JOURNAL OF CRUSTACEAN BIOLOGY, 20(4): 743–758, 2000

EVIDENCE FOR A NEW SPECIES OF RIVER CRAB (DECAPODA, BRACHYURA, POTAMONAUTIDAE) FROM THE DRAKENSBERG, SOUTH AFRICA

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A B S T R A C T

Investigations into morphological and genetic differentiation of the potamonautid fauna of southern Africa revealed the presence of two morphotypes collected from the Drakensberg, KwaZulu-Natal. Both forms have been regarded as *Potamonautes depressus* in museum collections. The taxonomic status of the two forms was investigated genetically and morphometrically using samples from five localities. The two morphotypes could be distinguished on the basis of colour, with the northern morphotype being bright orange in colour, slightly smaller, and with a more thickly set carapace than the brown to green-brown southern morphotype. Allozyme electrophoresis utilizing 21 presumptive loci showed the two morphotypes to separate at a genetic identity value of 0.599. Five loci proved to be diagnostic between the two forms, with strong heterogeneity present at a further three loci. Discriminant functions analysis of seven carapace variables showed the two morphotypes to be morphometrically distinct. The southern morphotype was identified as *Potamonautes depressus* s. str., and it was concluded that the northern morphotype represented an undescribed species, described here as *Potamonautes clarus* sp. nov. The new species is compared to known *Potamonautes* species from South Africa.

Freshwater crabs of the family Potamonautidae Bott, 1970, are widespread throughout the rivers of KwaZulu-Natal, South Africa (Stewart *et al.*, 1995). *Potamonautes sidneyi* Rathbun, 1904, is common in the rivers and streams of the low-lying Midlands region of KwaZulu-Natal (Barnard, 1950). *Potamonautes dentatus* Stewart, Coke, and Cook, 1995, is confined to the Mgeni and Inyamvubu rivers and tributaries of the Tugela river. *Potamonautes depressus* (Krauss, 1843) is found in the faster-flowing streams of the KwaZulu-Natal Midlands and the foothills of the Drakensberg (Stewart *et al.*, 1995). This latter species was described by Krauss (1843) as *Telphusa depressa* from a collection made in the Boschmannsrand area near Pietermaritzburg. Further specimens were documented from Weenen (Lenz-Lübeck, 1912) and the Wakkerstroom region (Barnard, 1935). Individuals of *P. depressus* are distinguished from *P. dentatus* by the lack of teeth on the anterolateral margins of the carapace; and from *P. sidneyi* by their relatively flat carapace, long slender limbs (merus 3–3.5 times longer than wide), the lack of granulated fields on the epibranchial region of the carapace, and the absence of a minute epibranchial tooth. A specimen from Durban, originally described as *Potamonautes inflatus* H. Milne Edwards, 1853, was thought to be a variation of *P. perlatus* H. Milne Edwards, 1837, by Barnard (1950). The species *P. inflatus* was later synonymised with *P. depressus* by Bott (1955). This latter identification has been confirmed by examination of the type material in the present study.

Bott (1955) included two subspecies under the species *Potamonautes depressus*: *P. depressus depressus* and *P. depressus dybowskii* Rathbun, 1905. The two subspecies are distinguished by the nature of the postfrontal crest, with the crest curving slightly forward near the anterolateral carapace margin in *P. depressus depressus*, and the index of the chelipeds, being narrower near its base in *P. depressus depressus* than in *P. depressus dybowskii* (see Bott, 1955). The species occurring in KwaZulu-Natal, as well as the *P. inflatus* individual, were identified as the nominal subspecies by Bott (1955).

The present investigation of genetic and morphological differentiation in the genus *Potamonautes* in KwaZulu-Natal forms part

of a larger systematic study of the potamonautid river crabs of southern Africa based on extensive field collections and the examination of existing museum and private collections. During this study two morphotypes of freshwater crabs in the Drakensberg area of KwaZulu-Natal have been identified, both of which were regarded as specimens of *Potamonautes depressus* in museum collections. Specimens of freshwater crabs collected by H. Lang in November 1930 at Tugela Gorge, below Mont-Aux-Sources, Basutoland (now Lesotho), and at Oliviershoek, near Van Reenen's Pass, in the Transvaal Museum collections were identified by Barnard (1935) as *P. depressus*. These crabs are generally smaller, with a slightly more inflated, thickly-set carapace than individuals collected in the more southern regions of the Drakensberg. The junction of the postfrontal crest with the anterolateral carapace margin forms a slight corner, which is less rounded than in the southern form. The two forms can also be distinguished on the basis of colour, with the northern form being characteristically bright orange and the southern form being brown to green-brown.

This paper investigates the genetic and morphological differentiation between these two morphotypes, an approach previously proven useful to delineate different species of potamonautid crabs from southern Africa (Stewart, 1997a, b; Stewart and Cook, 1998; Daniels *et al.*, 1999). Data is presented for the recognition of the two forms from the Drakensberg as separate species.

Materials and Methods

Collection.—Crabs were collected from boulder-strewn streams using hand-nets baited with ox-heart and were killed by overnight freezing. The five sampling localities (Fig. 1) included a stream along the Oliviershoek Pass; the Mahai stream in the Royal Natal National Park, below Mont-Aux-Sources; the headwaters of the Tugela river, near the Tendele Rest Camp, Royal Natal National Park; the Kamberg Nature Reserve; and the Coleford Nature Reserve.

Crabs belonging to the northern morphotype, characterized by a smaller body size and a slightly more inflated, more thickly-set, orange-coloured carapace, were found in museum collections from Oliviershoek and Mont-Aux-Sources and in new collections made at Oliviershoek, Mahai, and Tendele. The Kamberg and Coleford populations represent individuals of the southern morphotype characterised by a larger body size, with a flat carapace with rounded epibranchial corners and a typically greenish-brown to brown colour. These individuals were identified here as *P. depressus*, based on the species description by Krauss (1843) and on the descriptions and illustrations of Barnard (1935, 1950) and Bott (1955).

Genetic Analysis.—Genetic analysis was performed using allozyme electrophoresis. Muscle and digestive gland tissue was removed by dissection and stored in cryogenic tubes at –80°C until needed. Samples were homogenized in a 0.01 M Tris (pH 8) buffer, using a glass rod attached to a variable-speed electric motor. Prior to use, samples were centrifuged at 12,000 rpm for five min. Filter paper wicks (Whatmans #3) were inserted into the supernatant and inserted into the origin cut in the 13% hydrolysed starch gel (Sigma Chemicals Co., St. Louis, Missouri, U.S.A.). Three electrophoretic buffer systems were used in running the gels: (A) a discontinuous tris-citrate-borate-lithium hydroxide, gel buffer pH 8.7, electrode buffer pH 8.0 (Ridgeway *et al.*, 1970); (B) a continuous tris-borate-EDTA buffer system, pH 8.6 (Markert and Faulhaber, 1965); and (C) a continuous tris-citrate buffer system, pH 6.9 (Whitt, 1970). Gels were run at 40 mA inside a refrigerator (4°C). After 4–5 h, gels were removed, cut horizontally into 3 or 4 slices, and the sites of enzymatic activity were stained using a histochemical solution in a 2% agar overlay, according to the methods outlined by Shaw and Prasad (1970). For each locus, the most common allele in the Coleford population was assigned a value of 100, and the mobility of other alleles scored relative to this value. When more than 1 locus occurred, the most anodally migrating one was numbered 1, and the others were numbered sequentially. Twenty-one isozyme loci provided reliably interpretable banding patterns and were included in the analysis (Table 1).

Numerical analyses of the genetic data were performed using the BIOSYS-1 (Swofford and Selander, 1981) program. Allele and genotype frequencies were calculated. Observed genotype frequencies were compared with genotype frequencies expected under Hardy-Weinberg equilibrium, using a χ^2 goodness-of-fit test. The genotype frequencies of all heterozygous combinations were pooled if more than 2 alleles occurred at a locus. Percentage polymorphic loci were calculated for each population using no criterion (a locus was considered polymorphic if more than 1 allele was present at that specific locus). Mean expected heterozygosity (H) was calculated for each population according to Nei's (1978) unbiased estimates. Nei's (1978) unbiased genetic identity (I) and unbiased genetic distance (D) were calculated for each pairwise comparison of populations. The unbiased genetic identities (I) were then used to construct a dendrogram of genetic similarity using the UPGMA algorithm (Sneath and Sokal, 1973).

Morphometric Analyses.—Prior to dissection, crabs were sexed, weighed, and measured. Fifteen carapace and limb dimensions were measured using digital Vernier calipers, and the data were logged into a portable computer. All morphometric analyses were performed using log-transformed data (common logarithms) and StatSoft's Statistica (1996) program. Specimens of the northern morphotype included in the Transvaal Museum's collections were used in the morphometric analyses, effectively providing 71 individuals of the northern morphotype and 87 *P. depressus* individuals.

The carapace dimensions used in the morphological analyses included CL (the carapace length along the medial line), CWW (the carapace width at its widest point), CWP (the carapace width posteriorly), PFCD (the distance between the anterior margin and the postfrontal

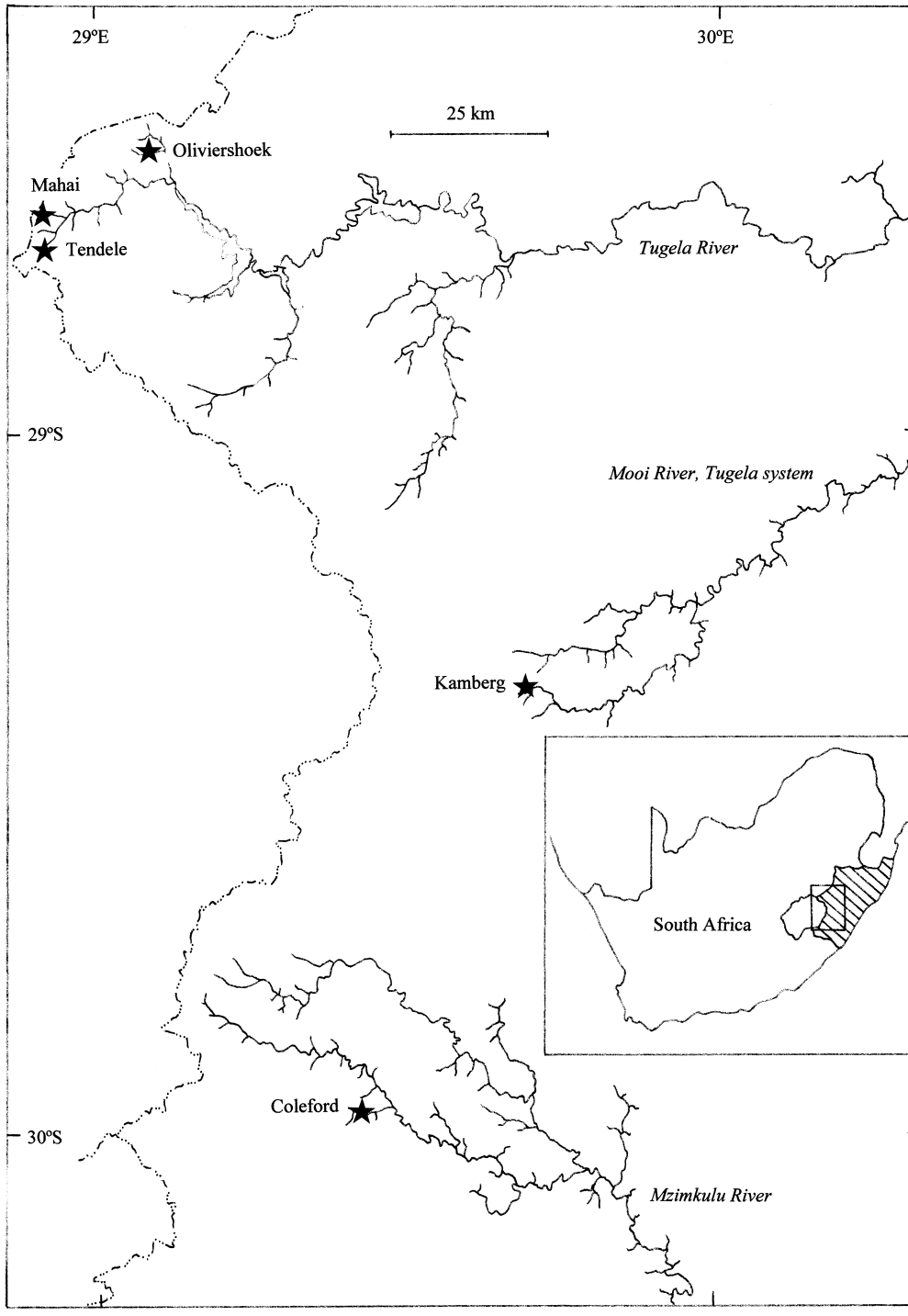


Fig. 1. Collection localities of the two *Potamonautes* morphotypes in KwaZulu-Natal, South Africa.

crest, taken medially), ED (the distance between the orbits), CWA (the distance between the exorbital teeth), and CH (the carapace height). Pereiopod dimensions included the width (P2PW) and the length (P2PL) of the propodus of the second pereiopod, the width (P2MW) and the length (P2ML) of the merus of the second pereiopod, and the same dimensions of the fifth pereiopod (P5PW, P5PL, P5MW, and P5ML).

Table 1. Enzymes and buffer systems used to determine genetic variation in the five populations of *Potamonautes* spp. studied. Buffer: (A) discontinuous tris-citrate-borate-lithium hydroxide buffer system (Ridgeway *et al.,* 1970); (B) continuous tris-borate-EDTA buffer system (Markert and Faulhaber, 1965); and (C) continuous tris-citrate buffer system (Whitt, 1970). Tissue: (m) muscle tissue and (h) hepatopancreas/digestive gland. E.C. = Enzyme Commission.

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 isomerase	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
Phosphogluconate dehydrogenase	PGD	1.1.1.44.	C	m	1

Morphometric differences between the 2 forms were investigated using stepwise discriminant functions analysis of the 7 carapace variables. A jackknife procedure (which eliminates biased group assignments) was used to calculate the classification function of each group, a linear combination of variables that best discriminates each group from the other. Classification information was determined, indicating the *a posteriori* probabilities of an individual belonging to a group. A frequency histogram was plotted of the scores of the individuals of the 2 groups along the first canonical variable calculated from the discriminant functions analysis.

Morphological differentiation between the 2 forms was also investigated by comparisons of the regressions of the various carapace variables over CL as well as regressions of the length over the width of the propodi and meri of the second and fifth pereiopods.

Qualitative Analyses.—Gonopods and mouthparts were removed by dissection, examined under a Leitz stereoscopic dissection microscope, and illustrated using a camera lucida.

RESULTS

Genetic Analyses

Allele frequencies (Table 2) and genotype frequencies were calculated from the 21 reliably scorable loci. Of these loci, nine (LT–1, ME, HEX, MDH–1, LDH, MPI, PGM–2, LGG–1, and LGG–3) were monomorphic across all the populations analysed. No single locus was polymorphic in all the populations studied. The number of alleles per locus varied from one in the monomorphic cases to two in ARK, IDH–2, GPI, PGM–1, GL, PGD, DIA, PHP and LGG–2, and to three in MDH–2, IDH–1 and LT–2. Genetic variability within populations was relatively low. Percentage polymorphic loci (no criterion) varied from 4.76% to 19.05%, in the Tendele, and the Mahai and Coleford populations, respectively. The highest unbiased estimated heterozygosity (0.030) was encountered in the Coleford population and the lowest (0.019) in the Tendele population. The mean number of alleles per locus varied from 1.05 in the Tendele population to 1.19 in the Mahai and Coleford populations. Of the 14 cases of polymorphism involving all loci and all populations, only one locus was found to show genotype frequencies that deviated significantly from frequencies expected under Hardy-Weinberg equilibrium. This was the LT–2 locus ($\chi^2 = 34.0$, $d.f. = 1$, $P < 0.001$) in the Coleford population and could be attributed to a deficiency of heterozygotes. For the remainder of the cases of polymorphism, a clear genetic interpretation of the results was possible.

The dendrogram (Fig. 2) constructed using Nei's (1978) genetic identity (I) values for all pairwise comparisons of populations (Table 3) showed that the five populations formed two distinct groupings. The two groups were (1) the populations of the southern morphotype (from Coleford and Kamberg, identified as *P. depressus*); and (2) the populations of the northern morphotype (from Oliviershoek, Mahai, and Tendele), separated at an I-value of 0.599 ($D = 0.513$). Five diagnostic loci (ARK, IDH–2, MDH–2, LT–2, and PHP) contributed significantly to the separation between the two forms. Individuals of the northern morphotype were fixed with the

Table 2. Allele frequencies at the twelve polymorphic loci studied to determine differentiation between five populations of *Potamonautes* from KwaZulu-Natal. *n* = sample size. Full enzyme names are given in Table 1.

Locus	Oliviershoek	Mahai	Tendele	Kamberg	Coleford
ARK					
n	24	43	12	18	24
100	0.000	0.000	0.000	1.000	1.000
75	1.000	1.000	1.000	0.000	0.000
IDH–2					
n	24	43	12	18	24
100	0.000	0.000	0.000	1.000	1.000
60	1.000	1.000	1.000	0.000	0.000
GPI					
n	24	43	12	18	24
195	0.000	0.000	0.000	0.278	0.000
100	1.000	1.000	1.000	0.722	1.000
MDH–2					
n	24	43	12	18	24
125	0.000	0.000	0.000	0.000	0.021
100	0.000	0.000	0.000	1.000	0.979
75	1.000	1.000	1.000	0.000	0.000
IDH–1					
n	15	38	12	18	24
135	0.033	0.000	0.000	0.000	0.000
120	0.967	0.987	1.000	0.000	0.000
100	0.000	0.013	0.000	1.000	1.000
LT–2					
n	24	43	12	18	34
250	0.000	0.000	0.000	0.000	0.029
100	0.000	0.000	0.000	1.000	0.971
–75	1.000	1.000	1.000	0.000	0.000
PGM–1					
n	24	43	12	18	24
100	0.000	0.012	0.000	1.000	1.000
90	1.000	0.988	1.000	0.000	0.000
GL					
n	22	42	12	18	24
110	0.523	0.607	0.250	0.000	0.042
100	0.477	0.393	0.750	1.000	0.958
PGD					
n	24	43	12	18	24
100	0.000	0.000	0.000	0.972	1.000
95	1.000	1.000	1.000	0.028	0.000
DIA					
n	24	43	12	18	35
100	0.979	1.000	1.000	1.000	1.000
90	0.021	0.000	0.000	0.000	0.000
PHP					
n	24	43	12	18	35
105	1.000	1.000	1.000	0.000	0.000
100	0.000	0.000	0.000	1.000	1.000
LGG–2					
n	24	43	12	18	35
100	1.000	0.988	1.000	1.000	0.671
90	0.000	0.012	0.000	0.000	0.329

alleles ARK^{75}, IDH–2^{60}, MDH–2^{75}, LT–2^{-75}, and PHP^{105} at these respective loci, while individuals of the southern morphotype were fixed for the alleles ARK^{100}, IDH–2^{100}, and PHP^{100}. At the MDH–2 and LT–2 loci, the alleles MDH–2^{100} and LT–2^{100} were fixed in the Kamberg population and were most abundant (at frequencies greater than 0.97) in the Cole-

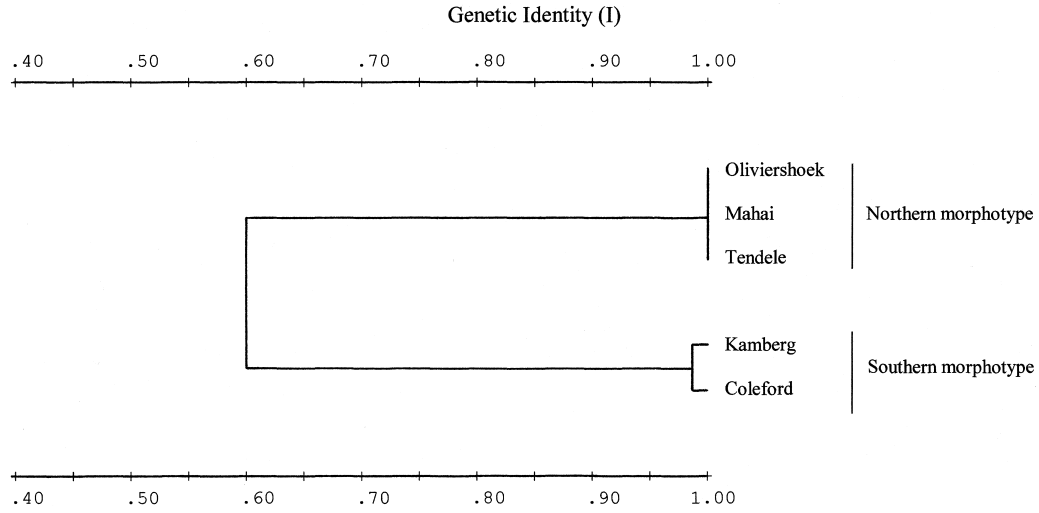


Fig. 2. Genetic similarity between the five *Potamonautes* populations studied, constructed from the matrix of Nei's (1978) unbiased genetic identities (*I*).

ford population, with MDH–2^{125} and LT–2^{250} present as rare alleles. Further, strong heterogeneity was observed between the northern and southern morphotypes at the IDH–1, PGM–1, and PGD loci.

Populations within each cluster were genetically homogenous. Genetic identities between populations of the northern morphotype varied from I = 0.994 to 1.000 (D = 0.000–0.006), while comparison of the two populations of the southern morphotype (*P. depressus*) revealed an *I*-value of 0.991 (D = 0.009).

Morphometric Analyses

The five populations of freshwater crabs fell into the same two groups that were revealed in the genetic analysis. Specimens from the Oliviershoek, Mahai, and Tendele populations were generally smaller, more thickly set, and with the junction of the postfrontal crest and anterolateral carapace margin being more angular than specimens from the Kamberg and Coleford populations. This division could also be made on the basis of colour, with the northern-morphotype individuals being characteristically bright orange and the southern morphotype individuals being brown to green-brown. The frequency histogram of the individual canonical variable scores of the two groups (Fig. 3) calculated from the discriminant functions analyses showed the two forms to be distinct with no overlap between the two groups. The mean canonical variable was 2.773 for *P. depressus* and –3.398 for the northern morphotype. Two of the carapace dimensions (CWW and PFCD) were excluded from the model. Of the remaining five dimensions, CWA, CH, and CL were found to be the most discriminating between the two groups. The classification function of the southern morphotype was Y = 2,844.9(CWA) – 2,931.23(CH) + 2,999.86(CL) – 397.22(CWP) – 1,672.14(ED) – 1,402.47, while the classification function of the northern morphotype was Y = 2,609.46(CWA) –

Table 3. Coefficients of Nei's (1978) unbiased genetic identity (above diagonal) and unbiased genetic distance (below diagonal) for pairwise comparisons of the five populations of *Potamonautes* sampled from KwaZulu-Natal.

Population	Oliviershoek	Mahai	Tendele	Kamberg	Coleford
Oliviershoek	–	1.000	0.997	0.597	0.595
Mahai	0.000	–	0.994	0.593	0.593
Tendele	0.003	0.006	–	0.608	0.605
Kamberg	0.516	0.522	0.498	–	0.991
Coleford	0.519	0.523	0.502	0.009	–

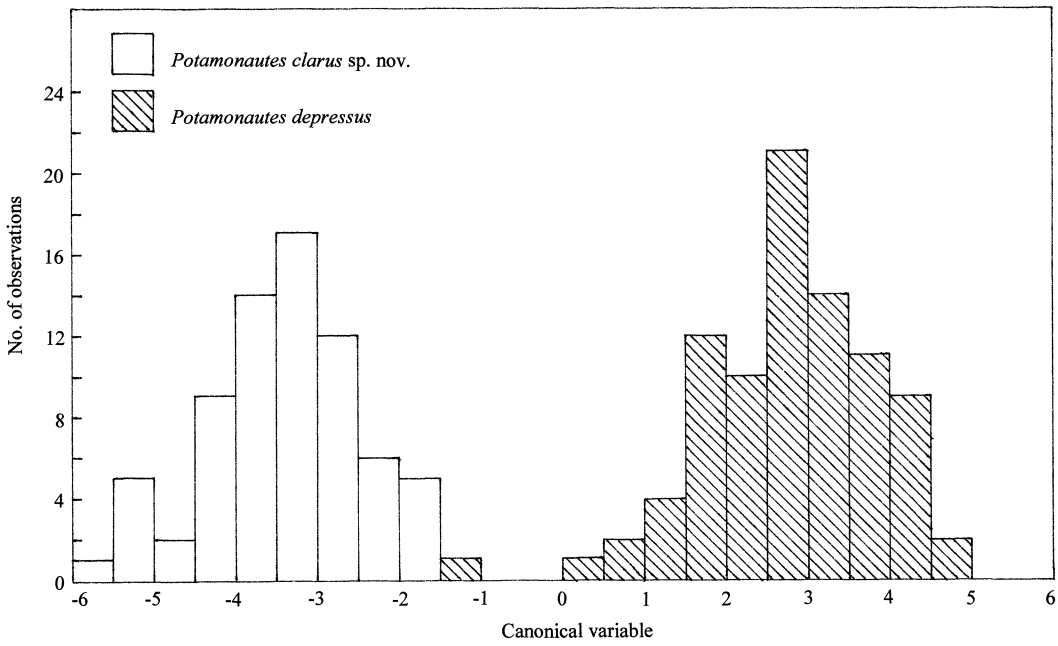


Fig. 3. Frequency of the individual scores of *P. depressus* (n = 87) and *P. clarus* sp. nov. (n = 71) along the canonical variable calculated from the discriminant functions analysis using seven carapace variables.

2,541.57(CH) + 2,600.23(CL) – 356.99(CWP) – 1,556.18(ED) – 1,112.91. The classification matrix (Table 4), based on the classification functions and the highest *a posteriori* probabilities, showed all the individuals of the northern morphotype (100%) to be correctly reassigned to that group. Eighty-six (98.85%) *P. depressus* individuals were reassigned to their group, with one individual being placed in the northern morphotype group.

All comparisons of the regressions involving carapace variables between the two groups showed significant differences (Fig. 4). These included CWW over CL (SS = 0.001, F = 9.295, $P < 0.005$), CWP over CL (SS = 0.061, F = 22.626, $P < 0.001$), PFCD over CL (SS = 0.017, F = 14.277, $P < 0.001$), ED over CL (SS = 0.003, F = 6.415, $P <$ 0.02), CWA over CL (SS = 0.002, F = 7.165, $P < 0.01$), and CH over CL (SS = 0.002, F = 7.412, $P < 0.01$). Of the comparisons of the regressions of pereiopod variables between the two forms, only P5ML regressed over P5MW showed a significant difference between the two forms (SS = 0.022, F = 12.784, $P < 0.001$). Using Student's t-test and CL as being representative of size, a significant size difference was observed between the two species (t = 11.971, $d.f.$ = 156, $P < 0.001$). The smallest mature female (having the abdomen overlapping the coxae of the pereiopods) of the southern morphotype (*P. depressus*) had a CL of 32 mm, while the largest immature female had a CL of 25.9 mm, indicating that the pubertal moult occurs roughly between the 26-mm and 30-mm (CL) size classes. The pubertal moult was determined to occur between the 16-mm and 20-mm size classes among females of the northern morphotype.

Table 4. Percentage of correct *a posteriori* classification to groups, based on classification functions calculated from seven carapace variables.

Species	Percen.t correctly classified	*P. depressus*	*P. clarus* sp. nov.
Potamonutes depressus	95.85	86	1
Potamonautes clarus sp. nov.	100	0	71

Qualitative Analyses

The gonopods, mandibular palp, and carapace of the northern and southern (*P. depressus*) morphotypes are illustrated in Fig. 5. No significant differences were observed between the third maxillipeds and the

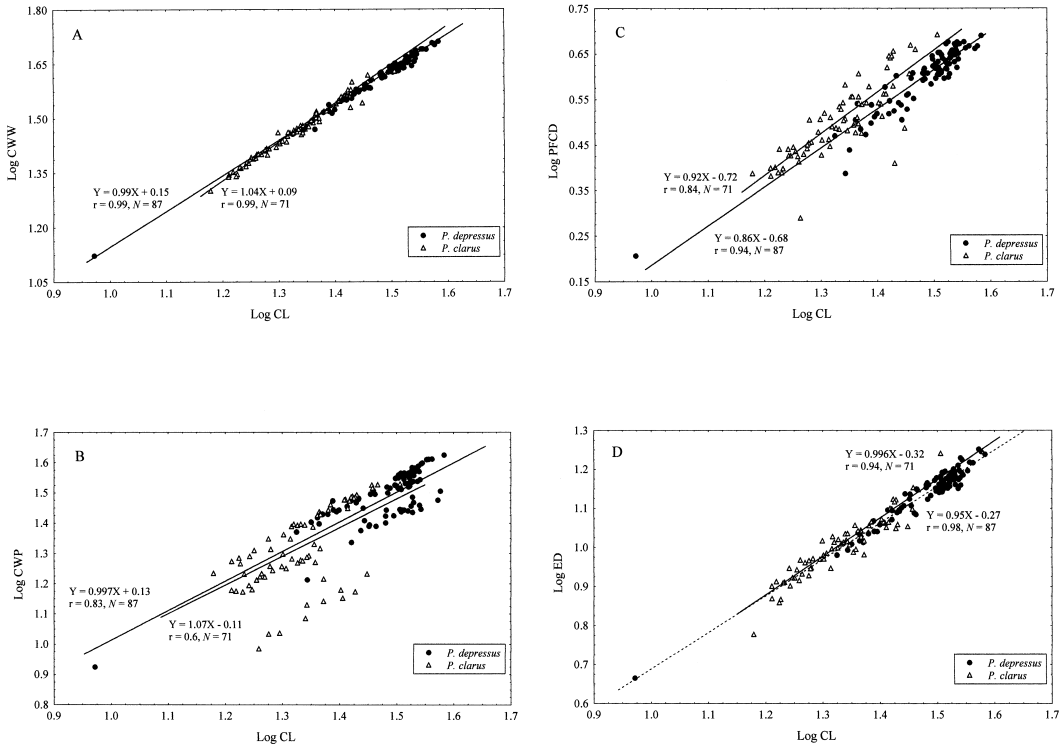
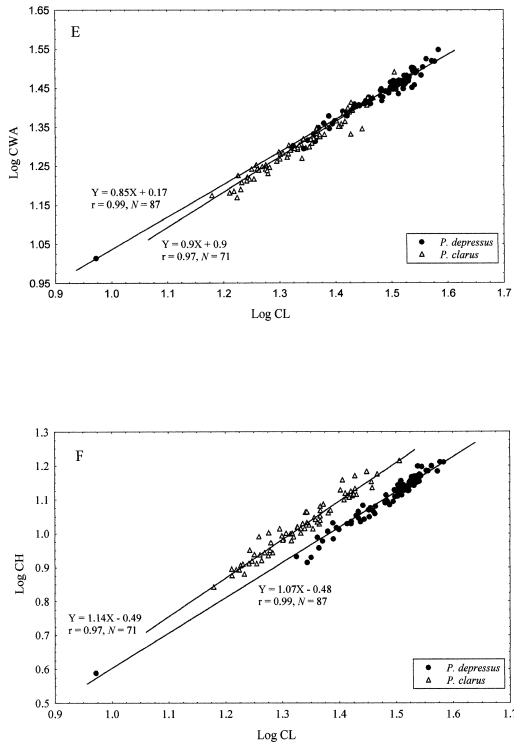


Fig. 4. Comparison of the regressions of (A) Log CWW (carapace widest width) over Log CL (carapace length); (B) Log CWP (carapace posterior width) over Log CL; (C) Log PFCD (postfrontal crest distance) over Log CL; (D) Log ED (orbital distance) over Log CL (carapace length); (continued, next page)

mandibular palps of the two forms. The cutting surface of the mandible appeared somewhat less rounded in *Potamonautes depressus* than in the northern morphotype. The terminal segment of gonopod 1 appeared to be slightly longer and more slender in the northern form than in the southern form.

Discussion

Genetic comparisons between populations of the two morphotypes revealed an average *I*-value of 0.599 (D = 0.512), which is substantially lower than *I*-values separating existing species within the genus *Potamonautes*. An *I*-value of 0.68 (D = 0.39) was used to distinguish *P. perlatus* from *P. parvispina* in the Western Cape (Stewart, 1997a). An *I*-value of 0.66 (D = 0.42) was shown to separate both *P. brincki* from *P. perlatus* in the Western Cape (Stewart, 1997b), and *P. unispinus* from *P. sidneyi* in Mpumalanga (Stewart and Cook, 1998). Higher *I*-values were presented for the specific delineation of *P. granularis* in the Western Cape (Daniels *et al.*, 1998a, 1999), which was separated from *P. perlatus* at an *I*-value of 0.88 (D = 0.13), and *P. sidneyi,* which was separated from an unidentified form from KwaZulu-Natal at 0.829 (D = 0.187) (Gouws *et al.*, in preparation). The *I*-value separating the two taxa in this study falls well within the range characterizing congeneric species specified by Thorpe (1982), who showed that 85% of congeneric *I*-values exceed 0.35, while 97% are below 0.85. The *I*-value obtained here also falls below the average of 0.66 for congeneric species differences (obtained in a survey of 28 species pairs of decapod Crustacea) presented by Hedgecock *et al.* (1982). Thorpe and Solé-Cava (1994) further stated that allopatric populations separated by an identity value of less than 0.85 were unlikely to be conspecific. Although no sympatric populations of the two forms were encountered, the presence of diagnostic fixed allele differences between the two taxa indicates a degree of genetic isolation, which would warrant specific recognition under Bock's (1986) interpreta-



(E) Log CWA (carapace anterior width) over Log CL; and (F) Log CH (carapace height) over Log CL, between *P. depressus* and *P. clarus* sp. nov. All comparisons are significantly different at the $P < 0.02$ level.

tion of Mayr's (1963) Biological Species Concept, whereby species are defined as groups of interbreeding or potentially interbreeding populations, reproductively or genetically isolated from other such groups.

Comparable *I*-values have been used to delineate species or have been found between nominally different species in a range of studies involving decapod Crustacea. *Uca speciosa* (Ives, 1891) and *U. spinicarpa* Rathbun, 1900, were determined to show a genetic distance (*D*) of 0.7, with a corresponding *I*-value of 0.5 (Salmon *et al.*, 1979). Within two genera of penaeid shrimp, *Penaeus* and *Metapenaeus*, species were separated with average *I*-values of 0.601 ($D = 0.509$) and 0.630 ($D = 0.462$), respectively (Tam and Chu, 1993). Identity values of 0.547 ($D = 0.604$) and 0.594 ($D = 0.52$) separated *Metanephrops thomsoni* Bate, 1888, from *M. japonicus* Tapparon Canefri, 1873, and *M. formosanus* Chan and Yu, 1987, respectively (Chu *et al.*, 1990). Similar values were obtained in the pairwise comparisons of the xanthid crabs *Trapezia cymodoce* Herbst, 1799, and *T. digitalis* Latreille, 1828, with six other members of the *Trapezia* species complex (Huber, 1985). Allegrucci *et al.* (1992) indicated that *Procambarus mirandai*

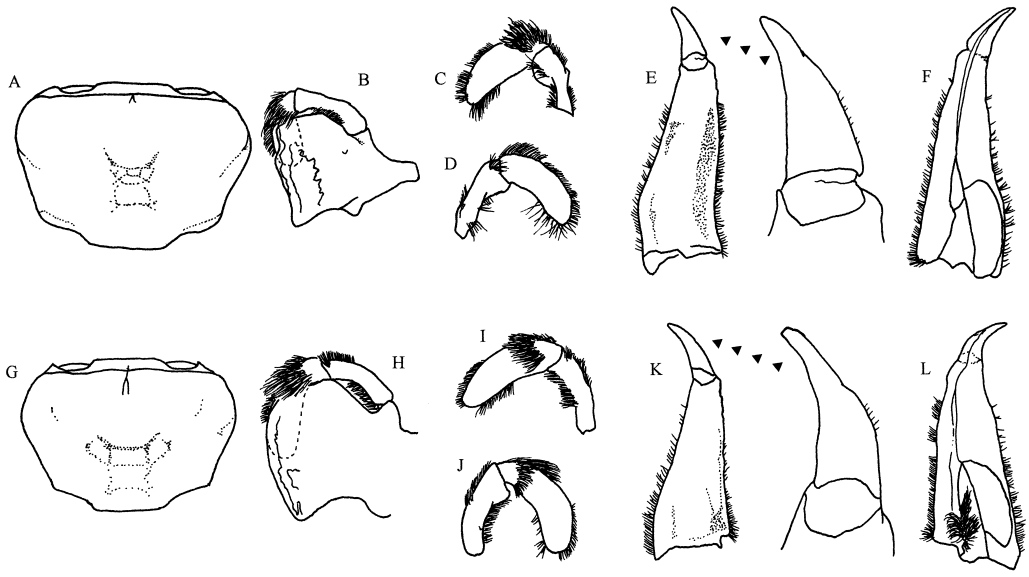


Fig. 5. A–F: *Potamonautes depressus* (unaccessioned specimens): A, carapace outline; B, left mandible and mandibular palp, posterior view; C, left mandibular palp, posterior view; D, left mandibular palp, anterior view; E, left gonopod 1, anterior view; and F, left gonopod 1, posterior view. G–L: *Potamonautes clarus* sp. nov., male holotype SAM A43550: G, carapace outline; H, left mandible and mandibular palp, posterior view; I, left mandibular palp, posterior view; J, left mandibular palp, anterior view; K, left gonopod 1, anterior view; and L, left gonopod 1, posterior view.

Villalobos, 1954, showed *I*-values of 0.625 and 0.631 (comparable *D*-values of 0.47 and 0.46) in comparisons with two undescribed *Procambarus* crayfish species, each representing a different colour morph. Comparable values have also been obtained in crustacean studies involving copepods (Boileau and Hebert, 1988) and isopods (Lessios and Weinberg, 1994).

Intraspecific genetic differentiation is generally low within the genus *Potamonautes*. Six studies (Stewart, 1997a, b; Daniels *et al.*, 1998b, 1999; Stewart and Cook, 1998; Gouws *et al.*, in preparation) involving seven *Potamonautes* species across South Africa revealed intraspecific *I*-values ranging between 0.902 and 1.000 (*D*-values between 0.000 and 0.103). The only notable exception was the occurrence of an *I*-value of 0.75 separating two groups of *P. brincki* populations across the Cape Flats and Cape Peninsula (Stewart, 1997b), which most probably alludes to a species-level difference. Thorpe and Solé-Cava (1994) contended that 93% of *I*-values between conspecific populations exceed 0.9. Intraspecific *I*-values within each taxon obtained in this study showed general agreement with the data presented above.

The most immediately apparent difference between individuals of the two taxa is the colour of the two morphotypes, with individuals from Oliviershoek, Mahai, and Tendele having a bright orange to orange-red carapace and limbs and individuals from Kamberg and Coleford with a yellow-brown to green-brown carapace. The causative mechanisms involved in colour polymorphism, be they genetic or ecological, have not been determined, although developmental stage, physiological condition, and season are known to effect body colour (Bert, 1986; Aotsuka *et al.*, 1995). Despite this, colour has been regarded as a reliable and useful feature in some taxonomic discourses involving decapod Crustacea (Knowlton, 1986; Knowlton and Mills, 1992; Daniels *et al.*, 1998a), as it is thought to be less influenced by environmental factors than in other groups. However, many recent studies delimiting species that use colour as a taxonomic character do so in addition to morphological or genetic data (e.g., Huber, 1985; Bert, 1986; Felder and Staton, 1994; Aotsuka *et al.*, 1995).

Morphologically, the individuals of the northern morphotype are generally slightly smaller than *P. depressus*. The carapace of the northern morphotype is higher, slightly narrower posteriorly, and has a slightly longer and wider frontal lobe than that of *P. depressus*. The forked mid-groove at the midpoint of the postfrontal crest of the northern morphotype is longer than in *P. depressus,* and the cardiac and urogastric grooves are more distinct.

Empirical evidence thus supports recognition of the northern morphotype (the orange crabs) as a new species, distinct from *P. depressus*. This new species is described here as *Potamonautes clarus*.

TAXONOMY

Potamonautes clarus, new species
Figs. 6A–C, 7A–L

Material Examined.—Holotype: South African Museum (SAM) A43550, 1 ♂ (CL = 30.3 mm), and paratypes: SAM A43551, 3 ♂♂, 2 ♀♀, Tributary of Mnjaneni River (28°34′25″S, 29°03′28″E), along Oliviershoek Pass, 3.5 km north of Drifter's Inn, collected on 21 and 22 January 1997 by B. A. Stewart, P. A. Cook, G. Gouws, and L. Hoenson. Other material examined: SAM A43552, 4 ♂♂, 2 ♀♀, Mahai stream, upstream of the Royal Natal National Park campsite (28°41′28″S, 28°56′40″E), collected on 22 January 1997 by B. A. Stewart, P. A. Cook, G. Gouws, and L. Hoenson. SAM A41312, 1 ♂, 1 ♀, Mahai stream, Royal Natal National Park (28°41′15″S, 28°56′30″E), collected on 4 December 1996 by E. Dickson. SAM A43553, 1 ♂, Sterkfontein dam, Free State, collected on 20 October 1993 by P. E. Reavell. Transvaal Museum TM5057–TM5059, 2 ♂♂, 1 ♀, Tugela Gorge, below Mont-Aux-Sources, Basutoland, collected November 1930 by H. Lang. TM5073–TM5074, 1 ♂, 1 ♀, Tugela Gorge, below Mont-Aux-Sources, Basutoland, collected November 1930 by H. Lang. TM5076–TM5079, 3 ♂♂, 1 ♀, Tugela Gorge, below Mont-Aux-Sources, Basutoland, collected November 1930 by H. Lang. TM5103–TM 5110, 4 ♂♂, 5 ♀♀, Oliviershoek, near Van Reenen's Pass, collected November 1930 by H. Lang. TM5112–TM 5113, 2 ♀♀, Oliviershoek, near Van Reenen's Pass, collected November 1930 by H. Lang.

Type Locality.—Tributary of Mnjaneni River, along Oliviershoek (Van Reenen's) Pass, 3.5 km north of Drifter's Inn, northern Drakensberg, KwaZulu-Natal, South Africa.

Distribution.—Found only in the higher tributaries of the Tugela River system, draining the northern Drakensberg, north of Cathedral Peak (South Africa), possibly extending into Lesotho, and Mpumalanga and Free State province of South Africa.

Diagnosis.—Carapace flattened and smooth, with rounded, smooth epibranchial corners.

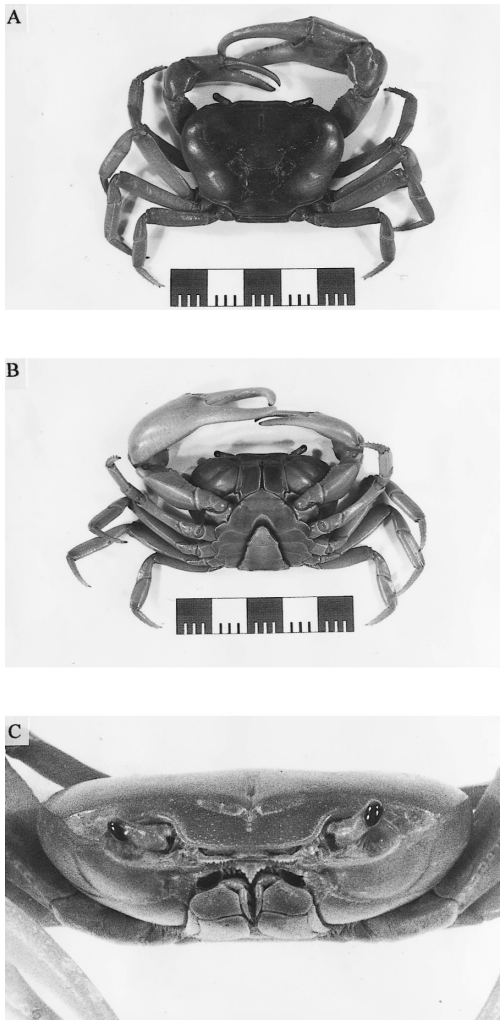


Fig. 6. *Potamonautes clarus* sp. nov. male holotype (CL = 30.3 mm). SAM A43550: A, whole animal, dorsal aspect; B, whole animal, ventral aspect; and C, cephalothorax, frontal view.

Postfrontal crest distinct, straight. Anterolateral carapace margin lacks teeth or granulations. Carapace and limbs characteristically bright orange in life, with lighter tips to chelipeds and legs. Chela of males highly arched. Legs long, slender. Terminal segment of gonopod 1 relatively long, slender, roughly subtriangular.

Description.—The description is based on the male holotype (SAM A43550); measurements of the holotype are presented in Table 5.

Carapace (Figs. 6A, C, 7A) and limbs bright orange when alive, with lighter cream to yellowish tips to chelipeds and pereiopods. Thoracic sternites, abdomen, and ventral portions of chelipeds and walking limbs cream to light brown, fades to duller orange-brown when preserved (70% ethanol). Cephalothorax ovoid, flattened dorsally, maximum height and width in anterior third (ratio CH/CL = 0.49, and CWW/CL = 1.41). Anterior margin straight. Frontal lobe relatively short. Carapace smooth, cardiac grooves moderately deep, distinct, urogastric grooves shallow, indistinct. Exorbital teeth small, blunt. Epibranchial teeth absent. Anterolateral margin posterior to postfrontal crest convex, smooth; margin curves inwards over branchial region surface. Postfrontal crest straight, distinct, with relatively long, forked mid-groove. Each carapace sidewall has longitudinal groove dividing subhepatic and pterygostomial regions, vertical groove extends from longitudinal groove, dividing dorsally to join lateral orbital margin and anterolateral margin behind postfrontal crest.

Thoracic sternites (Figs. 6B, 7B) 1 and 2 fused, no suture visible. Suture (first sternal groove) between sternites 2 and 3 complete, deep, shallower medially, suture (second sternal groove) between sternites 3 and 4 complete, deeper laterally, becoming shallower medially, sloping sharply towards abdominal cavity laterally, curving forward medially.

Third maxillipeds (Figs. 6C, 7E) fill entire buccal cavity, except for oval, efferent respiratory openings; relatively long flagellum present on exopod; vertical groove on ischium faint to absent. Inner margins of ischium and dactyl moderately setose.

Mandibular palp (Fig. 7F, G, H) 2-segmented, terminal segment undivided, with thick tuft of setae carried on small flange on posterior, proximal surface, margins of distal portion of terminal segment finely hirsute. Subterminal segment thickened distally.

Chelipeds (Figs. 6A, 7C, D) markedly unequal, dactylus of right cheliped highly arched, enclosing large, ovoid space, and 1.45 times length of dactylus of left cheliped. Cutting edges of both dactyli possess 16–18 small cutting teeth, with 3 to 4 being slightly larger, only largest readily visible on right dactylus. Propodus of right cheliped markedly swollen, 1.5 times length, 1.69 times width of left cheliped. Pollex with about 15 small cutting teeth, 5 of them being slightly larger. In both chelipeds, carpus with 1 sharp prominent spine and smaller, sharp, rudimentary

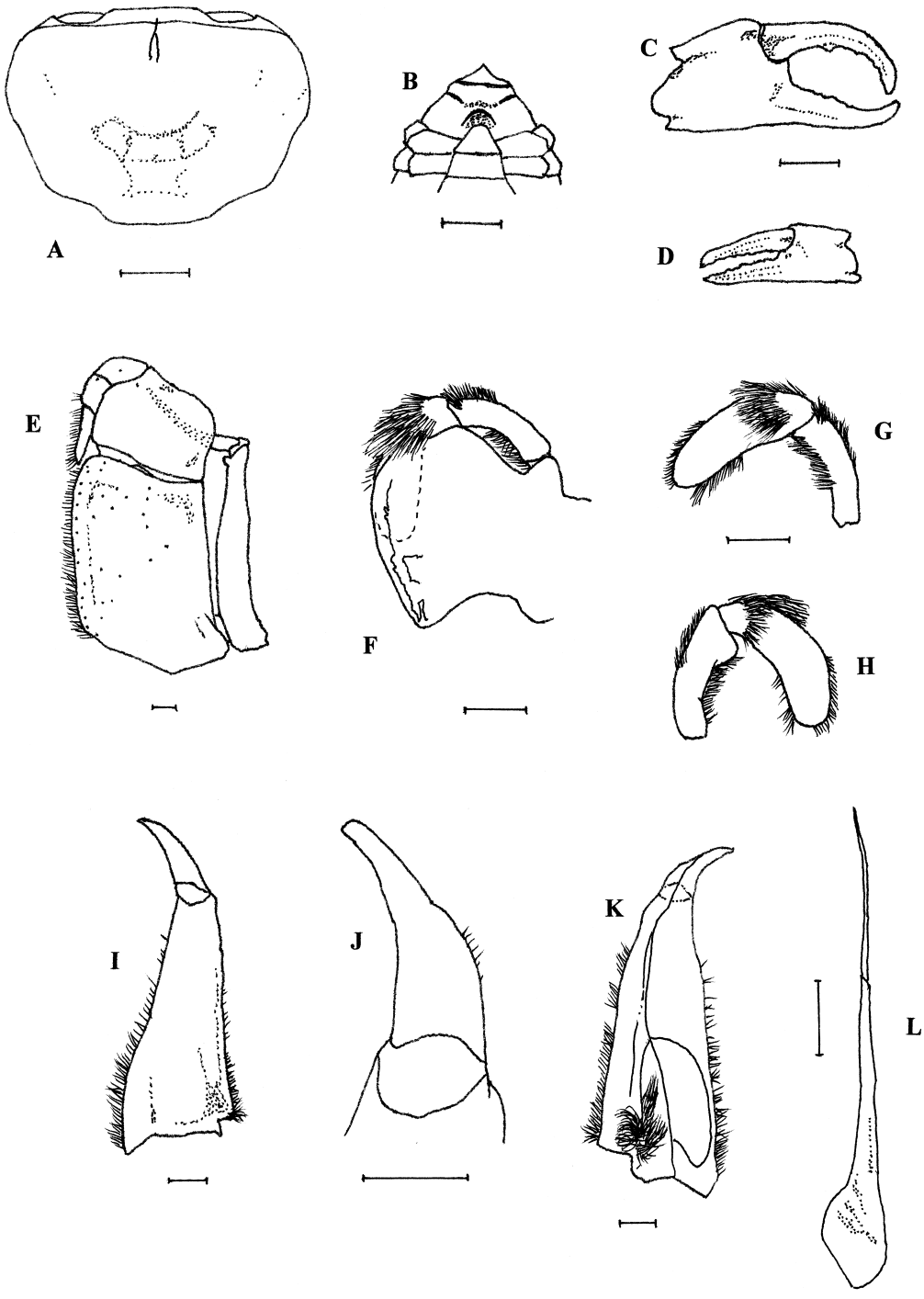


Fig. 7. *Potamonautes clarus* sp. nov. male holotype (CL = 30.3 mm) SAM A 43550, and unaccessioned specimens: A, carapace outline; B, thoracic sternum; C, right cheliped, dactylus and propodus; D, left cheliped, dactylus and propodus; E, left third maxilliped; F, left mandible and mandibular palp, posterior 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; and L, left gonopod 2, anterior view. Scale lines represent 1 mm, except for A–D, where they represent 10 mm.

Table 5. *Potamonautes clarus* sp. nov. Wet weight (g) and measurements (mm) for the male holotype and ranges for other individuals examined (maximum of 38 males and 37 females).

Variable	Abbreviation	Holotype	Males	Females
Wet weight	WW	21.9	2.3–20.3	2.3–20.0
Carapace length	CL	30.3	15.1–29.3	16.3–32.0
Carapace widest width	CWW	42.7	20.0–41.6	21.9–42.7
Carapace posterior width	CWP	31.9	9.7–33.7	12.2–34.0
Distance between postfrontal crest and anterior margin	PFCD	4.8	2.4–4.7	2.0–4.9
Distance between orbits	ED	13.9	6.0–14.2	7.2–17.4
Distance between exorbital teeth	CWA	28.6	15.0–26.7	14.8–31.0
Carapace height	CH	14.7	7.0–15.0	7.5–16.4
Width of sixth abdominal segment	AW6	9.1	3.9–8.4	5.9–25.6
Major cheliped propodus length	MCPL	43.5	11.2–41.4	11.8–27.0
Major cheliped propodus height	MCPH	16.8	4.6–17.2	4.8–10.9
Pereiopod 2, merus length	P2ML	21.1	8.9–17.7	7.8–15.3
Pereiopod 2, merus width	P2MW	5.0	3.1–5.2	2.8–7.5

spine. Meri of both chelipeds have weakly granulated margins, small blunt tooth beneath anterior-superior margin. No spine on anterior-inferior margin. Pereiopods long, slender (pereiopod 2, merus length/width = 4.22; pereiopod 5, merus length/width = 4.19). P3 longest, P5 shortest, anterior margins slightly concave, posterior margins slightly convex, margins smooth, dactyli of P2 to P5 possess sharp, spinelike bristles on margins, most prominent on P5.

Abdomen (Figs. 6B, 7B), first 5 segments short, wide, last 2 segments longer, telson triangular, rounded at distal end.

Pleopods (Fig. 7I, J, K), terminal segment of pleopod 1 (gonopod 1) short, 0.28 times length of subterminal segment, terminal segment curves sharply away from midline when viewed posteriorly, widest at base, ending in pointed tip. Subterminal segment of pleopod 1 tapers distally, margins moderately setose, posterior surface has twisting longitudinal groove, extending length of subterminal and terminal segments, anterior surface lacks longitudinal groove. Flanges and margins of groove may be finely hirsute. Terminal segment of pleopod 2 (gonopod 2) (Fig. 7L) relatively straight, threadlike, hollow, 0.6 times length of subterminal segment. Subterminal segment of pleopod 2 widest at base, tapering sharply inwards at approximately 0.3 length, forming long process supporting terminal segment.

Variation.—All specimens are characteristically bright orange in colour, with the thoracic sternites, abdomen, and undersides of the limbs being a cream-brown colour, and lighter cheliped and limb tips, although specimens become more orange-brown in the Tendele and Mahai populations. Chela of the females and the smaller males are not as highly arched as those of the holotype, rather closing tightly, with relatively thicker fingers and a less inflated pollex. Chelipeds of females and smaller males also appear, largely, equal or slightly subequal. In *P. clarus*, 77% of individuals showed right-handedness, 6% left-handedness, and 17% had chelipeds of equal or subequal (not differing by more than one millimeter) size. Right-handedness was exhibited by 85% of males and 67% of females, left-handedness by 6% of males and 7% of females, while 9% of males and 26% of females had equal or subequal chelae. Similarly, heterochely has been documented in *Potamonautes perlatus* (see Siegfried, 1972), *P. sidneyi* (see Raubenheimer, 1986), *P. dentatus* (see Stewart *et al.*, 1995), *P. parvispina* (see Stewart, 1997a), *P. granularis* (see Daniels *et al.*, 1998a), *P. unispinus* (see Stewart and Cook, 1998), and an undescribed species from KwaZulu-Natal (Gouws *et al.*, in preparation). The postfrontal crest, anterolateral margins and epibranchial corners are typically smooth in most individuals, while the curving of the anterolateral margin over the surface of the carapace in the branchial region is more distinct in the females and smaller males. The abdomen of mature females extends over the coxae.

Etymology.—From the Latin adjective "clarus", meaning "bright", referring to the

characteristic bright orange colour of the carapace and pereiopods.

Remarks.—Potamonautes clarus can readily be distinguished from the ten species of *Potamonautes* known to occur in South Africa. *Potamonautes warreni* Calman, 1918, *P. dentatus* Stewart, Coke, and Cook, 1995, *P. parvispina* Stewart, 1997a, and *P. unispinus* Stewart and Cook, 1998, either have an epibranchial tooth or a series of teeth or spines on the anterolateral margin of the carapace. *Potamonautes clarus* lacks an epibranchial tooth and the anterolateral carapace margin is smooth. *Potamonautes clarus* can be distinguished from an as yet undescribed species occurring in KwaZulu-Natal (Gouws *et al.*, in preparation) by the latter's highly inflated carapace, relatively small body size, incomplete postfrontal crest, and distinct orange-red carapace colour, with a characteristic dark silver-blue sheen. *Potamonautes brincki* Bott, 1960, is characteristically dark chocolate-brown to reddish-brown and has a finely granulated anterolateral margin, deep urogastric grooves, and a short forked mid-groove at the midpoint of the postfrontal crest (Stewart, 1997b), whereas the forked mid-groove in *P. clarus* is long, and the urogastric grooves shallow. Individuals of some *P. brincki* populations have a large, distinct flange on the terminal segment of the mandibular palp, which appears to be absent or diminished in *P. clarus*. Although the terminal segment of gonopod 1 in *P. clarus* curves away from the medial line, this curvature does not appear to be as pronounced as it is in *P. brincki* (Stewart, 1997b). *Potamonautes granularis* Daniels, Stewart, and Gibbons, 1998a, is distinguished from *P. clarus* by its possession of a distinct, granulated postfrontal crest. *Potamonautes sidneyi* Rathbun, 1904, has granulated anterolateral margins, and the epibranchial corners bear fields of granules or small ridges. The postfrontal crest is distinct and forms a sharp angle, often with a minute forward projecting epibranchial tooth, where it joins the anterolateral margin (Bott, 1955), whereas the epibranchial corners are smooth and more rounded in *P. clarus*. *Potamonautes clarus* has long, slender legs (P2ML/P2MW and P5ML/P5MW are roughly 2.8 and 3.0, respectively), whereas *P. sidneyi* has shorter, more stout legs (P2ML/P2MW = 2.5, and P5ML/P5MW = 2.6). The carapace of *P. sidneyi* and *P. perlatus* is distinctly vaulted (Bott, 1955), whereas that of *P. clarus* is relatively low and flat. The terminal segment of the gonopod 1 in *P. sidneyi* is relatively long, slender, and S-shaped. Similarly, the terminal segment of gonopod 1 of *P. dentatus* (see Stewart *et al.*, 1995), *P. parvispina* (Stewart, 1997a), and *P. unispinus* (Stewart and Cook, 1998) appears to be S-shaped, whereas that of *P. clarus* is straighter. In *P. clarus* this segment also lacks the rounded, inward-projecting lobe near the base of the ventral section as described and figured for *P. warreni* and *P. perlatus* H. Milne Edwards, 1837, by Bott (1955) and Stewart *et al.* (1995).

Of the South African species, the nominal subspecies of *P. depressus* Krauss, 1843, bears the closest resemblance to *P. clarus*, due to both possessing a flat carapace and long, slender limbs. *Potamonautes clarus* is, however, smaller and with a more vaulted carapace, and is characteristically brightly orange-coloured, with *P. depressus depressus* being darker yellow-brown to green-brown. The postfrontal crest is often directed forward near the anterolateral margin in *P. depressus depressus* (see Bott, 1955), but is straight in *P. clarus*. *Potamonautes depressus dybowskii* Rathbun, 1905, known from the French Congo, is, in turn, a larger crab than *P. d. depressus* (Bott, 1955). Bott (1955) figured and described the terminal gonopod 1 segments of both subspecies as being long and slender, whereas the terminal segments in both *P. clarus* and the *P. depressus* populations examined in this study were shorter, broader, and more triangular. The terminal segment of gonopod 1 in *P. clarus* was, however, slightly longer and more slender than that of *P. depressus depressus*.

Acknowledgements

Financial assistance was kindly provided by the World Wildlife Fund (WWF)–South Africa and the National Research Foundation. The KwaZulu-Natal Nature Conservation Services provided accommodation and logistical support. Liz Hoenson, Peter Cook and Tyrone Ridgway are thanked for their assistance in the field, and Michelle van der Merwe is thanked for assisting with the photography.

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RECEIVED: 21 July 1999.
ACCEPTED: 3 May 2000.