A NEW SPECIES OF FRESHWATER CRAB (DECAPODA, POTAMONAUTIDAE) FROM THE SWAMP FORESTS OF KWAZULU-NATAL, SOUTH AFRICA: BIOCHEMICAL AND MORPHOLOGICAL EVIDENCE

BY

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ABSTRACT

Potamonautid river crabs were collected from five localities in northeastern KwaZulu-Natal to determine biochemical and morphological differentiation among these populations. Two distinct forms were included in these collections. One of the forms could be identified as *Potamonautes sidneyi*, whereas the other was previously unknown. Biochemical analysis of 21 presumptive allozyme loci showed the two forms to be distinct, separating at a genetic identity value of 0.829. A fixed allele difference at the HEX locus in the sympatric populations of the two forms indicated that they were reproductively isolated. Morphometric analysis of seven carapace variables, by means of a discriminant function analysis, showed the two forms to be distinct. The unidentified form, which appears to be confined to patches of swamp forest in northeastern KwaZulu-Natal, is described in this paper as *Potamonautes lividus* sp. nov.

RÉSUMÉ

Des crabes de rivière du genre *Potamonautes* ont été récoltés dans cinq localités de la région nord-orientale du KwaZulu-Natal afin de déterminer les différences biochimiques et morphologiques entre les différentes populations. Deux formes distinctes étaient présentes dans ces récoltes. L'une de ces formes a pu être identifiée comme *Potamonautes sidneyi*, tandis que l'autre était inconnue jusqu'à présent. L'analyse biochimique de 21 loci enzymatiques a montré que les deux formes étaient distinctes, séparées par une valeur d'identité génétique de 0,829. Une différence allélique fixe au locus HEX dans les populations sympatriques des deux formes indique qu'ils sont isolés du point de vue reproductif. L'analyse morphométrique de sept variables de la carapace, au moyen d'une analyse discriminante, a montré que les deux formes étaient distinctes. La forme non-identifiée, qui apparaît confinée à des pans de forêt marécageuse au nord-est du KwaZulu-Natal, est décrite dans ce travail comme *Potamonautes lividus* sp. nov.

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INTRODUCTION

Freshwater crabs of the family Potamonautidae Bott, 1970 are common throughout the rivers of KwaZulu-Natal, South Africa. Of the three species documented from the province (Barnard, 1935, 1950), two, *Potamonautes sidneyi* Rathbun, 1904 and *Potamonautes dentatus* Stewart, Coke & Cook, 1995, are known to occur from the low-lying midlands to the coast.

The earliest records of *Potamonautes sidneyi* from the rivers of the province date back to 1910. Stebbing (1910) documented collections made at Port Natal, Lenz (1912) referred to collections made at Lake Sibayi and elsewhere in Zululand, and Barnard (1935) reported collections in the Transvaal Museum, made at Oliviershoek, in the northern Drakensberg. *Potamonautes dentatus*, however, has only recently been described from the Mgeni River, Inyamvubu River, and other tributaries of the Tugela River (Stewart et al., 1995).

Collections made near Empangeni and Richards Bay revealed specimens that could not be identified as any of the species known from the province of KwaZulu-Natal. The specimens were characterized by having a highly vaulted carapace; a medially reduced postfrontal crest; inflated, arched chelipeds; a downward projecting frontal lobe; rounded, downward sloping epibranchial corners; and a characteristic, silver-blue carapace sheen and bright orange to red chelipeds and pereopods. The specimens appear to exclusively inhabit small patches of swamp forest and associated herbaceous wetland, with characteristic swamp forest vegetation assemblages (Wessels, 1997) and spongy peat-soil, in which these crabs form shallow, U-shaped burrows, partly filled with water. The unique morphological characteristics and habitat requirements of this form raised the question whether it could be delineated as a species, separate to those previously known from the province.

This paper provides empirical morphological and biochemical evidence for a new species of potamonautid river crab from the swamp forests of northeastern KwaZulu-Natal, described as *Potamonautes lividus* sp. nov. Results are discussed against the broader evidence of variation and differentiation within the genus *Potamonautes* and the putative new species is compared to species known from South Africa and further afield.

MATERIALS AND METHODS

Collection. — Crabs were collected from five localities (fig. 1), using handnets baited with ox-heart. Individuals of the undescribed form (form A) were easily identifiable in the field due to the much inflated carapace, downward projecting

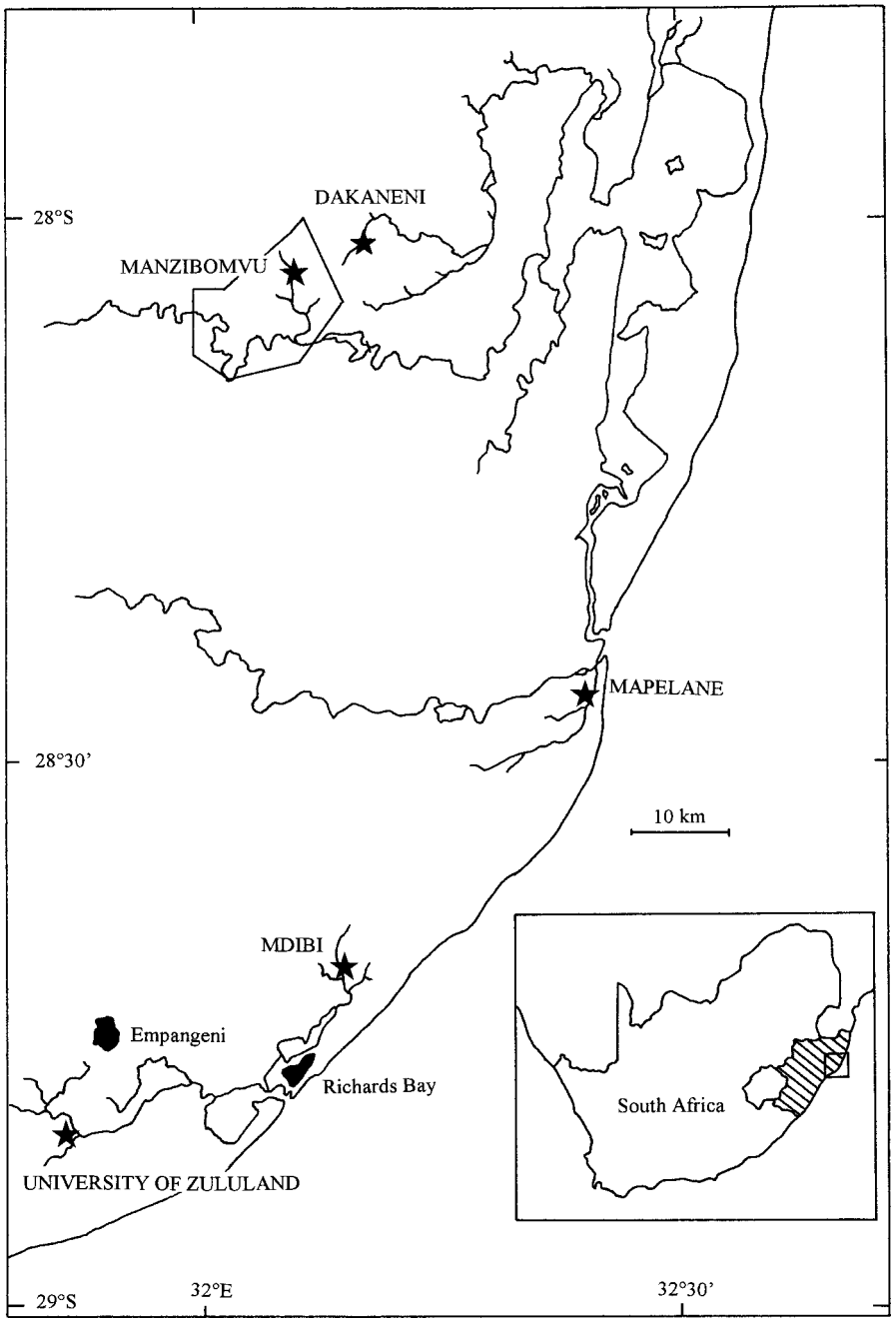

Fig. 1. Sampling localities in northeastern KwaZulu-Natal, South Africa.

frontal lobe and characteristic colouration. *Potamonautes sidneyi* (form B) individuals could be identified, following the descriptions and illustrations of Barnard (1950) and Bott (1955), by the presence of a sharp postfrontal crest, scabrosity of the epibranchial corners, and broad limbs. Individuals of the undescribed form (form A) were collected from a patch of swamp-forest, dominated by *Syzygium cordatum* Hochst. (common waterberry) trees and characterized by spongy, hydromorphic peat soil, at the University of Zululand campus; a similar forest along the Mdibi stream (an affluent of Lake Mzingazi), north of Richards Bay; and a *Ficus trichopoda* Bak. and *Barringtonia racemosa* (L.) Roxb. dominated swamp-forest near the Mapelane Nature Reserve. Populations of *P. sidneyi* were sampled from marshy grassland near Dakaneni village, outside Hluhluwe Game Reserve; and the upper reaches of the Manzibomvu River, within the Hluhluwe Game Reserve. *Potamonautes sidneyi* populations were also sampled from the University of Zululand campus and Mdibi swamp-forest, where they occur sympatrically with the undescribed form (form A). Crabs were killed by overnight freezing. Unfortunately, specimens of *P. sidneyi* from the Mdibi swamp-forest were unsuitable for genetic analysis, as specimens were processed days after capture and protein denaturation could not be avoided due to repeated freezing and thawing, during transportation.

Biochemical analysis. — Muscle and digestive-gland tissue (hepatopancreas) were removed from each specimen by dissection. Tissue samples were stored in cryogenic tubes at −80°C, until electrophoresis was ready to be performed. Tissue was homogenized in 0.01 M Tris buffer (pH 8), using a glass rod attached to a variable-speed, electric motor. The samples were centrifuged for five minutes at 12,000 rpm prior to use. Filter paper wicks (Whatmans #3) were dipped into the supernatant and inserted into the horizontal 13% starch gel (Sigma Chemicals Co., St. Louis, Missouri, U.S.A.).

Three electrophoretic buffer systems were used: (A) a discontinuous Tris-citrate-borate-lithium hydroxide buffer, with a gel buffer pH 8.7, and an electrode buffer pH 8.0 (Ridgeway, Sherburne & Lewis, 1970); (B) a continuous Tris-borate-EDTA buffer system, at pH 8.6 (Markert & Faulhaber, 1965); and (C) a continuous Tris-citrate buffer system, at pH 6.9 (Whitt, 1970). Gels were run inside a fridge (4°C) for 4 hours at 40 mA. These were then sliced horizontally into 3 or 4 slices and the sites of enzymatic activity histochemically stained in a 2% agar overlay (Shaw & Prasad, 1970).

Of 24 isozyme loci originally screened, 21 were reliably interpretable and included in the analyses (table I). A sample of *Potamonautes depressus* Krauss, 1843 from the Coleford Nature Reserve was included in each run as a reference population to provide continuity in scoring across all populations. For each locus, the most common allele of the Coleford population was assigned a value of 100

TABLE I

Enzymes and buffer systems used to determine biochemical variation in six populations of *Potamonautes* spp. studied

Enzyme	Abbreviation	E.C. Number	Buffer*	Tissue**	Loci
Arginine kinase	ARK	2.7.3.3.	A	m	1
Diaphorase	DIA	1.6.2.2.	A	h	1
Glucose-6-phosphate isomerase	GPI	5.3.1.9.	A	m	1
Hexokinase	HEX	2.7.1.1.	B	m	1
Isocitrate dehydrogenase	IDH	1.1.1.42.	C	m	2
Lactate dehydrogenase	LDH	1.1.1.27.	A	m	1
Malate dehydrogenase	MDH	1.1.1.37.	C	m	2
Malic enzyme	ME	1.1.1.40.	B	m	1
Mannose-phosphate isomerace	MPI	5.3.1.8.	B	m	1
Peptidase (glycyl-leucine as substrate)	GL	3.4.11.–	B	m	1
Peptidase (leucyl-glycyl-glycyl as substrate)	LGG	3.4.11.–	A	h	3
Peptidase (leucyl-tyrosine as substrate)	LT	3.4.11.–	A, B	m, h	2
Peptidase (phenylamine-proline as substrate)	PHP	3.4.11.–	B	h	1
Phosphoglucomutase	PGM	5.4.2.2.	B	m	2
Phosphoglusonate dehydrogenase	PGD	1.1.1.44.	C	m	1

* Buffer: (A) discontinuous Tris-citrate-borate-lithium hydroxide buffer system (Ridgeway et al., 1970); (B) continuous Tris-borate-EDTA buffer system (Markert & Faulhaber, 1965); and (C) continuous Tris-citrate buffer system (Whitt, 1970).

** Tissue: (m) muscle tissue and (h) hepatopancreas (digestive gland).

and all other alleles were scored relative to this value. Where more than one locus stained for a particular enzyme, the most anodally migrating one was labeled 1, and the others labeled sequentially.

All numerical analyses were performed using the BIOSYS-1 program (Swofford & Selander, 1981). Allele and genotype frequencies were calculated. Genotype frequencies were tested for deviation from frequencies expected under Hardy-Weinberg equilibrium by means of a χ^2-goodness-of-fit test. Where more than two alleles occurred at a locus, the genotype frequencies were pooled. Percentage polymorphic loci were calculated for each population, using no criterion (loci were considered polymorphic if more than one allele was present at a locus). The mean expected heterozygosity (H) was also calculated for each locus, using unbiased estimates (Nei, 1978). The mean unbiased genetic identity (I) and distance (D) were calculated for each pairwise comparison (Nei, 1978) and a dendrogram constructed using the measures of genetic identity and the UPGMA clustering algorithm (Sneath & Sokal, 1973).

Morphological analyses. — Prior to dissection, crabs were sexed and measured using digital Vernier-calipers and a portable computer. The populations used for the genetic analysis were supplemented with morphometric data from specimens

collected earlier at the above localities. Seven carapace variables were recorded for morphometric analyses. These were: carapace length along the medial line (CL); carapace width at the widest part (CWW); posterior carapace width (CWP); the distance between the postfrontal crest and the anterior carapace margin (PFCD); distance between the medial margins of the orbits (ED); distance between the exorbital teeth (CWA); and the carapace height (CH).

Data were log transformed (common logarithms) prior to analysis. All statistical analyses were performed using the Statistica Version 5.1 program (StatSoft Inc., 1996). Morphological variation between the two forms was determined by means of a stepwise discriminant function analysis, using seven carapace variables. Classification functions were calculated, using the jackknife procedure, ensuring an unbiased classification function for determining group assignment. Individuals were then reassigned to a group based on the highest a posteriori probability. A frequency histogram of individual canonical variable scores calculated from the discriminant function analysis was plotted to reveal discrimination between the two forms. Linear regression analyses were used to test for variation within specific variables between the two forms.

Gonopods and mouthparts were removed and examined, qualitatively, using a Leitz stereoscopic dissection microscope and illustrated using a camera lucida or photographed using a Wild MPS45 camera unit.

RESULTS

Biochemical analysis. — Of the 21 reliably scorable loci, nine (ARK, LT-1, ME, LDH, MPI, PGD, DIA, LGG-1, and LGG-3) were monomorphic across all populations analysed. Twelve loci were polymorphic (table II). The LT-2 locus was the only locus polymorphic in each population. The total number of alleles ranged from two in HEX, MDH-1, IDH-1, GL, PGM-2, PHP-2, and LGG-2, to five in GPI. Within populations the maximum number of alleles was four, found in GPI in the Manzibomvu population.

Out of thirty cases of polymorphism for all enzyme loci and populations, three (10%) were shown to deviate from genotype frequencies expected under Hardy-Weinberg equilibrium. The LT-2 locus in the University of Zululand A population ($\chi^2 = 9.468$, df $= 3$, $P = 0.024$), the PHP locus in the Mdibi population ($\chi^2 = 7.037$, df $= 1$, $P = 0.008$), and the PGM-2 locus in the Dakaneni population ($\chi^2 = 25.0$, df $= 1$, $P = 0.000$) were out of equilibrium due to a deficit of heterozygotes carrying the rare allele. All other loci conformed to Hardy-Weinberg expectations, thus supporting a purely genetic interpretation of the polymorphisms observed.

TABLE II

Distribution of allele frequencies at nine polymorphic loci in six populations of *Potamonautes* spp. studied. N = sample size; refer to table I for enzyme abbreviations

	Population					
Locus	Zululand A	Mdibi	Mapelane	Zululand B	Dakaneni	Manzibomvu
IDH-2						
N	41	24	25	13	25	13
120	0.012	0.729	0.380	0.000	0.000	0.000
80	0.988	0.229	0.000	1.000	1.000	1.000
45	0.000	0.043	0.620	0.000	0.000	0.000
GPI						
N	56	26	25	13	25	13
165	0.045	0.000	0.000	0.000	0.000	0.000
150	0.000	0.000	0.000	0.000	0.000	0.038
100	0.000	0.000	0.000	0.000	0.000	0.038
40	0.938	1.000	1.000	0.962	0.880	0.846
−80	0.018	0.000	0.000	0.038	0.120	0.077
HEX						
N	41	26	25	13	25	13
100	1.000	1.000	1.000	0.000	0.000	0.000
95	0.000	0.000	0.000	1.000	1.000	1.000
MDH-1						
N	41	26	25	13	25	13
100	0.988	1.000	1.000	1.000	1.000	1.000
90	0.012	0.000	0.000	0.000	0.000	0.000
MDH-2						
N	41	26	25	13	25	13
100	1.000	1.000	1.000	0.962	0.960	1.000
75	0.000	0.000	0.000	0.000	0.040	0.000
65	0.000	0.000	0.000	0.038	0.000	0.000
IDH-1						
N	41	26	25	13	23	7
150	0.000	0.000	0.000	1.000	0.891	0.929
135	1.000	1.000	1.000	0.000	0.109	0.071
LT-2						
N	41	26	25	13	25	13
250	0.049	0.019	0.020	0.000	0.000	0.000
100	0.854	0.885	0.840	0.923	0.980	0.769
0	0.098	0.096	0.140	0.077	0.020	0.231
PGM-1						
N	52	26	25	13	25	13
110	0.365	0.000	0.000	0.000	0.000	0.000
100	0.212	0.000	0.000	0.038	0.140	0.038
90	0.423	1.000	1.000	0.923	0.800	0.962
75	0.000	0.000	0.000	0.038	0.060	0.000

(continued overleaf)

TABLE II
(Continued)

Locus	Population					
	Zululand A	Mdibi	Mapelane	Zululand B	Dakaneni	Manzibomvu
GL						
N	41	26	25	13	25	13
90	1.000	0.962	1.000	1.000	1.000	1.000
75	0.000	0.038	0.000	0.000	0.000	0.000
PGM-2						
N	56	26	25	13	25	13
100	1.000	1.000	1.000	0.000	0.040	0.000
75	0.000	0.000	0.000	1.000	0.960	1.000
PHP						
N	41	26	25	13	25	13
105	1.000	0.808	1.000	1.000	1.000	1.000
90	0.000	0.192	0.000	0.000	0.000	0.000
LGG-2						
N	41	26	25	13	24	13
100	0.854	0.942	1.000	0.846	0.979	0.692
90	0.146	0.058	0.000	0.154	0.021	0.308

Genetic variability was low in all populations. The percentage polymorphic loci (no criterion) ranged from 9.5% to 33.3% in the Mapelane and Dakaneni populations, respectively. The mean unbiased expected heterozygosity varied from 0.034 (in the University of Zululand B population) to 0.063 (in the University of Zululand A and Manzibomvu populations). The mean number of alleles per locus ranged from 1.1 (in the Mapelane population) to 1.4 (in the Dakaneni and University of Zululand A populations).

The dendrogram constructed, based on the genetic identity values obtained by pairwise comparison of populations (table III), clearly distinguished two clusters (fig. 2). The group representing the form A populations clearly separated from the group representing the form B (*Potamonautes sidneyi*) populations, at a genetic identity value of $I = 0.829$ ($D = 0.187$). Three loci contributed significantly to this separation, one of them diagnostically. The HEX locus showed a fixed allele difference between the individuals of the two forms, with the form A individuals being fixed for the allele HEX^{100} and the form B (*P. sidneyi*) individuals fixed for the HEX^{95} allele. Strong heterogeneity was observed between the populations of the two forms at the IDH-1 and PGM-2 loci. With the exception of one individual in the Dakaneni population having a homozygote with the PGM-2^{100} allele, form A and form B were characterized by the fixation of alleles PGM-2^{100} and PGM-2^{75},

TABLE III

Coefficient matrix of Nei's (1978) unbiased genetic identity (above diagonal) and unbiased genetic distance (below diagonal) for pairwise comparisons of the six populations of *Potamonautes* studied

	Population					
	Zululand A	Mdibi	Mapelane	Zululand B	Dakaneni	Manzibomvu
Zululand A	*****	0.958	0.949	0.840	0.855	0.842
Mdibi	0.043	*****	0.986	0.821	0.832	0.822
Mapelane	0.052	0.014	*****	0.813	0.825	0.814
Zululand B	0.174	0.197	0.206	*****	0.998	0.999
Dakaneni	0.157	0.184	0.193	0.002	*****	0.994
Manzibomvu	0.172	0.196	0.206	0.001	0.006	*****

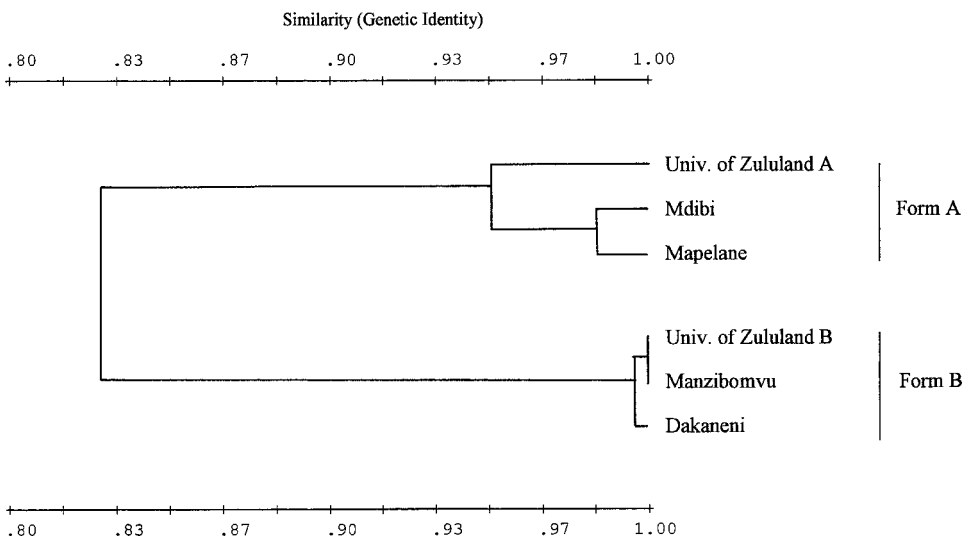

Fig. 2. UPGMA dendrogram constructed from the matrix of Nei's (1978) unbiased genetic identities (I), using allozyme data from 21 loci.

respectively. Identity-values for within form comparisons varied from 0.949 to 0.986 ($D = 0.014$ to 0.052); and 0.994 to 0.999 ($D = 0.001$ to 0.006) for form A and form B, respectively.

Morphometric analysis. — Individuals from the form A populations could easily be distinguished from the form B (*P. sidneyi*) individuals, by the possession of a much more vaulted carapace, inflated chelipeds, a postfrontal crest which diminishes medially; and the characteristic silver-blue carapace sheen and bright orange to red chelipeds and pereopods. Mature form B (*P. sidneyi*) individuals frequently attained a larger size class (with CL being representative of size) than did the mature form A individuals.

Three carapace variables (ED, CH, and CWP) contributed significantly to the discrimination between the two forms in the discriminant function analysis. The histogram plot of observed canonical variable scores (fig. 3) clearly separates the two forms, with the canonical variable means of the form A and form B individuals calculated at −0.996 and 1.965, respectively. The classification function (the linear function of variables which best discriminates the individuals of one group from the other specified groupings) was calculated for individuals of each form. The classification function of the form A group was:

$$Y = 306.704\ (\text{Log CWP}) - 26.103\ (\text{Log ED}) - 228.513\ (\text{Log CH}) - 64.156$$

and of the form B (*P. sidneyi*) group:

$$Y = 35.097\ (\text{Log ED}) - 315.543\ (\text{Log CH}) + 347.231\ (\text{Log CWP}) - 88.367$$

Using the classification functions, individuals were reassigned to groups based on highest a posteriori probabilities. Of the form A individuals 94.37% (134 of 142) were correctly classified with eight individuals being reassigned to the form B group. Of the form B individuals, 88.89% were correctly reassigned to the *P. sidneyi* group, with eight of the 72 individuals being reassigned to the form A group.

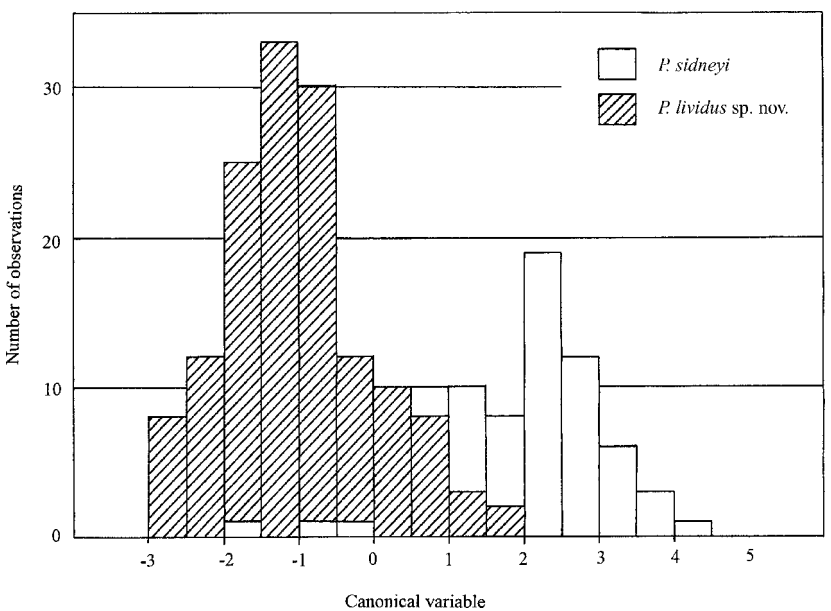

Fig. 3. Histogram of scores for specimens of *Potamonautes lividus* sp. nov. (form A) and *P. sidneyi* Rathbun, 1904 (form B), along the canonical variable calculated from a discriminant function analysis using seven carapace variables.

Comparison of regressions of the most discriminating variables between the two forms, regressed over CL, in turn, also showed significant differences between the two forms. CH regressed over CL showed highly significant differences between the two forms ($SS = 0.011$, $df = 1$, $F = 31.266$, $P < 0.001$); as did ED ($SS = 0.011$, $df = 1$, $F = 8.333$, $P < 0.005$) and CWP ($SS = 0.006$, $df = 1$, $F = 8.136$, $P < 0.005$). These represent the most discriminating variables and their regressions are represented graphically (fig. 4a, b, c). PFCD ($SS = 0.199$, $df = 1$, $F = 52.671$, $P < 0.001$), CWW ($SS = 0.003$, $df = 1$, $F = 30.963$, $P < 0.001$) and CWA ($SS = 0.001$, $df = 1$, $F = 6.182$, $P < 0.02$) also showed that there were significant differences between the two forms, but the discriminant function analysis showed them to be redundant in constructing the model.

Qualitative analysis. — Examination of gonopods and mouthparts revealed only slight differences in gonopod morphology. The terminal segment of gonopod 1 of the undescribed form was relatively short and triangular, whereas the terminal segment of gonopod 1 of *P. sidneyi* appears to be substantially longer and more slender. Although the terminal segment is bent away from the medial line in both forms, the terminal segment in *P. sidneyi* possesses a lobe or flange that extends inwards, giving the terminal segment an S-shaped appearance. The gonopods and mandibular palps of the two forms are illustrated in fig. 5.

DISCUSSION

The data presented above clearly indicate that the two forms of freshwater crabs from KwaZulu-Natal are distinct. The form A specimens are smaller, with a more vaulted carapace that is relatively narrower posteriorly, and that possesses a relatively narrower frontal lobe (ED) than that of the *Potamonautes sidneyi* individuals.

Not only are the two forms clearly distinct both biochemically and morphologically, but strong evidence suggests that they are reproductively isolated. The presence of a diagnostic locus, HEX, fixed for alternate alleles in the sympatric population of the two forms, indicates that a lack of gene flow and thus reproductive isolation exist between the two forms. This allows for species recognition under the Biological Species Concept (Mayr, 1970), under which species are defined as "groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups" (Mayr, 1963: 19) or "genetically isolated (in nature) from other such groups" (Bock, 1986: 33). In his model of allopatric speciation, Bock (1986) suggested that newly evolved species in sympatry would exert mutually selective demands on each other; by means of reproductive

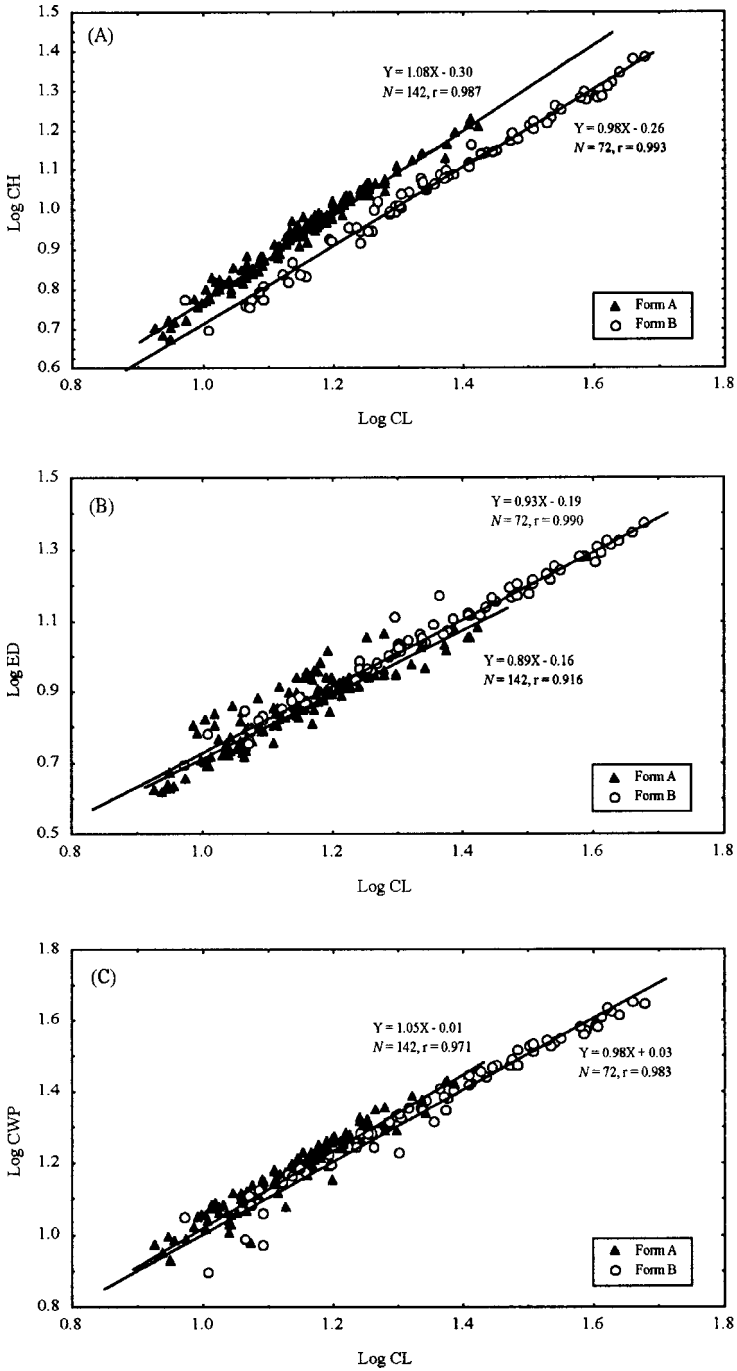

Fig. 4. Comparisons of the regressions of (A) Log CH (carapace height) over Log CL (carapace length), (B) Log ED (eye distance) over Log CL, and (C) Log CWP (posterior carapace width) over Log CL between form A (*Potamonautes lividus* sp. nov.) and form B (*P. sidneyi* Rathbun, 1904). All differences are significant (at the $P < 0.005$ level).

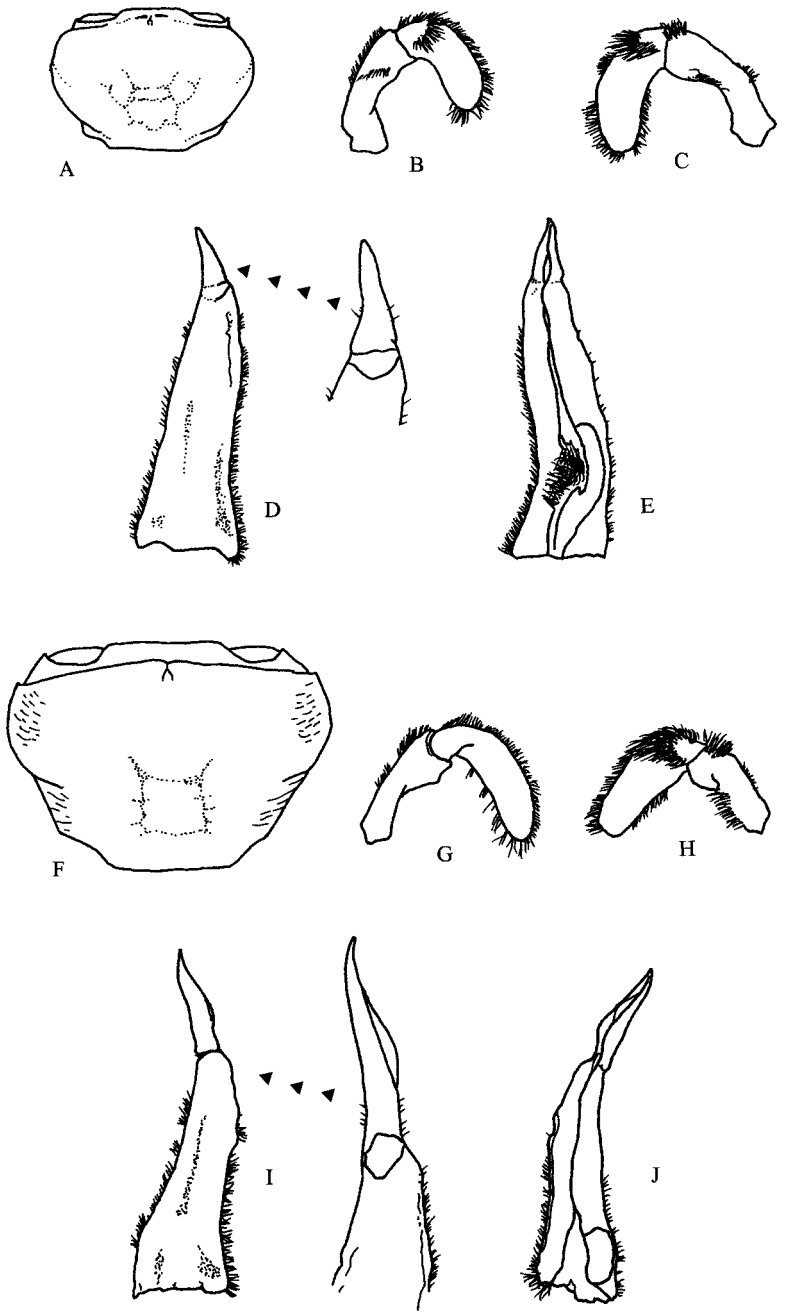

Fig. 5. A-E, form A (*Potamonautes lividus* sp. nov.; composite of male holotype, SAM A43540, and unaccessioned material): A, carapace outline; B, left mandibular palp, anterior view; C, left mandibular palp, posterior view; D, left gonopod 1, anterior view; and E, left gonopod 1, posterior view. F-J, form B (*Potamonautes sidneyi* Rathbun, 1904; unaccessioned specimen): F, carapace outline; G, left mandibular palp, anterior view; H, left mandibular palp, posterior view; I, left gonopod 1, anterior view; J, left gonopod 1, posterior view.

interference and ecological competition. This would lead to a divergence of morphological and behavioural aspects of feeding, reproduction, and other ecological features. The different gonopod morphologies of the two forms may reflect this.

The three form A populations clustered separately from the three *P. sidneyi* populations at a I-value of 0.829. Stewart (1997a) calculated the separation I-value between *P. parvispina* Stewart, 1997 and *P. perlatus* H. Milne Edwards, 1837 to be 0.68. An I-value of 0.66 separated both *P. perlatus* and *P. brincki* Bott, 1960 in the Western Cape (Stewart, 1997b), and *P. unispinus* Stewart & Cook, 1998 and *P. sidneyi* in Mpumalanga (Stewart & Cook, 1998). An I-value of 0.599 was obtained separating *P. clarus* Gouws, Stewart & Coke, 2000 and *P. depressus* (Gouws et al., 2000). The only species in the genus delineated using a higher I-value was *P. granularis* Daniels, Stewart & Gibbons, 1998, which was separated from *P. perlatus* in the Olifants River at a I-value of 0.88 (Daniels et al., 1998a, 1999). Although slightly higher than most interspecific values in the genus, the value obtained in this study nevertheless falls within the range (0.35 to 0.85) specified by Thorpe (1982) to indicate congeneric species differences. Allopatric populations showing I-values of less than 0.85 are unlikely to be conspecific (Thorpe & Solé-Cava, 1994). With the exception of two cases, all pairwise comparisons between allopatric populations of the two forms yielded I-values less than 0.85.

Identity-values were high for comparisons within both the form A (0.949 to 0.986) and *P. sidneyi* (0.994 to 0.999) groups. Intraspecific I-values are generally high in the genus *Potamonautes* and disjunct populations within a species tend to be genetically undifferentiated. Intraspecific I-values obtained in six studies (Stewart, 1997a, b; Stewart & Cook, 1998; Daniels et al., 1998b, 1999) involving six *Potamonautes* species varied from 0.902 to 1.000 ($D = 0.000$ to 0.103). An intraspecific I-value of 0.75 was obtained in a comparison of two groups of *P. brincki* populations across the Cape Peninsula and Cape Flats. However, this most probably indicates a species difference and requires further investigation (Stewart, 1997b). Given the high intraspecific I-values characteristic of the genus, and those obtained for comparisons within each form, the I-value obtained for the comparison between the two forms most likely represents a species difference.

Bock (1986) also defined a species as possessing a set of three distinct characteristics; genetic unity, reproductive unity, and ecological unity. Biochemical data provide support for compliance with the first two criteria for both forms. While no empirical evidence is as yet available confirming the third factor, a degree of macro- and microhabitat segregation, and thus ecological segregation, is evident between the two forms. *Potamonautes sidneyi* is widespread across the province (Stewart et al., 1995), while the other form appears to have a

more limited distribution in northeastern KwaZulu-Natal, restricted to patches of swamp forest with particular floral assemblages. The swamp forests are often characterized by *Barringtonia racemosa*, *Syzygium cordatum*, *Ficus trichopoda*, *Hibiscus tiliaceus* L., *Rauvolfia caffra* Sond., *Voacanga thouarsii* Roem. & Schult., *Raphia australis* Oberm. & Strey, and *Podocarpus falcatus* (Thunb.) R. Br. ex Mirb., with the fern, *Stenochlaena tenuifolia* (Desv.) T. Moore, often present in the understorey (Bruton, 1980; Moll, 1980). Within the swamp forests, the sympatric populations appear to show a degree of microhabitat segregation. Observations suggest that *P. sidneyi* has a closer association with water, as individuals were never collected great distances away from seepage pools and channels flowing through the forest. Conversely, individuals of the swamp-forest form were most often collected farther from water and their burrows were often among the vegetation. Observations suggest that this form is more terrestrial. Individuals were noticed to move to higher elevations when the water table rose through the hydromorphic peat soil, flooding their burrows and the surface, and they were also more active on cloudy, overcast days. Evidence of terrestrial adaptation is most likely to be found in the structure and histology of the organs and tissues involved in respiration: factors such as branchial chamber shape and volume, gill structure and formula, and the structure and vascularization of the branchiostegite lining, are known to reflect changes brought upon by the constraints of the terrestrial environment (Greenaway & Taylor, 1976; Díaz & Rodríguez, 1977; Taylor & Greenaway, 1979; Cumberlidge, 1986, 1991; Takeda et al., 1996). Although the availability of material prohibited the investigation of this aspect, future research may prove to be fruitful and highlight a greater distinction between the two forms.

Studies on terrestrial adaptation, supplemented by behavioural data that could illustrate microhabitat and ecological segregation, would quantify further the degree of differentiation between the two forms. However, two independent data sets, biochemical and morphological, present strong evidence for the specific status of the swamp-forest form.

TAXONOMY

Potamonautes lividus sp. nov. (figs. 6-7)

Material examined. — Holotype: South African Museum (SAM) A43540, one male, CL = 25.5 mm, *Ficus* and *Barringtonia* swamp forest, next to road, 8 km from rest camp, Mapelane Nature Reserve (28°27′11″S 32°24′18″E), coll. 27 January 1997 by B. A. Stewart, G. Gouws, and L. Hoenson. Allotype: SAM A43541, one female, CL = 24.5 mm, *Ficus* and *Barringtonia* swamp forest, next to road, 8 km from rest camp, Mapelane Nature Reserve (28°27′11″S 32°24′18″E), coll. 27 January 1997 by B. A. Stewart, G. Gouws, and L. Hoenson. Other material: SAM A43542, two males, *Ficus* and *Barringtonia* swamp forest, next to road, 8 km from rest camp, Mapelane Nature Reserve (28°27′11″S 32°24′18″E), coll. 27 January 1997 by B. A. Stewart, G. Gouws,

and L. Hoenson. SAM A43543, one female, Lake Nhlabane, north of Richards Bay (28°38′05″S 32°16′17″E), coll. 27 April 1993 by the University of Zululand Zoology Department. SAM A43544, three males, one female, Mdibi swamp forest, along Mdibi stream, 8 km north of Richards Bay (28°42′24″S 32°09′26″E), coll. 26 January 1997 by B. A. Stewart, P. A. Cook, M. Coke, P. E. Reavell, G. Gouws, and L. Hoenson. SAM A43545, one male, one female, *Syzygium* forest, University of Zululand campus (28°51′25″S 31°51′02″E), coll. 24 January 1997 by B. A. Stewart, P. A. Cook, P. E. Reavell, G. Gouws, and L. Hoenson. SAM A43546, two males, two females, University of Zululand, stream flowing through campus (28°55′S 31°41′E) coll. 10 September 1995 by P. E. Reavell. SAM A41313, one male, one female, swamp forest, Empangeni (28°51′25″S 31°51′02″E), coll. 2 August 1994 by M. Coke. SAM A41155, one male, University of Zululand, *Syzygium cordatum* swamp forest, holes in hydromorphic soil (28°45′S 31°45′E), coll. 10 December 1988 by P. E. Reavell. SAM A41153, three males, smaller holes in hydromorphic soil, *Syzygium cordatum* swamp forest, University of Zululand (28°45′S 31°45′E), coll. 20 March 1994 by P. E. Reavell. SAM A41156, one female, *Syzygium cordatum* swamp forest, University of Zululand (28°45′S 31°45′E), coll. 18 May 1992 by P. E. Reavell. SAM A41154, one male, one female, University of Zululand, *Syzygium cordatum* swamp forest, holes in hydromorphic soil (28°45′S 31°45′E), coll. 10 May 1990 by P. E. Reavell. SAM A43547, two males, one female, swamp forest, University of Zululand (28°45′S 31°45′E), coll. 1 April 1994 by P. E. Reavell. SAM A43548, one male, one female, *Syzygium* and *Bridelia* dominated swamp forest, University of Zululand (28°51′25″S 31°51′02″E), coll. 7 October 1996 by P. E. Reavell. SAM A43549, one female, Mdibi swamp forest, Lake Msingazi catchment area, north of Richards Bay (28°42′S 32°09′E), coll. 5 December 1996 by P. E. Reavell. Albany Museum — GEN910, one female, Mpolozi swamps, Mapelane (28°26′13″S 32°24′25″E), coll. 13 February 1991. Albany Museum — GEN965, two males, five females, N'Koninga Valley, Richards Bay (28°45′S 32°03′E), coll. 7 April 1988.

Other material. — The following specimens, included in the collections of the South African Museum, warrant further investigation as, although they bear some resemblance to the species described below, their identification is uncertain: SAM A41276, one male, one female, Mboneni dam, Mkuze Game Reserve (27°37′57″S 32°15′45″E), coll. 28 October 1994 by M. Coke; SAM A41299, two males, Nxwala stream, Mkuze Game Reserve (27°43′23″S 32°16′10″E), coll. 10 November 1994 by O. Bourquin; SAM A41277, one male, Rainpool, south of entrance, False Bay Park (28°00′01″S 32°21′21″E), coll. 26 October 1994 by M. Coke; SAM A41300, two males, Kangela rainpool, N2 near Mtubatuba (28°22′00″S 32°11′36″E), coll. 25 October 1994 by M. Coke; and SAM A41211, one male, Ntambanana (Ntrabanana), Zululand, coll. by H. H. Curson.

Type locality. — South Africa: KwaZulu-Natal, *Barringtonia* and *Ficus* swamp forest, next to road, 8 km from rest camp, Mapelane Nature Reserve (28°27′11″S 32°24′18″E).

Distribution. — At present known only from the swamp forests (characterized by *Barringtonia racemosa*, *Syzygium cordatum*, and *Ficus trichopoda* vegetation, and spongy, hydromorphic peat-soil) in the vicinity of Richards Bay, Empangeni, and the Mapelane Nature Reserve. It is probable that the distribution of the species may extend into low-lying areas to the east of the Drakensberg escarpment in the province of Mpumalanga and into Mozambique.

Etymology. — From the Latin "lividus", meaning "blue" or "light blue", referring to the characteristic silver-blue sheen of the carapace. The epitheton is an adjective agreeing in gender with the (masculine) generic name.

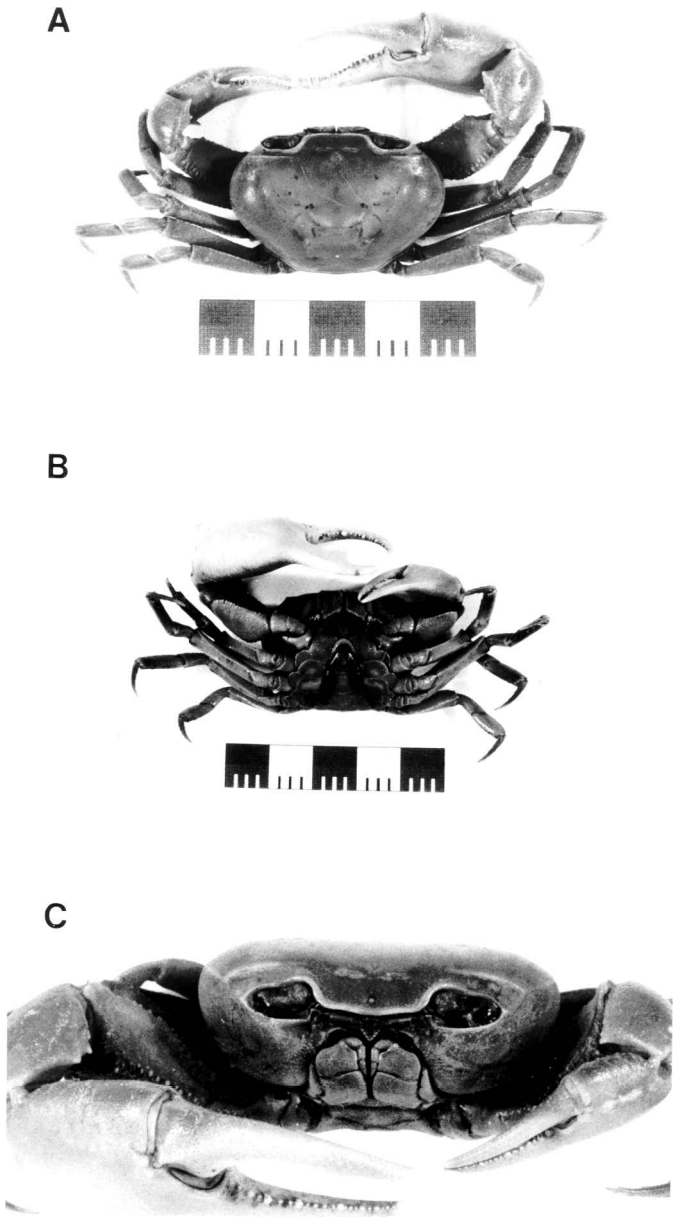

Fig. 6. *Potamonautes lividus* sp. nov., male holotype (CL = 25.5 mm). SAM A43540: A, whole animal, dorsal aspect; B, whole animal, ventral aspect; C, cephalothorax, frontal aspect.

Diagnosis. — Carapace distinctly vaulted and relatively narrow, posteriorly. Epibranchial teeth absent and anterolateral margin of the carapace smooth. Postfrontal crest indistinct medially, with frontal lobe projecting downwards. Carapace characteristically orange to red with silver-blue sheen. Chelipeds and limbs

bright orange to red, with lighter cheliped and dactyl tips. Chela inflated and highly arched.

Description of male holotype. — Size. Measurements of the holotype and the ranges of other specimens are given in table IV.

Carapace (figs. 6A, C, 7A). Carapace and limbs characteristically orange to red, with the carapace possessing a silver-blue sheen, when alive. Preserved specimens (70% ethanol) appear less orange, and the carapace retains a blue-grey colour. Cephalothorax ovoid, distinctly vaulted, branchial region highly convex (fig. 6A, C), maximum height and width at anterior third (ratio CH/CL = 0.65, CWW/CL = 1.45). Anterior margin straight. Urogastric grooves moderately well defined. Cardiac grooves shallow and poorly defined. Carapace otherwise smooth. Postfrontal crest smooth, curves forward medially. Postfrontal crest diminishes above inner orbit margins, though well defined immediately lateral to forked midpoint groove, by means of two indentations anterior to crest, indicating epigastric lobes. Exorbital teeth moderately sharp, but small. Epibranchial teeth absent. Anterolateral margin posterior to postfrontal crest smooth, margin curving in over carapace in branchial region, though indistinctly. Each flank has a longitudinal groove dividing the subhepatic from the pterygostomial region, with vertical groove running from longitudinal groove joining anterolateral margin between exorbital tooth and postfrontal crest.

Sternites (figs. 6B, 7B). Sternites 1 and 2 fused, no suture visible. First suture (sternal groove) between sternites 2 and 3 complete. Second sternal groove,

TABLE IV

Potamonautes lividus sp. nov.: wet weight (in g) and measurements (in mm) for the male holotype, and ranges for other individuals examined (maximum of 77 males and 70 females)

Variable	Abbreviation	Holotype	Males	Females
Wet weight	WW	24.2	0.5-21.4	0.7-18.3
Carapace length	CL	25.5	8.4-24.7	8.9-27.6
Carapace widest width	CWW	37.0	11.4-36.0	11.8-37.3
Carapace posterior width	CWP	28.1	9.0-26.9	8.6-30.6
Distance between postfrontal crest and anterior margin	PFCD	4.3	1.2-22.2	1.5-4.6
Distance between orbits	ED	11.4	4.2-11.9	4.3-12.3
Distance between exorbital teeth	CWA	25.9	9.6-25.4	9.6-26.2
Carapace height	CH	16.5	4.8-17.1	5.0-16.2
Width of sixth abdominal segment	AW6	7.5	2.8-7.4	3.1-24.0
Major cheliped propodus length	MCPL	42.7	7.1-40.2	7.6-29.1
Major cheliped propodus height	MCPH	19.8	2.8-18.9	2.8-14.2
Pereopod 2, merus length	P2ML	18.9	5.0-18.1	5.1-17.1
Pereopod 2, merus width	P2MW	5.8	1.9-5.6	1.8-5.9

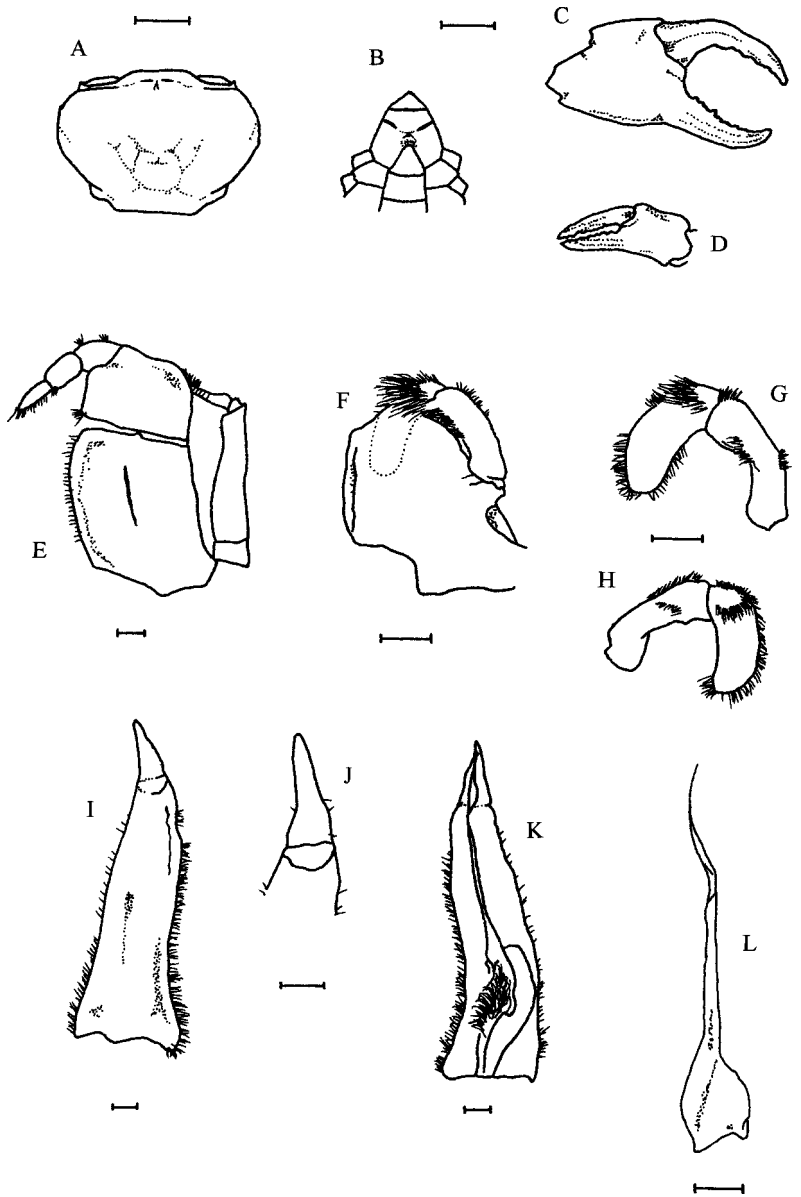

Fig. 7. *Potamonautes lividus* sp. nov., male holotype (CL = 25.5 mm). SAM A43540. A, carapace outline; B, thoracic sternum; C, right cheliped, dactylus and propodus; D, left cheliped, dactylus and propodus; E, left third maxilliped; F, left mandible and mandibular palp, posterior view; G, left mandibular palp, posterior view; H, left mandibular palp, anterior view; I, left gonopod 1, anterior view; J, terminal segment, left gonopod 1, anterior view; K, left gonopod 1, posterior view; L, left gonopod 2, anterior view. Scale lines represent 1 mm, except for A-D, where they represent 10 mm.

between sternites 3 and 4, complete laterally, becoming shallower medially, sloping sharply towards abdominopelvic region.

Third maxillipeds (figs. 6C, 7E). Filling entire buccal frame except for oval efferent respiratory openings; short flagellum present on exopods; ischium smooth, with moderately deep vertical groove.

Mandibular palp (fig. 7F, G, H). Mandibular palp with two segments, terminal segment undivided, with a dense tuft of setae on posterior surface of flange of terminal segment. Margins generally hirsute. Subterminal segment slightly enlarged distally.

Chelipeds and walking legs (figs. 6A, 7C, D). Chelipeds markedly unequal, dactylus of right cheliped strongly arched, 1.63 times length of dactylus of left cheliped. Both dactyli armed with up to 20 cutting teeth, three to four of which are slightly larger and more prominent. Propodus of right cheliped markedly inflated, 1.68 times longer and 2.0 times wider than that of left cheliped. Pollex possesses 20 cutting teeth, four to five of which are more prominent. Carpi of both chelipeds possess one prominent and three rudimentary teeth. Margins of meri granulose, small blunt spine on anterior surface, no spine on anterior-inferior margin. Pereopods generally slender (pereopod 2, merus length/width = 3.28, and pereopod 5, merus length/width = 2.71), P3 longest, P5 shortest, ventral margins of propodi and meri smooth, dorsal margin with fine sharp bristles, dactyli ending in sharp points, with margins bearing a series of sharp, spinelike bristles.

Pleon (figs. 6B, 7B). First five segments broad and short, last two segments longer, terminal segment rounded at distal end.

Pleopods (fig. 7I, J, K). Pleopod 1 (gonopod 1), terminal segment short, 0.25 times length of subterminal segment, terminal segment curves away from midline when viewed posteriorly, widest at base, ending in pointed tip. Subterminal segment of pleopod 1 tapers distally, inner lateral margin slightly irregular, outer lateral margin straighter, both margins hirsute. Posterior surface with twisting longitudinal groove, extending length of subterminal and terminal segment. Groove not visible on anterior surface. Posterior surface and margins of groove hirsute. Pleopod 2 (gonopod 2), terminal segment filament-like, 0.5 the length of subterminal segment, curving slightly towards medial line proximally, curves away from medial line distally (fig. 7L). Subterminal segment widest at base, tapering sharply inwards at 0.4 of length, forms narrow process supporting terminal segment.

Variation. — All individuals are characteristically dark orange to red, with a silver-blue sheen to the carapace, while the chelipeds vary in colour from bright orange-red to a lighter yellow-orange. All individuals are characterized by highly vaulted carapaces and downwardly projecting frontal lobes. The postfrontal crest, which is most pronounced laterally (from directly above the orbits to the anterolateral margin) and incomplete medially, is often more distinct in the smaller

individuals. Chelipeds are not always typically arched, enclosing a large ovoid space as in the holotype. Chelipeds of smaller individuals, although inflated, are less arched and often tight closing, with the small cutting teeth more obvious. Chelipeds of females and small males, in particular from the Mdibi and University of Zululand localities, have propodi that are relatively high, with inflated palms and relatively short, tightly closing chelae, which project slightly downwards. Heterochely has been demonstrated in most *Potamonautes*-species occurring in South Africa (Siegfried, 1972; Raubenheimer, 1986; Stewart et al., 1995; Stewart, 1997a, b; Stewart & Cook, 1998; Daniels et al., 1998a; Gouws et al., 2000). Within the species, 76% of the specimens were right-handed, with 11% being left-handed and 13% being of equal or subequal (differing by less than one millimeter) size. Of the males, 79% were right-handed, 8% left-handed and 13% equal or subequal. Females had 74% right-handedness, 13% left-handedness and in 13% of cases chelipeds were equal or subequal. The abdomen of mature females completely overlaps the coxae of the pereopods. The smallest mature female had a CL of 16.7 mm, with the largest immature female having a CL of 15.9, indicating that the pubertal moult occurs approximately between the 14 mm and 18 mm size classes.

Remarks. — *Potamonautes lividus* sp. nov. can readily be distinguished from the ten other species in the genus *Potamonautes* known to occur within South Africa. *Potamonautes warreni* Calman, 1918, *P. dentatus*, *P. parvispina*, and *P. unispinus* all bear a singular spine or a series of teeth on the anterolateral margins, whereas *P. lividus* sp. nov. is smooth. *Potamonautes perlatus*, *P. sidneyi*, and *P. granularis* are all larger crabs, with inflated, arched carapaces with complete, distinct postfrontal crests and scabrosity or granulation of the anterior margins, orbits, epibranchial corners or postfrontal crests. *Potamonautes lividus* sp. nov. has smooth carapace margins, and a incomplete postfrontal crest. The terminal segment of gonopod 1 in *P. lividus* sp. nov. is relatively short and triangular, and curves away from the medial line when viewed posteriorly, while that of *P. sidneyi* is relatively longer, more slender, and more S-shaped. The postfrontal crest of *P. sidneyi* may join the anterolateral margin at a relatively sharp angle and form a pronounced epibranchial corner along the anterolateral margin. The anterolateral margin in *P. lividus* sp. nov. is more rounded and the epibranchial corners slope down towards the flanks. *Potamonautes granularis* is chocolate brown with orange tips to the chelipeds. *Potamonautes brincki* is a smaller crab, with a smoother carapace, though also chocolate to reddish-brown in colour, with a finely granulated anterolateral margin, which does not curve in over the carapace in the branchial region (Stewart, 1997b). *Potamonautes depressus* and *P. clarus* both have rounded epibranchial corners and smooth carapaces, though these are relatively shallow and have flattened dorsal surfaces. Both are characterized by long, slender limbs. *Potamonautes depressus* is brown to green-brown in colour, while *P. clarus* is bright orange in colour.

A number of species from further afield bear a superficial resemblance to *P. lividus* sp. nov., particularly with regard to carapace morphology, though in most cases they can be readily distinguished. *Potamonautes pilosus* Hilgendorf, 1898 is known from East Africa and is a small crab, with a CWW of less than 25 mm. Specimens possess a weak postfrontal crest, which, along with the anterolateral margin, can be granulated (Hilgendorf, 1898; Bott, 1955). *Potamonautes neumanni* Hilgendorf, 1898 is known from East Africa, and has a characteristically vaulted carapace, without a complete, distinct postfrontal crest (Hilgendorf, 1898; Bott, 1955). The postfrontal crest is more distinct in *P. lividus*, particularly laterally, and the exorbital teeth are sharper and more defined in *P. lividus* than in *P. neumanni.* The terminal segment of gonopod 1 is longer and more slender in *P. neumanni* than in *P. lividus* and curves distinctly outwards. The subterminal segment of gonopod 1 is wider at its apex in *P. neumanni* than in *P. lividus*. Additionally, the carapace of preserved specimens of *P. neumanni* is typically grey in colour, while living specimens are violet in colour, with bright olive-green limbs and chelipeds (Hilgendorf, 1898). *Potamonautes ballayi acristatus* Bott, 1955 has an incomplete postfrontal crest, only present above the orbits, and small and blunt exorbital teeth. The major chela, although highly arched, and the minor chela are long and more slender than those of *P. lividus* sp. nov. The terminal segment of gonopod 1 of *P. granviki* Colosi, 1924 is curved towards the medial line when viewed posteriorly (Bott, 1955). The chelae of both *P. berardi berardi* Audouin, 1826 and *P. b. ignestii* Parisi, 1923 are relatively short and high, with stout, downward-projecting fingers. The anterior margin of the frontal lobe of *P. b. berardi* shows a distinct invagination and the anterolateral margin appears sharp and less rounded than in *P. lividus* sp. nov., while the postfrontal crest of *P. b. ignestii* is complete and has a fairly long medial groove. *Potamonautes emini emini* Hilgendorf, 1892 are generally small crabs, with a less inflated carapace and short, stout chelipeds. The subterminal and terminal segments of gonopod 1 are of more or less equal size (Bott, 1955). *Potamonautes e. semilunaris* Bott, 1955 and *P. luashiensis* Bott, 1955 are also small crabs (CWW < 20 mm) with the postfrontal crest only visible laterally and, in the case of *P. luashiensis*, the anterolateral margin and postfrontal crest may show granulation. *Potamonautes didieri* Rathbun, 1904 possesses a more complete, rounded postfrontal crest and shorter, more stout chelipeds (Bott, 1955).

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REFERENCES

BARNARD, K. H., 1935. Scientific results of the Vernay-Lang Kalahari expedition, March to September, 1930: Crustacea. Ann. Transvaal Mus., **16**: 481-492.

— —, 1950. Descriptive catalogue of South African decapod Crustacea (crabs and shrimps). Ann. South African Mus., **38**: 1-837.

BOCK, W. J., 1986. Species concepts, speciation, and macroevolution. In: K. IWATSUKI, P. H. RAVEN & W. J. BOCK (eds.), Modern aspects of species: 31-57. (University of Tokyo Press, Tokyo).

BOTT, R., 1955. Die Süsswasserkrabben von Afrika (Crust., Decap.) und ihre Stammesgeschichte. Ann. Mus. R. Congo Belge, (C, Zool.), **1** (3,3): 209-352, pls. 1-30.

BRUTON, M. N., 1980. An outline of the ecology of the Mgobezeleni lake system at Sodwana, with emphasis on the mangrove community. In: M. N. BRUTON & K. H. COOPER (eds.), Studies on the ecology of Maputaland: 408-426. (Rhodes University, Grahamstown).

CUMBERLIDGE, N., 1986. Ventilation of the branchial chambers in the amphibious West African freshwater crab, *Sudanonautes* (*Convexonautes*) *aubryi monodi* (Balss, 1929) (Brachyura, Potamonautidae). Hydrobiol., **134**: 53-65.

— —, 1991. The respiratory system of *Globonautes macropus* (Rathbun, 1898), a terrestrial freshwater crab from Liberia (Gecarcinucoidea, Gecarcinucidae). Crustaceana, **61** (1): 69-80.

DANIELS, S. R., B. A. STEWART & M. J. GIBBONS, 1998a. *Potamonautes granularis* sp. nov. (Brachyura, Potamonautidae), a new cryptic species of river crab from the Olifants River system, South Africa. Crustaceana, **71** (8): 885-903.

— —, — — & — —, 1998b. Genetic and morphometric variation in the potamonautid river crab *Potamonautes parvispina* (Decapoda: Potamonautidae) from two Western Cape rivers, South Africa. Journ. nat. Hist., London, **32**: 1245-1258.

— —, — — & — —, 1999. Genetic structure among populations of *Potamonautes perlatus* (Decapoda: Potamonautidae) from the Olifants River system in the Western Cape, South Africa. Journ. Zool., London, **249** (2): 137-142.

DÍAZ, H. & G. RODRÍGUEZ, 1977. The branchial chamber in terrestrial crabs: a comparative study. Biol. Bull., Woods Hole, **153**: 485-504.

GOUWS, G., B. A. STEWART & M. COKE, 2000. Evidence for a new species of river crab (Decapoda, Brachyura, Potamonautidae) from the Drakensberg, South Africa. Journ. Crust. Biol., **20** (4): 743-758.

GREENAWAY, P. & H. H. TAYLOR, 1976. Aerial gas exchange in Australian arid-zone crab, *Parathelphusa transversa* Von Martens. Nature, London, **262**: 711-713.

HILGENDORF, F., 1898. Die Land- und Süsswasser-Dekapoden Ostafrikas. Die Thierwelt Deutsch Ost-Afrikas, **4** (7): 1-37.

LENZ, H., 1912. Afrikanische Crustaceen aus schwedischen Sammlungen. Ark. Zool., **7** (29): 1-10.

MARKERT, C. L. & I. FAULHABER, 1965. Lactate dehydrogenase isozyme patterns of fish. Journ. exp. Zool., **159**: 319-332.

MAYR, E., 1963. Animal species and evolution: i-xiv, 1-797. (Harvard University Press, Cambridge, Massachusetts).

— —, 1970. Populations, species and evolution: i-xv, 1-453. (Harvard University Press, Cambridge, Massachusetts).

MOLL, E. J., 1980. Terrestrial plant ecology. In: M. N. BRUTON & K. H. COOPER (eds.), Studies on the ecology of Maputaland: 52-68. (Rhodes University, Grahamstown).

NEI, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583-590.

RAUBENHEIMER, C. D., 1986. Aspects of the biology of the freshwater crab, *Potamonautes sidneyi*, with particular reference to general seasonality and female aggression: i-xii, 1-106. (M.Sc. Thesis, Department of Biological Sciences, University of Natal, Durban).

RIDGEWAY, G. J., S. W. SHERBURNE & R. D. LEWIS, 1970. Polymorphism in the esterase of Atlantic herring. Trans. American Fish. Soc., **99**: 147-151.

SHAW, C. R. & R. B. PRASAD, 1970. Starch gel electrophoresis of enzymes — a compilation of recipes. Biochem. Genet., **4**: 297-320.

SIEGFRIED, W. R., 1972. Handedness in the Cape River crab, *Potamonautes perlatus* (M. Edw.). South African Journ. Science, **68**: 103-104.

SNEATH, P. H. A. & R. R. SOKAL, 1973. Numerical taxonomy: 1-573. (Freeman, London).

STATSOFT, Inc., 1996. STATISTICA for Windows. (Tulsa, Oklahoma, U.S.A.).

STEBBING, T. R. R., 1910. General catalogue of South African Crustacea. Ann. South African. Mus., **6**: 281-593.

STEWART, B. A., 1997a. Biochemical and morphological evidence for a new species of river crab *Potamonautes parvispina* sp. nov. (Brachyura, Potamonautidae). Crustaceana, **70** (6): 737-753.

— —, 1997b. Morphological and genetic differentiation between populations of river crabs (Decapoda: Potamonautidae) from the Western Cape, South Africa, with a taxonomic re-examination of *Gecarcinautes brincki*. Zool. Journ. Linnean Soc., London, **119**: 1-21.

STEWART, B. A., M. COKE & P. A. COOK, 1995. *Potamonautes dentatus*, new species, a freshwater crab (Brachyura: Potamoidea: Potamonautidae) from KwaZulu-Natal, South Africa. Journ. Crust. Biol., **15** (3): 558-568.

STEWART, B. A. & P. A. COOK, 1998. Identification of a new species of river crab (Decapoda: Brachyura: Potamonautidae) from South Africa using morphological and genetic data. Journ. Crust. Biol., **18** (3): 556-571.

SWOFFORD, D. L. & R. B. SELANDER, 1981. BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. (David L. Swofford, Illinois Natural History Survey).

TAKEDA, S., M. MATSUMASA, S. KIKUCHI, S. POOVACHIRANON & M. MURAI, 1996. Variation in the branchial formula of semiterrestrial crabs (Decapoda: Brachyura: Grapsidae and Ocypodidae) in relation to physiological adaptations to the environment. Journ. Crust. Biol., **16** (3): 472-486.

TAYLOR, H. H. & P. GREENAWAY, 1979. The structure of the gills and lungs of the arid zone crab, *Holthuisana* (*Austrothelphusa*) *transversa* (Brachyura: Sundathelphusidae) including observations on arterial vessels within the gills. Journ. Zool., London, **189**: 359-384.

THORPE, J. P., 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Ann. Rev. Ecol. Syst., **13**: 139-168.

THORPE, J. P. & A. M. SOLÉ-CAVA, 1994. The use of allozyme electrophoresis in invertebrate systematics. Zool. Scr., **23**: 3-18.

WESSELS, N. G., 1997. Aspects of the ecology and conservation of swamp forests in South Africa: 1-155. (M.Sc. (Technology) Thesis, Port Elizabeth Technikon, Port Elizabeth, South Africa).

WHITT, G. S., 1970. Developmental genetics of the lactate dehydrogenase isozymes of fish. Journ. exp. Zool., **175**: 1-35.

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