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# Renewed sampling of inland aquatic habitats in southern Africa yields two novel freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*)

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A recent survey of the freshwater streams of the Mecula and Yao mountains in the Niassa province of Mozambique resulted in the discovery of a new freshwater crab species. This species is genetically and morphologically distinct from described species from Mozambique or its neighbouring countries, and is described as *Potamonautes bellarussus* sp. nov. In addition, a new semi-terrestrial burrowing freshwater crab *Potamonautes flavusjo* sp. nov. from the Highveld of the Mpumalanga province in South Africa is described based on unique genetic and morphological characters. The phylogenetic affinities of the two new species in relation to the described eastern and southern African *Potamonautes* species is determined and the biogeographic implications are discussed.

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ADDITIONAL KEYWORDS: genetics – inland aquatic systems – mountainous – new species – systematics.

## INTRODUCTION

Afrotropical freshwater crabs as a whole are species-poor compared with other biogeographic regions, such as the Neotropical and Oriental areas (Yeo *et al.*, 2008; Cumberlidge *et al.*, 2009). Freshwater crab diversity in rivers, first- and second-order streams, and lakes in the Afrotropics is well documented, but aquatic habitats in remote mountainous regions have been neglected, hindering a more accurate reflection of species diversity and endemism. Recent sampling of freshwater habitats in the Afrotropical region has yielded several new species of potamonautid freshwater crab (for example, see Cumberlidge & Tavares, 2006; Cumberlidge & Dobson, 2008; Cumberlidge & Clark,

2010, 2012; Cumberlidge & Meyer, 2011; Duris & Koch, 2011; Meyer & Cumberlidge, 2011; Daniels & Bayliss, 2012; Phiri & Daniels, 2013). In southern Africa, Daniels & Bayliss (2012) discovered two new species of freshwater crabs from mountainous regions in Malawi and Mozambique. A recent survey of streams on the eastern Zimbabwean highlands resulted in the discovery of a new species of *Potamonautes* that is the first endemic species to be reported from Zimbabwe (Phiri & Daniels, 2013). Cumberlidge & Clark (2012) described the first cave-living potamonautid freshwater species from the Ethiopian highlands. Collectively, these results reiterate the renewed call for poorly sampled high-altitude freshwater systems in the Afrotropics to be a focal point of future systematic surveys.

The present work reports on the presence of a new freshwater crab species from the Mecula and Yao mountains, in the Niassa province in northern

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Mozambique, that were sent to the senior author for identification. These specimens were collected by J. Bayliss whilst undertaking an expedition to investigate unexplored mountainous regions (> 1500 m a.s.l.) in this region of Africa, and to determine the biogeographical linkages of species. Six freshwater crab species are known from Mozambique: *Potamonautes bayonianus* (Brito Capello, 1873); *Potamonautes calcaratus* (Gordon, 1929); *Potamonautes choloensis* (Chace, 1953); *Potamonautes namuliensis* (Daniels & Bayliss, 2012); *Potamonautes obesus* (H. Milne Edwards, 1868); and *Potamonautes sidneyi* (Rathbun, 1904). Only the recently described species *P. namuliensis* from the Namuli Mountains is endemic to Mozambique. Morphological comparisons of the Mecula and Yao mountain specimens with the six described Mozambican freshwater crab species, and with species from Malawi and Tanzania, suggest that the Mecula and Yao specimens represent an undescribed species.

In contrast to the Mozambican freshwater crab fauna, the freshwater crab systematics in neighbouring South Africa is well studied. The South African inland decapod fauna has been subjected to rigorous systematic scrutiny (using allozymes, mitochondrial DNA sequence data, morphology, and morphometrics), resulting in the description of seven novel species (Stewart, Coke & Cook, 1995; Stewart, 1997; Daniels, Stewart & Gibbons, 1998; Stewart & Cook, 1998; Gouws, Stewart & Coke, 2000; Daniels, Stewart & Burmeister, 2001; Gouws, Stewart & Reavell, 2001). South Africa's freshwater crab fauna currently comprise 13 described species: *Potamonautes brincki* (Bott, 1960); *Potamonautes calcaratus* (Gordon, 1929); *Potamonautes clarus* (Gouws *et al.*, 2000); *Potamonautes dentatus* (Stewart & Cook, 1995); *Potamonautes depressus* (Krauss, 1843); *Potamonautes granularis* (Daniels *et al.*, 1998); *Potamonautes lividus* (Gouws *et al.*, 2001); *Potamonautes parvispina* (Stewart, 1997); *Potamonautes parvicorpus* (Daniels *et al.*, 2001); *Potamonautes perlatus* (H. Milne-Edwards 1837); *Potamonautes sidneyi* (Rathbun, 1904); *Potamonautes unispinus* (Stewart & Cook, 1998); and *Potamonautes warreni* (Calman, 1918). More recently several cryptic lineages have been identified and are being described (E.E. Phiri, pers. comm.). The primary focus of these earlier systematic endeavours was to document the diversity of freshwater crabs in rivers and mountain streams; however, non-riverine habitats, such as vleis (wetland) areas, remained largely neglected.

Dr Johan Engelbrecht, formerly of the Mpumalanga Parks, sent an ethanol-preserved female specimen of an unidentified freshwater crab species from Verloren Vallei Nature Reserve to the first author for identification in December 2000. As the gonopods of male specimens are required for species identification, the female specimen could not be conclusively identified; however,

the female specimen was recently subjected to mitochondrial DNA (mtDNA) sequencing, and the resultant sequences were compared with all the described southern African freshwater crab species (Daniels *et al.* 2002a). Preliminary analyses of the mtDNA sequence unambiguously demonstrated that the Verloren Vallei Nature Reserve freshwater crab specimen represented a new species. More recently, Jerry Theron and Gerhard Diedericks sent a series of photographic images of a freshwater crab specimen similar in appearance to the female specimen from Verloren Vallei Nature Reserve that was collected in the Chrissiesmeer district. Both of the latter localities occur on the Highveld of the Mpumalanga province in South Africa. The Highveld is an inland plateau with an altitude above 1500 m a.s.l., but below 2100 m a.s.l. The presence of the undescribed Highveld freshwater crab species resulted in the senior author undertaking a sampling trip to the region to collect male specimens.

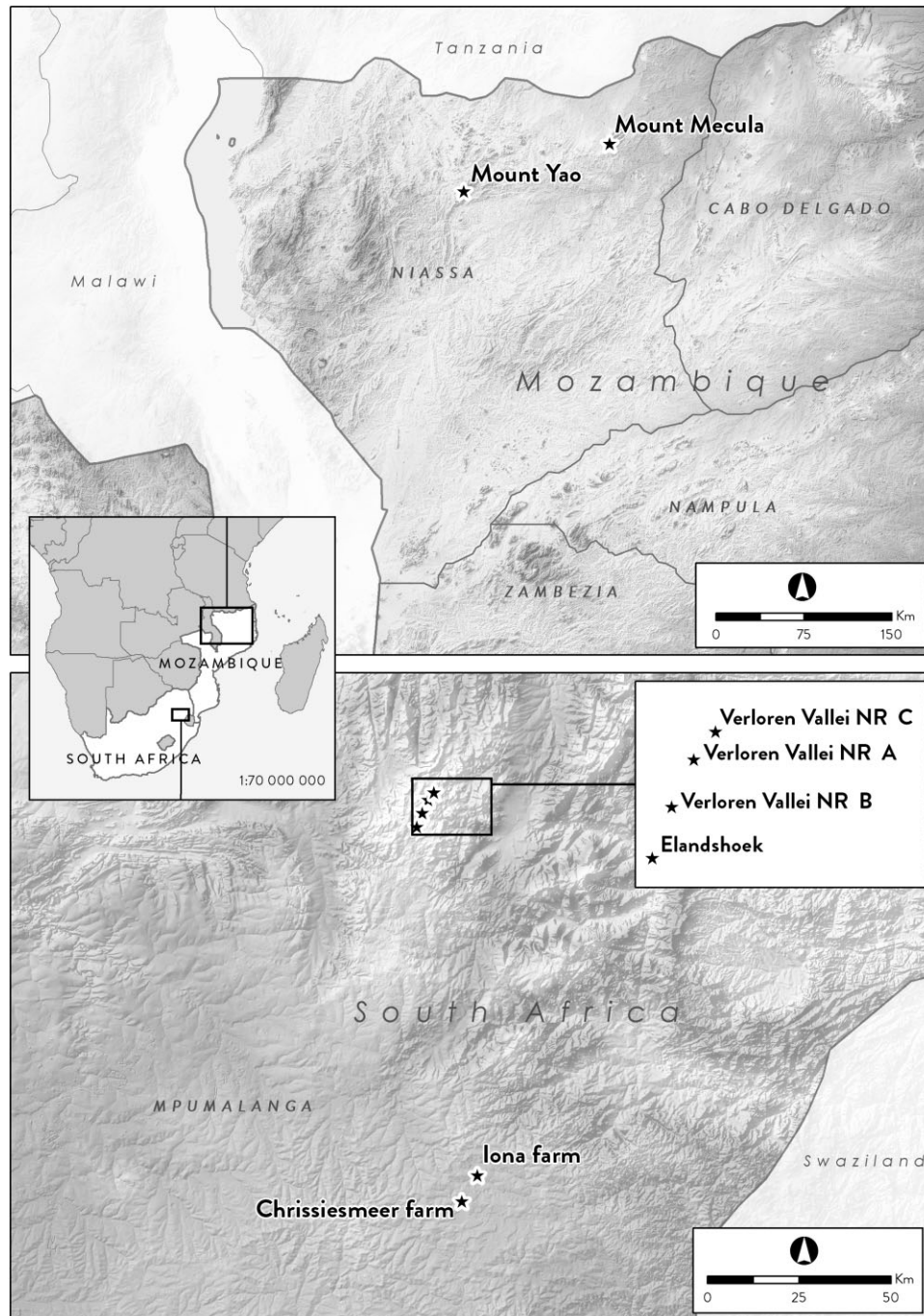
The objectives of the present study are twofold. Firstly, to diagnose and described the novel Mozambican and South African freshwater crab species in comparison with the known species from east and southern Africa. Secondly, to determine the phylogenetic placement of the two novel species in relation to the described species from east and southern Africa with the use of three partial mtDNA loci.

## MATERIAL AND METHODS

### SAMPLE COLLECTION

Freshwater crabs were collected from the Mecula ( $N = 4$ ) and Yao ( $N = 2$ ) mountains in the Niassa province of Mozambique (Fig. 1). These crabs were hand collected from large bolder-strewn mountain streams in forested areas. The latter undescribed freshwater crab species from Mozambique closely resembles *Potamonautes suprasulcatus* (Hilgendorf, 1898). Hence two specimens of *P. suprasulcatus* from Malagarasi in Tanzania were included in our study. The latter specimens were obtained from the British Natural History Museum, London, UK, and sent to the first author by Neil Cumberlidge for a larger phylogenetic study on the Afrotropical freshwater crabs.

The undescribed Highveld species was collected from three sample sites at Verloren Vallei Nature Reserve (A, B, and C), Elandshoek, as well as from two farms, Iona Farm and Miss Chrissie's Country House Farm, Chrissiesmeer, in the Lake Chrissiesmeer district of Mpumalanga province, South Africa. At the two Verloren Vallei Nature Reserve sample sites (B and C), as well as at the two farms in the Lake Chrissiesmeer district, the undescribed Highveld species was sympatric with *P. sidneyi*. A total of 18 *P. sidneyi* specimens



**Figure 1.** A map showing the two sampling sites, on Mecula and Yao mountains, Niassa province, Mozambique, and the six Highveld localities (Verloren Vallei Nature Reserve sites A, B, and C; Elandshoek; the Iona Farm; and Miss Chrissie's Country House Farm, Chrissiesmeer) in the Mpumalanga province of South Africa where the freshwater crabs were collected. The new Highveld species was collected sympatrically with *Potamonautes sidneyi* at four sample sites: Verloren Vallei Nature Reserve (NR) (sites B and C), and at Iona Farm and Miss Chrissie's Country House Farm, Chrissiesmeer.



and 28 specimens of the undescribed Highveld freshwater crab species were collected (Fig. 1). The undescribed Highveld freshwater crab species is a semi-terrestrial burrowing freshwater crab. Burrows were generally present under decaying Poaceae (grass) and Restionaceae (restios/reeds) in peat soil in close proximity to small streams. Active burrows were characterized by the deposition of moist soil at the burrow entrance and these burrows were frequently sealed with a peat plug. Freshwater crabs were dug from their burrows using a garden spade. The depth of the burrow depended on the depth of the water table, and was variable. A handheld GPS was used to record the latitude and longitude of the sites where samples were collected. Crabs were killed by freezing overnight and leg tissue was preserved in absolute ethanol for DNA analyses.

#### DNA EXTRACTION, POLYMERASE CHAIN REACTION, 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 polymerase chain reaction (PCR). Generally, a dilution of 1 µl of DNA in 19 µl of water was used. 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 mutation rate and had been successfully used for reconstructing evolutionary relationships among freshwater crabs (Daniels *et al.*, 2002a, 2006b; Daniels, Gouws & Crandall, 2006a; Daniels & Bayliss, 2012). The primer pairs used are outlined in Daniels *et al.* 2002a, 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. 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 mtDNA sequence data into a single data matrix and conducted all analyses on the combined data set. Maximum-parsimony (MP) and Bayesian inference (BI) approaches were used to estimate evolutionary relationships. MP analyses were executed in PAUP\* 4.0 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 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 < 75% were regarded as poorly resolved. Uncorrected sequence p distances were calculated in PAUP\* 4.0 beta 10 (Swofford, 2002). MODELTEST 3.06 (Posada & Crandall, 1998) was used to obtain the best-fitting substitution model for each gene locus. These substitution models were used in the partitioned BI analyses. The best-fitting 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 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 five million 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: pP < 1.00 was regarded as poorly resolved. The combined data were analysed using the mixed substitution models for each locus in the BI analyses.

#### OUT-GROUP SELECTION

Daniels *et al.* (2006a) demonstrated that *Liberonautes* (Bott, 1955) is sister to *Potamonautes*. Hence four *Liberonautes* (Bott, 1955) species were used as out-groups: *Liberonautes latydactylis* (De Man, 1903); *Liberonautes lugbe* (Cumberlidge, 1999); *Liberonautes nimba* (Cumberlidge, 1999); and *Liberonautes rubrigimanus* (Cumberlidge & Sachs, 1989). The GenBank accession numbers of the out-group species and the described South African freshwater crab species are provided in Daniels & Bayliss (2012). GenBank sequences for the newly described species *P. mutarensis* (Phiri & Daniels, 2013) from Zimbabwe are provided by Phiri & Daniels (2013).

#### MORPHOLOGY

Samples were divided into males and females, and the following measurements were taken with digital vernier callipers: CH, carapace height; CL, carapace length; CWP, width of the posterior margin of the carapace; CWW, carapace width at widest point; FW, frontal width

measured between the medial margins of the orbits; PFCD, distance between the postfrontal crest and the anterior margins of the carapace; length and width of merus of pereopods 2 and 5; and length of the dactylus 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, South Africa (SAM). Freshwater crabs were photographed with the use of a Nikon A300 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 stereomicroscope.

## RESULTS

### COMBINED PHYLOGENETIC ANALYSES MTDNA

The combined mtDNA data yielded a total of 1278 base pairs (bp), comprising 316, 362, and 600 bp for the 12S rRNA, 16S rRNA, and COI gene regions, respectively. A total of 156 mtDNA sequences were generated during the present study. Sequences of the three partial mtDNA gene fragments were deposited in GenBank: 12S rRNA (KJ 713394–KJ 713445), 16S rRNA (KJ 713446–KJ 713497), and COI (KJ 713498–KJ 713549). The MP analyses retrieved 116 trees, with consistency index (CI) = 0.40, retention index (RI) = 0.78, and with a tree length of 2149 steps from 426 parsimony informative characters. The three substitution models (used for the BI analyses) retrieved using MODELTEST are listed in Table 1. The tree topology for the two analytical methods (MP and BI) retrieved near-identical tree topologies, hence only the BI topology is shown

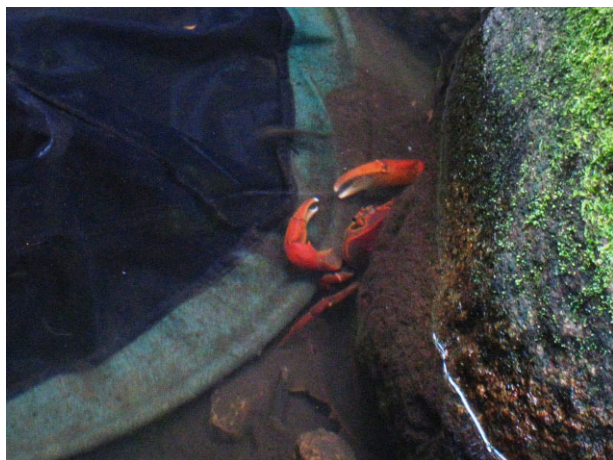
(Fig. 2). *Potamonautes* was retrieved as monophyletic (84%/1.00 pP) based on the present taxonomic sampling. A basal, statistically well-supported clade (84%/1.00 pP) comprising *P. obesus*, *P. namuleensis*, *P. calcaratus*, and *P. choloensis* was retrieved. All four species occur in Mozambique, whereas the specimens from the Mecula and Yao mountains in the Niassa province of Mozambique formed a distinct clade (90%/1.00 pP). The latter clade was distantly related to the four aforementioned freshwater crab species from Mozambique that composed the basal clade. Despite the morphological similarity of the freshwater crab specimens from the Mecula and Yao mountains to *P. suprasulcatus*, these two species were not closely related. *Potamonautes suprasulcatus* was retrieved as sister to *Potamonautes lirrangensis* Rathbun, 1904 with strong statistical support (96%/1.00 pP), whereas the latter clade was sister (90%/1.00 pP) to *Potamonautes platynotes* Cunningham, 1907 and *Potamonautes rayboldi* Cumberlidge & Vannini, 2004 (87%/1.00 pP), forming a distinct east African freshwater crab clade that is basal to the southern African freshwater crab taxa.

The specimens from the Mpumalanga Highveld in South Africa fell into two distinct clades. The 18 *P. sidneyi* specimens from the two sample sites (Verloren Vallei Nature Reserve sites B and C), together with specimens from Iona Farm and the single specimen from Miss Chrissie's Country House Farm, Chrissiemeer, formed a clade (clade B; 83%/1.00 pP). The latter clade was in turn sister to *P. sidneyi* (< 75%/1.00 pP). The entire *P. sidneyi* clade (clade B) was sister to all the large-bodied southern African riverine freshwater crab

**Table 1.** Substitution models and for each of the three mitochondrial DNA loci: 12S rRNA, 16S rRNA, and COI

Gene fragment	No. of base pairs	Model	Base pair frequencies	Rate matrix	G distribution shape parameter	Proportion of invariable sites (I)
12S rRNA	316	K81uf + I + G	A = 40.51% C = 06.16% G = 15.62% T = 37.71%	R (a) [AC] = 1.00 R (b) [AG] = 6.15 R (c) [AT] = 0.53 R (d) [CG] = 0.53 R (e) [CT] = 6.15 R (f) [GT] = 1.00	0.84	0.41
16S rRNA	362	TIM + I + G	A = 38.48% C = 07.36% G = 13.78 % T = 40.38%	R(a) [AC] = 1.00 R(b) [AG] = 11.14 R(c) [AT] = 1.99 R(d) [CG] = 1.99 R(e) [CT] = 6.48 R(f) [GT] = 1.00	0.43	0.21
COI	600	GTR + I + G	A = 37.96% C = 15.24% G = 17.65% T = 29.16%	R(a) [AC] = 0.17 R(b) [AG] = 9.14 R(c) [AT] = 1.01 R(d) [CG] = 0.39 R(e) [CT] = 2.10 R(f) [GT] = 1.00	1.01	0.50





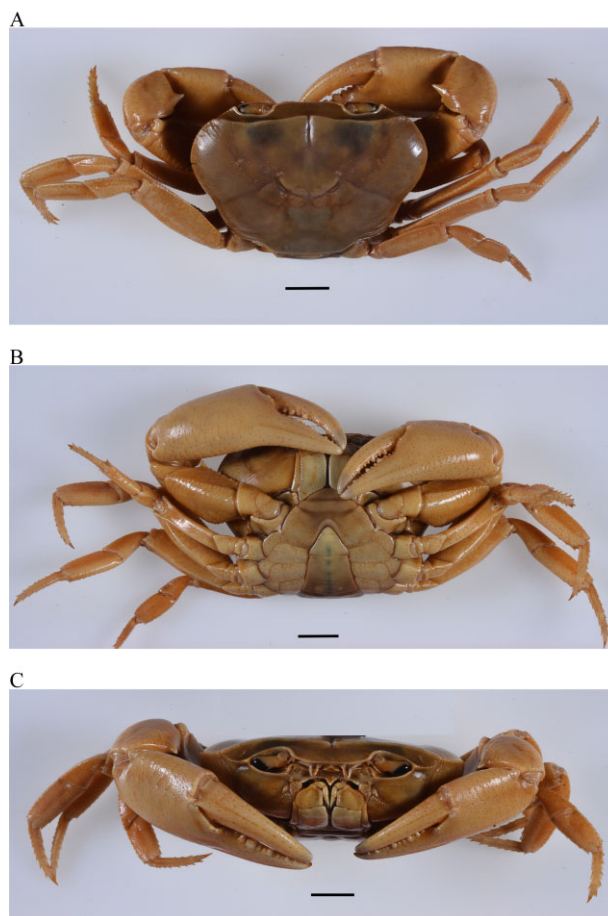
**Figure 3.** *Potamonautes bellarussus* sp. nov., female from the Mecula mountains in the Niassa province, Mozambique, showing the bright-red colour of the freshwater crab while alive.

*Paratype:* One male specimen (SAM A 48213), Yao Mountain, with the same collection information as the holotype.

*Additional material examined:* One female (SAM A 48217), Yao Mountain, 1045 m a.s.l., 12°27'36" S, 36°32'42" E, Niassa province, northern Mozambique, collected on 13 May 2012 by Julian Bayliss, one female (SAM A 48218), Yao Mountain, 1045 m a.s.l., 12°27'36" S, 36°32'42" E, Niassa province, northern Mozambique, collected on 13 May 2012 by Julian Bayliss; three females from the Mecula Mountain, Niassa province, 1046 m a.s.l. (SAM A 48214, A 48215, and A 48216), at 12°43'39" S, 37°38'49" E, northern Mozambique, collected on 9 May 2012 by Julian Bayliss.

*Diagnosis:* Flat freshwater crab species, postfrontal crest deep and well defined, exorbital tooth prominent, anterolateral margins granulate (Figs 3, 4A–C). Carapace, periopods, chelipeds blood red when alive (colour faded in preserved specimens).

*Description:* See Table 2 for the measurements of the holotype. Postfrontal crest deep, distinct, crossing the entire carapace, groove at posterior part of carapace deep. Colour faded in preserved specimens. Carapace flat (CWW/CH = 3.07), narrow posteriorly (CWP/CL = 0.44). Exorbital epibranchial tooth prominent, anterolateral margin behind epibranchial tooth heavily granulated. Anterolateral margins heavily granulated, urogastic and cardiac regions deep, well-defined, subhepatic region of carapace sidewall faintly granulated. Sternites of sulcus s3/s4 well defined. Dactylus of major cheliped with series of well-defined teeth, dactylus not arched, tips white. First carpal tooth on



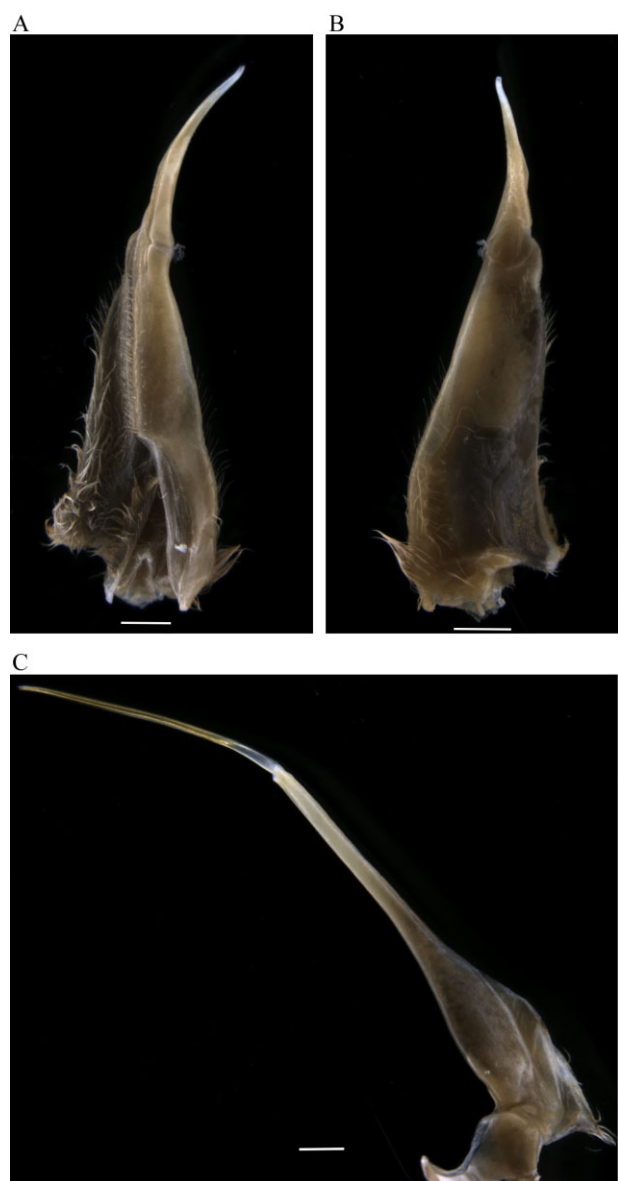
**Figure 4.** *Potamonautes bellarussus* sp. nov., male holotype (carapace length = 39.29 mm) from the Yao Mountains, 1045 m a.s.l., 12°27'276" S, 36°32'260" E, Niassa province, Mozambique (SAM A 48212): A, whole animal, dorsal aspect; B, whole animal, ventral aspect; C, cephalothorax, frontal aspect. Scale bars: 10 mm.

carpus of cheliped with large prominent and sharp spine, second carpal tooth smaller, third small carpal tooth behind the second tooth is present but reduced in size. Terminal article of gonopod 1 long, slim, curving to the right, and comprising nearly half of gonopod 1 and nearly half of the length of gonopod 1 (Fig. 5A, B). Gonopod 2 is thin, long, and filamentous, with the terminal article long and slender, and with a slight curve (Fig. 5C).

*Distribution:* Endemic to Mecula and Yao Mountains in the Niassa province of northern Mozambique.

*Remarks:* *Potamonautes bellarussus* sp. nov. resembles *P. suprasulcatus* in morphology. *Telphusa suprasulcata* var. *pseudoperlata* (Hilgendorf, 1898) and *Telphusa mrogoroense* (Hilgendorf, 1898) are considered here to be junior subjective synonyms of





**Figure 5.** *Potamonantes bellarussus* sp. nov., male holotype from the Yao Mountains, Niassa province, Mozambique (SAM A 48212): A, left gonopod 1, anterior view; B, left gonopod 1 posterior view; C, left gonopod 2 anterior view. Scale bars: 10 mm.

*P. suprasulcatus*; however, phylogenetically these two species are distantly related. *Potamonantes bellarussus* sp. nov. formed a distinct cladem whereas *P. suprasulcatus* was sister to *P. lirrangensis*. In addition, *P. suprasulcatus* has a sharp carpal tooth on the carpus of cheliped and a second carpal tooth smaller spine, with no other teeth behind it, whereas a tooth is present in *P. bellarussus* sp. nov. In addition, *P. bellarussus* sp. nov. and *P. suprasulcatus* are ecologically distinct, with *P. bellarussus* sp. nov. living in

mountain streams whereas *P. suprasulcatus* lives in major rivers (Reed & Cumberlidge, 2006).

The novel Mozambican freshwater crab species was also phylogenetically distantly related to the six described freshwater crab species from Mozambique (*P. bayonianus*, *P. choloensis*, *P. calcaratus*, *P. obesus*, *P. namuliensis*, and *P. sidneyi*). Notably, the four east African freshwater crab species present in Mozambique (*P. choloensis*, *P. calcaratus*, *P. obesus*, and *P. namuliensis*) formed a distinct basal clade. The two remaining species (*P. bayonianus* and *P. sidneyi*), characterized by wide distribution ranges in southern Africa, belonged to a clade comprising the large-bodied freshwater crab species. Superficially, *P. bellarussus* sp. nov. also resembles *Potamonantes unisulcatus* (Rathbun, 1933). The latter species is endemic to the Uluguru Mountains in northern Tanzania, and gonopod 1 of *P. bellarussus* sp. nov. is different from *P. unisulcatus* (Reed & Cumberlidge, 2006). In addition, in *P. bellarussus* sp. nov. three carpal teeth are present, whereas in *P. unisulcatus* only two teeth are present.

**Etymology:** The name *P. bellarussus* sp. nov. is an arbitrary combination of two aspects. 'Bellus' is Latin for beautiful, and is in honour of Dr Bella Davies for her dedication to freshwater ecology in the UK and Africa, whereas the Latin 'russus' refers to the blood-red colour of living specimens. The name is used as a noun in apposition.

#### **POTAMONAUTES FLAVUSJO SP. NOV.**

(FIGS 6, 7A–C, 8A–C)

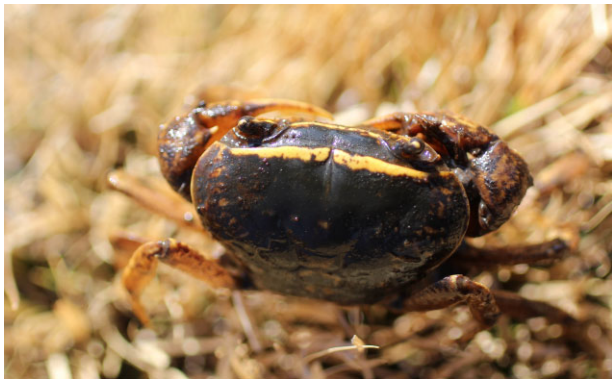
**Holotype:** One male specimen (SAM A 48203), Verloren Vallei Nature Reserve (site B), 2000 m a.s.l., 25°20'19" S, 30°07'32" E, Mpumalanga province of South Africa, collected on 27 June 2013 by Savel Daniels, Fawzia Gordon, and Piet Makuwa.

**Paratype:** Six male and one female specimens, (SAM A 48204) Verloren Vallei Nature Reserve (site A), 2018 m a.s.l., 25°18'25" S, 30°08'25" E, Mpumalanga province of South Africa, collected 28 June 2013 by Savel Daniels, Fawzia Gordon, Mr Mkwana, and Gerhard Diedericks.

**Additional material examined:** Six females (SAM A 48206), with the same locality and collection information as the holotype. One female (SAM A 48207), Verloren Vallei Nature Reserve (site A), 2016 m a.s.l., 25°18'25" S, 30°08'25" E, Mpumalanga province of South Africa, collected on 27 June 2013 by Savel Daniels, Fawzia Gordon, and Piet Makuwa. One male (SAM A 48208), Verloren Vallei Nature Reserve (site C), 2065 m a.s.l., 25°17'18" S, 30°09'18" E, Mpumalanga province of South Africa, collected on 28 June 2013 by

**Table 2.** *Potamonautes bellarussus* sp. nov. – measurements (in mm) of the holotype and ranges for additional material examined

Variable	Abbreviation	Holotype	Males	Females
Carapace length	CL	39.29	22.91–33.31	18.07–35.73
Carapace width at widest point	CWW	58.42	32.71–47.98	25.18–48.64
Carapace posterior margin	CWP	17.54	12.32–16.73	9.75–18.71
Frontal width	FW	19.90	10.83–15.37	8.29–16.76
Distance between postfrontal crest and anterior margin	PFCD	6.01	3.03–4.16	2.59–4.20
Carapace height	CH	19.03	10.57–15.22	8.19–16.82
Major cheliped propodus length	MCPL	49.79	19.66–21.10	6.30–21.98
Pereiopod 2, merus length	P2ML	22.40	13.63–19.64	10.21–18.85
Pereiopod 2, merus width	P2MW	8.47	5.15–7.84	3.92–7.72
Pereiopod 5, merus length	P5ML	18.25	11.53–19.01	10.58–18.85
Pereiopod 5, merus width	P5MW	6.24	4.46–7.79	4.10–8.02



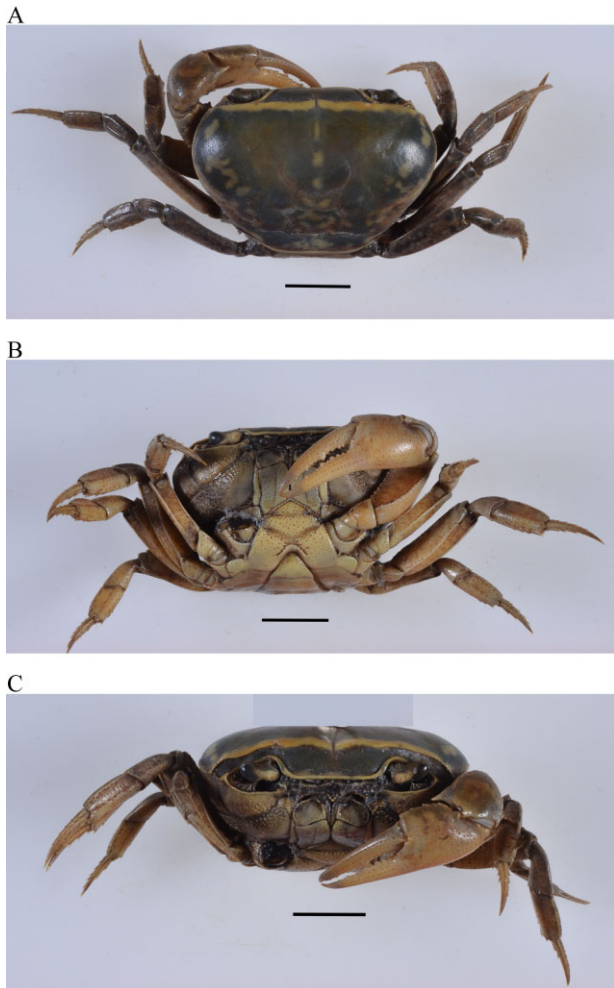
**Figure 6.** Live image of a female *Potamonautes flavusjo* sp. nov., from Elandshoek, 1987 m a.s.l. (SAM A 48209), Mpumalanga province of South Africa, demonstrating the bright-yellow postfrontal crest margin. Photo taken by Gerhard Diedericks.

Savel Daniels, Fawzia Gordon, Mr Mkwana, and Gerhard Diedericks. One female (SAM A 48209), Elandshoek, 1987 m a.s.l., 25°22'33" S, 30°06'45" E, Mpumalanga province of South Africa, collected on 28 June 2013 by Savel Daniels, Fawzia Gordon, and Gerhard Diedericks. Nine specimens (SAM A 48205), Miss Chrissie's Country House Farm, Chrissiesmeer, next to Lake Chrissiesmeer, 1665 m a.s.l., 26°17'33" S, 30°13'24" E, Mpumalanga province of South Africa, collected on 30 June 2013 by Savel Daniels and Fawzia Gordon. Five specimens (SAM A 48210), Iona Farm, in the Lake Chrissiesmeer district, 1648 m a.s.l., 26°13'46" S, 30°10'40" E, Highveld of the Mpumalanga province of South Africa, collected on 2 July 2013 by Savel Daniels, Fawzia Gordon, Hannes Marais, and Ursula Franke.

**Diagnosis:** Carapace highly vaulted, postfrontal crest margin, pereiopods, and chelipeds bright yellow, fading

to dull yellow upon preservation in absolute ethanol. Anterolateral margin of carapace smooth, lacking dentition, exorbital tooth low but well defined (Fig. 7A–C). Ventral carapace surface and ventral chelipeds light yellow.

**Description:** See Table 3 for measurements of the holotype. Live specimens of *P. flavusjo* sp. nov. have a distinct yellow postfrontal crest band and dull mottled yellow marked cephalograstic and anterolateral margin region. Chelipeds and ventral surface of pereiopods light yellow, dorsal surface of the pereiopods light brown (Fig. 7A, B, C). Colour fades upon preservation. Carapace distinctly vaulted ( $CWW/CH = 2.33$ ) narrow posteriorly ( $CWP/CL = 0.51$ ). Anterolateral margin smooth. Postfrontal crest deep, well defined, and curves forward medially. Exorbital tooth small, moderately sharp. Urogastric and cardiac grooves defined. Sternites 1 and 2 fused. First suture between sternites 2 and 3 complete. Second sternal groove between sternites 3 and 4 complete. Third maxillipeds fill the entire buccal frame, except for respiratory opening. Mandibular palp two segments, terminal segment undivided, sense tuft of setae on posterior surface of flange. Chelipeds generally unequal, in the holotype the right cheliped is broken off. Dactyli armed with 18 small cutting teeth. Carpi of chelipeds possess one prominent tooth and two small teeth. Pereopods slender, pereiopod 3 is the longest, pereiopod 5 is the shortest. Dorsal margins of pereiopods with fine sharp bristles, dactyli ending in sharp points, margins possessing spine-like bristles. Pleopod 1 (gonopod 1), terminal segment short, 0.24 times length of subterminal segment, terminal segment curves away from midline when viewed posteriorly, widest at base ending in point. Subterminal segment tapers distally (Fig. 8A, B). Pleopod 2 (gonopod 2) terminal filament-like, 0.5 times the length of subterminal segment (Fig. 8C).



**Figure 7.** *Potamonautes flavusjo* sp. nov., male holotype (carapace length = 24.02 mm) Verloren Vallei Nature Reserve (site B), 2000 m a.s.l., 25°20'333" S, 30°07'546" E, Mpumalanga province of South Africa (SAM A 48203): A, whole animal, dorsal aspect; B, whole animal, ventral aspect; C, cephalothorax, frontal aspect. Scale bars: 10 mm. The right cheliped was broken off prior to capture.

**Distribution:** Endemic to the Highveld region in the Mpumalanga province of South Africa, where the species occurs exclusively in vleis (wetland) areas next to small streams where they burrow into peat soils. Burrows are always covered by grassy pools and reed beds.

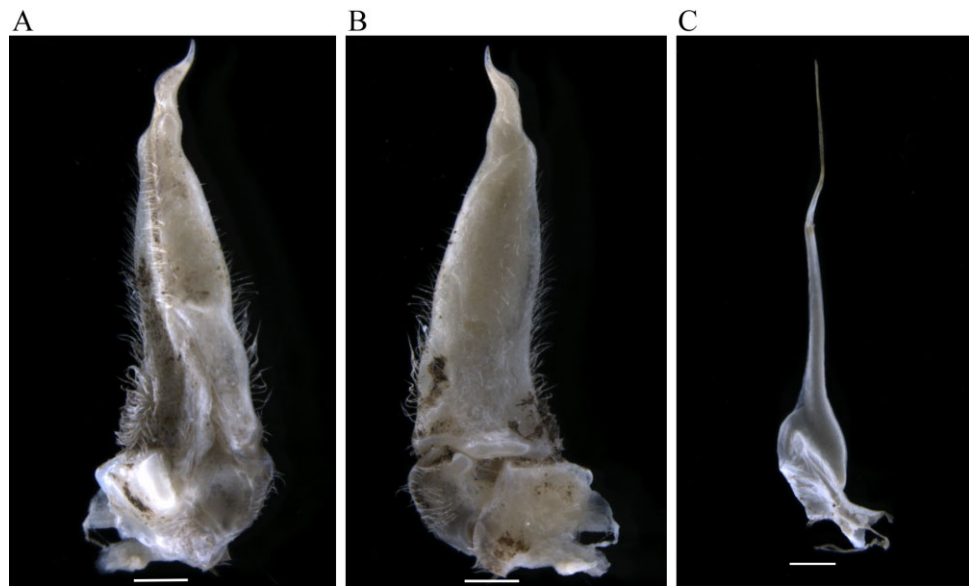
**Remarks:** Although frequently collected sympatrically with *P. sidneyi*, *P. flavusjo* sp. nov. is genetically and morphologically distinct. Phylogenetically, *P. flavusjo* sp. nov. is sister to *P. mulanjeensis* from Mount Mulanje in Malawi and *P. mutareensis* from the Nyanga mountain range in the Eastern Highlands of Zimbabwe, based on mtDNA evidence. These three species can easily be distinguished morphologically.

*Potamonautes mutareensis* shows a moderately arched right dactylus, a characteristic common in most small-bodied freshwater crab living in mountainous regions. This feature is absent in *P. flavusjo* sp. nov. (since the right chelae is broken off), but the dactylus is not arched in any of the other specimens. In addition, the two species are also distinct on the basis of carapace colour, with *P. flavusjo* sp. nov. possessing a bright-yellow postfrontal crest margin and yellow margins of the carapace, whereas these features in *P. mutareensis* are all brown. *Potamonautes mulanjeensis* is a similar sized species (CWW = 34.81 mm) to *P. flavusjo* sp. nov. Neither of the two species sister to *P. flavusjo* sp. nov. have a burrowing lifestyle.

Superficially, *P. flavusjo* sp. nov. is morphologically similar to the two South African freshwater crab species that are semi-terrestrial: *P. calcaratus* and *P. lividus*. All three freshwater crab species are characterized by a vaulted carapace, which is an adaptation to a semi-terrestrial lifestyle away from permanently flowing rivers and streams. In both *P. lividus* and *P. calcaratus* the dactylus of the right cheliped is arched, whereas in *P. calcaratus* the dactylus of the right chela is flattened, which is an adaptation for burrowing. *Potamonautes calcaratus* has a small but distinct tooth on the anterolateral margin of the carapace, a near-flat right cheliped, and a slate-black carapace. The carapace of *P. lividus* when alive is blue with a light-blue shine, whereas the chelipeds and limbs are orange or red. No carapace dentition is present in either *P. lividus* or *P. flavusjo* sp. nov.

In contrast, in *P. flavusjo* sp. nov. the major cheliped shows no special adaptation for a burrowing mode of life. *Potamonautes lividus* does not burrow to the same depths as *P. flavusjo* sp. nov. or *P. calcaratus*. All three of these species are ecologically distinct, with *P. flavusjo* sp. nov. being exclusively associated with peat soils in vleis areas on the Mpumalanga Highveld, where it occurs under grass and reed banks. Burrows of *P. flavusjo* sp. nov. are generally straight and vertical, and typically stretch up to 1 m into the peat soil; however, the burrow depth is dependent on the depth of the water table (S.R.D., pers. observ.). Burrows always have a single opening used for both entry and exit, with the crab generally occupying a small round chamber at the bottom of the burrow that is full of freshwater (pH 7.38, near neutral; conductivity 103  $\mu\text{S cm}^{-1}$ ; temperature of the water at bottom of the burrow 14.6°C, recorded at the Iona Farm sample site in the district of Chrissiesmeer only). In contrast, *P. lividus* forms U-shaped burrows, < 30 cm deep (G. Gouws, pers. comm.), and is endemic to hydromorphic peat swamp forest areas (composed of *Barringtonia*, *Ficus*, and *Syzygium* trees) in north-eastern KwaZulu-Natal (Gouws *et al.*, 2001). More recently a population of *P. lividus* was discovered at Dwessa Forest in





**Figure 8.** *Potamonautes flavusjo* sp. nov., male holotype, Verloren Vallei Nature Reserve (site B), Mpumalanga province of South Africa (SAM A 48203). A, left gonopod 1, anterior view; B, left gonopod 1, posterior view; C, left gonopod 2, anterior view. Scale bars: 10 mm.

**Table 3.** *Potamonautes flavusjo* sp. nov. – measurements (in mm) of the holotype and ranges for additional material examined

Variable	Abbreviation	Holotype	Males	Females
Carapace length	CL	24.02	15.67–20.89	14.96–23.74
Carapace width at widest point	CWW	34.75	21.74–29.24	20.89–33.68
Carapace posterior margin	CWP	12.31	8.16–10.53	8.62–13.39
Frontal width	FD	14.20	9.67–12.75	8.49–14.32
Distance between postfrontal crest and anterior margin	PFC	4.13	2.64–3.21	2.68–4.23
Carapace height	CH	14.87	9.05–12.70	9.52–14.78
Major cheliped propodus length	MCPL	22.86	12.35–18.70	11.91–20.79
Pereiopod 2, merus length	P2ML	11.82	8.27–11.09	8.08–12.23
Pereiopod 2, merus width	P2MW	4.50	3.30–4.78	3.67–5.20
Pereiopod 5, merus length	P5ML	11.56	8.30–10.23	8.76–12.11
Pereiopod 5, merus width	P5MW	4.47	4.28–4.43	3.80–4.97

the Eastern Cape province of South Africa, approximately 750 km from its previously known distribution range in KwaZulu-Natal (S.R.D., pers. observ.). Unpublished mtDNA sequence data reveal that the Eastern Cape and KwaZulu-Natal specimens are genetically nearly identical (S.R.D., pers. observ.). *Potamonautes lividus* has also been collected under decaying logs of wood in forested areas, particularly following episodes of heavy rains (S.R.D., pers. observ.). *Potamonautes calcaratus* is exclusive to the Kruger National Park in South Africa, although the species is also present in southern Mozambique and Zimbabwe (Reed & Cumberlidge, 2004). *Potamonautes calcaratus* can typically be found around ephemeral pans where

they burrow into the banks of the pans. A single burrow opening is present in this species, and the burrows can extend deep into the soil around the ephemeral pans.

**Etymology:** The name *P. flavusjo* sp. nov. is an arbitrary combination of two aspects. The species name takes ‘flavus’, Latin for yellow, as a reference to the distinct canary yellow postfrontal crest margin (Fig. 6), whereas ‘jo’ is added in honour of Dr Johan Engelbrecht, recently deceased, for his unwavering dedication and commitment to freshwater research in the Mpumalanga province of South Africa, and for sending the authors the first female specimen of the species. The name is used as a noun in apposition.



## DISCUSSION

Our results indicate that even well-sampled countries such as South Africa can still yield novel freshwater crab species. Other Afrotropical countries that have not recently been subjected to surveys of their inland freshwater regions are similarly likely to harbour a wealth of undocumented freshwater crab diversity. For example, in Mozambique, where in the last two years two novel endemic species have been recorded from remote areas (*P. bellarussus* sp. nov. and *P. namuliensis*), the distribution of another species (*P. choloensis*) has been extended, and a new species from Gorongosa National Park in Mozambique is currently being described (N. Cumberlidge, pers. comm.). Similar hidden diversity patterns are likely to be true for Zimbabwe following the recent discovery of that country's first endemic freshwater crab species, *P. mutarensis*. A systematic focus on inland freshwater systems, such as ephemeral pans, caves, remote inaccessible mountain streams, and waterfalls in poorly sampled areas could potentially yield several novel freshwater crab species, as well as other freshwater taxa, such as freshwater fishes, dragonflies, and molluscs. The East African Rift Valley has a tremendous diversity of freshwater crab species, particularly in Kenya and Tanzania, and has historically been well sampled (Reed & Cumberlidge, 2006; Cumberlidge & Dobson, 2008; Cumberlidge & Clark, 2010; Cumberlidge & Meyer, 2011). The southern regions of the mountain ranges in Mozambique and most of the adjoining Zimbabwe Highlands are among the most poorly sampled areas on the continent, and are likely to harbour equally high levels of endemism.

The distinctiveness together with the marked genetic divergence of *COI* among the sympatric *P. sidneyi* and *P. flavusjo* sp. nov. at four of the sample sites on the Highveld corroborates the distinctiveness of the species. Where *P. sidneyi* with *P. flavusjo* sp. nov. are sympatric, no haplotypes were shared between the two species, providing evidence of reproductive maternal isolation between the two species, when invoking the biological species concept. Furthermore, the two species exhibit fixed morphological differences in gonopods 1 and 2 and carapace morphology, providing additional support for the phylogenetic species concept. In addition, *P. flavusjo* sp. nov. and *P. sidneyi* are ecologically distinct. *Potamonautes flavusjo* sp. nov. is an extensive burrowing species that occurs exclusively in peat soils where restios (reeds) and grass pools are present, whereas *P. sidneyi* is widely distributed in rivers, streams and wetlands from the Eastern Cape, KwaZulu-Natal, Mpumalanga, and Limpopo provinces of South Africa, and only digs shallow burrows. For a burrowing species, *P. flavusjo* sp. nov. exhibits low levels of genetic variation over the sampled areas in the Highveld. The

shallow genetic differentiation is rather surprising considering that the two burrowing Afrotropical freshwater crab species that have been subjected to population-level genetic analyses [*P. calcaratus* and *Seychellum alluaudi* (A. Milne-Edwards and Bouvier, 1893)] exhibit marked genetic differentiation at small spatial scales (Daniels, Stewart & Cook, 2002b; Daniels, 2011). These results suggest the presence of unsampled subpopulations of *P. flavusjo* sp. nov. within the distribution range on the Highveld, or alternatively are indicative of a highly amphibious capability for this semi-terrestrial species in the absence of major barriers to gene flow. We are unable to differentiate between these two hypotheses; however, it is likely that during periods of heavy rain during the summer months the species is capable of widespread overland dispersal, considering its adaptation to a semi-terrestrial mode of life. Ecological validation of the latter hypotheses are required.

Interestingly, *P. sidneyi* is a morphologically variable taxon: for example, the Verloren Vallei *P. sidneyi* specimens were chocolate brown in colour, with a slightly vaulted carapace (probably resulting from its burrowing mode of life), whereas the Chrissiesmeer district *P. sidneyi* specimens exhibited a near purple carapace margin and chelipeds, and a flat carapace, typical of river-living animals. Despite the variation in morphology these specimens formed a statistically well-supported clade (clade B), suggesting that morphological characters alone should be treated with caution when inferring species boundaries.

Phylogenetically, *P. flavusjo* sp. nov. from the Highveld in Mpumalanga is not closely related to any of the described South African species; instead, it is sister to *P. mutarensis* from Zimbabwe and *P. mulanjeensis* from Malawi. Biogeographically the north-eastern parts of southern Africa is well known to harbour species with a strong tropical affinity, and the close phylogenetic relationship of *P. flavusjo* sp. nov. with taxa outside of South Africa corroborates evidence for a tropical ancestry. The north-eastern region of South Africa (including the KwaZulu-Natal, Limpopo, and Mpumalanga provinces) represents the southern distribution limits of numerous tropical species.

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