MtDNA lineage diversity of a potamonautid freshwater crab in KwaZulu-Natal, South Africa

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Five species of freshwater crab (genus *Potamonautes*) are known from KwaZulu-Natal, South Africa, whilst a sixth (*Potamonautes isimangaliso*) was recently described from the iSimangaliso Wetland Park. Earlier molecular studies of crab diversity in the province were largely limited in geographic scope or employed genetic markers, ill-suited for identifying intraspecific diversity. Possible species-level diversity or cryptic taxa may have thus remained undetected. In this study, lineage diversity was examined in a widespread species, *Potamonautes sidneyi*, using mitochondrial sequence data, to determine whether this species harbours cryptic diversity that could be of conservation importance in the province, particularly with respect to the iSimangaliso Wetland Park. The taxonomic status of *P. isimangaliso* was also assessed. Mitochondrial sequence data were generated and analysed to identify unique lineages and to examine their distributions. Phylogenetic analyses were used to determine whether these lineages represented known or potentially novel species, using comparative data from southern African *Potamonautes* species. Seven independent networks were identified within *P. sidneyi* and substantial structure was observed amongst sampling localities. Phylogenetic analyses revealed two distinct, divergent lineages in *P. sidneyi*. One was positively assigned to *P. sidneyi*, whereas the placement of the other suggested a novel species. These results suggested possible species diversity within *P. sidneyi*, with one lineage occurring in the north-east of the province, around the iSimangaliso Wetland Park. *Potamonautes isimangaliso* was clearly allied to *Potamonautes lividus*, but genetic divergences suggested that *P. isimangaliso* is a distinct taxon and that *P. lividus* may represent a species complex.

Conservation implications: This study confirmed unique freshwater crab diversity, both within KwaZulu-Natal and associated with the iSimangaliso Wetland Park.

Introduction

An intensive, dedicated, large-scale systematic study into the diversity of South Africa's freshwater crab fauna was launched during the 1990s (Hart, Stewart & Bickerton 2001; Stewart 1997a, 1997b; Stewart, Coke & Cook 1995). Through this, seven new species were described (Daniels, Stewart & Burmeister 2001; Daniels, Stewart & Gibbons 1998a; Gouws, Stewart & Coke 2000; Gouws, Stewart & Reavell 2001; Stewart & Cook 1998; Stewart 1997a; Stewart *et al.* 1995) and one re-described (Stewart 1997b). At its conclusion, 13 species of *Potamonautes* MacLeay, 1838, the only genus of freshwater crabs occurring in South Africa, were recognised (Cumberlidge *et al.* 2009; Daniels, Phiri & Bayliss 2014; Gouws & Stewart 2001; Hart *et al.* 2001). More than 50% of these are endemic (Cumberlidge *et al.* 2009). Subsequently, ongoing research has identified and described four new species (e.g. Daniels *et al.* 2014; Peer *et al.* 2015; Phiri & Daniels 2014a) throughout the country.

Along with the Western Cape province of South Africa, the province of KwaZulu-Natal (KZN) was a substantial focus of this research programme. Previously, only two species were known to occur in KZN: *Potamonautes sidneyi* (Rathbun, 1904) and *Potamonautes depressus* (Krauss, 1843) (Gouws & Stewart 2001).

Potamonautes sidneyi is a widely distributed species, occurring across the eastern part of South Africa and extending northwards, at least into Zimbabwe (Gouws, Daniels & Stewart 2002). It is generally widespread across KZN, from the foothills of the Drakensberg Mountains through the lower-lying midlands to the coast (Gouws & Stewart 2001). *Potamonautes depressus* occurs in the upper, faster-flowing tributaries that drain the Drakensberg and its foothills (Gouws & Stewart 2001). A number of accounts of unexpected species or enigmatic records are known from the province, which likely reflect taxonomic uncertainty and/or the assignment of known (extralimital) species epithets to the then-undescribed species (see Gouws & Stewart 2001; Gouws *et al.* 2002).

With dedicated sampling and systematic investigation using morphometric and genetic approaches, three new species were described from KZN. *Potamonautes dentatus* Stewart, Coke & Cook, 1995 was described from sections of the Mngeni and Thukela rivers by Stewart *et al.* (1995). Gouws *et al.* (2000) described *Potamonautes clarus* Gouws, Stewart & Coke, 2000 from the headwaters of the Thukela River along the Drakensberg in the north-western part of the province. This species was delineated from *P. depressus*, which occurs along the Drakensberg to the south (Gouws & Stewart 2001). Daniels *et al.* (2003) later revealed *P. clarus* and *P. depressus* to be a complex of considerable cryptic diversity.

Potamonautes lividus Gouws, Stewart & Reavell, 2001, a morphologically distinct species associated with swamp forests dominated by *Barringtonia racemosa* and *Ficus tricopoda*, was described from a few localities in the north-eastern region of KZN (Gouws *et al.* 2001). More recently, *Potamonautes isimangaliso* Peer & Gouws, 2015 was described from the iSimangaliso Wetland Park in the north-eastern part of the province. This ecologically unique, burrowing species, occurring in ephemeral pans, was primarily delineated from the burrowing and morphologically similar *P. lividus* (Peer *et al.* 2015). Although the programme in KZN was underpinned by a substantial sampling effort, there were still notable sampling gaps (particularly in terms of specimens examined genetically), especially in the south and north-east of the province (Gouws & Stewart 2001). The iSimangaliso Wetland Park, an area of considerable biological, cultural and socio-economic importance, is found in the latter. This park is South Africa's first United Nations Educational, Scientific and Cultural Organization World Heritage Site and includes three Ramsar Wetlands of International Importance, namely the St Lucia estuarine lake, Lake Sibaya and Kosi Bay (Perissinotto, Stretch & Taylor 2013).

Although genetic approaches, specifically allozyme electrophoresis, were used to identify and delineate two of the species from the province (Gouws *et al.* 2000; Gouws *et al.* 2001), these studies were limited in their geographic or taxonomic focus (i.e. in terms of specific species compared to the putatively new species).

A wider geographic consideration of genetic diversity and structure amongst and within the species of KZN was attempted, again using allozymes (Gouws & Stewart 2001). However, it has been consistently recognised that allozyme variation in southern African potamonautid crabs is limited and that these markers may be too conservative to detect finer genetic diversity (Daniels 2003; Daniels, Gibbons & Stewart 1999; Daniels, Gouws & Crandall 2006; Daniels, Stewart & Gibbons 1998b; Daniels, Stewart, Ridgway & Florence 2001; Gouws & Stewart 2001; Gouws *et al.* 2001; Gouws *et al.* 2002).

Aside from studies of cryptic diversity and species delineation within the *P. clarus* – *P. depressus* complex (Daniels *et al.* 2003; Phiri & Daniels 2014b), DNA sequence data have generally not been used in studies of KZN crabs and there has been no published DNA-based survey of lineage diversity within the most widespread species in the province. The extent of divergence amongst mitochondrial DNA sequences generated for *P. isimangaliso* and published data for *P. lividus* (Daniels *et al.* 2002) provided initial evidence of the separation of these species (Peer *et al.* 2015). However, the phylogenetic relationships between these species, and between *P. isimangaliso* and other KZN species, were not considered.

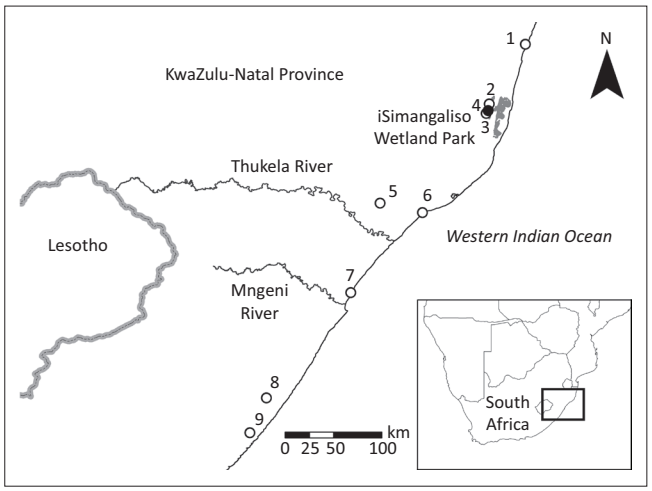
The primary aim of this study is to provide a coarse-grained and broad-scale snapshot of mitochondrial DNA lineage diversity along the coastal zone in the KZN province for the most widespread species, *P. sidneyi*, and to consider aspects of its biogeography in the region.

Highlighting unique diversity within the province would be of interest to provincial conservation (Ezemvelo KZN Wildlife) and regional management (e.g. the iSimangaliso Wetlands Park Authority) agencies. Documenting undescribed diversity (novel species or unique genetic lineages) in the area around the iSimangaliso Wetland Park would be noteworthy and add to the conservation value of the park, considering the recent description of *P. isimangaliso*. This species is presently believed to be endemic to the park (Peer *et al.* 2015) and there is a growing number of cryptic taxa and unique lineages recently documented in this region (see Maake *et al.* 2013). The study also seeks to examine the relationship between *P. isimangaliso*, the morphologically similar *P. lividus*, and other species from the province and southern African region to support or refute its specific status.

Methods

Potamonautes sidneyi and *P. isimangaliso* were sampled between February 2012 and June 2013 from nine localities across KZN, South Africa (Figure 1), and were identified using published keys (Gouws & Stewart 2001; Hart *et al.* 2001; Peer *et al.* 2015). Four of these localities – Lake Sibaya (Site 1), Mpophomeni Stream (Site 2), Dukandlovu Pan (Site 3) and Hluhluwe (Site 4) (Figure 1) – were from the north-eastern part of KZN, within or in close proximity to the iSimangaliso Wetland Park. The remaining localities were distributed along a coastal transect extending to the south of the province.

Total genomic DNA was extracted from pereiopod muscle tissue from each specimen using a Thermo Fisher Scientific GeneJET Genomic DNA Purification Kit (Massachusetts, USA) following the manufacturer's protocols, except that extracted DNA was eluted in a final volume of 100 µL. A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by a polymerase chain reaction. Each 25 µL reaction contained 1 × buffer, 3 mM $MgCl_2$, 0.2 µM of each of Folmer *et al.*'s (1994) primers (LCOI-1490 and HCOI-2198), 0.2 mM of each deoxynucleotide, 0.5 U *Taq*-polymerase (Southern Cross Biotechnology, South Africa) and 3 µL template DNA, made up to the final volume with ultrapure water.



Source: Drafted by Willem Coetzer; South African Institute for Aquatic Biodiversity

FIGURE 1: Sampling localities of *Potamonautes sidneyi* (open circles) and *Potamonautes isimangaliso* (filled circle) in KwaZulu-Natal, South Africa: (Site 1) Lake Sibaya, (Site 2) Mpophomeni Stream, (Site 3) Hluhluwe, (Site 4) Dukandlovu Pan, (Site 5) Entumeni, (Site 6) Siyayi, (Site 7) Mhlanga, (Site 8) Oribi Gorge and (Site 9) Mtamvuna.

The thermocycling regime for the amplification of this gene fragment included an initial denaturing step of 2 min at 95 °C, followed by 35 cycles of denaturing (95 °C for 30 s), annealing (44 °C for 40 s) and extension (72 °C for 1 min). This was followed by a final extension step of 72 °C for 10 min. A fragment of the 16S ribosomal DNA (rDNA) was amplified from representative individuals (see below), using the primers (16Sar and 16Sbr) of Palumbi *et al.* (2007) and the above polymerase chain reaction recipe and thermocycling regime, but with annealing performed at 50 °C.

To confirm successful amplification, products were visualised on an ultraviolet transilluminator, following electrophoresis in 1% agarose gels stained with ethidium bromide. Amplicons were sent to a commercial sequencing facility (Macrogen Inc., South Korea), where they were purified, sequenced using standard BigDye v3.1 (Applied Biosystems, Austin, Texas, USA) terminator chemistry and analysed on an ABI 3730XL (Applied Biosystems) automated sequencer.

Sequences were checked against their respective chromatograms for ambiguities, misreads and sequencing errors using Chromas Lite (Technelysium Pty Ltd., South Brisbane, Australia). Alignment and further editing were performed using DNASTAR® Lasergene SeqMan Pro 11 (Madison, Wisconsin, USA). The final alignments of the COI and 16S data sets were produced using ClustalX2 (Larkin *et al.* 2007) and MAFTT version 6 (Katoh & Toh 2008), respectively, with an iterative refinement strategy (L-INS-i) (Katoh *et al.* 2005) for the latter.

Data analyses proceeded in two stages. Firstly, to understand diversity within and amongst sampling localities of *P. sidneyi* and *P. isimangaliso*, and the diversity of lineages within *P. sidneyi*, analyses were conducted with the full set of COI sequences generated. To examine genealogical and geographic relationships, a 95%-probability parsimony network was generated using TCS1.21 software (Clement, Posada & Crandall 2000). A midpoint-rooted neighbour-joining tree (Saitou & Nei 1987), based on uncorrected sequence divergences, was generated in PAUP*4b10 (Phylogenetic Analysis Using Parsimony) software (Swofford 2002). Uncorrected sequence divergences within and amongst identified lineages were examined using PAUP.

In a second set of analyses, the phylogenetic placement of, and divergences amongst, the above lineages were contextualised and an attempt was made to confirm or assign species identifications to each. Representatives of the unique lineages identified were included in a matrix containing published data, sourced from GenBank® (Table 1), of other southern and eastern African *Potamonautes* species. Data from the COI and 16S fragments were analysed as a combined data set and phylogenetic analyses were conducted by unweighted parsimony and Bayesian inference. For the parsimony analysis, a heuristic tree search was conducted in PAUP*4b10, using tree bisection and reconnection branch swapping of a tree obtained by a random stepwise addition of taxa, employing 1000 such iterations. Nodal support was evaluated by bootstrapping of the data set (Felsenstein 1985), using 1000 pseudoreplicates.

Prior to the Bayesian analysis, the optimal model of nucleotide substitution for each partition (COI and 16S rDNA) of the combined data set was identified using jModelTest 2.1.4 (Darriba *et al.* 2012) with the choice of model determined by the Akaike (1974) Information Criterion. Bayesian inference was conducted in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Four simultaneous, independent analyses, each employing three heated and one cold Markov chains, were run for 10^7 generations, sampling every 2000 generations. The chosen models were specified for each partition, with Mr Bayes estimating the model parameters. Default, unlinked priors were used. The standard deviation of split frequencies was observed to monitor convergence of the analyses on a similar part of the posterior distribution. Stationarity and effective sample size (ESS > 200; such that sampling of the various parameters from the posterior distribution was sufficient) were determined using Tracer 1.5 software (Rambaut & Drummond 2009). A majority rule consensus tree was constructed from the 18 000 trees retained from the four analyses combined, once a 10% burn-in was discarded in each; the frequency of retrieval of particular nodes representing the Bayesian Posterior Probabilities (BPPs) for those relationships.

Liberonautes rubigimanus Cumberlidge & Sachs, 1989 and *Sudanonautes floweri* (De Man, 1901) were included as outgroups in these analyses. Previous multi-locus phylogenies (Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006; Daniels *et al.* 2015) revealed *Liberonautes* Bott, 1955, and a *Sudanonautes* Bott, 1955 and *Potamonemus* clade to be sister taxa to the *Potamonautes* A. Milne-Edwards, 1887 clade that nests both *Erimetopus* Rathbun, 1894 and *Platythelphusa* A. Milne-Edwards, 1887. Representatives of *Liberonautes* and *Sudanonautes* have

TABLE 1: Southern and Eastern African *Potamonautes* species included in the phylogenetic analysis. *Liberonautes latidactylus* and *Sudanonautes aubryi* were included as outgroup taxa. GenBank® accession numbers for the cytochrome *c* oxidase subunit I and 16S ribosomal DNA sequences are included and the data sources provided.

Species	GenBank® number		Source
	Cytochrome *c* oxidase subunit I	16S	
Potamonautes barbarai	AF494023	AF493165	Daniels 2002; Phiri & Daniels 2014a
Potamonautes barnardi	AF494028	AF493171	Daniels 2002; Phiri & Daniels 2014a
Potamonautes bayonianus	AF510868	AY042243	Daniels *et al.* 2002
Potamonautes bellarussus	KJ713548	KJ713496	Daniels *et al.* 2014
Potamonautes brincki	AF510875	AY042244	Daniels *et al.* 2002
Potamonautes calcaratus (1)	AF510867	AY042242	Daniels *et al.* 2002
Potamonautes calcaratus (2)	JF799190	JF199118	Daniels & Bayliss 2012
Potamonautes choloensis	JF799203	JF799137	Daniels & Bayliss 2012
Potamonautes clarus	AF510872	AY042241	Daniels *et al.* 2002
Potamonautes dentatus	AF510878	AY042246	Daniels *et al.* 2002
Potamonautes depressus	AF510877	AY042247	Daniels *et al.* 2002
Potamonautes flavusjo	KJ713524	KJ713473	Daniels *et al.* 2014
Potamonautes granularis	AF510876	AY042254	Daniels *et al.* 2002
Potamonautes isimangaliso	KR137640	KR137641	Peer *et al.* 2015
Potamonautes lirrangensis	AY803568	AY803534	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Potamonautes lividus: South Africa	AF510879	AY042248	Daniels *et al.* 2002
Potamonautes lividus: Swaziland	JF799194	JF799150	Daniels & Bayliss 2012
Potamonautes mulanjeensis	JF799201	JF799139	Daniels & Bayliss 2012
Potamonautes mutareensis	KC768296	KC768270	Phiri & Daniels 2013
Potamonautes namuliensis	JF799198	JF799142	Daniels & Bayliss 2012
Potamonautes obesus	JF799188	JF799126	Daniels & Bayliss 2012
Potamonautes odhneri	AY803571	AY803538	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Potamonautes parvicorpus	AF510869	AY042252	Daniels *et al.* 2002
Potamonautes parvispina	AF510873	AY042253	Daniels *et al.* 2002
Potamonautes perlatus	AF494024	AF493172	Daniels 2003; Phiri & Daniels 2014a
Potamonautes platynotus	AY803572	AY803539	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Potamonautes raybouldi	AY803573	AY803540	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Potamonautes sidneyi	AF510871	AY042245	Daniels *et al.* 2002
Potamonautes subukia	AY803569	AY803535	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Potamonautes unispinus	AF510870	AY042250	Daniels *et al.* 2002
Potamonautes warren	AF510880	AY042251	Daniels *et al.* 2002
Outgroups	-	-	-
Sudanonautes floweri	AY803574	AY803541	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006
Liberonautes rubigimanus	AF399978	-	Plachetzki & Cumberlidge 2001
Liberonautes rubigimanus	-	AY803543	Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006

Note: Please see the full reference list of the article, Gouws, G., Peer, N. & Perissinotto, R., 2015, 'MtDNA lineage diversity of a potamonautid freshwater crab in KwaZulu-Natal, South Africa', *Koedoe* 57(1), Art. #1324, 12 pages. http://dx.doi.org/10.4102/koedoe.v57i1.1324, for more information

been used as outgroups in other phylogenetic studies of *Potamonautes* (Daniels & Bayliss 2012; Daniels *et al.* 2014; Phiri & Daniels 2013). Uncorrected sequence divergences amongst known species and the representatives of the respective lineages were calculated in PAUP.

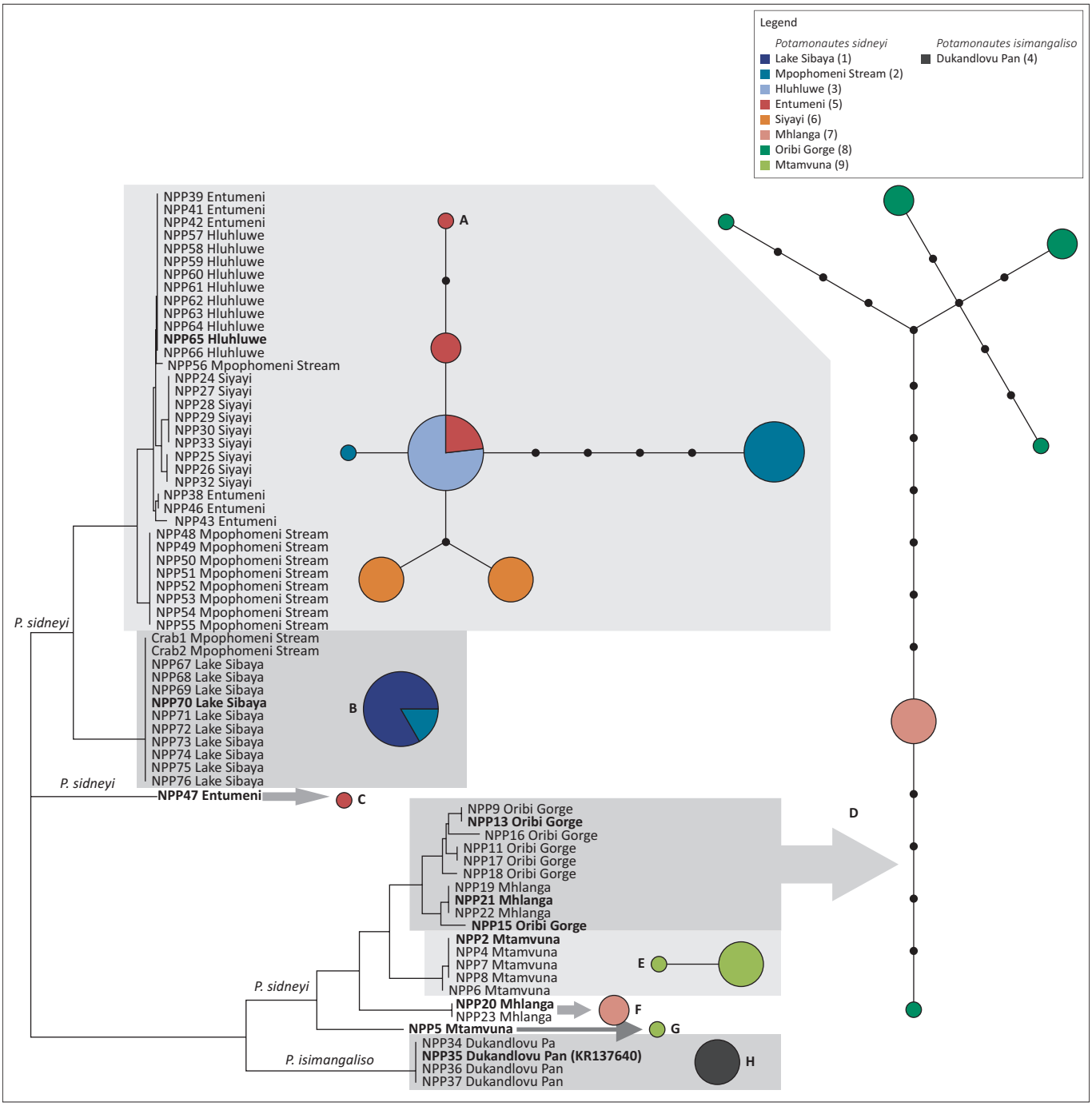
Results

Specimens of *P. isimangaliso* were sampled only from Dukandlovu Pan (Figure 1, Site 4), whilst specimens from all the other localities were positively identified as *P. sidneyi*.

All COI sequences were submitted to GenBank® under accession numbers KT275869 to KT275936. The full COI alignment of all 69 specimens was 639 nucleotides in length. Upon translation to amino acids, there was no evidence of stop codons in the alignment and it was concluded that the data represented functional proteins and were of mitochondrial origin.

One haplotype was found amongst the four *P. isimangaliso* individuals from Dukandlovu Pan, including the individual sequenced by Peer *et al.* (2015). Nineteen unique haplotypes were identified within *P. sidneyi* (Figure 2). The distribution of haplotypes within this species indicated substantial genetic structuring amongst sampling localities. Eight individuals possessed unique haplotypes, while nine haplotypes were found in multiple individuals from the same locality. The remaining two haplotypes were shared amongst sampling localities and only amongst two localities in each instance: amongst the proximate Lake Sibaya (Site 1) and Mpophomeni Stream (Site 2) localities, and amongst the geographically more distant Hluhluwe (Site 3) and Entumeni (Site 5) localities, respectively.

The 20 haplotypes observed in this study were placed in 8 independent networks that could not be linked with 95% confidence. Five networks (Figure 2, Networks C, E, F, G and H), all but one consisting of single haplotypes, represented single localities. Two larger networks (Networks A and D)



Source: Drafted by Gavin Gouws; South African Institute for Aquatic Biodiversity

The midpoint-rooted neighbour-joining tree (left) has species identifications for clades indicated on the branches. Eight independent 95%-credible parsimony networks are labelled A–H. The size of the nodes are proportional to the frequency of the haplotypes, whilst colours indicate their relative occurrence at the various localities. Each branch represents one mutational step, with small black dots indicating intermediate or unsampled haplotypes. Grey shading indicates the corresponding occurrence of individuals and haplotypes between the tree clusters and the networks. Bold terminal names indicate those sequences that were included in the subsequent phylogenetic analyses.

FIGURE 2: Relationships amongst cytochrome *c* oxidase subunit I mtDNA haplotypes found amongst *Potamonautes sidneyi* and *Potamonautes isimangaliso* individuals sampled from KwaZulu-Natal.

and one consisting of a single haplotype (Network B), found in both Lake Sibaya (Site 1) and Mpophomeni Stream (Site 2), represented multiple localities. Haplotypes of Network A were found in four localities from the north-eastern to central parts of the province: Mpophomeni Stream site (Site 2), Hluhluwe (Site 3), Entumeni (Site 5) and Siyayi (Site 6). Those of Network D were found in the localities to the south of KZN: Mhlanga (Site 7) and Oribi Gorge (Site 8). Several localities possessed haplotypes of more than one network: Entumeni (Site 5), Mpophomeni Stream (Site 2), Mhlanga (Site 7) and Mtamvuna (Site 9).

Uncorrected sequence divergences among haplotypes belonging to different networks ranged from 2.8% to 14.7% (with a mean of 10.7%). The two haplotypes of Network E were 0.2% divergent. Network A showed the greatest diversity (seven haplotypes), with divergences amongst the constituent haplotypes ranging from 0.2% to 1.3% (with a

mean of 0.6%). Greater divergences were observed within Network D (from 0.6% to 2.5%; mean 1.5%).

The diversity and the geographic structure observed were substantiated by the neighbour-joining tree (Figure 2). Two distinct clusters were retrieved, with a single sample from Entumeni (NPP47) placed outside of these. The first cluster, corresponding to Networks A and B, contained samples identified as *P. sidneyi* from the north-eastern and central parts of the province: Lake Sibaya (Site 1), Mpophomeni Stream (Site 2), Hluhluwe (Site 3), Entumeni (Site 5) and Siyaya (Site 6).

The second cluster contained individuals identified as *P. sidneyi*, as well as the *P. isimangaliso* specimens from Dukandlovu Pan (Site 4). These formed separate sub-clusters and corresponded to separate networks (Networks D to G for *P. sidneyi* and Network H for *P. isimangaliso*). The *P. sidneyi* individuals in this cluster were from the three sites towards the south of the province: Mhlanga (Site 7), Oribi Gorge (Site 8) and Mtamvuna (Site 9).

For the phylogenetic analyses, a 16S rDNA fragment was sequenced for a single representative of each of seven networks (Networks A–C, and E–H) and three representatives of Network D (representing the three divergent groups of haplotypes in this network). These 16S rDNA sequences were lodged under GenBank® accession numbers KT275860 to KT275868. This fragment was analysed in combination with the COI data for each of these specimens and data downloaded from GenBank®.

Once sequences were trimmed to equal length, the COI and 16S alignments were 362 and 401 nucleotides in length, respectively, yielding a combined alignment of 763 nucleotides. The parsimony analysis, based on 207 parsimony informative characters (97 from the COI data and 110 from the 16S rDNA), yielded a single most parsimonious tree (Figure 3a: 1106 steps; consistency index = 0.326; retention index = 0.543; rescaled consistency index = 0.177).

Hasegawa, Kishino and Yano's (1985) HKY model, with unequal base frequencies (A = 0.332, C = 0.206, G = 0.077 and T = 0.385), a transition to transversion rate of 4.826, a proportion of invariable sites (I = 0.487) and a gamma-distribution (α = 0.707) of rate variation was determined to be the most appropriate for the COI partition. The model chosen for the 16S partition included unequal base frequencies (A = 0.394, C = 0.078, G = 0.136 and T = 0.393), mostly independent transition and transversion rates ($R_{[A\leftrightarrow C]}$ = 0.364, $R_{[A\leftrightarrow G]}$ = 7.317, $R_{[A\leftrightarrow T]}$ = $R_{[G\leftrightarrow T]}$ = 1.000, $R_{[C\leftrightarrow G]}$ = 0.675 and $R_{[C\leftrightarrow T]}$ = 2.893), a proportion of invariable sites (I = 0.169) and a gamma-distribution (α = 0.443) of rate variation.

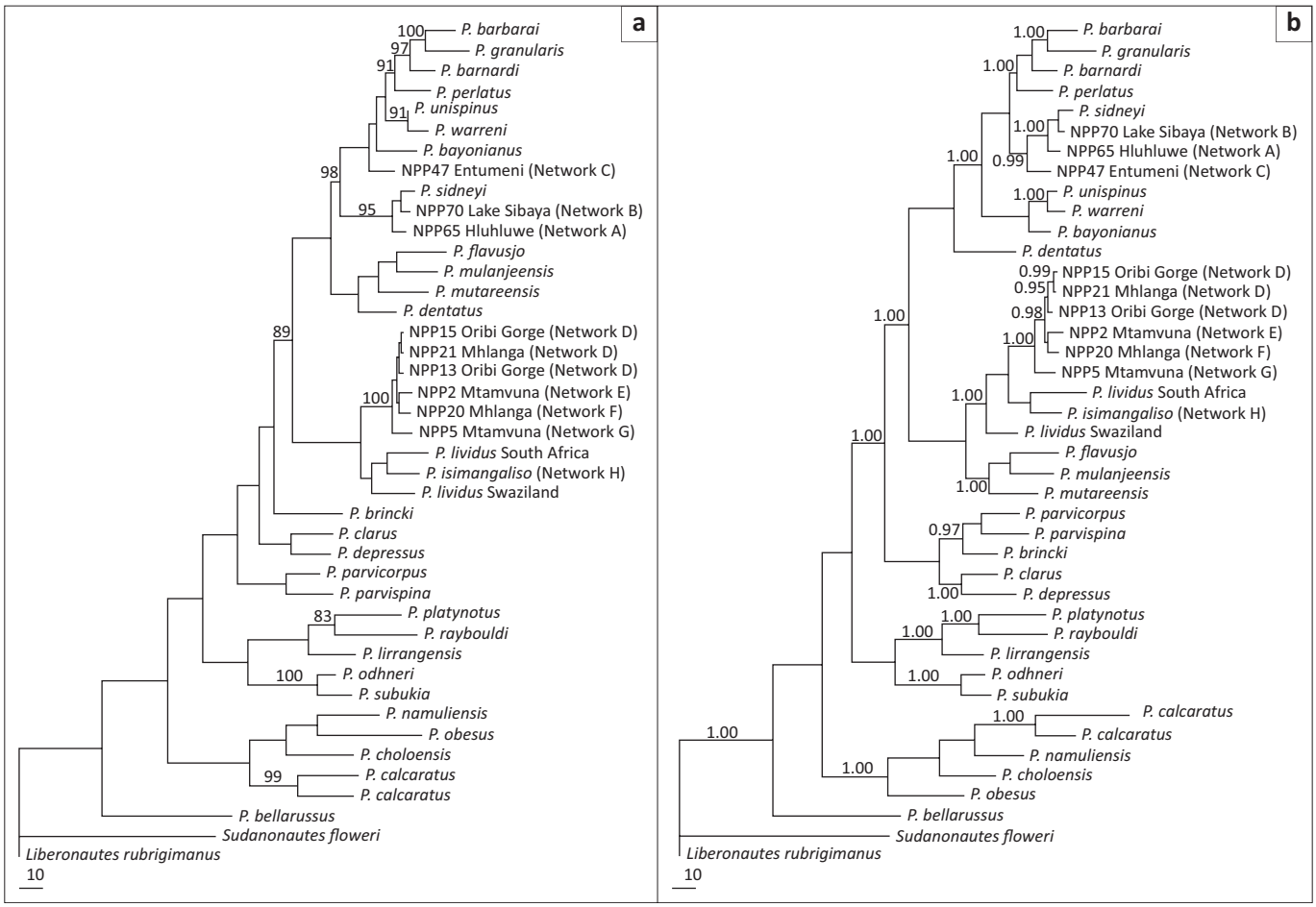
The most likely topology (-lnL = 7841.114) obtained through the Markov chain Monte Carlo searches across the four Bayesian analyses is presented in Figure 3b. Both phylogenetic trees showed certain similarities to published phylogenies (Daniels & Bayliss 2012; Daniels, Cumberlidge, Pérez-Losada, Marijnissen & Crandall 2006; Daniels *et al.* 2014; Phiri & Daniels 2013) relating to the species represented in the major clades retrieved, but many of the relationships within and among these clades were not consistent. Nonetheless, it was possible to resolve, in either of the two analyses, the placement of representatives of the unique lineages with high support.

Like the neighbour-joining tree of the COI data, the representatives of *P. sidneyi* were placed in two distinct and well-separated clades in both analyses. The representatives of the lineages from the north-eastern and central parts of the province (NPP65 Hluhluwe, representing Network A; and NPP70 Lake Sibaya, representing Network B) formed a clade with the published *P. sidneyi* sequence with high support (95% bootstrap; 1.00 BPP). NPP47 Entumeni (Network C) was well-supported as a sister-taxon to this group (0.99 BPP) in the Bayesian analysis. Although this specimen was not associated with this clade in the parsimony analysis, the alternative placement was not supported (Figure 3a). Regardless, these specimens were all placed in a larger clade containing a number of large-bodied riverine species (*sensu* Daniels *et al.* 2002; *Potamonautes barbarai* Phiri & Daniels, 2014, *Potamonautes barnardi* Phiri & Daniels, 2014, *Potamonautes bayonianus* [Brito-Capello, 1864], *Potamonautes granularis* Daniels, Stewart & Gibbons, 1998, *Potamonautes perlatus* [H. Milne-Edwards, 1837], *P. sidneyi*, *Potamonautes unispinus* Stewart & Cook, 1998 and *Potamonautes warreni* [Calman, 1918]) with strong support (98%; 1.00 BPP).

The second group of individuals identified as *P. sidneyi* (NPP13 Oribi Gorge, NPP15 Oribi Gorge and NPP21 Mhlanga, representing Network D, and the representatives of Networks E, F and G) from the south of the province were placed in a clade containing two published *P. lividus* sequences and the *P. isimangaliso* (Network H) representative, with 1.00 BPP. The *P. isimangaliso* specimen was consistently the sister-taxon to the sequence of *P. lividus* from South Africa, to the exclusion of the representative from Swaziland.

Sequence divergences amongst all included, recognised species and representatives of the lineages are presented in the Appendix, Table 1. Divergences amongst known species ranged from 2.3% (*P. barbarai* versus *P. granularis*) to 21.5% (*Potamonautes calcaratus* [Gordon, 1929] versus *P. lividus* from South Africa), with a mean of 13.5%. Those specimens of *P. sidneyi* from the north-eastern and central parts of the province, placed in a clade with *P. sidneyi* and the large-bodied riverine species, were 1.8% – 4.8% divergent from the published *P. sidneyi* sequence. These individuals (and *P. sidneyi* itself) were, in turn, 9.2% – 11.8% divergent from the representatives of the second clade of *P. sidneyi* individuals from the south of the province.

The divergences amongst these two groups were substantially higher than the divergences within (0.4% – 4.8%). This southern clade showed a marginally closer relationship to *P. lividus* and *P. isimangaliso* (6.7% – 10.0% divergence) than to the north-eastern *P. sidneyi* clade. Considering the combined COI and 16S rDNA data, the *P. isimangaliso* representative



Source: Drafted by Gavin Gouws; South African Institute for Aquatic Biodiversity

FIGURE 3: The phylogenetic placement, based on the parsimony and Bayesian analyses of combined cytochrome *c* oxidase subunit I and 16S ribosomal DNA sequence data partitions, of unique KwaZulu-Natal *Potamonautes sidneyi* lineages and *Potamonautes isimangaliso*, relative to Southern and Eastern African *Potamonautes* species: (a) the single most parsimonious tree (1106 steps, consistency index = 0.326, retention index = 0.543, rescaled consistency index = 0.177) and (b) the most likely (-lnL = 7841.114) topology obtained across the four Bayesian analyses. Numbers on the branches indicate bootstrap support (only values > 75% are shown) and Bayesian Posterior Probabilities (only Bayesian Posterior Probabilities ≥ 0.95) for relationships in A and B, respectively.

was 7.4% and 7.8% divergent from the Swaziland and South African representatives of *P. lividus*, respectively.

Discussion

The analysis of the COI data reveals substantial genetic diversity and structure within *P. sidneyi* in KZN; 7 divergent, unlinked networks were retrieved (with an additional network representing *P. isimangaliso*) and only 2 of 19 haplotypes are shared amongst sampling localities.

For the most part, the data show a clear isolation and lack of gene flow amongst most sampling localities, even amongst those that are geographically proximate. This is contrary to expectations for a species that is assumed to be highly dispersive (Daniels 2003; Daniels *et al.* 1998a, 1998b; Daniels *et al.* 1999; Daniels, Gouws & Crandall 2006; Daniels, Stewart, Ridgway & Florence 2001) and follows more closely patterns observed in certain primary, obligate freshwater fishes from South Africa, for example *Galaxias* (Chakona, Swartz & Gouws 2013). The patterns observed reflect not only the isolation of individual drainages, but often the isolation of localities within the same system (e.g. amongst Hluhluwe and Mpophomeni streams, which flow into Lake St Lucia).

In the similarly widespread *Potamonautes perlatus sensu lato*, extensive sharing of haplotypes was observed amongst localities, but these were within the same catchments (Daniels, Gouws & Crandall 2006; Phiri & Daniels 2014a). Very few haplotypes were shared amongst currently isolated systems, much like the present case. The isolation of the individual localities and systems is perhaps facilitated by the hydrology of KZN. In the subtropical to tropical north-east of the province, drainages are generally mature, widely separated and flow over low relief floodplains (Skelton 2001). Geographic distance may prohibit dispersal amongst systems, despite the area being flood-prone and the presence of ephemeral wetlands (Skelton 2001), which should facilitate dispersal. Towards the south of the province, in the montane escarpment region, a dense network of shorter, high-gradient, deeply-incised catchments fragment the landscape (Skelton 2001). Dispersal may be hindered across higher drainage divides.

Two divergent phylogenetic lineages were found in *P. sidneyi*, with one of these showing a possible closer affinity to *P. lividus* and *P. isimangaliso*. These two lineages were restricted to the north-eastern and central, and southern parts of the province, respectively. The distribution of these lineages and the division amongst them reflects the two aquatic ecoregions mentioned above, as well as the separation of the Sibayi and Zululand from the Mngeni and Mzimkhulu aquatic bioregions (see Rivers-Moore, Goodman & Nkosi 2007); these regions defined by ecology, species diversity and endemism.

Previous studies considering the genetic diversity or structure in *P. sidneyi* in KZN provided conflicting patterns. An allozyme study, delineating *P. lividus* from *P. sidneyi*, found low levels of diversity and only shallow structure in the latter (Gouws *et al.* 2001). However, localities from which *P. sidneyi* was sampled were from the north-eastern region of KZN and may represent only one of the lineages identified in the current study. With slightly wider sampling, a study of morphometric and allozyme variation within and amongst all *Potamonautes* species in KZN provided some evidence of genetic structure and differentiation within *P. sidneyi* (Gouws & Stewart 2001).

Two distinct genetic clusters were identified: one from the central and north-east of the province and the other from the high-lying Drakensberg in the extreme west (Gouws & Stewart 2001). Whether these correspond to the two lineages of the present study or represent additional lineage diversity remains to be determined as no *P. sidneyi* populations from the Drakensberg were included in this study. Nonetheless, the genetic differentiation of *P. sidneyi* from the Drakensberg was confirmed in a subsequent study (Gouws *et al.* 2002).

These populations form a distinct genetic cluster separate to samples from elsewhere in the province; the latter showed closer genetic similarity to *P. sidneyi* samples from other provinces, that is, Mpumalanga, Eastern Cape and Limpopo (Gouws *et al.* 2002). Interestingly, the latter study showed genetic differentiation amongst populations of *P. sidneyi* from different reaches of the same river system, the Thukela. This reflects a longitudinal pattern of differentiation involving the phylogenetic separation (see Daniels *et al.* 2002) of high-altitude, small-bodied species (members of the *P. depressus* – *P. clarus* complex in this system) in the upper reaches from the robust species of the middle to lower reaches (*P. sidneyi*) and then possible longitudinal genetic structure within the latter.

The co-occurrence of multiple, genetically independent networks at single localities, particularly in the south of the province where drainage is steep and with potential for isolation along the river course, attests to the diversity in the species and may perhaps reflect such longitudinal or altitudinal genetic structure. As the present study focused on sampling in the coastal zone only, this requires further investigation in the Thukela River and these southern systems. Nonetheless, *P. sidneyi* clearly shows a complex pattern of lineage diversity and structure within KZN and beyond. This is currently being investigated through a much broader phylogeographic study of this species across its full South African distribution.

The taxonomic status of the two *P. sidneyi* lineages requires consideration. The sequence divergence amongst these lineages (9.2% – 11.8%) was more extensive than was observed amongst other species (*P. barbarai*, *P. barnardi*, *P. bayonianus*, *P. granularis*, *P. perlatus*, *P. unispinus* and *P. warreni*) in the group of large-bodied, riverine species from South Africa, and amongst these species and the reference *P. sidneyi* individual (2.3% – 8.3%).

Furthermore, the higher values from individual comparisons amongst the two lineages approached the mean divergence amongst all valid species included in the study. *Potamonautes sidneyi* in KZN shows comparable diversity to *P. perlatus* (see Phiri & Daniels 2014a), which, along with *P. sidneyi*, is one of the most widely distributed South African freshwater crab species (Daniels 2003; Daniels, Gouws & Crandall 2006; Gouws *et al.* 2002; Phiri & Daniels 2014a). However, a deeper divergence was observed between lineages of *P. sidneyi* in KZN than was observed amongst the lineages of *P. perlatus*. Comparisons amongst representatives of the two *P. sidneyi* lineages yielded sequence divergences of 6.2% – 9.3% for the 16S data, and 12.2% – 14.3% for COI, which exceeded the maximum values of 6% and 10% for 16S and COI, respectively, in *P. perlatus* (Daniels 2003; Daniels, Gouws & Crandall 2006).

Two new species (*P. barbarai* and *P. barnardi*) were delineated, using genetic evidence alone, amongst the three lineages in *P. perlatus sensu lato* (Phiri & Daniels 2014a). The extent of divergence, the phylogenetic placement of lineages identified as *P. sidneyi* in the present phylogenetic analyses, results from past analyses (Gouws & Stewart 2001; Gouws *et al.* 2002) and the precedent set by Phiri and Daniels (2014a) suggests that there may be additional species-level diversity with *P. sidneyi*, requiring recognition.

The identification and description of new species within *P. sidneyi* is deferred at present, for two reasons. The first is the uncertainty over which lineage or clade represents the 'true' *P. sidneyi*. Rathbun (1905), when describing the species, indicated the type locality as being 'Natal', without providing a more specific locality within the province. The species were described from material collected by Sarah Abraham in 1871 (Rathbun 1905) and archival research may be required to determine the type locality more accurately. Secondly, the wider phylogeographic study and a more rigorous consideration of morphological variation across the species' range would provide greater insight into the taxonomic status of these and any other lineages.

While *P. isimangaliso* shows a clear resemblance to *P. lividus* (Peer *et al.* 2015), the lack of the distinctive colouration of the latter (Gouws & Stewart 2001; Gouws *et al.* 2001) aided the identification of *P. isimangaliso* specimens in the current

study. However, recent collections of *P. lividus* (Daniels *et al.* 2014) suggest that colour may be variable in this species (S.R. Daniels, pers. comm.).

Phylogenetically, the representative *P. isimangaliso* specimen was associated with *P. lividus* from South Africa (KZN), with the representative from Swaziland sister to these (or even placed outside of a clade formed by these and one of the *P. sidneyi* lineages). The phylogenetic placement of *P. isimangaliso* suggests that it may be conspecific to *P. lividus* from KZN. However, the *P. isimangaliso* specimen was substantially (~ 7.5%) and roughly equally divergent from each of the *P. lividus* representatives.

Recently, Daniels *et al.* (2014) reported the occurrence of *P. lividus* from Dweza Forest in the Eastern Cape province. Together with the specimens from Swaziland (see Daniels & Bayliss 2012), this represents a significant range extension for the species. However, unpublished sequence data revealed almost no differentiation among *P. lividus* from KZN and from the Eastern Cape (Daniels *et al.* 2014). This suggests a genetic cohesion within *P. lividus* over a range of some ~ 750 km (Daniels *et al.* 2014) and indicates that both *P. lividus* from Swaziland and *P. isimangaliso* represent unique lineages, both warranting recognition at species level.

The description by Peer *et al.* (2015) and support for *P. isimangaliso* as a valid taxon are significant because Gouws *et al.* (2001) highlighted a number of specimens in museum collections that resembled *P. lividus*. However, identifications of these specimens could not be confirmed at the time as the seemingly diagnostic colouration (see above) was not preserved. Repeated sampling from these localities, which were not typical swamp forests, was unsuccessful. The possibility that these morphotypes may correspond to *P. isimangaliso*, *P. lividus* or additional taxa within a possible complex require further investigation.

Conclusion

The present study highlights the unique diversity within the north-eastern region of KZN, in and around the iSimangaliso Wetland Park. A unique lineage of *P. sidneyi* is found here, which is separate to a lineage occurring to the south of the province and possibly an additional lineage occurring along the Drakensberg to the west (Gouws & Stewart 2001).

Whether this north-eastern lineage is restricted to this area or has a more extensive distribution throughout the range of *P. sidneyi* remains to be determined. Allozyme invariance among populations from this area and from further afield, including other provinces, indicates that this may be the case (Gouws *et al.* 2002). However, the conservative nature of these markers in potamonautid crabs (see Introduction) may obscure true lineage diversity.

Potamonautes isimangaliso is currently endemic to the iSimangaliso Wetland Park (Peer *et al.* 2015), but possibly occurs at other localities in the north-east (Gouws *et al.* 2001), and clearly represents a unique species within the *P. lividus* – *P. isimangaliso* complex. These findings suggest a somewhat unique potamonautid fauna within this region, adding to the conservation value of this ecologically-, culturally- and socio-economically important system. The iSimangaliso Wetland Park was proclaimed a United Nations Educational, Scientific and Cultural Organization World Heritage Site in 1999 for three outstanding universal values, one of which is biodiversity (Perissinotto *et al.* 2013). The findings of this study confirm that the park truly represents a hotspot of biological diversity.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

The study was jointly conceived, conceptualised and designed by N.P. (Nelson Mandela Metropolitan University), R.P. (Nelson Mandela Metropolitan University) and G.G. (South African Institute for Aquatic Biodiversity). Fieldwork and sampling were undertaken by N.P. and R.P. Data were generated by N.P. and G.G., and analysed by G.G. G.G. took the lead in writing the manuscript, with significant editorial input and direction from R.P. and N.P. The research was supported, financially and logistically, by grants made to R.P.

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Appendix 1

TABLE 1-A1: Sequence divergences among Southern African *Potamonautes* species and unique lineages found in KwaZulu-Natal. Divergences are uncorrected sequence divergences, calculated from the combined 16S ribosomal DNA and cytochrome *c* oxidase subunit I mitochondrial DNA sequence data.

***Potamonautes* species and unique lineages**	**Taxon**															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
(1) *P. barbarai*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(2) *P. barnardi*	0.035	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(3) *P. bayonianus*	0.059	0.049	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(4) *P. bellarussus*	0.147	0.141	0.132	-	-	-	-	-	-	-	-	-	-	-	-	-
(5) *P. brincki*	0.109	0.103	0.101	0.123	-	-	-	-	-	-	-	-	-	-	-	-
(6) *P. calcaratus* 1	0.201	0.203	0.195	0.175	0.187	-	-	-	-	-	-	-	-	-	-	-
(7) *P. calcaratus* 2	0.161	0.160	0.159	0.147	0.147	0.117	-	-	-	-	-	-	-	-	-	-
(8) *P. choloensis*	0.151	0.154	0.147	0.155	0.148	0.184	0.138	-	-	-	-	-	-	-	-	-
(9) *P. clarus*	0.122	0.116	0.108	0.117	0.096	0.194	0.158	0.152	-	-	-	-	-	-	-	-
(10) *P. dentatus*	0.091	0.075	0.088	0.140	0.108	0.201	0.173	0.149	0.119	-	-	-	-	-	-	-
(11) *P. depressus*	0.124	0.116	0.110	0.128	0.104	0.191	0.158	0.163	0.080	0.128	-	-	-	-	-	-
(12) NPP82 Network D	0.124	0.120	0.118	0.129	0.107	0.194	0.155	0.157	0.077	0.130	0.033	-	-	-	-	-
(13) NPP85 Network E	0.104	0.099	0.097	0.109	0.086	0.189	0.153	0.146	0.073	0.106	0.070	0.070	-	-	-	-
(14) *P. flavusjo*	0.119	0.113	0.109	0.132	0.118	0.192	0.165	0.161	0.133	0.088	0.142	0.137	0.129	-	-	-
(15) *P. granularis*	0.023	0.046	0.077	0.158	0.113	0.206	0.166	0.165	0.134	0.104	0.132	0.131	0.110	0.125	-	-
(16) *P. lirrangensis*	0.155	0.152	0.151	0.143	0.122	0.191	0.164	0.154	0.135	0.154	0.145	0.141	0.122	0.158	0.164	-
(17) *P. isimangaliso*	0.119	0.111	0.107	0.127	0.112	0.195	0.169	0.151	0.128	0.091	0.126	0.129	0.112	0.089	0.128	0.143
(18) *P. lividus* SA	0.133	0.122	0.126	0.150	0.128	0.215	0.176	0.183	0.137	0.110	0.137	0.145	0.131	0.116	0.142	0.165
(19) *P. lividus* Swaziland	0.097	0.100	0.094	0.129	0.110	0.187	0.159	0.146	0.122	0.088	0.131	0.130	0.102	0.087	0.109	0.150
(20) *P. mulanjeensis*	0.129	0.122	0.107	0.138	0.120	0.194	0.170	0.155	0.130	0.098	0.137	0.141	0.129	0.080	0.139	0.151
(21) *P. mutareensis*	0.110	0.112	0.112	0.144	0.114	0.195	0.166	0.162	0.130	0.094	0.132	0.125	0.123	0.092	0.121	0.157
(22) *P. namuliensis*	0.150	0.147	0.142	0.142	0.140	0.173	0.120	0.117	0.161	0.154	0.145	0.136	0.143	0.159	0.159	0.131
(23) *P. obesus*	0.174	0.175	0.175	0.166	0.169	0.202	0.163	0.150	0.168	0.164	0.186	0.186	0.173	0.189	0.178	0.158
(24) *P. odhneri*	0.150	0.141	0.144	0.134	0.139	0.207	0.176	0.165	0.125	0.147	0.133	0.133	0.130	0.152	0.155	0.143
(25) *P. parvicorpus*	0.111	0.108	0.102	0.119	0.074	0.190	0.156	0.157	0.092	0.115	0.099	0.094	0.076	0.123	0.116	0.130
(26) *P. parvispina*	0.115	0.113	0.100	0.131	0.087	0.190	0.157	0.155	0.109	0.111	0.114	0.113	0.084	0.136	0.121	0.130
(27) *P. perlatus*	0.048	0.035	0.053	0.149	0.105	0.200	0.161	0.152	0.127	0.082	0.121	0.128	0.101	0.109	0.061	0.158
(28) *P. platynotus*	0.166	0.165	0.158	0.152	0.138	0.213	0.175	0.167	0.138	0.168	0.157	0.149	0.143	0.175	0.176	0.118
(29) *P. raybouldi*	0.163	0.155	0.158	0.146	0.139	0.195	0.168	0.158	0.152	0.158	0.153	0.146	0.134	0.168	0.166	0.118
(30) *P. sidneyi*	0.063	0.054	0.058	0.140	0.111	0.201	0.165	0.141	0.123	0.081	0.126	0.130	0.114	0.103	0.083	0.161
(31) NPP65 Network A	0.065	0.058	0.056	0.138	0.108	0.203	0.172	0.146	0.118	0.083	0.115	0.119	0.113	0.101	0.084	0.158
(32) NPP70 Network B	0.057	0.055	0.055	0.139	0.105	0.201	0.172	0.140	0.124	0.083	0.122	0.124	0.114	0.104	0.079	0.157
(33) NPP47 Network C	0.059	0.055	0.052	0.134	0.105	0.194	0.162	0.154	0.115	0.082	0.121	0.121	0.104	0.103	0.079	0.149
(34) NPP13 Network A	0.105	0.102	0.101	0.126	0.116	0.195	0.166	0.156	0.118	0.087	0.126	0.124	0.110	0.097	0.116	0.146
(35) NPP15 Network A	0.104	0.101	0.100	0.127	0.114	0.191	0.162	0.152	0.117	0.087	0.122	0.120	0.109	0.094	0.117	0.144
(36) NPP21 Network A	0.105	0.102	0.101	0.126	0.116	0.192	0.164	0.152	0.117	0.083	0.121	0.121	0.110	0.098	0.118	0.145
(37) NPP2 Network F	0.107	0.106	0.108	0.130	0.120	0.202	0.170	0.158	0.123	0.091	0.133	0.126	0.116	0.099	0.117	0.152
(38) NPP20 Network G	0.106	0.104	0.104	0.127	0.117	0.192	0.166	0.156	0.124	0.090	0.132	0.129	0.116	0.097	0.117	0.150
(39) NPP5 Network H	0.103	0.100	0.105	0.134	0.111	0.202	0.166	0.162	0.127	0.084	0.126	0.127	0.114	0.100	0.113	0.149
(40) *P. subukia*	0.153	0.139	0.145	0.134	0.142	0.209	0.169	0.172	0.115	0.147	0.137	0.129	0.130	0.148	0.158	0.143
(41) *P. unispinus*	0.062	0.042	0.044	0.141	0.099	0.197	0.162	0.153	0.124	0.077	0.109	0.118	0.097	0.111	0.077	0.152
(42) *P. warren*	0.062	0.047	0.048	0.138	0.101	0.203	0.163	0.150	0.113	0.080	0.112	0.113	0.098	0.108	0.077	0.150

***Potamonautes* species and unique lineages**	**Taxon**															
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
(17) *P. isimangaliso*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(18) *P. lividus* SA	0.078	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(19) *P. lividus* Swaziland	0.074	0.097	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(20) *P. mulanjeensis*	0.098	0.120	0.096	-	-	-	-	-	-	-	-	-	-	-	-	-
(21) *P. mutareensis*	0.103	0.136	0.096	-	-	-	-	-	-	-	-	-	-	-	-	-
(22) *P. namuliensis*	0.154	0.174	0.156	0.095	-	-	-	-	-	-	-	-	-	-	-	-
(23) *P. obesus*	0.191	0.192	0.181	0.154	0.144	-	-	-	-	-	-	-	-	-	-	-
(24) *P. odhneri*	0.154	0.171	0.156	0.183	0.173	0.140	-	-	-	-	-	-	-	-	-	-
(25) *P. parvicorpus*	0.115	0.130	0.110	0.159	0.148	0.166	0.181	-	-	-	-	-	-	-	-	-
(26) *P. parvispina*	0.120	0.141	0.117	0.130	0.127	0.138	0.172	0.132	-	-	-	-	-	-	-	-
(27) *P. perlatus*	0.108	0.117	0.094	0.131	0.140	0.142	0.181	0.127	0.074	-	-	-	-	-	-	-
(28) *P. platynotus*	0.175	0.186	0.160	0.112	0.116	0.145	0.176	0.154	0.114	0.115	-	-	-	-	-	-
(29) *P. raybouldi*	0.157	0.187	0.163	0.172	0.168	0.164	0.178	0.159	0.138	0.145	0.177	-	-	-	-	-

Table 1-A1 continues on the next page →

TABLE 1-A1 (Continues...): Sequence divergences among Southern African *Potamonautes* species and unique lineages found in KwaZulu-Natal. Divergences are uncorrected sequence divergences, calculated from the combined 16S ribosomal DNA and cytochrome *c* oxidase subunit I mitochondrial DNA sequence data.

***Potamonautes* species and unique lineages**	**Taxon**															
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
(30) *P. sidneyi*	0.108	0.122	0.099	0.161	0.172	0.155	0.183	0.153	0.139	0.133	0.160	0.121	-	-	-	-
(31) NPP65 Network A	0.108	0.128	0.097	0.101	0.108	0.145	0.174	0.156	0.120	0.121	0.059	0.168	0.152	-	-	-
(32) NPP70 Network B	0.109	0.127	0.103	0.106	0.103	0.150	0.185	0.161	0.113	0.128	0.064	0.168	0.158	0.031	-	-

***Potamonautes* species and unique lineages**	**Taxon**									
	33	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
(33) NPP47 Network C	-	-	-	-	-	-	-	-	-	-
(34) NPP13 Network A	0.094	-	-	-	-	-	-	-	-	-
(35) NPP15 Network A	0.092	0.012	-	-	-	-	-	-	-	-
(36) NPP21 Network A	0.094	0.011	0.004	-	-	-	-	-	-	-
(37) NPP2 Network F	0.103	0.022	0.026	0.024	-	-	-	-	-	-
(38) NPP20 Network G	0.095	0.019	0.020	0.019	0.023	-	-	-	-	-
(39) NPP5 Network H	0.094	0.031	0.033	0.031	0.037	0.037	-	-	-	-
(40) *P. subukia*	0.149	0.139	0.139	0.140	0.146	0.144	0.156	-	-	-
(41) *P. unispinus*	0.048	0.093	0.092	0.093	0.102	0.094	0.094	0.148	-	-
(42) *P. warren*	0.057	0.098	0.097	0.098	0.105	0.101	0.098	0.143	0.024	-

P., Potamonautes