Allozyme variation amongst populations of the freshwater crab, *Potamonautes perlatus* (Decapoda: Potamonautidae) in the Berg River system, Western Cape

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The Cape river crab, *Potamonautes perlatus*, is widely distributed in streams and rivers of the Western Cape where it exhibits considerable morphological variation. Recent genetic work on populations in the Olifants River system has demonstrated the existence of a new cryptic species of river crab, while populations of *P. perlatus* in the nearby Berg River system remained uninvestigated. Six populations of *P. perlatus* were collected from along the length of the Berg River system (125 km) and the genetic structure was investigated using allozyme electrophoresis. Results from 14 allozyme loci showed that the populations were genetically invariant across the river system. It is suggested that gene flow may be responsible for the poor genetic differentiation amongst populations in the Berg River system. The implication for future management of the system is briefly discussed.

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Introduction

The classification of aquatic inland invertebrates has received much systematic attention. Despite such efforts, however, the diversity of most taxa remains poorly documented. The classification of freshwater crabs in South Africa is relatively recent and little systematic work has been undertaken since the earlier work of Barnard (1935, 1950) and Bott (1955, 1960). Barnard (1935) remarked that most populations of freshwater crab species exhibit considerable morphological variation and that the occurrence of transitional forms between certain species further complicates the delineation of species boundaries among taxa. In an attempt to map the systematics of freshwater crabs in South Africa, a sampling program was initiated in 1992. In the Western Cape, only one species of river crab *Potamonautes perlatus* (H. Milne Edwards 1837) was thought to occur in river systems in the area (Harrison & Elsworth 1958, Harrison & Agnew 1962). The new sample program initially led to the discovery of two new species *P. brincki* (erroneously placed in the *Gecarcinautes*, Bott 1960) and *P. parvispina* in the Western Cape (Stewart 1997a, 1997b). During the course of this study B.A. Stewart (unpublished data) noted a sharp genetic discontinuity among population of *P. perlatus* in the Olifants River system. This matter was further investigated by Daniels, Stewart & Gibbons (in press) who concluded that a narrow hybrid zone was present between two genetically distinct population groups. These authors identified and described a new cryptic river crab species *P. granularis* from the lower parts of the Olifants River system (Daniels *et al.* 1998a).

The discovery of three new river crab species in the Western Cape clearly indicate that our understanding of the biodiversity in fresh water systems is poor. The discovery of a cryptic species of river crab in the Olifants River system, a relatively well-studied system, is surprising and shows that further research is required if the biodiversity of inland systems is to be mapped accurately. The genetic structure of populations of *Potamonautes perlatus* in the Berg River was examined in an attempt to further explain the diversity exhibited by this species of river crab.

Materials and methods

Six populations of *Potamoanutes perlatus* were sampled from the Berg River system (Figure 1). The procedure and data analysis followed that by Daniels *et al.* (1998b). Briefly, crabs were killed by freezing overnight at –20 ^{0}C and tissue samples extracted, and stored at –80 ^{0}C in liquid nitrogen. Samples were then homogenized and the supernatant electrophoresed in a 13% starch gel with three buffer systems, and 14 loci were stained for. Alleles were scored and numerical analyses were performed using the computer program BIOSYS 1 (Swofford & Selander 1981).

Results

Of the 14 loci studied 12 were monomorphic (ARK-1, IDH-2, GAP-1, LT-1, ME-1, MDH-1, LT-2, LDH-2, PGM-1, GL-1, MPI-1, PGM-2) and two were polymorphic (GPI-1 and MDH-2) (Table 1). The number of alleles ranged from two in MDH-2 to three in GPI-1. No single locus was polymorphic in all populations. All instances of polymorphism were in Hardy-Weinberg equilibrium, thus allowing for the clear interpretation of the results. The alleles frequencies were used to calculate the genetic distance (D) and genetic identity (I) (Nei 1978) between the populations (Table 2). The I values obtained for the six population groups ranged from 0.998–1.00 (D = 0.002-0.00). The UPGMA dendrogram (Figure 2) demonstrates the close genetic similarity between population groups.

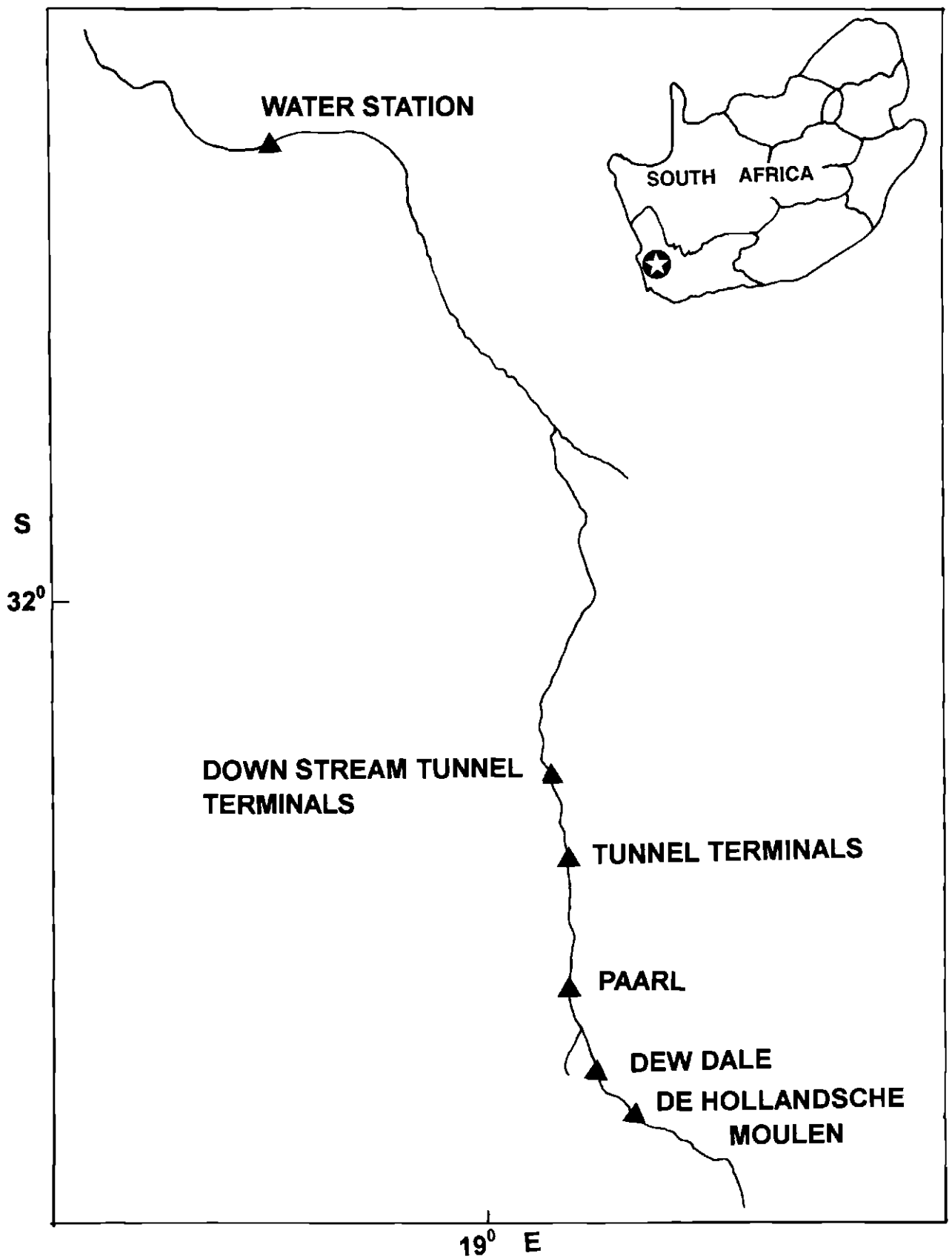

Figure 1 Sample sites along the Berg River system

The observed heterozygosity (H_o) and expected heterozygosity (H_e) were low and ranged from 0.00–0.029 and 0.00–0.024 respectively. The percentage of polymorphic loci ranged from 0.0–7.1% and the mean number of alleles ranged from 1.0 -1.1. The pairwise $F_{(ST)}$ amongst all the samples ranged from 0.063–0.172, with a mean of 0.136. The mean

Table 1 Distribution of alleles over the two polymorphic loci amongst the six population groups (N = sample size)

	Populations					
	Paarl	De Hollandsche Moulen	Tunnel terminals	Downstream tunnel terminals	Dew Dale	Water Station
Locus						
GPI-1						
N	15	3	5	10	7	20
B	1.000	1.000	1.000	0.900	1.000	1.000
C	0.000	0.000	0.000	0.050	0.000	0.000
D	0.000	0.000	0.000	0.050	0.000	0.000
MDH-2						
N	15	3	5	10	7	20
A	0.800	1.000	1.000	1.000	1.000	1.000
B	0.200	0.000	0.000	0.000	0.000	0.000

Table 2 Coefficient of unbiased genetic identity (I – above diagonal) and unbiased genetic distance (D – below diagonal) between the six population groups

	Paarl	De Hollandsche Moulen	Tunnel terminals	Downstream tunnel terminals	Dew Dale	Water Station
Paarl	*****	0.998	0.998	0.997	0.998	0.998
De Hollandsche Moulen	0.002	*****	1.000	1.000	1.000	1.000
Tunnel terminals	0.002	0.000	*****	1.000	1.000	1.000
Downstream tunnel terminals	0.003	0.000	0.000	*****	1.000	1.000
Dew Dale	0.002	0.000	0.000	0.000	*****	1.000
Water Station	0.002	0.000	0.000	0.000	0.000	*****

$F_{(IS)}$ was low at –0.188 and the mean $F_{(IT)}$ was at –0.027.

Discussion

Populations of *Potamonautes perlatus* exhibited no apparent genetic structure along the length of the Berg River system. Similar results have been reported in other conspecific freshwater crab species. For example, populations of *P. parvipsina* from the Berg and Olifants River systems had separated at genetic identity (I) values > 0.998 (Daniels *et al.* 1998b). Stewart & Cook (1998) obtained I > 0.90 for populations of *P. sidneyi* in Mpumalanga, while populations of *P. unispinus* had I > 0.97 in the Northern Province and Mpumalanga. Stewart (1997b) also showed that conspecific I values for populations of *P. perlatus* were > 0.97 in the Liesbeek River. These values clearly indicate that the high genetic I value obtained for intraspecific freshwater crab populations over a broad range is relatively low. The data agrees well with that reported for a wide range of conspecific populations, and supports Thorp's (1982) conclusion that 85% of I values between congeneric species exceed 0.35 and that 97% of conspecific populations usually have I > 0.85. The data from this article also confirm that intraspecific geographic variation in crustacean populations are almost non existent (Hedgecock *et al.* 1982). It must be noted, however, that genetic I values have relatively large standard errors associated with them and that the small sample size from the present study is too restricted to reveal any fine scale genetic structure amongst the populations (Nei 1978). The large standard error of the small sample size, and I, argues for a quantitative rather than a qualitative interpretation of our data. A larger sample size and more loci should be screened to obtain better insight into the genetic structure of *P. perlatus* in the Berg River.

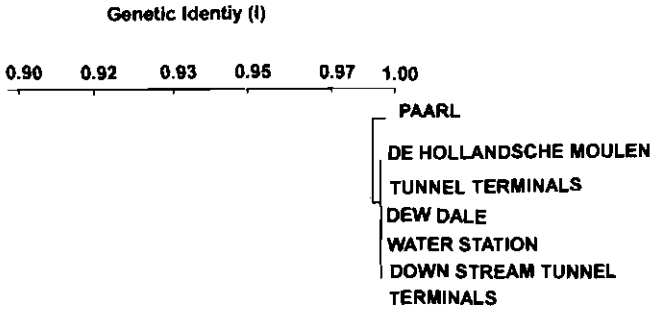

Figure 2 Dendrogram generated from matrix of Nei's (1978) genetic identities and the UPGMA algrithm. Data based on 14 loci

The low observed heterozygosity observed in this study compares well with those of other freshwater crab species, for example heterozygosty ranged from 0.006–0.022 in *P. parvispina*, and from 0.006–0.042 in *P. brincki* (Daniels *et al.* 1998c, B.A. Stewart unpublished). The results indicate that heterozygosity levels in freshwater crab species are low. The heterozygosity reported here is in agreement with the mean

heterozygosity of 0.048 that was calculated for decapod crustaceans (Hedgecock *et al.* 1982). An estimation of the heterozygosity is however dependent on both the sample size and the number of loci screened. Heterozygosity deficits were detected over all loci, including those from Group 1 allozymes (enzymes that play a central role in metabolism) such as PGM and GPI (Creasey *et al.* 1997). The major cause of this low heterozygosity may be attributed to inbreeding or the mixing of two or more population groups, which is corroborated by the low and negative $F_{(IS)}$ value.

The absence of a significant genetic structure suggests that relatively high levels of gene flow are occurring amongst populations. If drift and migration are balanced among populations the number of dispersing individuals between populations (Nm) can be calculated as $F_{(ST)} = 1/(4Nm + 1)$ being equal to two migrant individuals per generation. Slatkin & Barton (1989) suggest that Nm values above one are sufficient to prevent genetic structuring amongst populations. High levels of gene flow amongst freshwater crabs in the same river systems are likely to be common, as individuals can move freely in the water environment, and dispersal may occur as adults or as juveniles during flooding events. A recent study by Daniels *et al.* (1998b) reported that up to seven migrant individuals occurs amongst populations of *P. parvispina*. Although the dispersal capacity of river crabs has been little studied, it is likely to be high where the nature of the environment is continuous, such as in rivers. Freshwater crabs have also been observed walking short distances over land during the rainy season, a further indication that they can migrate (Barbaresi *et al.* 1997). What is interesting about this study is that there is no apparent genetic differentiation between the populations in the Berg River system when compared with that of the populations of *P. perlatus* which occurs in the upper section of the Olifants River system. If the genetic distance (D) is linearly related to the time of divergence (assuming that the rate of codon substitution is constant and alleles selectively neutral) and the D value of 1 equated with a divergence of 5 m.y (Nei & Roychoudhury 1974), the results from this study ($D < 0.001$) indicates that the time of separation between the populations from the two rivers was recent. This may be attributed to a recent split between the two drainages (that may be attributed to river capture) and/or a high dispersal capacity amongst these river crabs. The true causative factors remains elusive and thus needs to be investigated further. The present study also shows that *P. granularis* (Daniels *et al.* 1998a) is endemic to the lower reaches of the Olifants River system. The allozyme data demonstrate a close genetic similarly between populations of *P. perlatus* in the Berg River and Olifants River systems – this may be attributed to the fact that allozymes are relatively conserved structurally. Allozymes may thus not be very effective in detecting genetic structure among conspecific populations. The use of an alternative method such as mitochondrial (mt) DNA sequencing (of a protein coding gene such as cytochrome b) or micro-satellites may yield some degree of genetic structuring. The use of such methods has been shown to be of particular interest when studying population genetics as it often reveals patterns of genetic structuring amongst populations that are generally thought to be panmictic (homogenous) (Palumbi *et al.* 1991).

The results from this study clearly shows that there is a need to characterize the South African aquatic inland invertebrate fauna if the diversity among taxa is to be mapped accurately. There is increasing evidence to suggest that the freshwater invertebrate fauna may be more diverse and speciose than previously thought. Such research will become imperative in future if river systems are to manage as ecologically sustainable units. The Berg River system has in recent years come under increased pressure from industry, agriculture and a rapidly expanding urban population. The ineffective management of this system may have disastrous ecological consequences for a number of species. Already there are plans to dam the Berg River system, which could pose a serious threat to the low lying fynbos and to the high mountain stream invertebrates, many of which are endemic.

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