



Neglected refugia of biodiversity: mountainous regions in Mozambique and Malawi yield two novel freshwater crab species (Potamonautidae: *Potamonautes*)

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Phylogenetic relationships amongst the southern African freshwater crab fauna are reinvestigated following the recent collection of morphologically distinct *Potamonautes* specimens from remote mountainous regions in Malawi and Mozambique. Specimens were subjected to DNA sequencing of three mtDNA loci, cytochrome *c* oxidase subunit I (COI), 12S rRNA, and 16S rRNA and compared to the 14 described species from the region. Phylogenetic analysis using maximum parsimony and Bayesian inference revealed the presence of two novel evolutionary lineages. The phylogeny demonstrates that *Potamonautes obesus* (A. Milne-Edwards, 1868) is sister to a morphologically distinct novel species from Mount Namuli in Mozambique. Two sympatric and genetically distinct species from Mount Mulanje, in Malawi (forms A and B) were recognized. Form B is sister to the large-bodied South African riverine freshwater crabs and represents a novel lineage whereas the remaining species (form A) from Mulanje, in Malawi was sister to samples from Mounts Inago and Mabu, and in Mozambique was identified as *Potamonautes choloensis* (Chace, 1953). The two novel evolutionary lineages were genetically distinct and morphologically different from the described species in each of the respective regions. Two new freshwater crab species *Potamonautes namuliensis* sp. nov. and *Potamonautes mulanjeensis* sp. nov., are described in the present study. The samples from Mount Mulanje in Malawi, and Mounts Mabu and Inago in Mozambique represent new distribution records for *Potamonautes choloensis*.

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INTRODUCTION

Freshwater crabs are the largest macro invertebrates of inland aquatic systems throughout the Afrotropical region. Despite their key roles in freshwater ecosystems, as detritivores and prey items for birds, fish, and crocodiles, the taxonomy of freshwater crabs has until recently remained in flux. The Potamonautinae Bott, 1970, is the most diverse family on the African

continent and contains seven genera (Cumberlidge, von Sternberg & Daniels, 2008; Ng, Guinot & Davie, 2008). Genera can be easily differentiated morphologically but subgenera, species, and subspecies delineation can often be problematic (Daniels *et al.* 2002, Daniels, Gouws & Crandall, 2006a). *Potamonautes* MacLeay, 1838 is the most speciose genus and contains in excess of 73 described species (Cumberlidge *et al.*, 2008; Ng *et al.*, 2008). The genus is widely distributed throughout the continent from the southern Sahara desert southwards to South Africa. Species inhabit a diversity of inland aquatic systems

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ranging from high altitude mountain streams, rivers and lakes, to ephemeral pans. In the past, species boundaries in *Potamonautes* have been poorly defined. For example, only six freshwater crab species [*Potamonautes brincki* (Bott, 1960), *Potamonautes calcaratus* (Gordon, 1929), *Potamonautes depressus* (Krauss, 1843), *Potamonautes perlatus* (H. Milne Edwards, 1837), *Potamonautes sidneyi* (Rathbun, 1904), and *Potamonautes warreni* (Calman, 1918)] were historically known to occur in South Africa (Barnard, 1935, 1950). Recent systematic studies of South African freshwater crabs have resulted in the doubling of the number of species with the recognition of seven new species from that country (Stewart, Coke & Cook, 1995; Stewart, 1997; Daniels, Stewart & Gibbons, 1998, Stewart & Cook, 1998; Gouws, Stewart & Coke, 2000; Gouws, Stewart & Reavell, 2001; Daniels, Stewart & Burmeister, 2001). These results demonstrated high levels of endemism among mountain-living species of freshwater crabs, suggested that highland areas need to be targeted during systematic surveys (Daniels *et al.*, 2003). For example, small-bodied mountain-living species are restricted to first and second order streams whereas large-bodied species occur in the larger river systems. Furthermore, these results indicate that species with widespread distributions may include cryptic species and these need to be incorporated into systematic studies (Daniels *et al.*, 2003, 2006a, b).

The freshwater crab fauna of Malawi (formerly Nyasaland) and Mozambique (formerly Portuguese, East Africa) is poorly studied, and appears to be species depauperate, however freshwater bodies in these countries have been devoid of recent sampling and may well harbour undescribed species. Five freshwater crab species are known from Malawi, these include *Potamonautes choloensis* (Chace, 1953), *Potamonautes lirrangensis* (Rathbun, 1904), *Potamonautes montivagus* (Chace, 1953), *Potamonautes obesus* (A. Milne-Edwards, 1868), and *Potamonautes suprasulcatus* (Hilgendorf, 1898). In neighbouring Mozambique four species are present, *Potamonautes bayonianus* (Brito Capello, 1873), *P. calcaratus*, *P. sidneyi*, and *P. obesus* (Barnard, 1950; Chace, 1953; Bott, 1955; Reed & Cumberlidge, 2004, 2006; Cumberlidge & Daniels, 2008). Bott (1955) treated *P. choloensis* as a junior synonym of *Potamonautes* (*Orthopotamonautes*) *depressus dybowskii*. Notably, *P. choloensis* is absent from the Systema Brachyurorum (Ng *et al.*, 2008) leading to further confusion over its taxonomic validity. However, Cumberlidge *et al.* (2009) recognized it as a valid species and this opinion is accepted here. Bott (1955) referred to *P. obesus* as *Potamonautes* (*Obesopotamonautes*) *obesus obesus* (A. Milne-Edwards, 1868). This species is treated here as *P. obesus* following Reed & Cumberlidge (2004), who

regarded *Potamonautes bottegoi* (De Man 1898) as a junior synonym. These taxonomic synonymies have contributed to the instability and confusion of species boundaries within *Potamonautes*. Notably, a number of these freshwater crab species have wide distribution ranges coupled with marked levels of intraspecific morphological variation (Barnard, 1950; Daniels *et al.*, 2002; Reed & Cumberlidge, 2004). For example, *P. obesus* is found in Somalia, Kenya, Tanzania (including the islands of Zanzibar and Pemba), Malawi, Zimbabwe, and Mozambique (Barnard, 1950; Reed & Cumberlidge, 2004, 2006). The latter authors reported marked variation amongst *P. obesus* specimens and noted the presence of two distinct morphs within this species. *Potamonautes lirrangensis* is also widely distributed in rivers and lakes in the Democratic Republic of the Congo, Malawi, and Tanzania (Reed & Cumberlidge, 2006).

Recently, a large-scale sampling initiative funded by the Darwin Initiative was launched to document the biodiversity of poorly sampled mountainous areas in northern Mozambique and the adjacent interior of southern Malawi. The project was a collaborative venture between the Royal Botanic Gardens Kew and BirdLife International in the United Kingdom, the Instituto de Investigação Agrária de Mozambique (IIAM), the Mulanje Mountain Conservation Trust (MMCT) in Malawi, and the Forest Research Institute of Malawi (FRIM). The project was led by the Royal Botanic Gardens Kew. To date this study has yielded over 20 species new to science, including four chameleons, a viper, two plants, as well as five butterflies (Branch & Bayliss, 2009; Branch & Tolley, 2010; J. Bayliss pers. observ.). Freshwater crabs were collected from a number of high altitude mountainous areas in Malawi and Mozambique and sent to the first author for identification. Upon examination of the sampled material it became apparent that several specimens were morphologically distinct from known species in the respective regions. Consequently a molecular systematic study was initiated to examine and compare the phylogenetic relationship between specimens from the newly collected localities and known southern African species.

MATERIAL AND METHODS

SAMPLE COLLECTION

Freshwater crabs were collected from mountains in northern Mozambique (Mount Mabu, $N = 6$; Mount Inago, $N = 6$; and Mount Namuli, $N = 4$) and bordering southern Malawi (Mount Mulanje $N = 4$) (Fig. 1). Crabs were killed by freezing and preserved in absolute ethanol for DNA analyses. Samples were identified using Chace (1953), Bott (1955), and Reed &

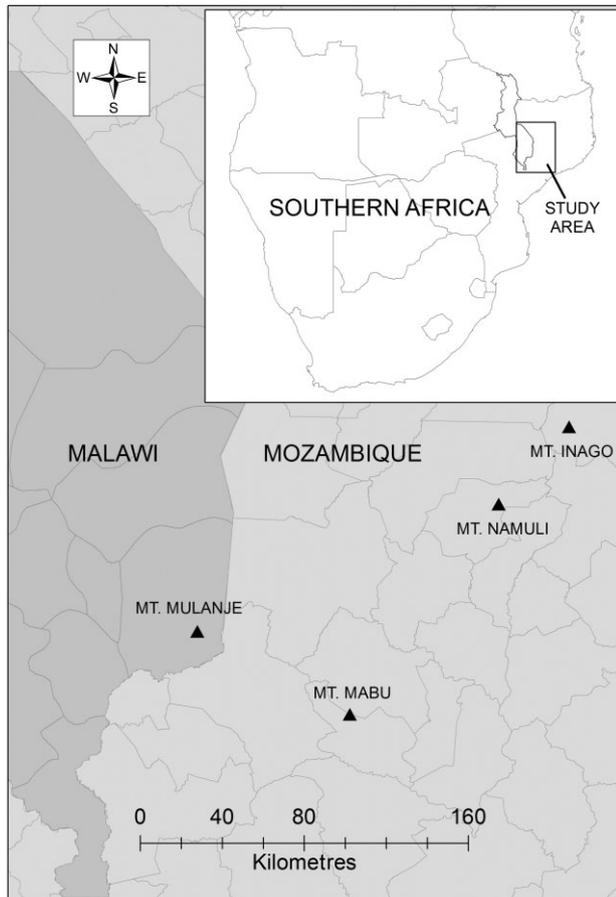


Figure 1. A map showing the three sample sites in central and northern Mozambique and the one sample site in southern Malawi where freshwater crabs were collected.

Cumberlidge (2006). In addition we also included samples of *P. obesus* from Zanzibar ($N = 6$), the type locality of the species, and one sample from Malawi. Two GenBank sequences of *P. obesus* from Kenya and Tanzania were also included (Klaus, Schubart & Brandis, 2006; Daniels *et al.*, 2006a). Specimens of *P. calcaratus* ($N = 4$) from Mozambique and two *Potamonautes lividus* Gouws *et al.*, 2001 specimens from Swaziland were also included. Sequences for all 14 of the described southern African freshwater crab species, as well as seven East African potamonautid crab species were downloaded from GenBank (see Table 1 for details) and included to validate the phylogenetic placement of the newly collected specimens (Daniels *et al.*, 2002, 2006a; Klaus *et al.*, 2006).

DNA EXTRACTION, PCR, AND SEQUENCING

Muscle tissue extracted from walking legs was subjected to DNA extraction using a Qiagen DNEasy kit, following the manufacturer's protocol. Extracted DNA

was stored in a refrigerator until required for PCR. Generally, a 1 μ l DNA in 19 μ l water dilution was performed prior to use. Three partial gene fragments were selected for the present study: cytochrome *c* oxidase subunit I (COI), 12S rRNA, and 16S rRNA.

These three loci were selected because each has a different mutational rate and have been successfully used for reconstructing evolutionary relationships amongst freshwater crabs (Daniels *et al.*, 2002, 2006a, b; Klaus *et al.*, 2006; Shih *et al.*, 2006). Primer pairs are outlined in Daniels *et al.* 2002, 2006a). Standard PCR conditions were followed for amplification and DNA sequencing protocols were followed (Daniels *et al.*, 2006a).

PHYLOGENETIC ANALYSES

SEQUENCE NAVIGATOR (Applied Biosystems) was used to compute a consensus sequence from forward and reverse strands. The COI sequences were aligned manually. No insertions or deletions were evident for the protein coding COI locus and sequences for this locus were aligned manually. The 12S rRNA and 16S rRNA loci were aligned using CLUSTAL X (Thompson *et al.*, 1997). As all three partial fragments occur on the mitochondria and are linked, we combined the DNA sequence data for the three into a single data matrix and conducted all analyses on the combined data set. Maximum parsimony (MP) and Bayesian approaches were used to estimate evolutionary relationships. 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 tree bisection-reconnection branch swapping using 100 random taxon additions, with gaps treated as fifth characters in the 12S rRNA and 16S rRNA. Phylogenetic confidence in the nodes recovered from MP was estimated by bootstrapping (Felsenstein, 1985), analysing 1000 pseudoreplicates of data sets. Bootstrap values for nodes of $< 70\%$ were regarded as poorly resolved. Uncorrected sequence ('p') distances were calculated in PAUP*4 v. beta 10 (Swofford, 2002). MODELTEST v. 3.06 (Posada & Crandall, 1998) was used to obtain the best-fit substitution model for each gene locus. These substitution models were used in the partitioned Bayesian analyses. The best-fit maximum likelihood score was chosen using the Akaike information criterion (AIC; Akaike, 1973), as this reduces the number of parameters that contribute little to describing the data by penalizing more complex models (Nylander *et al.*, 2004; Posada & Buckley, 2004). Bayesian inferences were used to investigate optimal tree space using the program MRBAYES 3.0b4 (Ronquist & Huelsenbeck, 2003). For each analysis, four Markov chains were run, with each chain starting from a random tree and run for

Table 1. List of GenBank species and mtDNA sequences used during the present study. A dash indicates the absence of sequence data for that specific locus

Species	Reference study	GenBank accession numbers		
		12S rRNA	16S rRNA	COI
<i>Liberonautes rubrigimanus</i>	Daniels <i>et al.</i> (2006a)	–	AY803543	–
<i>Potamonautes bayonianus</i>	Daniels <i>et al.</i> (2002)	AY042321	AY042243	AF510868
<i>Potamonautes brincki</i>	Daniels <i>et al.</i> (2002)	AY042322	AY042244	AF510875
<i>Potamonautes calcaratus</i>	Daniels <i>et al.</i> (2002)	AY042323	AY042242	AF510867
<i>Potamonautes clarus</i>	Daniels <i>et al.</i> (2002)	AY042320	AY042241	AF510872
<i>Potamonautes dentatus</i>	Daniels <i>et al.</i> (2002)	AY042324	AY042246	AF510878
<i>Potamonautes depressus</i>	Daniels <i>et al.</i> (2002)	AY042325	AY042247	AF510877
<i>Potamonautes emini</i>	Daniels <i>et al.</i> (2006a)	AY803493	AY803533	–
<i>Potamonautes granularis</i>	Daniels <i>et al.</i> (2002)	AY042326	AY042254	AF510876
<i>Potamonautes lirrangensis</i>	Daniels <i>et al.</i> (2006a)	AY803494	AY803534	AY803568
<i>Potamonautes lividus</i>	Daniels <i>et al.</i> (2002)	AY042327	AY042248	AF510879
<i>Potamonautes obesus</i>	Daniels <i>et al.</i> (2006a)	AY803497	AY803537	AY803647
<i>Potamonautes obesus</i>	Klaus <i>et al.</i> (2006)	–	AM234646	–
<i>Potamonautes odhneri</i>	Daniels <i>et al.</i> (2006a)	AY803498	AY803538	AY803571
<i>Potamonautes parvicorpus</i>	Daniels <i>et al.</i> (2002)	AY042328	AY042252	AF510869
<i>Potamonautes parvispina</i>	Daniels <i>et al.</i> (2002)	AY042329	AY042253	AF510873
<i>Potamonautes perlatus</i>	Daniels <i>et al.</i> (2002)	AY042330	AY042249	AF510874
<i>Potamonautes platynotus</i>	Daniels <i>et al.</i> (2006a)	AY803499	AY803539	AY803572
<i>Potamonautes sidneyi</i>	Daniels <i>et al.</i> (2002)	AY042331	AY042245	AF510871
<i>Potamonautes subukia</i>	Daniels <i>et al.</i> (2006a)	AY803495	AY803535	AY803569
<i>Potamonautes raybouldi</i>	Daniels <i>et al.</i> (2006a)	AY803540	AY803500	AY803573
<i>Potamonautes unispinus</i>	Daniels <i>et al.</i> (2002)	AY042332	AY042250	AF510870
<i>Potamonautes warreni</i>	Daniels <i>et al.</i> (2002)	AY042333	AY042251	AF510880
<i>Potamonemus sachsii</i>	Daniels <i>et al.</i> (2006a)	AY803490	AY803530	–
<i>Sudanonautes aubryi</i>	Daniels <i>et al.</i> (2006a)	AY803502	AY803542	AY803575

5 000 000 generations, sampling each chain every 10 000th tree. This process was repeated four times to ensure that trees converged on the same topology. A 50% majority rule consensus tree was generated from the trees retained (after the burn-in trees were discarded – using likelihood plots) with posterior probabilities (pP) for each node estimated by the percentage of time the node was recovered. Posterior probabilities values $< 0.95 pP$ were regarded as poorly resolved. The combined data were analysed using the mixed substitution models for each locus in the Bayesian analyses.

OUTGROUP SELECTION

Daniels *et al.* (2006a) demonstrated that *Potamonautes* is sister to *Potamonemus* (Cumberlidge & Clark, 1992), *Liberonautes* (Bott, 1955) and *Sudanonautes* (Bott, 1955) allowing species from these three genera to be used as outgroup taxa. Hence, *Potamonemus sachsii* (Cumberlidge & Clark, 1992), *Liberonautes rubrigimanus* (Cumberlidge & Sachs, 1989), and *Sudanonautes aubryi* (H. Milne Edwards, 1853) were

used as outgroups. See Table 1 for the downloaded GenBank accession numbers of the outgroup species.

MORPHOLOGY

Samples were divided into males and females and the following measurements were taken with digital vernier callipers. The carapace length (CL); the carapace widest at width point (CWW); the width of the posterior margin of the carapace (CWP); the distance between the postfrontal crest and the anterior margins of the carapace (PFCD); the frontal width measured between the medial margins of the orbits (FW); the distance between the exorbital teeth (CWA); and the carapace height (CH) were measured. Pereiopod measurements were also taken: length and width of merus of pereiopods 2 and 5, and the length and height of the major cheliped. All measurements are given in mm. Samples have been deposited in the South African Museum of Natural History, Iziko Museums of Cape Town (SAM). Freshwater crabs were photographed with the use of a Nikon A 300 digital camera. In addition, the structure of gonopods

1 and 2 were photographed with a Leica MZ 75 digital camera, attached to a Leica EC 3 X stereo-microscope.

RESULTS

PHYLOGENETIC ANALYSES

The partial fragments amplified were 596, 259, and 354 bp for COI, 12S rRNA, and 16S rRNA, respectively. Hence, the combined mtDNA data set comprised of 1182 bp. Newly generated sequences are deposited in GenBank (COI accession numbers JF 799183–JF 799215; 12S rRNA accession numbers JF 799152–JF 799182; 16S rRNA accession numbers JF 799118–JF 799151). The AIC substitution models for the three loci used during the partitioned Bayesian analyses are as follows: for COI, the substitution model was general time reversible + I + G (-lnL = 5116.99; AIC = 10253.98), the base frequencies are A = 38.34%, C = 14.48%, G = 18.48%, and T = 28.71%, the rate matrix was R(a)[A-C] = 0.36, R(b)[A-G] = 8.93, R(c)[A-T] = 0.90, R(d)[C-G] = 0.32, R(e)[C-T] = 2.81 and R(f)[G-T] = 1.00, and the proportion of invariable sites (I) was 0.50, with a gamma shape distribution of 1.12; for 12SrRNA the substitution model was K81uf + G (-lnL = 1867.97; AIC = 3747.95), the base frequencies are A = 36.83%, C = 5.89%, G = 17.42%, and T = 39.86%, the rate matrix was R(a)[A-C] =, R(f)[G-T] = 1.00, R(b)[A-G] = R(e)[C-T] = 5.55 and R(c)[A-T] = R(d)[C-G] = 0.61, and the gamma shape distribution was 0.37; and for 16S rRNA the substitution model was HKY (Hasegawa, Kishino & Yano, 1985) + I + G (-lnL = 2741.55; AIC = 5495.11), the base frequencies are A = 37.08%, C = 7.29%, G = 16.95%, and T = 38.69% and the transition/transversion ratio was 2.06, and the proportion of invariable sites (I) was 0.15 with a gamma shape distribution of 0.48. For the MP analyses 410 characters were found to be informative, the consistency index was 0.38, and the retention index was 0.76, and six trees were recovered with a tree length of 1729 steps. The Bayesian analyses recovered a near identical tree topology with strong statistical support for the same clades evident in the MP analyses (Fig. 2). The monophyly of *Potamonautes* was retrieved with strong statistical support (73%/1.00 pP). The specimens from Zanzibar, the type locality for *P. obesus*, were sister to specimens of this species from Tanzania, Kenya, and Malawi with strong statistical support (94%/1.00 pP). The latter clade was sister to samples from Mount Namuli, Mozambique albeit with poor statistical support (< 70%/< 0.95 pP). Samples from Mount Mabu (Mozambique), Mount Mulanje (Malawi) (form A), and Mount Inago (Mozambique) formed a well-supported clade (88%/1.00 pP). The samples from the latter three localities conform to the diagnosis of *P. choloensis*. These specimens have been

deposited in SAM (accession numbers SAM A46802, SAM A46803, SAM A46804, SAM A46805, SAM A46806, and SAM A46807). The phylogeny retrieved the monophyly of the South African freshwater crab species (73%/0.98 pP). As in the earlier two studies by Daniels *et al.* (2002, 2006a) two distinct clades were present that comprised small-bodied, mountain-living species and large-bodied riverine species. The small-bodied, mountain-living species comprised two clades, one grouping specimens of *Potamonautes clarus* sister to *P. depressus* from the Drakensberg Mountains, with strong statistical support (100%/1.00 pP); and the second all the Western Cape small-bodied, mountain-living species (*P. brincki*, *Potamonautes parvispina*, and *Potamonautes parvicarpus*) with strong statistical support (84%/1.00 pP). The clade comprising the large-bodied riverine species was statistically well supported (90%/1.00 pP) and was sister to a novel undescribed large-bodied species from Mount Mulanje (form B) in Malawi. Potamonautid species from East Africa were basal to the monophyletic southern African freshwater crab clade albeit with poor statistical support (< 70%/< 0.95 pP).

For the COI locus, sequence divergence values within *P. obesus* (Zanzibar, Tanzania, Kenya, and Malawi) were 12.31%. The two genetically distinct groups from Mount Mulanje (form A, representing *P. choloensis* and form B representing an undescribed taxon) were characterized by a 16.16% sequence divergence for the COI locus, demonstrating genetic isolation and the presence of two distinct maternal gene pools at this sympatric locality. Although we hesitate to subscribe to the application of sequence divergence values as indicators of species status, the marked genetic divergence, coupled with distinct morphological characters and their phylogenetic placement provide evidence for the presence of two novel lineages, which are here recognized as distinct species within *Potamonautes*, one from Mount Namuli in Mozambique, and one from Mount Mulanje in Malawi.

TAXONOMY

- SUBORDER BRACHYURA LINNAEUS, 1758
 SUPERFAMILY POTAMOIDEA ORTMANN, 1896
 FAMILY POTAMONAUTIDAE BOTT, 1970
 SUBFAMILY POTAMONAUTINAE BOTT, 1970
 GENUS *POTAMONAUTES* MACLEAY, 1838
***POTAMONAUTES NAMULIENSIS* SP. NOV.**

(FIGS 3A–C, 4A–C)

Holotype: Manho forest 1500 m above sea level (a.s.l.) 15°21'30"S, 37°03'40"E, Mount Namuli, Zambézia Province, central Mozambique SAM A46796, 1 male

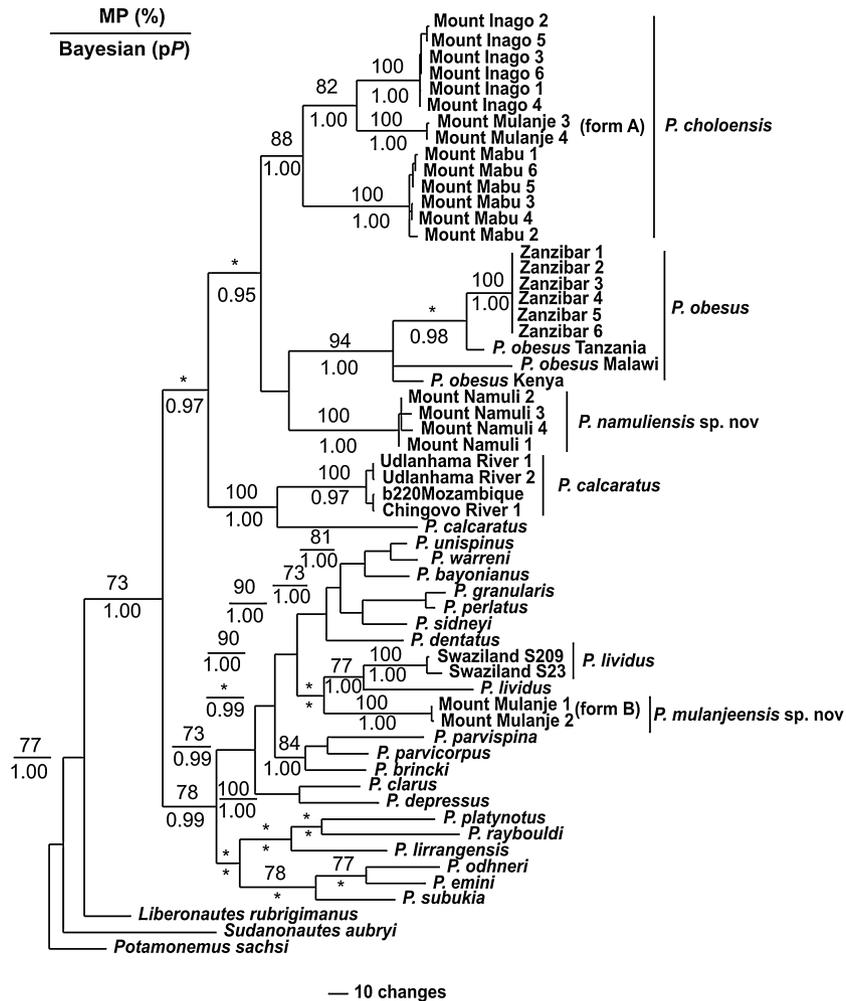


Figure 2. A Bayesian phylogram for the combined 12S rRNA + 16S rRNA + COI mtDNA sequence data. Statistical values above the nodes represent values for bootstrapping for maximum parsimony (MP). Values below each node represent the posterior probability (pP) values for the Bayesian analyses. Only bootstrap values > 70% and pP values > 0.95 are shown.

collected 25.xi.2008 by Julian Bayliss. Endemic to Mount Namuli and appears to be restricted to boulder strewn mountain streams with closed canopy forest. Samples were collected from an upland flowing stream in open habitat but also bordering riverine forest, and crabs were found hiding in vegetation overhanging the stream, or in mud on the stream banks, as well as under rocks.

Paratype: Manho forest 1500 m a.s.l., Mount Namuli, central Mozambique SAM A46797, 1 male collected 25.xi.2008 by Julian Bayliss.

Additional material examined: Manho forest 1500 m a.s.l., Mount Namuli, central Mozambique SAM A46798, 1 male and 2 females (one gravid) collected 25.xi.2008 by Julian Bayliss.

Description and diagnosis: Measurements of the holotype are provided in Table 2. Carapace of living specimens light brown to orange, chelipeds with hints of red, particularly on dactylus, tip of propodus. Cephalothorax ovoid, smooth, flattened, maximum height (CH/CL = 0.48), posterior margin width (CWP/CL = 1.42). Exorbital tooth smooth, nearly continuous with epibranchial corner, postfrontal weak, short fork at midpoint. Urogastric grooves moderately deep, cardiac grooves deep (Fig. 3A). Sternites 1 and 2 fused, no suture discernible, suture between sternites 2, 3, and 4 complete, deep. Third maxillipeds filling entire buccal frame except for small, oval respiratory opening; flagellum present on exopod; ischium with faint distinct groove. Two segmented mandibular palps with dense setae, terminal segment undivided and characterized by dense setae on posterior

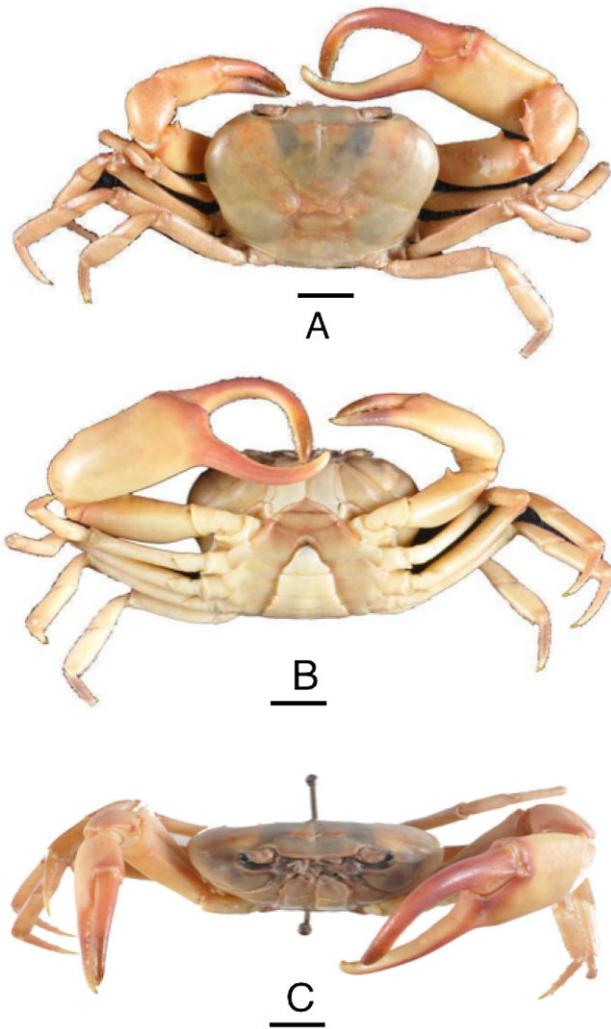


Figure 3. *Potamonautes namuliensis* sp. nov. Male holotype (carapace length = 21.55 mm) from Manho forest 1500 m a.s.l., Mount Namuli, Zambézia Province, central Mozambique SAM A46796. A, whole animal dorsal aspect; B, whole animal ventral aspect; C, cephalothorax, frontal aspect. Scale bars = 10 mm.

proximal surface. Chelipeds markedly unequal, enlarged highly arched right dactylus, both dactyli armed with several small cutting teeth (Fig. 3B). Heterochely appears to be associated with the three male samples; the two females appear to have chelipeds of equal size. Propodus of right chelipeds also markedly arched. In both chelipeds, carpus with two prominent teeth. Pereiopods slender, pereopod 3 longest, pereopod 5 shortest, dactyli ending in sharp point, margins bearing sharp, spine-like bristles. First five segments of abdomen broad short, last two segments longer, terminal segment pointed at distal end. Gonopod 1, terminal segment short, curving away from midline when viewed posteriorly, widest at base, ending in

pointed tip (Fig. 4A, B). Subterminal segment of gonopod 1 tapering distally, inner margin slightly irregular. Basal margins of subterminal segment of gonopod 1 with setae. Gonopod 2, terminal segment filamentous, about half the length of subterminal segment. Subterminal segment of gonopod 2 widest at base, tapering inward at 0.5 times length, narrow upright process supporting terminal segment (Fig. 4C).

Distribution: This species has only been collected from high mountain streams (>1500 m a.s.l.) in Mount Namuli, central Mozambique, and appears to be endemic to the region, suggesting that it may be of conservation concern considering the anthropogenic impacts such as deforestation in the region.

Remarks: The species is superficially similar to other mountain-living species in South Africa including *P. parvicorpus* Daniels *et al.* 2001 from Table Mountain, *P. brincki* (Bott, 1960) from the Hottentots Holland Mountains, and *P. depressus* and *P. clarus* Gouws *et al.* 2001 from the Drakensberg Mountains. In addition, *P. namuliensis* sp. nov. also resembles both *Potamonautes infravallatus* (Hilgendorf, 1898) and *Potamonautes xiphoidus* Reed & Cumberlidge, 2006 from the Western Usambara Mountains in Tanzania. All six of these species are small-bodied, characterized by a smooth carapace, lack dentition on the anteriolateral margins, have the major cheliped highly arched, and are endemic to mountainous areas. A similar pattern has also been documented for certain freshwater crab species found in the Rift Valley in East Africa (Reed & Cumberlidge, 2006). Despite the superficial similarity in carapace and cheliped morphology, the species are distinct based on first gonopod structure and all the known species having well-developed, strong postfrontal crest regions.

Etymology: The species is named after Mount Namuli, Mozambique, where it was first collected.

POTAMONAUTES MULANJEENSIS SP. NOV.

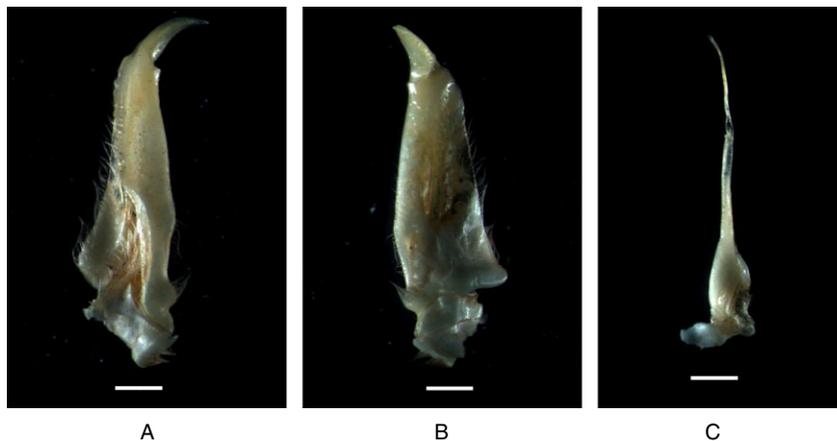
(FIGS 5A–C, 6A–C)

Holotype: Madzeka hut 1800 m a.s.l., 15°50'00"–16°03'00"S, 35°30'00"–35°4'00"E, Mount Mulanje (form B on tree topology, Fig. 2), southern Malawi, SAM A46799, 1 male collected v.2009 by Julian Bayliss. The samples were collected from a pool underneath rocks and from a lowland stream.

Paratype: Lichenya hut, Mount Mulanje, southern Malawi, SAM A46800, 1 juvenile male collected vi.2009 by Julian Bayliss.

Table 2. *Potamonautes namuliensis* sp. nov. Measurements (in mm) of the holotype and ranges for additional material measured

Variable	Abbreviation	Holotype	Males	Females
Carapace length	CL	21.55	19.84–21.24	18.62–18.41
Carapace width at widest point	CWW	30.7	29.31–30.47	25.45–27.0
Carapace posterior margin	CWP	12.17	11.77–11.89	11.55–12.17
Frontal width	FW	9.7	8.55–9.77	8.4–8.78
Distance between postfrontal crest and anterior margin	PFCD	2.84	2.67–2.9	2.42–2.54
Carapace height	CH	10.33	9.45–9.75	8.59–9.1
Major cheliped propodus length	MCPL	36.66	30.81–30.49	14.37–15.12
Major cheliped propodus height	MCPH	13.79	12.39–11.71	6.40–5.90
Pereiopod 2, merus length	P2ML	14.47	13.0–13.11	9.63–10.83
Pereiopod 2, merus width	P2MW	3.3	3.52–3.53	3.33–3.59
Pereiopod 5, merus length	P5ML	12.44	11.4–11.47	8.85–10.65
Pereiopod 5, merus width	P5MW	3.19	3.08–3.4	3.06–3.48

**Figure 4.** *Potamonautes namuliensis* sp. nov. male holotype, SAM A46796. A, left gonopod 1, anterior view; B, left gonopod 1 posterior view; C, left gonopod 2 anterior view. Scale bars = 10 mm.

Additional material examined: Mount Mulanje 2000 m a.s.l., southern Malawi, SAM A46801, 2 juvenile males, 3 juvenile females, collected vi.2009 by Julian Bayliss.

Description and diagnosis: Measurements of the holotype are provided in Table 3. Postfrontal crest present and strongly developed, with short forked region at midpoint. Carapace of living specimens light brown. Cephalothorax ovoid, granulated on the anteriolateral margins, and flattened, maximum height (CH/CL = 0.49) posterior margin width (CWP/CL = 1.51). Urogastric grooves moderately deep, cardiac grooves deep (Fig. 5A). Sternites 1 and 2 fused, no suture discernible, suture between sternites 2, 3, and 4 complete, deep. Third maxillipeds filling entire buccal frame except for small, oval respiratory opening; flagellum present on exopod; ischium with faint distinct

groove. Two segmented mandibular palps, terminal segment undivided and characterized by dense setae on posterior proximal surface. Chelipeds markedly unequal, arched right dactylus, both dactyli armed with several small cutting teeth (Fig. 5B). Heterochely appears to be associated with the adult male holotype, the remaining specimens are juvenile and are not heterochelous. In both chelipeds, carpus with two prominent teeth. Pereiopods slender, pereiopod 3 longest, pereiopod 5 shortest, dactyli ending in sharp point, margins bearing sharp, spine-like bristles. First five segments of abdomen broad and short, last two segments longer, terminal segment pointed at distal end. Gonopod 1, terminal segment short, curving away from midline when viewed posteriorly, widest at base, ending in pointed tip (Fig. 6A, B). Subterminal segment of gonopod 1 tapering distally, inner margin slightly irregular. Basal margins of

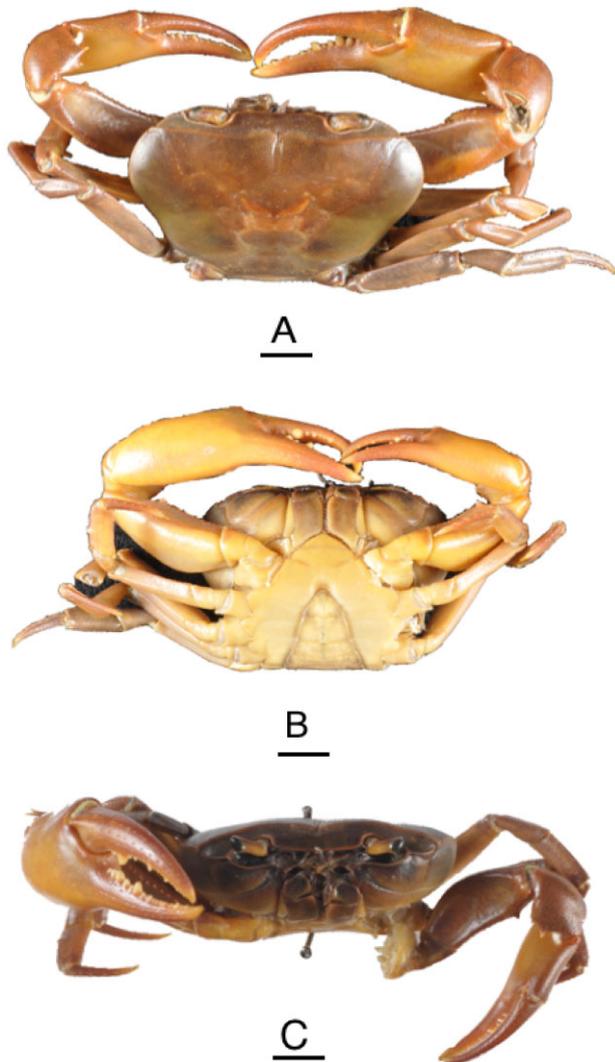


Figure 5. *Potamonautes mulanjeensis* sp. nov. Male holotype (carapace length = 25.38 mm) Madzeka hut 1800 m a.s.l., Mount Mulanje (form B, Fig. 2), southern Malawi, SAM A46799. A, whole animal dorsal aspect; B, whole animal ventral aspect; C, cephalothorax, frontal aspect. Scale bars = 10 mm.

subterminal segment of gonopod 1 with setae. Gonopod 2 is broken off in the holotype and hence not described. The paratype is a juvenile male and hence was not used to describe gonopod 2.

Distribution: This species has only been collected from high mountain streams (> 1800 m a.s.l.) on Mount Mulanje in southern Malawi and appears to be endemic. The species may be of conservation concern given its current small distribution range.

Remarks: The carapace characters of *P. mulanjeensis* sp. nov. such as the flat and granulated anteriolateral

margins resemble those of *P. montivagus* (Chace, 1953). The species bears superficial resemblances to other large-bodied southern African freshwater crab species such as *P. sidneyi*, *P. perlatus*, and *Potamonautes granularis*, but was found here to be genetically distinct. The close phylogenetic relationship between *P. mulanjeensis* sp. nov. and the large-bodied South African riverine species suggests a close biogeographical affinity between the Malawian large-bodied freshwater crab species and the southern African species.

Etymology: The species is named after Mount Mulanje, Malawi, where it was first collected.

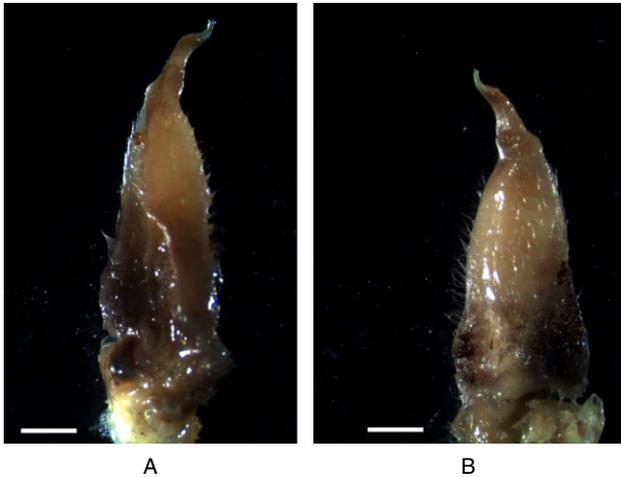
DISCUSSION

The present study reports on the discovery of two new freshwater crab species from central Mozambique and southern Malawi. There are two genetically and morphologically distinct taxa present at Mount Mulanje that clearly represent two reproductively isolated units, as evidenced from the sympatric occurrence of *P. choloensis* and *P. mulanjeensis*. *Potamonautes mulanjeensis* is the sister taxon to large-bodied South African freshwater crab species. This species superficially resembles *P. montivagus* but the two species differ morphologically. The description of *P. namulienensis* represents the first new freshwater crab species from Mozambique in over 80 years. Similarly, the description of *P. mulanjeensis* from Malawi represents the first new species from this country in over 50 years. These results clearly indicate that both countries probably harbour additional undescribed freshwater crab species and inland aquatic regions in the area should be targeted during future sampling. In general, species of freshwater crabs with a wide geographical range should be targeted for systematic studies given the presence of cryptic freshwater crab lineages in the Afrotropical region (Daniels *et al.*, 2003, 2006a). The Rift Valley of East Africa and the eastern Congo basin are biodiversity hotspots for freshwater crabs and potentially harbour a large number of undescribed species.

The phylogenetic results also provide novel insights into the biogeographical affinities of the southern African freshwater crab fauna. The tree topology places a number of the east African potamonautid species as basal to the southern African freshwater crab species. It indicates the monophyly of large-bodied freshwater crab species, with *P. mulanjeensis* most basal in that clade. In addition, the phylogenetic results demonstrate that the mountain-living ecomorphotype, characterized by a generally oval carapace, a lack of ornamentation of the anterolateral

Table 3. *Potamonautes mulanjeensis* sp. nov. Measurements (in mm) of the holotype and ranges for additional material measured

Variable	Abbreviation	Holotype	Males	Females
Carapace length	CL	25.38	10.81–17.57	12.51–16.4
Carapace width at widest point	CWW	38.49	14.54–24.47	15.93–23.82
Carapace posterior margin	CWP	12.84	5.93–9.61	5.95–10.28
Frontal width	FD	12.41	5.44–8.54	6.73–7.88
Distance between postfrontal crest and anterior margin	PFCD	3.78	1.94–3.0	2.86–5.12
Carapace height	CH	12.87	5.04–8.22	5.7–7.64
Major cheliped propodus length	MCPL	38.66	8.13–14.95	9.38–13.29
Major cheliped propodus height	MCPH	15.02	5.93–2.98	5.12–3.33
Pereiopod 2, merus length	P2ML	17.01	6.0–10.03	6.98–9.1
Pereiopod 2, merus width	P2MW	4.78	2.12–3.75	2.56–3.46
Pereiopod 5, merus length	P5ML	13.69	6.61–9.68	7.11–9.0
Pereiopod 5, merus width	P5MW	4.06	2.1–3.49	2.32–3.23

**Figure 6.** *Potamonautes mulanjeensis* sp. nov. male holotype, SAM A46799. A, left gonopod 1, anterior view; B, left gonopod 1 posterior view. Scale bars = 10 mm.

carapace margins, and a highly arched cheliped, has evolved independently on several occasions, as mountain-living species are not monophyletic. Such results suggest that a cladistic morphological analysis may provide limited insight into phylogenetic patterning within *Potamonautes*, considering the widespread convergence in morphological characters. Mountain-living species are generally basal taxa in our phylogenetic results. However, a complete phylogeny of *Potamonautes* species is required to understand both taxonomic affinities and biogeographical patterning in the genus. DNA-based phylogenies will provide considerable value in detecting not only novel evolutionary lineages, but also in validating existing taxonomic units and synonymies as demonstrated during the present study.

The study further re-iterates the need for renewed sampling effort in remote areas that have not been previously sampled, as these areas are likely to harbour significant numbers of undocumented species. Large areas of the continental Afrotropical region remain unsampled and require prioritization for sample collection in order to document biodiversity. Renewed systematic biological surveys will undoubtedly yield invaluable information on the alpha, beta-, and gamma diversity of these regions. These data are important in the determination of the IUCN Red listing of African freshwater crabs. For example, *P. choloensis* was regarded as vulnerable (VU) during its assessment (Cumberlidge *et al.*, 2009). However, considering the current findings and the now broader distribution of the species evident from the present study, the listing should probably be updated. Similarly, *P. lividus* is also listed as vulnerable (VU) but the recent discovery of the species in Swaziland during the present study suggests a broader distribution of the taxon and a re-evaluation of the species IUCN status (Cumberlidge *et al.*, 2009).

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