotropic, Mesoamerican, Gabon and Oriental regions whereas the Peripatopsidae have a Southern Hemisphere distribution and are present in Australia, Chile, New Zealand and South Africa, exhibiting a distinct Gondwanic distribution. The Peripatopsidae are currently thought to comprise 42 genera and approximately 110 species.

Velvet worms are stealthy predators that entrap their prey items by spraying glue that contains digestive enzymes from slime papilla lateral to the head and start to eat the prey item immediately. Reproduction in velvet worms is characterised by a diversity of strategies. While two distinct sexes are present (only one species is parthenogenic), the animals lack the typical lock-and-key copulation mechanism. Instead, the most common form of reproduction is dermal insemination. Typically, males bite into the cuticle of females and place small packets of sperm on the dermis. Haemocytes attack the sperm packets, dissolve the content and release the sperm. The sperm then swings through the body cavity (haemocoel) and reaches the uterus. In other species, sperm is transferred via head copulatory organs via species-specific cephalic pits. Species can be oviparous, viviparous or ovoviviparous.

Although the alpha taxonomy of velvet worms has received attention, the paucity of material, the considerable variability in assumed diagnostic features and the pronounced level of morphological stasis and convergence all suggest that traditional taxonomic characteristics may have underestimated the diversity

harboured several geographically distinct clades. The species complexes included *P. balfouri*, *P. capensis*, and *P. moseleyi* (Daniels and Ruhberg, 2010; Daniels et al., 2013; McDonald and Daniels, 2012). Extensive sampling of all three these species complexes was undertaken, and species boundaries were investigated with the use of molecular and morphological datasets. For most lineages, the species complexes exhibited clear morphological characters that could delineate the novel lineages.

Evolutionary relationships in the widely distributed velvet worm *P. balfouri* sensu lato species complex were examined using molecular and gross morphology and scanning electron microscopy (SEM). Sequence data were generated for the COI mtDNA and the 18S nDNA loci. Phylogenetic analyses of the combined mtDNA and nDNA sequence data revealed that *P. clavigera* specimens from the Southern Cape were sister to *P. balfouri* sensu lato specimens from the Cederberg Mountains. Within the main *P. balfouri* sensu lato species complex, three additional clades could be discerned. The phylogenetic position of the obligatory troglobitic species *P. alba* was equidistant between the Cape Peninsula and adjacent interior and the two Boland and Hottentots Holland Mountain clades. On the Cape Peninsula, *P. stelliporata* specimens nested among the sympatric *P. balfouri* sensu lato specimens. The Cape Peninsula specimens were sister to samples from Jonkershoek Site 1, Kogelberg and Simonsberg. Two Boland clades were retrieved, comprising Du Toit's Kloof, Bain's Kloof and Mitchell's Pass and sister to specimens from the Boland and adjacent Hottentots Holland Mountains. These results revealed complex biogeographic patterning in the *P. balfouri* sensu lato species complex. The presence of sympatric yet genetically discrete species pairs at six of the sample localities (Du Toit's Kloof, Simonsberg, Jonkershoek sites 1 and 2, Kogelberg and Landroskop) suggests that there is reproductive isolation among the lineages. Divergence time estimations suggest a Miocene/Pleistocene cladogenesis. A taxonomic revision of the *P. balfouri* sensu lato species complex was undertaken to stabilise the taxonomy. *P. clavigera* is monophyletic and retained for the Southern Cape specimens, *P. balfouri* sensu strictu is now confined to the Cape Peninsula and adjacent interior with *P. stelliporata* regarded as a junior synonym of the latter taxon, and *P. alba* is endemic to the Wynberg Cave systems on the Cape Peninsula. Three novel species (*P. bolandi*, *P. cederbergiensis*, and *P. purpureus*) were described based on these results.

Similarly, a phylogeographic study of the *P. capensis* sensu lato species complex, using COI and 18S nDNA sequence data, revealed three geographically discrete clades that were characterised by marked COI sequence

divergence values with subtle morphological differences (McDonald and Daniels, 2012). Divergence time estimations using mutation rates in arthropods revealed a Pleistocene divergence among the three geographically discrete clades. Two of the three clades were described as novel species (McDonald et al., 2012).

Within the third species complex, systematics of *P. moseleyi* sensu lato, a widely distributed species, was examined to test the occurrence of cryptic lineages. A total of 81 specimens of *P. moseleyi* were collected from 12 localities throughout its known distribution in the Eastern Cape and KwaZulu-Natal provinces of South Africa. All specimens were sequenced for a 631 base pair fragment of the mitochondrial COI locus while a 717 base pair fragment of the 18S nDNA locus was sequenced for a single sample for each of the clades evident from the COI topology. DNA sequence data were analysed using maximum parsimony and Bayesian inferences while a haplotype network was constructed and an analysis of molecular variation was conducted. Gross morphological characteristics, such as the number of pregenital leg pairs, the genital areas and colour variation in each sample locality, were examined. Moreover, the characters of the antennae, the shape of the main dermal papillae and the male genital pad features were investigated. Topologies derived from the combined DNA analyses (COI and 18S nDNA) were congruent and revealed that *P. moseleyi* was a complex comprised of five genetically distinct clades. These clades have subsequently been described as novel species (Ruhberg and Daniels, 2013). Currently a formal International Union for Conservation of Nature Red Listing is being undertaken for *Peripatopsis* in order to formulate better conservation management plans for this faunal group.

Cryptic speciation and biogeographic patterning were examined in the geographically widely distributed South African velvet worm *Opisthopatus cinctipes* under a null hypothesis that multiple cryptic lineages were nested within the species. A total of 184 *O. cinctipes* specimens, together with its two point endemic sister species (*O. roseus* and *O. herbertorus*), were collected from 49 sample localities throughout the forest archipelago along the Indian Ocean Coastal Belt forest and Afrotemperate forest patches in the Eastern Cape, KwaZulu-Natal and Mpumalanga provinces in South Africa. All specimens were sequenced for two partial mtDNA fragments (COI and 12S rRNA) while a single specimen from each locality was sequenced for the 18S nDNA locus. Gross morphological characters together with SEM data were collected for a single male specimen per locality. Evolutionary relationships were assessed

using maximum likelihood and Bayesian inferences while population genetic structure was conducted for the COI locus. Phylogenetic results revealed seven distinct statistically defined clades, suggesting the presence of several novel lineages (Fig. 2). Clades exhibited strong geographic patterning that was irreconcilable with the existing taxonomy within *Opisthopatus*. Population results demonstrated marked genetic structuring and indicated the absence of shared haplotypes among sample localities. Specimens confined to Afrotropical forests in the interior were basal while specimens from the Indian Ocean Coastal Belt were derived. Divergence time estimations were conducted for *Opisthopatus* and the results revealed an Eocene divergence among the basal clades. Both the gross morphological analyses and the SEM revealed limited diagnostic morphological differences among the genealogically discreet clades. The absence of diagnostic morphological differences among the clades is problematic in defining traditional species boundaries. Collectively, these results suggest higher levels of endemism within the assumed widely distributed *O. cinctipes* species complex (Fig. 3). Sedentary habitat specialist invertebrates restricted to are likely to exhibit similar patterns, accentuating the conservation value of these habitats in an attempt to optimise the protection of genetic diversity.

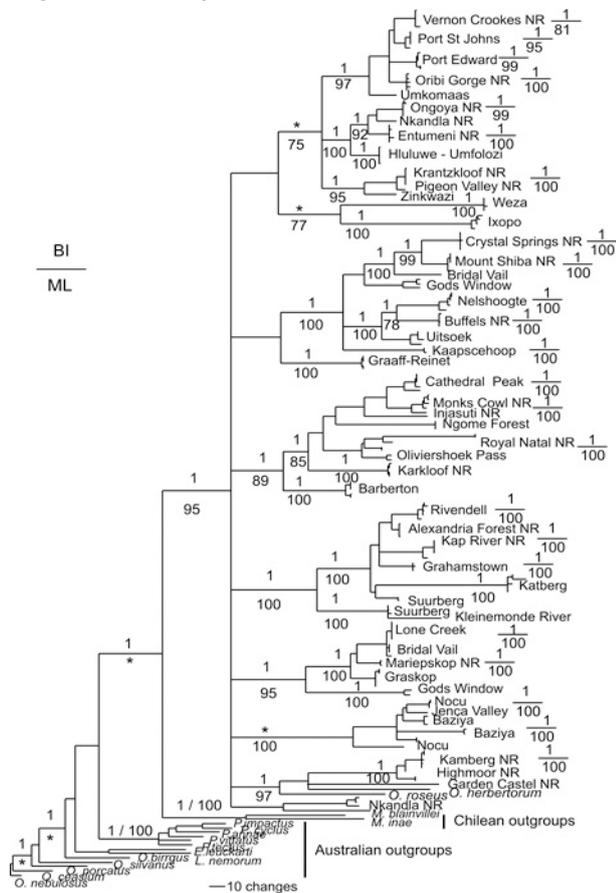


Figure 2. A maximum likelihood tree topology derived from two mitochondrial DNA loci (COI and 12S rRNA) demonstrates divergences within *Opisthopatus cinctipes*.

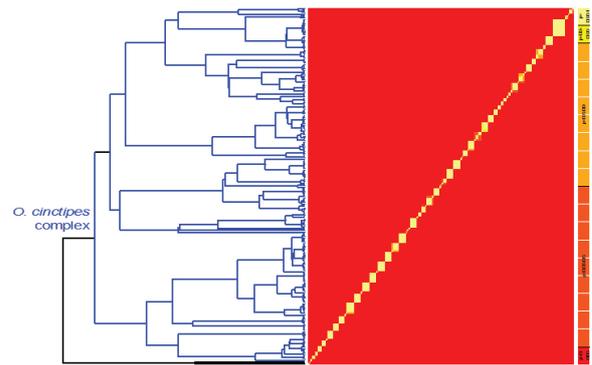


Figure 3. A Bayesian tree derived from the combined mtDNA loci demonstrating the presence of cryptic lineages within *Opisthopatus cinctipes*.

Given the antiquity of velvet worms, their habitat specificity and their inability to traverse xeric geographic barriers make them excellent organisms with which to test biogeographic hypotheses. The distribution of Onychophora across the southern continents has long been contentious, with some authors considering the fauna to be the result of historical vicariance events. However, it has recently been hypothesised that New Zealand was completely inundated during the late Oligocene (25–22 Ma) and that the entire biota therefore is the result of several recent long-distance dispersals from Australia. Together with colleagues from New Zealand, we explored this assumption using phylogenetic and molecular dating of DNA sequence data from Onychophora. The phylogenetic trees revealed that the New Zealand genera *Ooperipatellus* and *Peripatoides* together with selected Australian genera (*Euperipatoides*, *Phallocephale* and an undescribed genus from Tasmania) formed a monophyletic group that was the sister group to genera from Chile (*Metaperipatus*) and South Africa (*Peripatopsis* and *Opisthopatus*). The relaxed-clock dating analyses yielded mean divergence times from 71.3 to 78.9 Ma for the split of the New Zealand *Peripatoides* from their Australian sister taxa. The posterior intervals were very broad and ranged from 24.5 to 137.6 Ma, depending on the prior assumptions. The mean divergence of the New Zealand species of *Ooperipatellus* from the Australian species *O. insignis* was estimated at between 39.9 and 46.2 Ma, with posterior intervals ranging from 9.5 to 91.6 Ma. The age of *Peripatoides* is consistent with long-term survival in New Zealand and implies that New Zealand was not completely submerged during the Oligocene. *Ooperipatellus* is less informative on the question of continuous land in the New Zealand region because we cannot exclude a post-Oligocene divergence. The great age of *Peripatoides* is consistent with a vicariant origin of this genus resulting from the rifting of New Zealand from the eastern margin

of Gondwana and supports the assumptions of previous authors who considered the Onychophora to be a relict component of the New Zealand biota (Allwood et al., 2010).

Phylogenetic affinities among the global velvet worm fauna have remained unexplored. The contemporary distributions of widespread groups of terrestrial animals and plants are supposedly the result of a mixture of vicariance due to continental drift and more recent transoceanic dispersal. For organisms exhibiting a vicariant biogeographic pattern – achieving their current distribution by riding on the plates of former supercontinents – this view is largely inspired by the belief that Pangaea lacked geographic or ecological barriers or that extinctions and dispersal would have erased any biogeographic signal since the early Mesozoic. We undertook a time-calibrated molecular phylogeny of Onychophora. Our data demonstrated that transoceanic dispersal could not be invoked to explain contemporary distributions and revealed that the early diversification of the group predated the breakup of Pangaea while maintaining regionalisation even on landmasses that had remained contiguous throughout the history of the group. These results corroborate a growing body of evidence from palaeontology, paleogeography and palaeoclimatic modelling, depicting ancient biogeographic regionalisation over the continuous landmass of Pangaea (Fig. 4) (Murrienne et al., 2014).

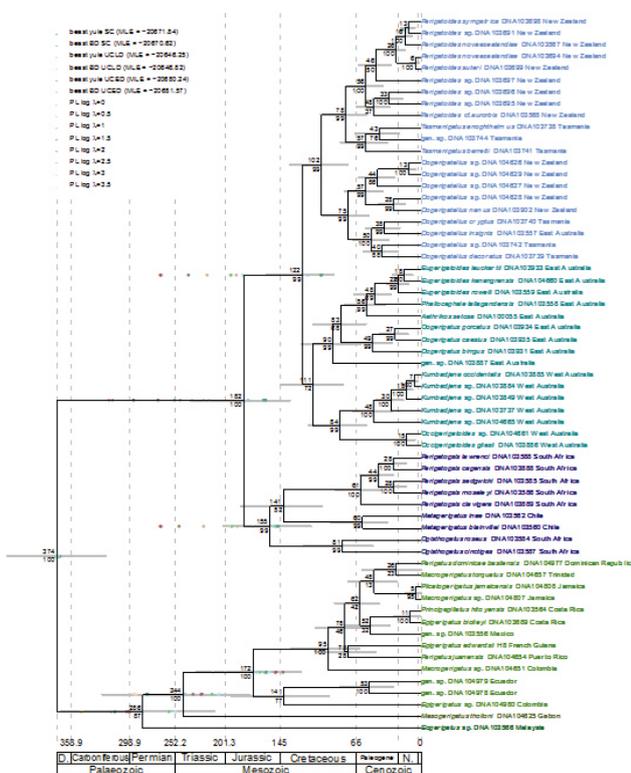


Figure 4. A maximum likelihood tree topology for representatives of the global velvet worm fauna demonstrating evolutionary relationships and divergence time estimations.

FRESHWATER DECAPODS

Freshwater decapods, such as crabs and crayfish, have been demonstrated to be excellent taxa to correlate phylogenetic and biogeographic patterning. Freshwater crabs (Potamoidea) are distributed circumtropically, have fastidious habitat requirements, exhibit high levels of species diversity and endemism, exhibit direct development, lack a dispersive larval phase and complete their entire life cycle in freshwater. Freshwater crabs comprise in excess of 1 320 extant species and represent the largest group within the Brachyura. Primary freshwater crabs are assigned to five families, four of which are probably monophyletic (Gecarcinidae, Potamidae, Potamonautidae and Pseudothelphusidae) while the fifth family, the Neotropical Trichodactylidae, represents a separate invasion of freshwaters. In the Afrotropics, freshwater crabs are the largest inland aquatic invertebrates and are critical to ecosystem function and structure. Freshwater crabs in Africa are medically important because they are vectors in the transmission of river blindness and paragonimiasis in humans. Most species of Afrotropical freshwater crabs are riverine, but there are a significant number of species that have adapted to live in nearly all inland aquatic 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.

Despite their importance as disease vectors, the alpha taxonomy of Afrotropical freshwater crabs has remained limited. Consequently, a systematic study of the Southern African fauna was initiated to document and describe the alpha taxonomy of the group. This study has led to the description of several novel species, with several still awaiting formal description (see for example Cumberlidge and Daniels, 2014; Daniels et al., 1998, 2001, 2014; Daniels and Baylis 2012; Phiri and Daniels, 2013, 2014). The inability of freshwater crabs to traverse freshwater systems together with the fact that freshwater systems are generally island like renders them useful organisms with which to explore biogeographic and paleodrainage interconnectivity.

The endemic, monotypic freshwater crab species *Seychellum alluaudi* was used as a template to examine the initial colonisation and evolutionary history among the major islands in the Seychelles Archipelago. Five of the 'inner' islands in the Seychelles Archipelago, including Mahé, Praslin, Silhouette, La Digue and Frégate, were sampled. Two partial mtDNA fragments, 16S rRNA and COI were sequenced for 83 specimens of *S. alluaudi*. Evolutionary relationships among populations

were inferred from the combined mtDNA dataset using maximum parsimony, maximum likelihood and Bayesian inferences. Analysis of molecular variance was used to examine genetic variation among and within clades. A haplotype network was constructed and divergence time estimation was employed to date the colonisation and divergence of lineages on the islands. Phylogenetic analyses of the combined mtDNA dataset of 1103 base pairs retrieved a monophyletic *S. alluaudi* group comprised of three statistically well-supported monophyletic clades. Clade 1 was exclusive to Silhouette, Clade 2 included samples from Praslin, sister to La Digue, while Clade 3 comprised samples from Mahé, sister to Frégate. The haplotype network corresponded to the three clades. Within Mahé, a substantial phylogeographic substructure was evident. Analysis of molecular variance results revealed limited genetic variation within localities with most variation occurring among localities. Divergence time estimations predated the Holocene sea level regressions and indicated a Pliocene/Pleistocene divergence among the three clades evident within *S. alluaudi*. The monophyly of each clade suggests that transoceanic dispersal is rare. The absence of shared haplotypes among the three clades, coupled with marked sequence divergence values, suggests the presence of three allopecies within *S. alluaudi* (Cumberlidge and Daniels, 2014).

Phylogenetic studies of freshwater crab relationships globally have largely concentrated on regionally discrete geographic areas (for example Southern Africa, Indo-China, the Aegean, the Seychelles Archipelago and Borneo) and have generally focused on a specific ingroup (Daniels, 2011; Daniels et al., 2002, 2006). 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. Hence, I initiated a phylogenetic study that focused exclusively on the Afrotropical freshwater crab fauna. During this study, phylogenetic reconstruction, divergence time estimation 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, COI and the histone 3 gene). This study represented the most comprehensive taxonomic sampling of any freshwater crab family globally and explored the impact of paleodrainage interconnectivity on cladogenesis among freshwater crabs.

Phylogenetic analyses of the total evidence data using maximum likelihood, maximum parsimony and Bayesian inference produced a robust statistically well-supported multilocus topology that reaffirmed the monophyly of the Afrotropical freshwater crab fauna (Fig. 5). The estimated divergence times suggest that the Afrotropical Potamonautidae diverged during the Eocene (Fig. 6). 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. Within the East African clade, we observed two independent transoceanic dispersal events, one to the Seychelles Archipelago and a second to Madagascar. 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. 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 wide-ranging taxonomic implications of the phylogenetic results will be discussed. The results from the present study reveal the importance of historical paleodrainage connectivity together with contemporary dispersal events, geotectonic uplift and transoceanic dispersal in sculpting the contemporary biogeographic patterning among Afrotropical freshwater crabs (Daniels et al., 2015). Future endeavours will examine evolutionary relationships among other neglected Afrotropical freshwater decapods such as prawns, a group that has remained understudied throughout the continent.

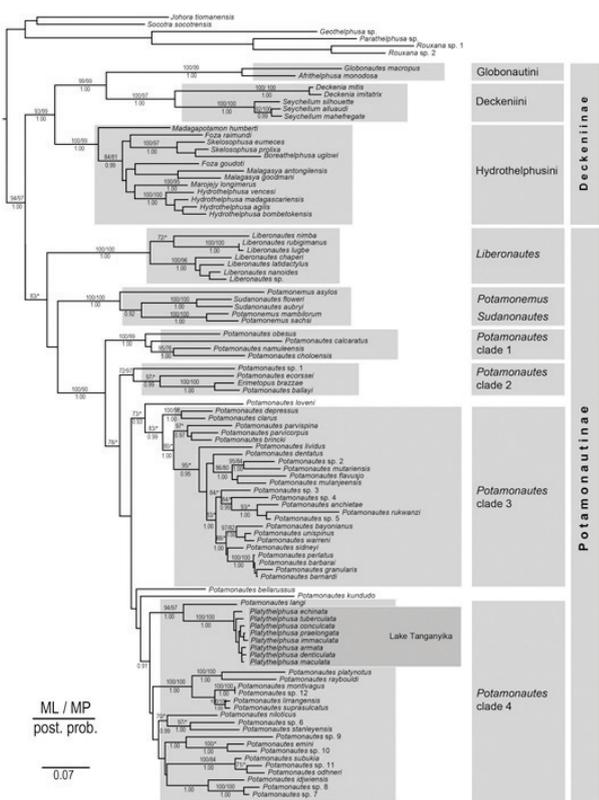


Figure 5. A maximum likelihood tree topology derived from four partial DNA sequence loci (COI, 12S rRNA, 16S rRNA and histone 3) demonstrating the evolutionary relationships among Afrotropical freshwater crabs.

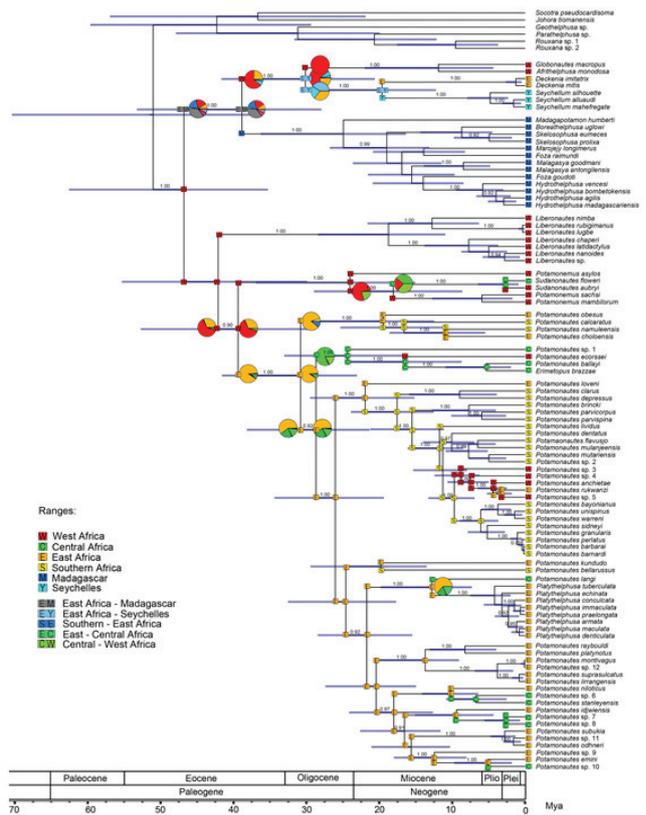


Figure 6. Divergence time estimations derived from a BEAST analyses for the Afrotropical freshwater crab fauna.

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