Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda, Potamonautidae) with the description of a new species from mountain streams in the Western Cape, South Africa

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Accepted: 4 November 2000

Daniels, S. R., Stewart, B. A. & Burmeister, L. (2001). Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda, Potamonautidae) with the description of a new species from mountain streams in the Western Cape, South Africa. — *Zoologica Scripta*, *30*, 181–197.

Recent systematic research has revealed that *Potamonautes brincki* comprises two genetically and morphologically distinct population groups. The systematic affinities between these population groups have remained uncertain. In the present study, the relationship between the population groups was examined. Eleven populations were collected from high mountain streams in the Western Cape, South Africa and used in the genetic and morphological analyses. Allozyme electrophoresis of 13 protein coding loci separated two main population groups: group A (Cape Peninsular groups) and group B (Hottentot's Holland) at $I = 0.73$. Two additional genetic groups were evident, with group B being conspecific to group A, and group D being conspecific to group C. Morphological examination of pleopod 1 and the terminal segment of the mandibular palp showed considerable differences between the two main population groups, with groups A and B being similar and groups C and D being similar. The morphometric data for the four main groups were examined using discriminant functions analysis and the two main groups were compared using analyses of covariance. Discriminant functions analysis showed a moderate degree of overlap between the groups. Additional morphometric data showed a clear discrimination between the two main population groups. The genetic and morphometric data sets exhibited congruent patterns of variation and the data showed the presence of a species boundary. A new freshwater crab species, *P. parvicorpus* sp. n., is described. The results of the present study are discussed in the light of historical and contemporary factors that are likely to have contributed to speciation.

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Introduction

Four river crab species are known to occur in the Western Cape, South Africa. *Potamonautes perlatus* (Milne-Edwards, 1837) was, until recently, thought to be the only river crab that occurred in this area, where it is widespread and is found from Clanwilliam to Port Elizabeth in the Eastern Cape, typically in upper, middle and lower reaches of rivers (Barnard 1950). The remaining three species are endemic to the region. *Potamonautes granularis* Daniels, Stewart & Gibbons, 1998 is known only from the lower reaches of the Olifants River system. *Potamonautes parvispina* Stewart, 1997a is endemic to high mountain streams in the Berg and Olifants River systems, while *P. brincki* (Bott, 1960) occurs in high mountain streams on the Cape Peninsula, the Hottentot's Holland Mountains and the Kleinriver Mountains (Stewart 1997b). These results indicate that the freshwater crab fauna of the region is considerably more species rich than previously thought (Barnard 1950). With the exception of *P. brincki*, allozyme studies of these freshwater crab species have revealed the general absence of population structuring (Daniels *et al*. 1998, 1999a).

Stewart (1997b) reported substantial genetic and morphological divergence amongst allopatric populations of *P. brincki* and identified two distinct population groups. One group occurred on the Cape Peninsula, mainly on Table Mountain, with a second group occurring further eastwards on

the Hottentot's Holland Mountains and the Kleinriver Mountains. Allozyme data demonstrated that *P. brincki* is a highly structured genetic entity (genetic identity, $I = 0.75$) with substantial fixed allelic differences between these two population groups. The extent of the genetic differentiation recorded amongst *P. brincki* populations is exceptional. Intraspecific genetic differentiation amongst freshwater crab species has been found to be low, usually on the order of 0.88–1.00 (Daniels *et al.* 1999b). The results obtained by Stewart (1997b) thus possibly provide evidence for a species boundary between these *P. brincki* populations.

Morphologically, these two groups exhibit variation in the structure of the mandibular palp and the terminal segment of pleopod 1 (gonopod). The Cape Peninsula populations all have a simple terminal segment of the mandibular palp, which is covered by a dense tuft of setae (Stewart 1997b). However, in specimens from the Hottentot's Holland and the Kleinriver Mountains, the terminal segment of the mandibular palp is divided and characterized by a dense tuft of setae that arises from a ridge or a flange. The inner margin of the subterminal segment of pleopod 1 is markedly irregular in the Cape Peninsula populations and appears to differ in shape from that of the Hottentot's Holland and Kleinriver Mountains populations. These differences, along with the strong genetic discontinuity, led Stewart (1997b) to suggest that a species boundary may be present between these two population groups. However, she suggested that, before such a species boundary could be confidently identified, further samples needed to be collected from the region geographically situated between these two main population groups.

The primary objectives of the present study are fourfold. Firstly, to investigate the genetic relationship of populations geographically intermediate to the two main groups defined by Stewart (1997b). Secondly, to examine the structure of the terminal segment of the mandibular palp and the structure of pleopod 1 in all populations. Thirdly, to examine population structuring within and between population groups and, fourthly, to revisit the possibility of the existence of a species boundary between the Cape Peninsula and the Hottentot's Holland groups.

Materials and methods

Sample collection

Specimens were collected from 11 first- and second-order mountain streams in the Western Cape (Fig. 1). The geographically intermediate populations were collected from the Lang River, a tributary of the Eerste River (Jonkershoek), and unnamed tributaries of the Lourens and the Sir Lowry's Rivers. In addition, samples were re-collected from the same localities on the Cape Peninsula, Hottentot's Holland Mountains and Kleinriver Mountains (Fernkloof) as used by Stewart (1997a,b). Voucher specimens of the Cape Peninsular form were deposited at the South African Museum (SAM), Cape Town, South Africa. Crabs were caught with handnets following their attraction to baited (ox heart) lines. On capture, crabs were transferred alive to the laboratory and killed by freezing at –20 or –80 °C for 24 h prior to measurement and tissue extraction.

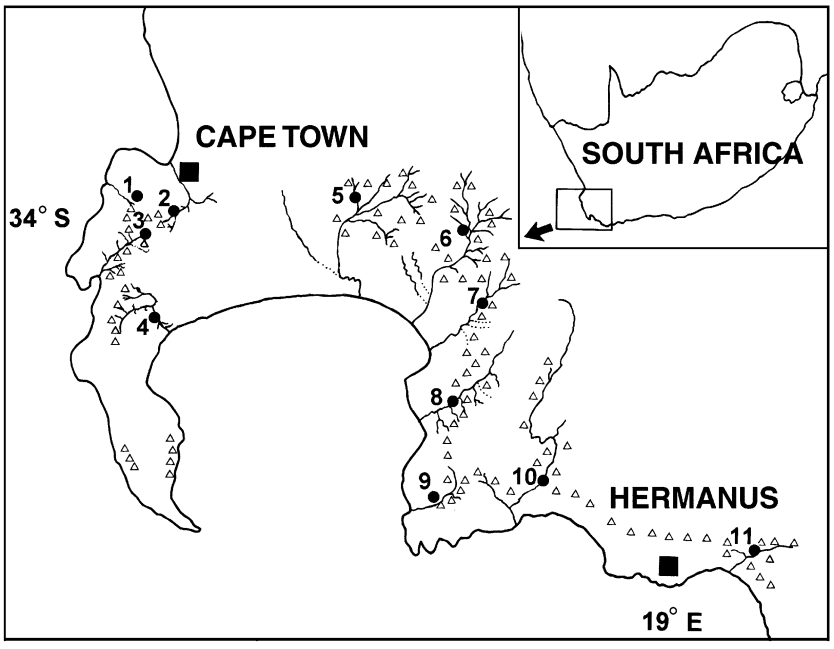

Fig. 1 Localities sampled: 1, Blinkwater; 2, Orange Kloof; 3, Kirstenbosch; 4, Silvermine; 5, Jonkershoek; 6, Lourens River; 7, Sir Lowry's River; 8, Steenbras; 9, Rooiels; 10, Palmiet; 11, Fernkloof. Open triangles represent mountain ranges.

Table 1 Enzyme and buffer systems used during electrophoresis. *N* = the number of loci.

Enzyme	Abbreviation	Buffer	E.C. number	*N*
Arginine kinase	ARK	A	2.7.3.3	1
Glucose phosphate isomerase	GPI-1	A	5.3.1.9	1
Isocitric dehydrogenase	IDH	B	1.1.1.42	2
Lactate dehydrogenase	LDH	B	1.1.1.27	1
Peptidase (glycyl leucine as substrate)	GL	B	3.4.11-	1
Peptidase (leucine tyrosine as substrate)	LT	A	3.4.11-	1
Malate dehydrogenase	MDH	C	1.1.1.37	2
Malic enzyme	ME	C	1.1.1.40	1
Mannose phosphate isomerase	MPI	C	5.3.1.8	1
Phosphoglucomutase	PGM	A	2.7.5.1	2

Genetics

Muscle tissue was removed from each specimen after all morphological measurements had been taken. Tissue samples were placed in cryotubes and stored in liquid nitrogen prior to freezing at –80 °C on return to the laboratory. The genetic variation at 18 isozyme loci was examined on a 13% starch gel (Sigma Chemicals Co., St Louis, MO, USA). Only 13 allozyme loci were consistently scored for all the populations, and analysis has thus been confined to these. Three electrophoretic buffer systems were used: (A) a discontinuous tris-citrate–borate–lithium hydroxide buffer, gel buffer pH 8.7, electrode buffer pH 8.0 (Ridgeway *et al.* 1970); (B) a continuous tris-borate–ethylenediamine tetraacetic acid (EDTA) buffer system, gel and electrode buffer at pH 8.6 (Markert & Faulhaber 1965); and (C) a continuous tris-citrate buffer system, gel and electrode buffer at pH 6.9 (Whitt 1970).

Tissue samples were placed in Eppendorf tubes and homogenized in 0.01 M Tris buffer (pH 8). Water-soluble proteins were separated from the homogenate by centrifugation at 2500 *g* for 5 min. Filter paper (Whatman's #3) wicks were dipped into the supernatant and the wicks were inserted into a horizontal starch gel. Red food colour dye was used as a marker on gels. Gels were run for between 2.5 and 5 h at 30–50 mA at 4 °C in a fridge. Thereafter, gels were divided into 3–4 slices, and stained for enzymatic activity by applying specific chemical reagents in a 2% agar overlay (Shaw & Prasad 1970). The enzymes stained for and the buffer system used are provided in Table 1.

The numerical analyses were performed using the BIOSYS-1 program (Swofford & Selander 1981). Allelic and genotype frequencies were computed. Chi-square analyses were used to test if populations were in Hardy–Weinberg equilibrium. Leven's (1949) correction for small sample size was used. The mean heterozygosity per locus (Ho) for each population was calculated using Nei's (1978) unbiased estimates. The percentage of polymorphic loci in each population was determined. Loci were considered polymorphic if the frequency of the most common allele did not exceed 0.99. The mean unbiased genetic identity (*I*) and genetic distance (*D*) among the populations were calculated from the allelic frequencies according to Nei (1978).

Values of $F_{(ST)}$ can be considered to be a measure of the proportion of the genetic variation among populations. Little genetic differentiation is evident when the $F_{(ST)}$ value falls between 0 and 0.05, moderate genetic differentiation is indicated by values between 0.05 and 0.15, while values between 0.15 and 0.25 indicate great genetic differentiation and values > 0.25 very great genetic differentiation (Wright 1978). The *F* statistics, including $F_{(IS)}$ (the mean value of genetic differentiation or inbreeding coefficient within subgroups), $F_{(IT)}$ (the mean value of genetic differentiation over the entire population) and $F_{(ST)}$ (the genetic differentiation between any two subpopulations), were calculated to determine the degree of genetic differentiation amongst the populations (Wright 1965). $F_{(ST)}$ values were tested for significance using the formula given in Waples (1987), $\chi^2 = 2NF_{(ST)}(k-1)$, where N = the total number of individuals sampled and k = the number of alleles at the locus. The degrees of freedom are equal to $(k-1)(r-1)$, where r is the number of populations. Levels of differentiation were examined between the Cape Peninsula group and the Hottentot's Holland and the intermediate groups. The Hottentot's Holland group was in turn compared to the Kleinriver Mountains population (Fernkloof) and to the intermediate group. In addition, genetic differentiation within the Cape Peninsula group (Blinkwater, Silvermine, Kirstenbosch and Orange Kloof), the intermediate group (Jonkershoek, Lourens River and Sir Lowry's Pass) and the Hottentot's Holland group (Steenbras, Rooiels and Palmiet) were examined. The mean $F_{(ST)}$ for all the polymorphic loci was calculated across all the 11 populations.

Morphometrics

The carapace and limbs were measured to the nearest 0.1 mm using a digital calliper attached to a portable computer. The following carapace measurements were taken: the carapace length measured along the medial line (CL); the carapace

width at the widest part (CWW); the width of the posterior margins of the carapace (CWP); the distance between the postfrontal crest and the anterior margins of the carapace (PFCD); the distance between the medial margins of the orbits (ED); the distance between the exorbital teeth (CWA); and the carapace height (depth) (CH). The following non-carapace variables were measured: the length of the propodus of pereopod 2 (P2PL); the width of the propodus of pereopod 2 (P2PW); the length of the merus of pereopod 2 (P2ML); the width of the merus of pereopod 2 (P2MW); the length of the propodus of pereopod 5 (P5PL); the width of the propodus of pereopod 5 (P5PW); the length of the merus of pereopod 5 (P5ML); and the width of the merus of pereopod 5 (P5MW). The carapace and pereopod variables were analysed separately using discriminant functions analysis following logarithmic transformation of the variables. Groups were defined according to the genetic clusters evident from the genetic analysis. The jack-knife method was followed to calculate the classification functions. Bivariate scatter plots were drawn based on selected carapace and non-carapace variables between the two putative species identified in this study. The slopes were statistically compared using analysis of covariance (ANCOVA) in the STATISTICA software package (Stat Soft Inc.).

Qualitative analyses

Pleopod 1 and the structure of the mandibular palp were examined in all populations and drawn from samples representing each of the four population groups using a camera lucida attached to a Wild stereo microscope.

Table 2 The distribution of allele frequencies over the polymorphic loci for the 11 populations (*N* = sample size).

	Population										
Locus	1	2	3	4	5	6	7	8	9	10	11
ARK-1											
(*N*)	47	10	8	13	30	28	20	5	22	11	35
A	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
GPI-1											
(*N*)	47	10	8	13	30	28	20	5	23	11	34
B	0.968	1.000	1.000	1.000	0.983	1.000	0.913	0.000	0.000	0.000	0.000
D	0.032	0.000	0.000	0.000	0.017	0.000	0.087	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.956
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044
ME-1											
(*N*)	46	4	5	9	18	28	20	5	19	11	28
A	0.000	0.750	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000
C	1.000	0.250	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000
MDH-2											
(*N*)	45	5	8	13	25	27	20	5	23	11	29
A	0.978	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.828
B	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.155
LDH-2											
(*N*)	43	9	8	13	29	28	20	5	18	11	32
A	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–
C	1.000	0.556	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
D	0.000	0.444	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1											
(*N*)	47	9	8	13	25	28	20	5	19	11	29
A	0.000	0.278	0.250	0.192	0.000	0.000	0.000	1.000	1.000	1.000	1.000
B	1.000	0.722	0.750	0.808	1.000	1.000	1.000	0.000	0.000	0.000	0.000
GL-1											
(*N*)	42	10	2	13	30	28	20	5	23	10	35
A	0.024	0.850	1.000	0.769	0.883	0.808	1.000	1.000	0.826	1.000	0.929
B	0.976	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000
D	0.000	0.150	0.000	0.231	0.117	0.192	0.000	0.000	0.087	0.000	0.071

Table 3 The mean number of alleles per locus, the percentage of the loci that were polymorphic and the mean heterozygosity observed (Ho) amongst the 11 populations.

Population	Mean sample size	Mean number of alleles per locus	Percentage of loci polymorphic	Ho
1. Blinkwater	43.5	1.2	23.1	0.008
2. Orange Kloof	7.8	1.3	30.8	0.050
3. Kirstenbosch	7.2	1.1	7.7	0.038
4. Silvermine	12.7	1.2	15.4	0.053
5. Jonkershoek	25.0	1.2	15.4	0.010
6. Lourens River	20.0	1.1	7.7	0.006
7. Sir Lowry's River	22.5	1.1	7.7	0.013
8. Steenbras	5.0	1.0	0.0	0.000
9. Rooiels	18.6	1.2	7.7	0.007
10. Palmiet	9.5	1.0	0.0	0.000
11. Fernkloof	31.4	1.3	23.1	0.020

Results

Genetics

The allele frequencies for the seven polymorphic loci are presented in Table 2. Of the 13 loci examined, six were monomorphic (IDH-2, MDH-1, IDH-1, LT-2, MPI-1, PGM-2). The seven polymorphic loci were ARK-1, GPI-1, ME-1, MDH-2, LDH-2, PGM-1 and GL-1. The number of alleles ranged from two in ARK-1, ME-1, MDH-2, LDH-2 and PGM-1, to four in GL-1 and GPI-1. No single locus was polymorphic in all the populations. Of the 18 polymorphic cases, seven (38%) were out of Hardy–Weinberg equilibrium ($P < 0.05$), which was attributed to a deficit of heterozygotes containing rare alleles. The following populations were out of Hardy–Weinberg equilibrium for the locus identified: at GL-1 ($\chi^2 = 83.01$) in Blinkwater; at ME-1 ($\chi^2 = 7.2$) and GL-1 ($\chi^2 = 5.67$) in Orange Kloof; at GL-1 ($\chi^2 = 9.42$) in Jonkershoek; at GL-1 ($\chi^2 = 9.14$) in Lourens River; at GL-1 ($\chi^2 = 44.31$) in Rooiels; and at MDH-2 ($\chi^2 = 12.08$) and GL-1 ($\chi^2 = 26.78$) in Fernkloof. The mean number of alleles ranged from 1 to 1.3 and the percentage of polymorphic loci ranged from 0 to 30.8%, while the heterozygosity values ranged from 0 to 0.053 (Table 3).

The allele frequencies were used to determine the degree of genetic similarity between populations (Table 4). The genetic similarity between the populations was used to construct a UPGMA dendrogram. The dendrogram separated the populations into two distinct clusters at $I = 0.73$ (Fig. 2), coinciding with the geographical distribution of the populations, and it was clear that two main groups could be discerned. Three loci, ARK-1, GPI-1 and ME-1, were fixed for alternative alleles in these two population groups. Each of these two major clusters in turn consisted of two distinct subclusters. Genetic identity values within each of these two subclusters were > 0.88. The intermediate group (group B) separated from the Cape Peninsular group (group A) at $I = 0.89$, while the Fernkloof population (group D) separated from the Hottentot's Holland (group C) group at $I = 0.92$.

Population substructuring as derived from the $F_{(ST)}$ values was calculated (Table 5). When groups A and C were compared, highly significant $F_{(ST)}$ values were obtained at all loci, ranging from 0.019 to 1.00 with a mean of 0.825. Similarly, when groups A and B were compared, highly significant $F_{(ST)}$ values were obtained, ranging from 0.019 to 1.00 with a mean of 0.655. A comparison between groups B and C was significant for all loci, ranging from 0.087 to 1.00 with a mean of 0.899. Groups C and D showed significant $F_{(ST)}$ values, ranging from 0.033 to 1.00 with a mean of 0.659. Values of $F_{(ST)}$ for the four Cape Peninsula populations (group A) were considerable, with ME-1, LDH-2, PGM-1 and GL-1 showing significant $F_{(ST)}$ values ranging from 0.024 to 0.692. In the geographically intermediate group (group B), there was limited genetic structuring, and $F_{(ST)}$ values ranged from 0.043 to 0.068 with GL-1 being statistically significant. In the Hottentot's Holland population group (group C), moderate genetic structuring was evident, and a significant result was obtained for GL-1 with a mean $F_{(ST)}$

Table 4 Coefficient of unbiased genetic identity (I — above diagonal) and unbiased genetic distance (D — below diagonal) between the populations.

Population	1	2	3	4	5	6	7	8	9	10	11
1. Blinkwater	*****	0.869	0.921	0.936	0.855	0.859	0.848	0.619	0.628	0.619	0.697
2. Orange Kloof	0.141	*****	0.942	0.942	0.854	0.854	0.852	0.776	0.775	0.776	0.858
3. Kirstenbosch	0.082	0.060	*****	0.998	0.916	0.914	0.917	0.723	0.718	0.723	0.798
4. Silvermine	0.066	0.060	0.002	*****	0.918	0.919	0.914	0.708	0.708	0.708	0.787
5. Jonkershoek	0.156	0.158	0.087	0.086	*****	1.000	0.999	0.767	0.766	0.767	0.686
6. Lourens River	0.152	0.158	0.089	0.085	0.000	*****	0.997	0.764	0364	0.764	0.683
7. Sir Lowry's River	0.165	0.160	0.087	0.090	0.001	0.003	*****	0.774	0.770	0.774	0.692
8. Steenbras	0.479	0.253	0.325	0.345	0.265	0.269	0.256	*****	0.999	1.000	0.919
9. Rooiels	0.465	0.255	0.332	0.345	0.267	0.270	0.261	0.001	*****	0.999	0.918
10. Palmiet	0.479	0.253	0.325	0.345	0.265	0.269	0.256	0.000	0.001	*****	0.919
11. Fernkloof	0.360	0.154	0.226	0.240	0.377	0.381	0.369	0.084	0.085	0.084	*****

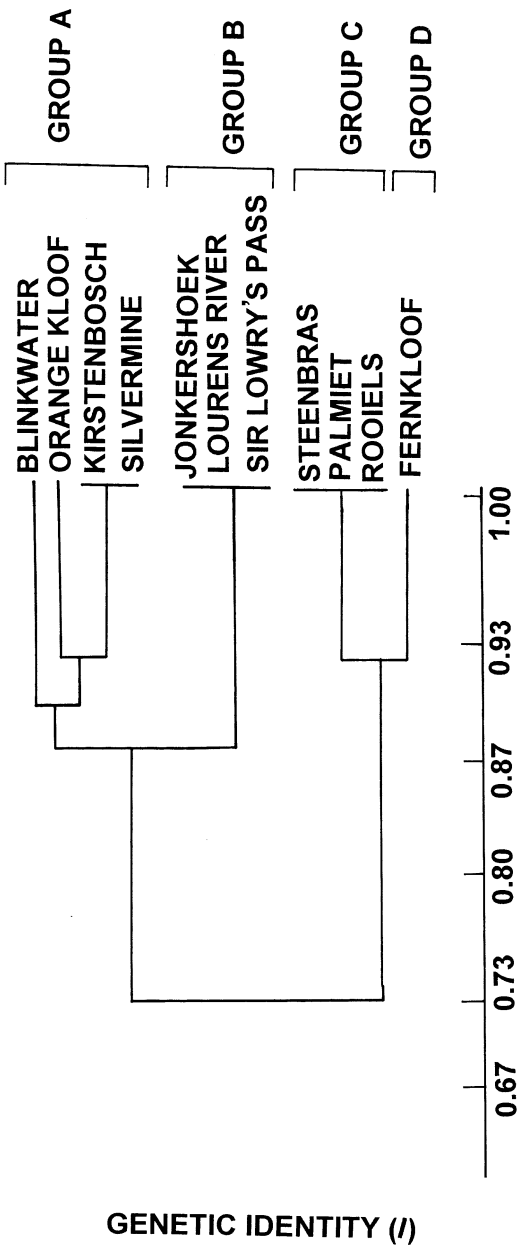

Fig. 2 UPGMA analysis based on Nei's genetic distance.

of 0.085. The pairwise $F_{(ST)}$ amongst all the 11 populations ranged from 0.129 (MDH-2) to 1.00 (ARK-1), with a mean at 0.838. This indicates considerable genetic differentiation between the populations. The mean $F_{(IS)}$ value between all the 11 population groups was 0.367. $F_{(IT)}$ ranged from 1.00 (ARK-1 and ME-1) to 0.524 (MDH-2) with a mean of 0.897.

When geographical distance (km) between population sites was plotted against genetic identity (*I*) values for the two main population groups (Fig. 3), a clear relationship was found between genetic and geographical distance for the Cape Peninsula and the intermediate groups (group 1) ($Y = 0.0467X + 0.002$; $r = 0.026$; $P < 0.05$). However, no genetic patterns existed with geographical distance for the Hottentot's Holland and Fernkloof groups (group 2) ($Y = 0.372X - 0.024$; $r = -0.026$; $P > 0.05$).

Morphology

A two-dimensional plot of the individual scores along the first two canonical variables based on the logarithmically transformed carapace measurements for the four genetically defined population groups showed that four distinct groupings were present (Fig. 4). The first two canonical variables contributed 90.03% to the total variation between groups (Table 6).

The classification functions for the four population groups are presented in Table 7, and give a moderate degree of support that the four genetically distinct population groups can be distinguished using morphometric criteria. In addition, a two-dimensional plot of the individual scores along the first two canonical variables based on the logarithmically transformed pereopod measurements for the four population groups showed differentiation (Fig. 5). The first two canonical variables contributed 97.55% to the total variation between groups (Table 8). The classification functions for the four population groups showed a moderate degree of differentiation (Table 9).

The slopes of the regression of carapace width anteriorly (CWA) against carapace length (CL) were significantly different ($F = 152.46$; $P < 0.01$), with group C (*P. brincki*) being broader than specimens of the same length in group A. The slopes of the regressions for the carapace height (CH) against carapace length (CL) for the two taxa could be discerned statistically ($F = 110.07$; $P < 0.01$), with *P. brincki* being proportionally deeper bodied than group A. However, group A was proportionally broader in its carapace width posteriorly (CWP) ($F = 123.82$; $P < 0.01$) and in the distance between the medial margins of the orbits (ED) ($F = 11.84$; $P < 0.01$) relative to CL when compared to group C (*P. brincki*) (Fig. 6). When the slope of the carapace widest width (CWW) was compared between the two taxa relative to CL, the slope was significantly different ($F = 39.12$; $P < 0.01$). Group C (*P. brincki*) specimens are larger in CWW relative to specimens of a similar length in group A. The distance between the postfrontal crest and the anterior margin of the carapace (PFCD) was plotted against the carapace length (CL) and compared between the two groups (*P. brincki* and group A). No significant differences in the slopes of these regressions could be detected ($F = 0.010$; $P > 0.05$).

The slopes of the regression for the width of the merus of pereopod 2 (P2MW) regressed over the length of the merus of pereopod 2 (P2ML) were statistically different ($F = 23.89$;

Table 5 $F_{(ST)}$ values within and amongst the three population groupings (NS = not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

	Locus							
Group	ARK-1	GPI-1	ME-1	MDH-2	LDH-2	PGM-1	GL-1	Mean
Cape Peninsula and Hottentot's Holland Group	1.00 ***	0.982 ***	0.892 ***	0.019 NS	0.407 ***	0.688 ***	0.623 ***	0.825 ***
Cape Peninsula and intermediate group	1.00 ***	0.047 **	0.720 ***	0.019 NS	0.407 ***	0.159 ***	0.570 ***	0.655 ***
Intermediate group and Hottentot's Holland group	—	0.938 ***	1.00 ***	—	—	1.00 ***	0.087 **	0.899 ***
Hottentot's Holland group and Fernkloof	1.00 ***	0.033 NS	—	0.122 ***	—	—	0.065 ***	0.659 ***
Cape Peninsula	—	0.024 NS	0.692 ***	0.017 NS	0.375 **	0.080 *	0.668 ***	0.485 ***
Intermediate group	—	0.043 NS	—	—	—	—	0.068 *	0.061 ***
Hottentot's Holland	—	—	—	—	—	—	0.085 ***	0.085 ***
Overall	1.00 ***	0.936 ***	0.931 ***	0.129 ***	0.421 ***	0.798 ***	0.571 ***	0.838 ***

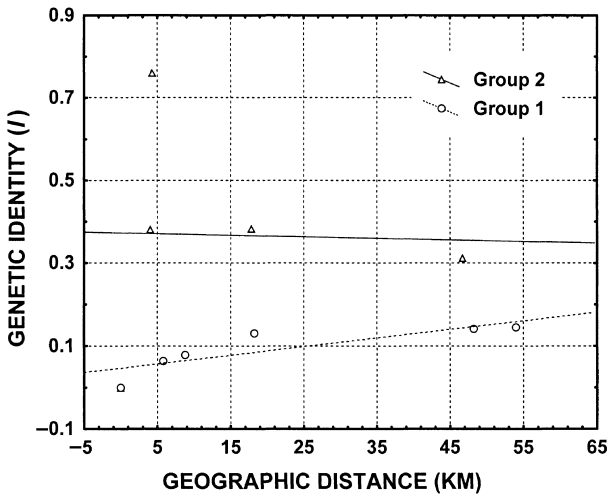

Fig. 3 Scatter plot of geographical distance (km) against Nei's (1978) genetic distance.

$P < 0.01$), with group C specimens being broader than specimens of group A (Fig. 7). The width of the propodus of pereopod 2 (P2PW) was compared against the length of the propodus of pereopod 2 (P2PL), and was found to be statistically significant ($F = 216.44$; $P < 0.01$), with group A being proportionally larger than group C (*P. brincki*) specimens of a similar size. The slopes of the regressions for the merus width of pereopod 5 (P5MW) were compared to the merus length of pereopod 5 (P5ML) and were found to be statistically significant ($F = 6.52$; $P < 0.05$) with group C (*P. brincki*) specimens being larger than those in group A.

Table 6 Relative contributions of the three canonical variables, calculated for the discriminant functions analysis based on the carapace variables for the four genetically defined population groups.

Canonical variable	Cumulative percentage	Eigen value
Canonical variable 1	59.62	1.276
Canonical variable 2	90.03	0.903
Canonical variable 3	100.00	0.206

Table 7 Percentage correct *a posteriori* classification to groups based on the morphometric classification function of the carapace variables for the four genetically defined population groups.

	Population group				
Group	A	B	C	D	Correct classification (%)
Group A	81	2	13	1	83.50
Group B	10	71	17	1	71.71
Group C	8	0	90	10	83.33
Group D	2	0	13	20	57.14

When the width of the propodus of pereopod 5 (P5PW) ($F = 108.76$; $P < 0.01$) was compared against the length of the propodus of pereopod 5 (P5PL), group A specimens were proportionally larger than specimens of group C (*P. brincki*).

Qualitative analyses

Mandibular palp. All specimens falling into groups A and B had similar mandibular palps, with the terminal segment

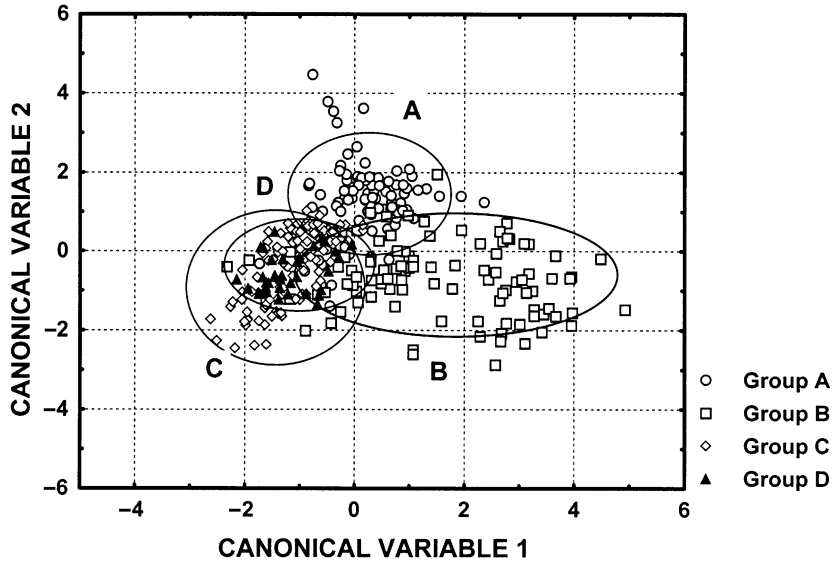

Fig. 4 Polygons encompassing the individual scores for the four genetically distinct population groups based on the logarithmically transformed carapace variables. Group A (Cape Peninsular group); group B (intermediate group); group C (Hottentot's Holland group); group D (Fernkloof population).

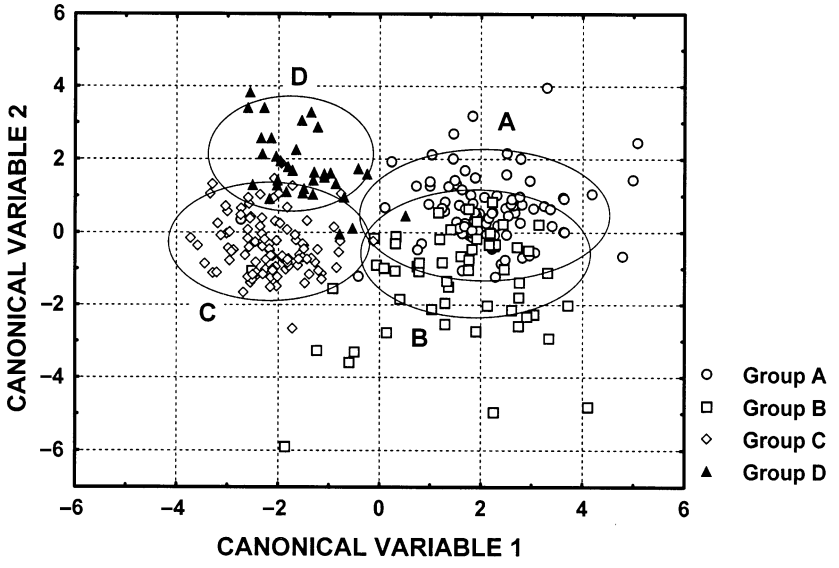

Fig. 5 Polygons encompassing the individual scores for the four genetically distinct population groups based on the logarithmically transformed pereopod variables. Group A (Cape Peninsular group); group B (intermediate group); group C (Hottentot's Holland group); group D (Fernkloof population).

Table 8 Relative contributions of the three canonical variables, calculated for the discriminant functions analysis based on the pereopod variables for the four genetically defined population groups.

Canonical variable	Cumulative percentage	Eigen value
Canonical variable 1	78.54	3.856
Canonical variable 2	97.55	0.933
Canonical variable 3	100	0.120

bearing a dense tuft of setae on the proximal third of the segment. There was no ridge on the terminal segment. However, in the Hottentot's Holland group (group C) and Fernkloof group (group D), the terminal segment consistently contained a dense tuft of setae arising from a flange or a ridge on the proximal third of the segment (Fig. 8).

Pleopod (gonopod) 1. The structure of pleopod 1 was similar in the Cape Peninsula group (group A) and the intermediate group (group B). Pleopod 1 was characterized by a smooth terminal segment curving away at the midpoint when viewed posteriorly, widest at the base, ending in a pointed tip that curved slightly. The terminal segment was more strongly curved in groups A and B than in groups C and D (Fig. 9). In addition, the inner lateral margin of the subterminal segment of pleopod 1 was markedly irregular in groups A and B, while it was only slightly irregular in groups C and D.

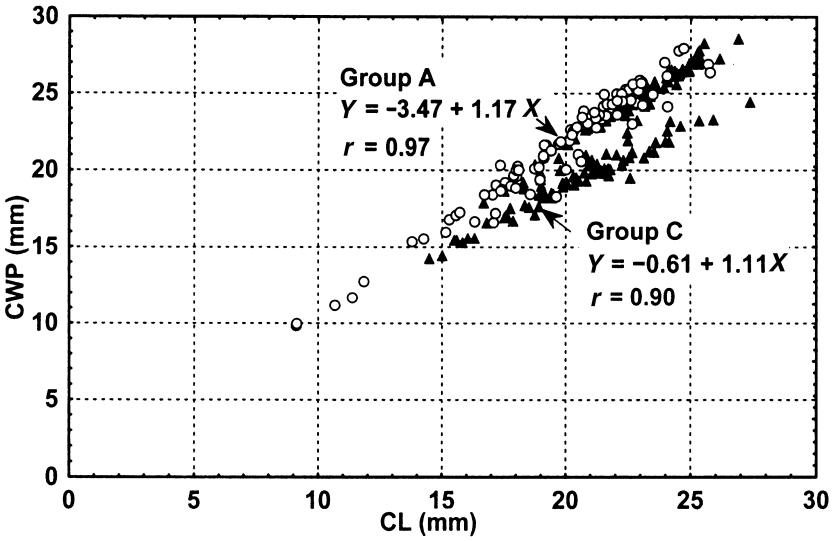

Fig. 6 Comparison of the regression of CWP (carapace width posterior) over CL between groups A and C (*P. brincki*).

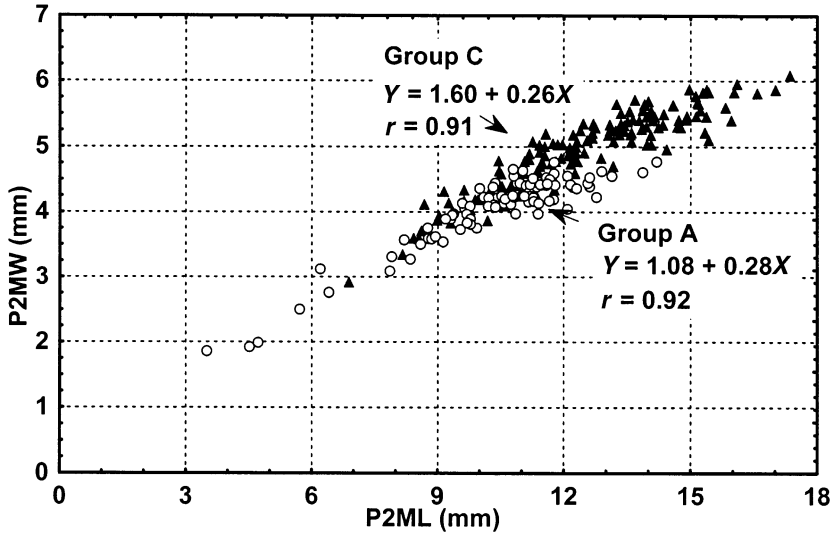

Fig. 7 Comparison of the regression of P2MW (pereopod 2 merus width) over P2ML (pereopod 2 merus length) between groups A and C (*P. brincki*).

Table 9 Percentage correct *a posteriori* classification to groups based on the morphometric classification function of the pereopod variables for the four genetically defined population groups.

	Population group				
Group	A	B	C	D	Correct classification (%)
Group A	80	7	2	1	88.88
Group B	14	38	4	0	67.85
Group C	0	0	100	3	97.08
Group D	0	1	5	28	82.35

Discussion

Congruent patterns of genetic and morphological data strongly suggest the presence of two distinct species. Not only do these two main groups A (Cape Peninsula) and C (Hottentot's Holland) separate at a genetic *I* of 0.73, but their genetic distinctiveness is further corroborated by the high mean $F_{(ST)}$ values. The presence of fixed allelic differences at three loci (ARK-1, GPI-1 and ME-1) between groups A and C indicate that they are genetically isolated, with no gene flow occurring presently. Furthermore, the presence of a distinct flange on the proximal third of the terminal segment of the mandibular palp in one of the putative species and the occurrence of considerable differences in the structure of pleopod 1 between the two putative species provide further support for the recognition and delimitation of a species boundary. Gouldstein *et al.* (2000) argue that the delineation of species boundaries should be robust and include as many fixed characters as are available. This is clearly the case in the present study, with both the genetic and morphological data strongly supporting the presence of two distinct species.

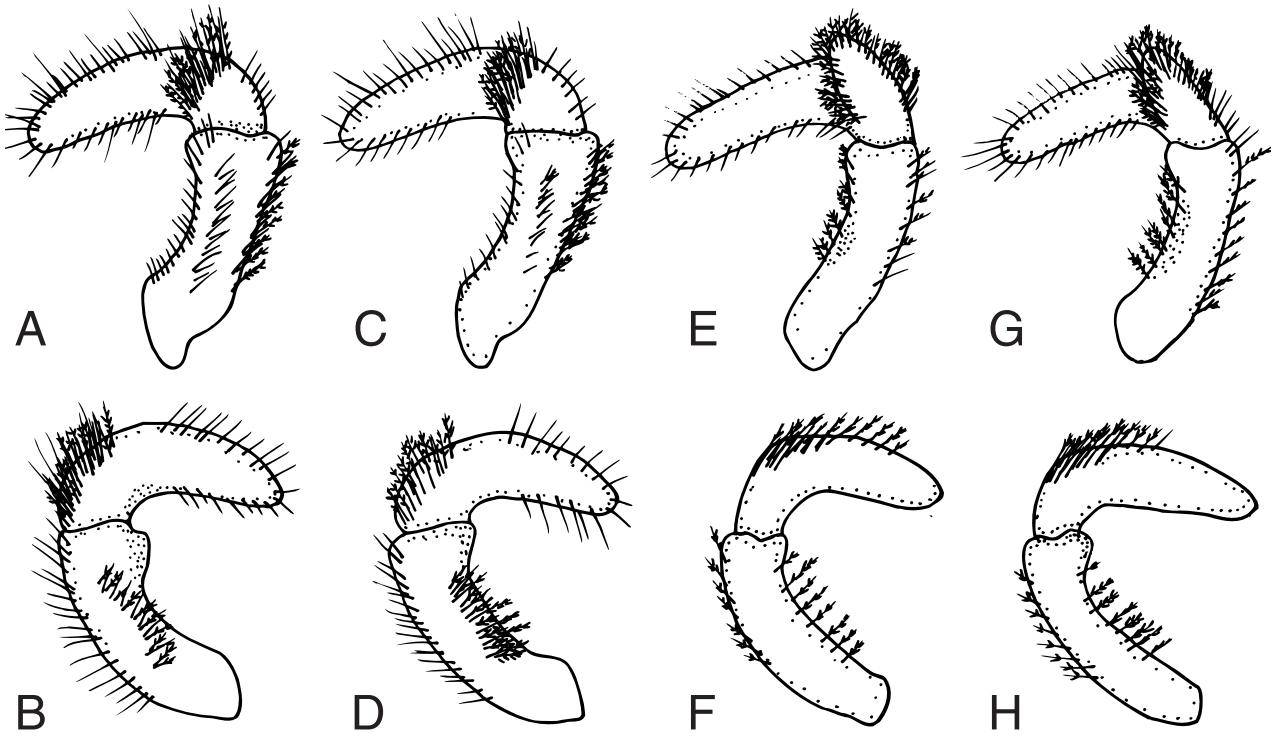

Fig. 8 A–H. Left mandibular palp. —A, B. Blinkwater. —A. Posterior view. —B. Anterior view. —C, D. Jonkershoek. —C. Posterior view. —D. Anterior view. —E, F. Rooiels. —E. Posterior view. —F. Anterior view. —G, H. Fernkloof. —G. Posterior view. —H. Anterior view.

Populations collected from the geographically intermediate group B (Jonkershoek, Lourens River, Sir Lowry's Pass) were genetically (I = 0.88) and morphologically similar to group A (Cape Peninsula). This group lacked the flange on the terminal segment of the mandibular palp, and had a very irregular margin on pleopod 1. However, groups A and B were fixed for an alternative allele at ARK-1. Group C populations (Hottentot's Holland) were genetically (I = 0.91) and morphologically similar to group D (Fernkloof) with all specimens possessing the distinct flange or ridge on the terminal segment of the mandibular palp, and having a relatively smooth inner margin on pleopod 1. A fixed allelic difference at ARK-1 is also present between groups C and D.

Interspecific genetic identity values obtained for freshwater crabs studied thus far have generally been less than 0.85. The I value obtained in the current study (I = 0.73) falls within the range reported for congeneric taxa. For example, the genetic I between *P. perlatus* and *P. parvispina* was at 0.68 (Stewart 1997b), between *P. unispinus* Stewart & Cook, 1998 and *P. sidneyi* (Rathbun, 1904) at 0.66 (Stewart & Cook 1998), between *P. sidneyi* and *P. lividus* Gouws, Stewart & Reavell, 2001 at 0.82 and between *P. depressus depressus* (Krauss, 1843) and *P. clarus* Gouws, Stewart & Coke, 2000 at 0.59 (Gouws 1999). Suzuki & Okano (2000) recently reported that three freshwater crab species in the genus *Geothelphusa* Stimpson, 1859 could also be separated at a genetic $I < 0.85$. Similar results have been reported for other marine decapod crustaceans. For example, Bert (1986) reported a genetic I of 0.46 for two allopatric stone crab species of the genus *Menippe*, Sbordoni *et al.* (1990) reported a mean I value of 0.79 for two genetically divergent populations of the cave-dwelling shrimp *Troglocaris anophthalmus* (Kollar, 1848), while Thompson (1996) reported an I value of 0.759 for two species of rock lobster of the genus *Panulirus*. Thorpe & Sole Cava (1994) showed that congeneric invertebrate taxa have I values that range between 0.34 and 0.85. The high $F_{(ST)}$ value reported in this study indicates that there is substantial genetic differentiation between the 11 populations examined. The high mean of 0.838 indicates that 83.8% of the total genetic variation results from differences between populations, while 16.2% results from variation within populations. Mean interspecific $F_{(ST)}$ values reported between freshwater crab species generally exceed 0.60. For example, the mean $F_{(ST)}$ value between populations of *P. perlatus* and *P. granularis* was 0.645 (Daniels *et al.* 1999a,b); between *P. sidneyi* and *P. perlatus* it was 0.672 (Gouws 1999); between *P. sidenyi* and *P. lividus* it was 0.645 (unpublished data) and between *P. depressus depressus* and

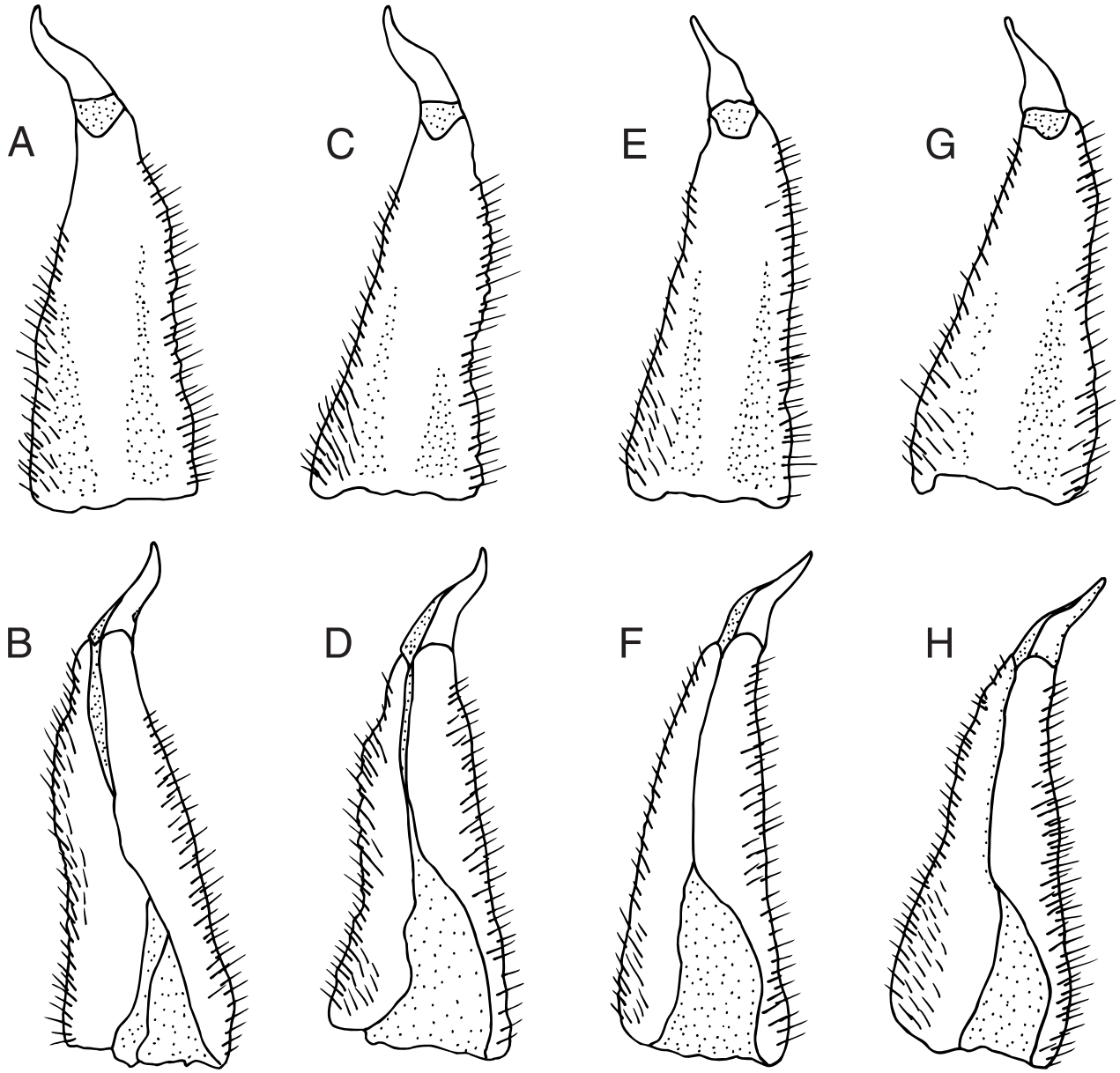

Fig. 9 A–H. Left pleopod 1. —A, B. Blinkwater. —A. Posterior view. —B. Anterior view. —C, D. Jonkershoek. —C. Posterior view. —D. Anterior view. —E, F. Rooiels. —E. Posterior view. —F. Anterior view. —G, H. Fernkloof. —G. Posterior view. —H. Anterior view.

P. clarus it was 0.872 (Daniels 2001, unpublished data). The mean $F_{(ST)}$ value obtained between the Cape Peninsula and the Hottentot's Holland populations clearly falls within the range reported for congeneric taxa.

Intraspecific levels of genetic differentiation for freshwater crabs are generally low and have been found to be greater than $I = 0.85$ (Daniels *et al*. 1998, 1999a,b). Conspecific populations generally show slight allele frequency differences at a few loci, while congeneric taxa are fixed for alternative alleles at certain loci. Limited genetic variation as derived from the genetic I values is evident within each of the four groups. However, $F_{(ST)}$ values within each of the four groups showed that some genetic structuring was present. In group A, a moderate degree of genetic structuring was evident, while in groups B and C limited genetic structuring was observed. $F_{(ST)}$ values between catchments for three mountain stream river crabs were at 0.032, 0.085 and 0.127 for *P. parvispina*, *P. clarus* and *P. depressus depressus*, respectively (Daniels *et al*.

1998; 2001, unpublished data). These results indicate that genetic variation in mountain stream taxa may be moderate to high. Hughes *et al.* (1995) reported that the $F_{(ST)}$ values vary in the freshwater shrimp *Paratya austarliensis* and are largely dependent on the spatial level examined, with populations within streams having an $F_{(ST)}$ of 0.06 while those from different catchments having a mean of 0.57. The large $F_{(ST)}$ values obtained in the present study suggest that limited dispersal is occurring between populations. More recently, Wishart & Hughes (2001) reported that populations of the net-winged midge, *Elporia barnardi*, distributed on Table Mountain had a mean $F_{(ST)}$ of 0.24, while those at Jonkershoek had a mean of 0.02, with the total variation between the two groups being at 0.39. These results are particularly interesting because the freshwater crabs examined from these two areas, Table Mountain and Jonkershoek, showed very similar patterns of genetic differentiation, with a high mean $F_{(ST)}$.

Generally, intraspecific $F_{(ST)}$ values have been reported to be considerably low for crustaceans. For example, Creasey *et al.* (1997) reported that in the majid spider crab, *Encephaloides armstrongi* (Wood Mason, 1891), the mean $F_{(ST)}$ was at 0.005. Similarly, two studies on the giant tiger prawn, *Penaeus monodo* (Fabricius, 1798), by Benzie *et al.* (1992) and Forbes *et al.* (1999), reported mean values of 0.031 and 0.007, respectively. Passamonti *et al.* (1997) reported a mean $F_{(ST)}$ of 0.059 for the Norwegian lobster, *Nephrops norvegicus* (L). These results are not surprising considering that all the above-mentioned decapods are marine species, and the marine environment is generally more continuous as opposed to freshwater environments.

Heterozygosity values for the freshwater crabs were low within each of the groups, and ranged from 0.002 in group C, to 0.009 in group B, to 0.020 in group D to 0.037 in group A. Low heterozygosity values are well documented amongst potamonautid crabs (Daniels *et al.* 1998, 1999a,b) and are generally below the mean of 0.048 calculated for decapods (Hedgecock *et al.* 1982).

The question now arises as to whether the two groups A and C are sufficiently divergent to be two separate species. When species are in sympatry, a single fixed locus can indicate that interbreeding is not occurring. Richardson *et al.* (1986) suggested that allopatric populations fixed at more than 20% of all loci can be regarded with confidence as separate biological species. In the present study, 43% (three of seven) of all loci examined between groups A and C were fixed, thus supporting the idea that group A is a new and as yet undescribed species. Defining populations that are in allopatry as distinct species is often met with difficulty, as the potential for gene exchange (reproduction) cannot truly be assessed. The biological species concept defines a species as 'a group of interbreeding or potentially interbreeding populations, reproductively or genetically isolated from other such groups' (Mayr 1964). Bock (1992) identifies three major properties which are possessed by fully evolved species, namely genetic, reproductive and ecological isolation. He further argues that some species may not have all of these properties totally developed, and that it is not always necessary to demonstrate reproductive isolation for all species. Groups A and C are genetically isolated from each other, thus complying with one of the criteria as proposed by Mayr (1964), and should thus be considered as distinct species. Group B is genetically conspecific to group A, and morphologically similar to this group, while group D is also conspecific to group C and morphologically similar to this group. These results may thus indicate that groups B and D may have diverged recently from groups A and C, respectively, as only a single fixed allelic difference exists between these population groups, indicating a lack of gene flow. The phylogenetic species concept defines a species as 'the smallest diagnosable cluster of individual organisms with which there is a parental pattern of descent' (Cracraft 1989). Arguably, the flange or ridge on the terminal segment of the mandibular palp represents such a diagnostic heritable trait. However, the monophyly of this trait is unknown. Considering the congruence between the genetic and morphological data sets, these distinct allopatric lineages should best be regarded as separate species (Sites & Crandall 1997).

Spatial patterns of genetic variation are generally at present not environmentally dependent and are thus likely to reflect historical and contemporary factors that affected the population structure (Riddle 1996). Using the genetic *I* values, and two molecular clocks for allozyme data (Nei & Roychoudhury 1974; Yang *et al.* 1974), the time of divergence between groups A and C falls between the Pliocene/Miocene, while the divergence between groups A and B and groups C and D falls between the Pleistocene/late Pliocene. Climatological and geological changes that have occurred during this time period are likely to have resulted in the contraction and expansion of inland taxa. Throughout the Miocene, the climate oscillated between warmer and cooler phases, associated with eustatically induced transgressions (higher sea levels) and regressions (lower sea levels) of sea level, respectively. Sea level changes altered the climate, mainly affecting rainfall and temperature. Transgressions were typically associated with higher temperatures and an increase in rainfall; regressions were characterized by increased aridity and lower temperatures (Deacon 1985). During the late Miocene, the climate became cooler and progressively more arid. These xeric climatic conditions were further aided by the development of the Benguela current. At the beginning of the Pliocene, sea levels started to rise again, although not as high as in the Miocene. By the late Pliocene, sea levels were more or less similar to those in the

Pleistocene (Tyson 1986). Groups A and C may have speciated during the regression that occurred at the end of the late Miocene/early Pliocene. Subsequent changes in sea level and climate during the late Pliocene/early Pleistocene resulted in more arid conditions and led to the fragmentation between groups A and B and between groups C and D.

The endemic aquatic invertebrates in the Western Cape typically occupy palaeogenic zones (upper reaches of forest streams, riverine forest and caves). Evidence is emerging that suggests that these high mountain stream invertebrates are highly specious, and that each mountain possesses a unique biota. Studies on the population genetic structure of mountain stream invertebrates have reported considerable genetic divergence between taxa from the Cape Peninsula Mountains and the Hottentot's Holland Mountains. The consistent west/east split suggests that an environmental barrier(s) may exist that prevents gene flow between mountain ranges. For example, the data from the present study, as well as data on the freshwater amphipod genus *Paramelita* (Schellenberg, 1926), studies on the net-winged midge species, *Elporia barnardi*, and recent work on the freshwater isopod genus, *Mesamphisopus* (Nicholls, 1943), support the hypothesis that at least two distinct biogeographical areas exist on mountain chains in the Western Cape (Stewart 1992; Wishart & Hughes 2001; G. Gouws 2001, personal communication). These results support the hypothesis that considerable speciation has occurred between the freshwater mountain stream species found on Cape Peninsula and those on Hottentot's Holland Mountains. Although the effect of climatological and geological change had a profound influence on the population genetic structure and distribution of these aquatic organisms, the fact that the three freshwater crustacean species (*Mesamphisopus*, *P. brincki* and *Paramelita*) so far examined are direct developers may further have contributed to the pattern of genetic differentiation that is being observed.

Morphometrically and morphologically, groups A and C are distinct, as evident in the structure of the mandibular palp and the consistent difference in the structure of pleopod 1. Morphometrically the two main population groups can be distinguished easily, corroborating the genetic distinctiveness of groups A and C. As *P. brincki* was described from a population collected on the Hottentot's Holland Mountains (group C), the Cape Peninsula form, group A, is thus a new species, the description of which follows.

Description of taxa

Genus *Potamonautes* MacLeay, 1838

Potamonautes parvicorpus sp. n. (Figs 10, 11)

Holotype. 1♂, SOUTH AFRICA, Liesbeek River, 30 January 1990, M. Hill (SAM A 44166).
Paratypes. Same data as for holotype, 5♂ 4♀ (SAM A 41141).
Other material. Blinkwater Ravine, 3♂ 3♀, 3 May 1993, B. A. Stewart & A. Mader (SAM A 41136); Blinkwater Ravine, 1♀, November 1971, anonymous collector (SAM A 41190); Orange Kloof, Table Mountain, 5♂ 5♀, 28 September 1994, J. Hulley (SAM A 41195); Noordhoek, De Goede Hoop River, 2♂ 4♀, 26 July 1996, A. Mader (SAM A 44167); Platteklip, Table Mountain, 4♂ 5♀ 1 juvenile, November 1999, M. Wishart (SAM A 44172); Silvermine, 1♀, November 1999, M. Wishart (SAM A 44168); Vishoek, 5 juveniles, November 1999, M. Wishart (SAM A 44171); Skeleton Gorge, 2♂ 1♀ 4 juveniles, September 1999, M. Wishart (SAM A 44173); Disa Stream, 1♂ 2♀2 juveniles, September 1999, M. Wishart (SAM A 44169).
Etymology. The specific epithet has been taken from the Latin, 'parvus', meaning small, alluding to the small body of the animal, while the Latin, 'corpus' refers to the body. The specific name hence is an adjective, agreeing in gender with the (masculine) generic name.

Description

Male (holotype). Measurements of the holotype are given in Table 10. Carapace and limbs dark chocolate brown fading to a lighter shade of brown when preserved (Fig. 10A–C). Cephalothorax ovoid, distinctly arched, maximum height and width at anterior third (ratio CH/CL = 0.54, CWW/CL = 1.38) (Fig. 10A). Anterior margin of front slightly indented. Carapace smooth, urogastric and cardiac groove moderately deep. Exorbital teeth blunt, epibranchial teeth absent. Anterolateral margin between exorbital teeth and postfrontal crest finely granulated, lacking teeth. Anterolateral margins posterior to postfrontal crest finely granulated, margin not curving inward over the surface of carapace in branchial region. Postfrontal crest poorly developed posterior to eye orbits fading at midpoint, with a short-forked groove at midpoint. Each flank with longitudinal groove. Sternites 1 and 2 fused, no suture present. Suture between sternites 2 and 3 complete, deep suture between 3 and 4 complete, shallow lateral part sloping sharply towards abdomen. The third maxilliped fills the buccal frame except for a small oval efferent respiratory opening (Fig. 11B). Flagellum present on exopod; ischium smooth, with faint vertical groove.

Mandibular pulp with two segments, terminal segment undivided, with an extremely dense tuft of pinnate setae on the proximal third of the segment (Fig. 11C,D). Chelipeds markedly unequal, dactylus of right cheliped slightly arched, both dactyli armed with several small to medium cutting teeth (Fig. 11H,I). Propodus of right cheliped more swollen than the left, pollex armed with small cutting teeth. In both chelipeds, carpus with one large prominent spine and two rudimentary teeth, anteroinfero margin of merus with no spine. Pereopodus slender, pereopods armed with sharp point, margins of dactyli of P2–P5 smooth.

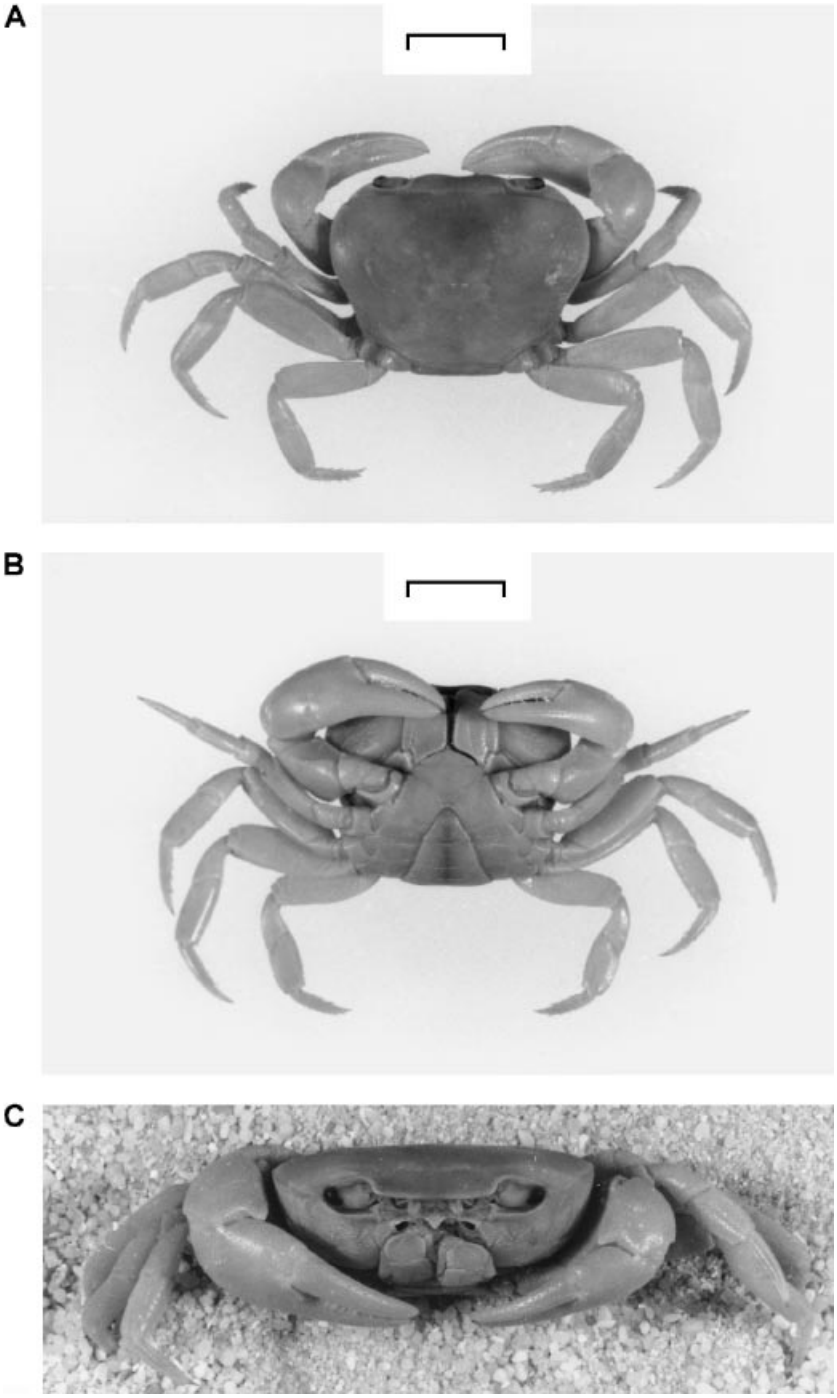

Fig. 10 A–C. *Potamonautes parvicorpus* sp. n. Male (M = male), holotype, Liesbeek River, Cape Peninsula, South Africa (CL = 18.36 mm) (SAM A 44166). —A. Whole animal, dorsal aspect. —B. Whole animal, ventral aspect. —C. Cephalothorax, frontal aspect. Scale bar = 10 mm.

Pleopod 1 (gonopod 1), terminal segment short, 0.25 length of the subterminal segment, terminal segment curving away from the midline when viewed posteriorly, widest at base, ending in a pointed tip (Fig. 11E,F). Subterminal segment of pleopod 1 tapering distally, inner lateral margin slightly. Irregular, posterior surface with twisting longitudinal groove, running length of both subterminal and terminal segments, anterior surface lacking longitudinal groove. Pleopod 2 (gonopod 2), hollow, about 0.65 length of subterminal segment, widest at base, tapering sharply inward at 0.4 times length, forming a narrow upright process supporting terminal segment (Fig. 11G).

Variation. The anterolateral margins of juveniles may be beaded, but become smooth as the crab matures. Both sexes exhibit heterochely, with 86.84% of males being right

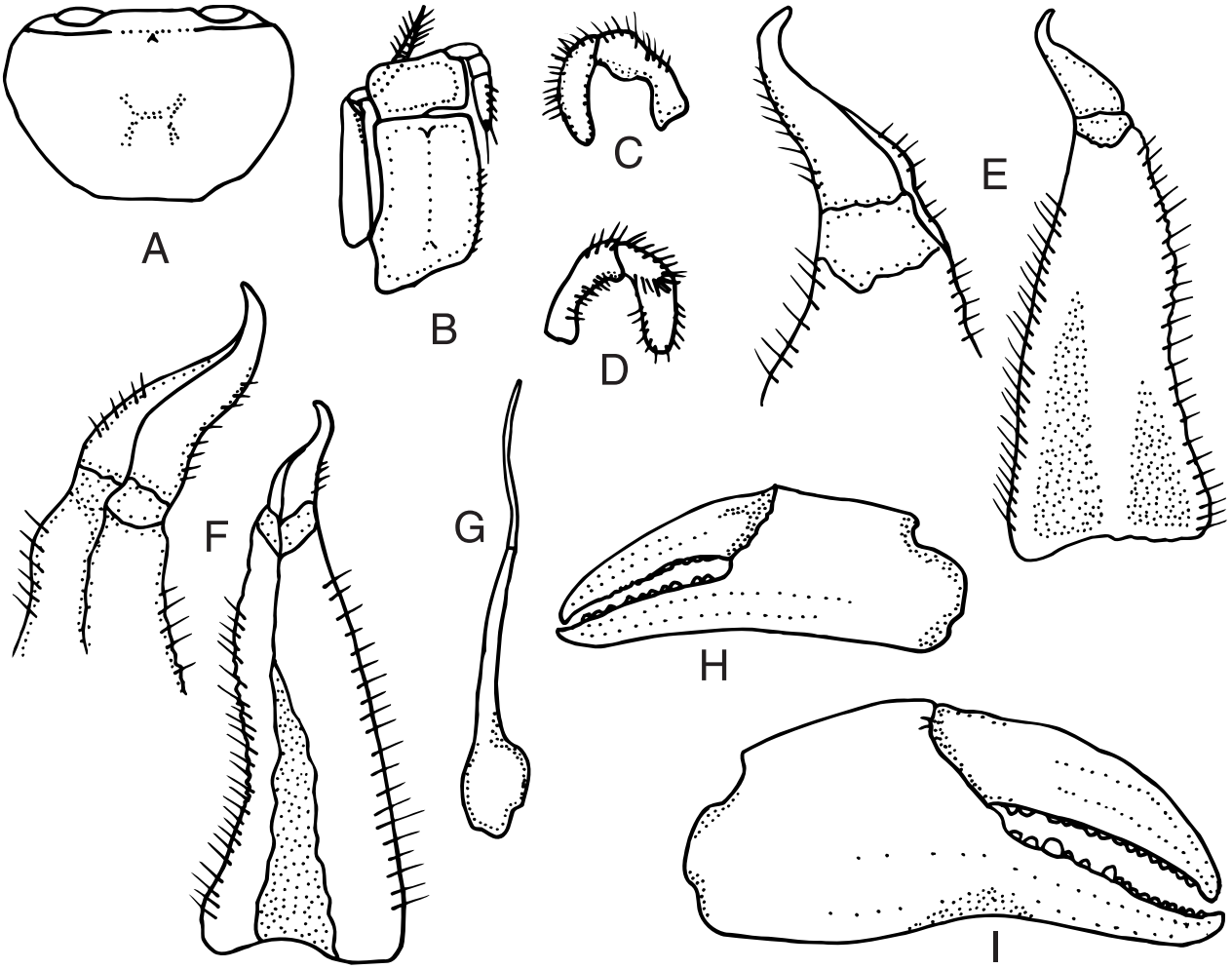

Fig. 11 A–I. *Potamonautes parvicorpus* sp. n. Male (M = male), holotype. —A. Carapace outline. —B. Right third maxilliped. —C. Right mandibular palp, anterior view. —D. Right mandibular palp, posterior view. —E. Right pleopod 1, posterior view. —F. Right pleopod 1, anterior view. —G. Right pleopod 2, anterior view. —H. Left cheliped, dactylus and propodus. —I. Right cheliped, dactylus and propodus. Scale bar = 10 mm.

Table 10 Measurements (in mm) of the holotype and ranges of measurements for *Potamonautes parvicorpus* sp. n.

Variable	Abbreviation	Holotype	Males	Females
Carapace length	CL	18.36	24.08–11.85	24.71–9.13
Carapace widest width	CWW	25.31	36.24–15.9	34.99–11.41
Carapace width posteriorly	CWP	17.33	26.38–12.75	27.95–9.84
Carapace height	CH	10.02	13.48–5.68	13.12–4.47
Distance between postfrontal crest and the anterior margins of the carapace	PFCD	2.72	4.27–2.06	3.82–1.42
Distance between orbits	ED	10.07	13.28–7.03	14.07–4.73
Distance between exorbital teeth	CWA	11.57	24.09–12.57	23.84–9.27
Width of sixth abdominal segment	AW6	3.26	7.63–3.62	21.57–3.10
Major cheliped propodus length	MCPL	15.73	25.08–9.60	19.15–6.59
Major cheliped propodus height	MCPH	5.93	12.81–3.34	10.84–2.41
Pereopod 2, merus length	P2ML	5.94	14.20–6.21	12.62–1.92
Pereopod 2, merus width	P2MW	2.92	4.77–2.76	4.44–1.92

handed and 13.15% being left handed; in females, 4% were homochelic, 78.87% right handed while 17% were left handed.

Remarks. Collected from the Liesbeek River system, Table Mountain, Cape Town, South Africa. This species is known from high mountain streams on the Cape Peninsular where it prefers unpolluted streams with leaf cover and small boulders.

P. parvicorpus sp. n. bears superficial resemblance to other South African freshwater crabs. The new species can easily be distinguished from the five freshwater crab species with toothed epibranchial corners: these are *P. parvispina* Stewart, 1997b, *P. dentatus* Stewart, Coke & Coke, 1995, *P. warreni* Calman, 1918, *P. unispinus* and *P. obesus calcaratus* (Bott, 1955). In *P. parvispina*, a small but distinct tooth is present on the epibranchial corner and this species is restricted to upper tributaries of the Berg and Olifants River systems. In *P. dentatus*, the anterolateral margin is characterized by a series of teeth, and this species is endemic to KwaZulu-Natal. *P. warreni* is distinct from the new species as it possesses a single tooth or a series of well-defined teeth on the anterolateral margins of the carapace, and is restricted to the Orange and Vaal River systems. *P. unispinus* is characterized by the presence of a single spine on the anterolateral margins of the carapace. In *P. obesus calcaratus*, the anterolateral margin possesses a single spine-like tooth. All these species, with the exception of *P. parvispina* and *P. obesus calcaratus*, occur in large river systems. *P. parvicorpus* sp. n. bears a number of similarities to *P. brincki* (Stewart 1997a); however, the new species does not have a flange on the terminal segment of the mandibular palp, and is genetically distinct from *P. brincki*. In addition, the structure of pleopod 1 is markedly different between *P. brincki* and *P. parvicorpus* sp. n. In both *P. depressus depressus* and *P. clarus*, the limbs are slender, and both are genetically distinct from the new species. *P. depressus depressus* is brown to green–brown in colour, while *P. clarus* is bright orange in colour. *P. lividus*, a species restricted to swamp forests, is orange to red with a silver–blue sheen and is characterized by an inflated carapace, with highly arched chela. *P. perlatus*, *P. sidneyi* and *P. granularis* are all large species, where the postfrontal crest is well defined and beaded. These species generally live in larger river systems, and are often absent from high mountain streams.

Further afield, a number of freshwater crab species bear superficial resemblance to *P. parvicorpus* sp. n. For example, *Potamonautes pilosus* (Hilgendorf, 1898) and *P. neumanni* (Hilgendorf, 1898) are restricted to East Africa, with *P. pilosus* possessing a depressed carapace while *P. neumanni* has a vaulted carapace. In *P. ballayi adentatus* (Bott, 1955), the postfrontal crest is complete and individuals possess large gaping chela with slender fingers. The terminal segment of pleopod 1 in both *P. loveridgei* (Rathbun, 1933) and *P. macrobrachii* (Bott, 1953) is markedly different from that of *P. parvicorpus* sp. n. *P. suprasulcatus suprasulcatus* (Hilgendorf, 1898) possesses large, stout chelipeds and a finely granulated anterolateral margin. In both *P. walderi* (Colosi, 1924) and *P. bipartitus* (Hilgendorf, 1898), the medial invagination and postfrontal crest are concave.

Acknowledgements

Thanks to the National Research Foundation, WWF-South Africa and the University of Stellenbosch for financial support, and the latter institute for providing a vehicle to collect crabs. Thanks are also due to the private landowners for allowing us to collect crabs on their property. Alex Searby and Gavin Gouws helped to collect crabs at Jonkershoek and Fernkloof, while Marcus Wishart collected additional material of the Cape Peninsula species. Liz Hoenson is thanked for her help at the South African Museum, and Michelle van der Merwe for photographing the holotype.

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