ARTICLE IN PRESS

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Molecular Phylogenetics and Evolution xxx (2005) xxx–xxx

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

Lack of taxonomic differentiation in an apparently widespread freshwater isopod morphotype (Phreatoicidea: Mesamphisopidae: *Mesamphisopus*) from South Africa

Gavin Gouws [a,*], Barbara A. Stewart [b], Conrad A. Matthee [a]

[a] *Evolutionary Genomics Group, Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[b] *Centre of Excellence in Natural Resource Management, University of Western Australia, 444 Albany Highway, Albany, WA 6330, Australia*

Received 20 December 2004; revised 2 June 2005; accepted 2 June 2005

Abstract

The unambiguous identification of phreatoicidean isopods occurring in the mountainous southwestern region of South Africa is problematic, as the most recent key is based on morphological characters showing continuous variation among two species: *Mesamphisopus abbreviatus* and *M. depressus*. This study uses variation at 12 allozyme loci, phylogenetic analyses of 600 bp of a COI (cytochrome *c* oxidase subunit I) mtDNA fragment and morphometric comparisons to determine whether 15 populations are conspecific, and, if not, to elucidate their evolutionary relationships. Molecular evidence suggested that the most easterly population, collected from the Tsitsikamma Forest, was representative of a yet undescribed species. Patterns of differentiation and evolutionary relationships among the remaining populations were unrelated to geographic proximity or drainage system. Patterns of isolation by distance were also absent. An apparent disparity among the extent of genetic differentiation was also revealed by the two molecular marker sets. Mitochondrial sequence divergences among individuals were comparable to currently recognized intraspecific divergences. Surprisingly, nuclear markers revealed more extensive differentiation, more characteristic of interspecific divergences. This disparity and the mosaic pattern of differentiation may be driven by stochastic population crashes and genetic bottlenecks (caused by seasonal habitat fluctuations), coupled with genetic drift.

Keywords: Phreatoicidea; *Mesamphisopus*; COI; Allozymes; Western Cape; South Africa

1. Introduction

The isopodan suborder Phreatoicidea is represented in southern Africa by the single, endemic genus *Mesamphisopus* (Kensley, 2001; Nicholls, 1943). This genus probably occupies a basal position among studied phreatoicidean genera (Wilson and Keable, 1999) and contains four described species, all occurring within the Western Cape Province, South Africa: *M. capensis*, collected from Table Mountain on the Cape Peninsula; *M. penicillatus*, collected from a type locality near Hermanus; and *M. abbreviatus* and *M. depressus*, collected from proximate localities in the Hottentot's Holland Mountains (the northern slopes of the Kogelberg and the Steenbras Valley, respectively) (Barnard, 1914, 1927, 1940; Kensley, 2001; Nicholls, 1943). A recent investigation, however, identified an additional four cryptic species, morphologically similar to *M. capensis*, and it was furthermore suggested that many individuals sampled from the southwestern part of South Africa and kept in museum collections[1] had been incorrectly identified as

* Corresponding author. Fax: +61 8 9892 8547.
E-mail address: ggouws@cyllene.uwa.edu.au (G. Gouws).

[1] For example, South African Museum accessions A3992, A3993, A4006, A4181–A4183, A4186, A4187, A6052, A6932–A6934, and A6950.

1055-7903/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.06.002

M. capensis (Gouws et al., 2004). Following the morphological key of Kensley (2001), it was clear that many of these specimens and others collected more recently from proximate localities do not belong to *M. capensis* or the four cryptic species identified by Gouws et al. (2004)—these undescribed species and *M. capensis* lack a pair of subapical robust setae dorsally on the pleotelson. Neither can they be identified as *M. penicillatus*, which possesses characteristically heavily setose antennal peduncles (Barnard, 1940; Kensley, 2001). This leaves only *M. abbreviatus* and *M. depressus* as possibilities for identification. The majority of these specimens cannot be unambiguously assigned, using the available diagnoses and descriptions (Barnard, 1927; Kensley, 2001; Nicholls, 1943) to either of these two species. These works document subtle differences in pereon, telson and gnathopod shape, and coloration between *M. abbreviatus* and *M. depressus*, with the two species primarily distinguished by the degree of setation of the head and pereon, these being more setose in *M. depressus* (Barnard, 1927; Kensley, 2001; Nicholls, 1943). However, characters such as gnathopod and telson shape are known to exhibit within-population variation (Barnard, 1927), while setation, in particular, is of only limited systematic importance (Wilson and Keable, 1999, 2001). The identification of *M. abbreviatus* and *M. depressus*, described from geographically proximate type localities (Barnard, 1927), as separate species is questioned in light of this morphological plasticity. Kensley's (2001) key is also of limited use, relying on characters showing continuous variation, such as the extent of setation. As a result, specimens are easily and equivocally identified as either species. The broader limitations of the key (and further evidence of morphological plasticity) are illustrated by the fact that the presence of the pair of robust subapical setae dorsally on the pleotelson, used to distinguish *M. capensis* from *M. abbreviatus*, *M. depressus*, and *M. penicillatus*, appears to be a variable character, with these setae present in frequencies between 0.03 and 1.00 in many of these misidentified collections (unpublished data).

The use of molecular data to resolve such taxonomic difficulties and to identify and delineate cryptic species has become increasingly widespread in crustacean systematics (King and Hanner, 1998; Larsen, 2001; Sarver et al., 1998; Schubart et al., 1998). Genetic differentiation, in addition to inferred reproductive isolation and evidence of morphometric differences, has already been presented as an argument for the delimitation of cryptic species within the genus *Mesamphisopus* (Gouws et al., 2004). However, the establishment of an interspecific "standard" for the genus or species of interest is critical in this regard. Underscoring this is an understanding of the extent of, and patterns of, intraspecific genetic differentiation present among individual populations. A scenario under which the populations identified as *M. abbreviatus* or *M. depressus* is found to be conspecific would enable the examination of intraspecific genetic differentiation. Alternatively, should more than one species be present among the sampled populations, individual distributions may be far-ranging enough to enable this.

Classical population genetic theory predicts that taxa with limited dispersal capabilities will show great levels of genetic differentiation, resulting from mutation and genetic drift in the absence of gene flow and selection. Even sessile or relatively sedentary species, with narrow individual ranges, may show only limited genetic differentiation between geographically disjunct populations due to the occurrence of vagile life history stages, where a negative relationship between the extent of genetic differentiation and the duration and dispersal efficiency of free-swimming larval stages has been documented or is expected (Bohonak, 1999; Burton and Feldman, 1982; Sponer and Roy, 2002).

Whereas the marine environment has traditionally been thought to present few obvious physical barriers to gene flow (Bohonak, 1999) and genetic connectivity among conspecific populations are regarded as high (Bahri-Sfar et al., 2000; Bucklin et al., 1997; Rodriguez-Lanetty and Hoegh-Guldberg, 2002), freshwater environments are often more complex with habitats essentially existing as "islands" within the broader terrestrial environment (Michels et al., 2003; Wishart and Hughes, 2003). Accordingly, more complex patterns of differentiation are expected among freshwater isopod populations than among populations of marine or terrestrial isopods, where high levels of gene flow and isolation by distance have often been documented (Beck and Price, 1981; Wang and Schreiber, 1999). General patterns of restricted gene flow and high levels of genetic differentiation among geographically separated populations have been reported for riverine organisms (Meyran et al., 1997; Woolschot et al., 1999), while genetic structure within riverine systems has been proposed to represent a nested hierarchy (Meffe and Vrijenhoek, 1988), with populations nested within subcatchments and larger catchments. As such, and given their predominant restriction to high-altitude streams in broad, mature valleys (Barnard, 1927), *Mesamphisopus* populations occurring within the same drainage system may be expected to be genetically more similar, with greater genetic differentiation observed among populations situated in different drainage systems. These among-drainage relationships, in turn, may be expected to reflect a pattern of isolation by distance, as chance of genetic exchange (migrations) among adjacent drainage systems would be more likely than gene flow among geographically widely separated drainages. From the outset, however, genetic differentiation among populations is expected, and patterns perhaps complicated by the fact that within the Isopoda, as in other peracarid Crustacea, young are brooded within a marsupium and free-swimming larval stages do not occur.

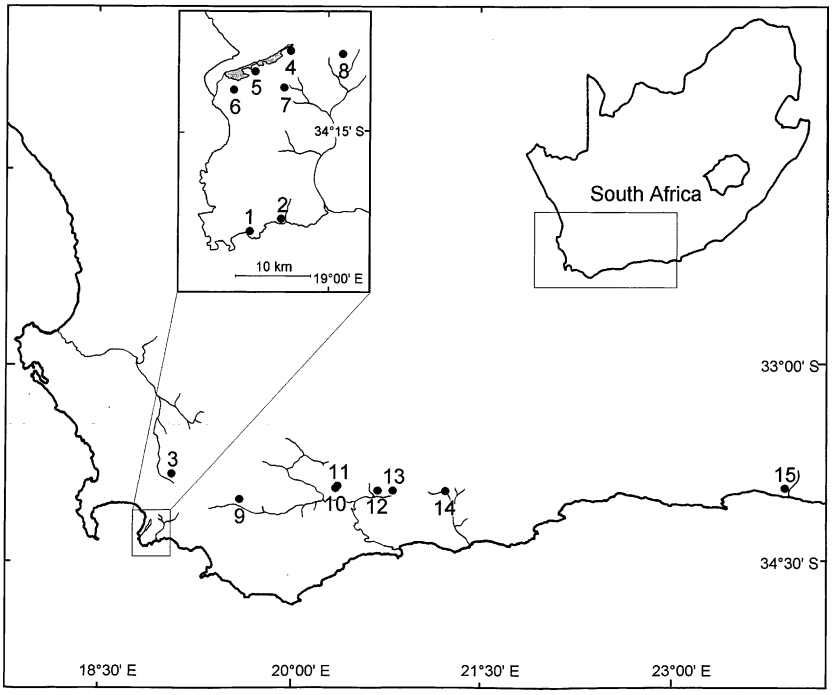

Fig. 1. Collection localities of the 15 studied populations, with individuals tentatively identified as *Mesamphisopus abbreviatus* or *M. depressus* in the Western Cape Province and Eastern Cape Province, South Africa. Localities included (with drainage systems in parentheses): (1) Betty's Bay A (Betty's Bay marshland), (2) Betty's Bay B (Disa Stream/Betty's Bay marshland), (3) Wemmershoek (Berg River), (4) Steenbras A (Steenbras River), (5) Steenbras B (Steenbras River), (6) Steenbras C (Steenbras River), (7) Kogelberg (Palmiet River), (8) Grabouw (Palmiet River), (9) Greyton (Breede River), (10) Protea Valley (Breede River), (11) Barrydale (Breede River), (12) Tradouw Pass (Breede River), (13) Grootvadersbos (Breede River), (14) Riversdale (Vet River), and (15) Tsitsikamma (Storms River).

The aims of the present study are thus twofold. First, to determine whether populations tentatively identified as either *M. depressus* or *M. abbreviatus* could reasonably be regarded as conspecific. To address this, specimens were collected from a large geographic range, encompassing the type localities of both species and the collection localities of the above-mentioned, misidentified museum specimens. As the primary focus of this study is to address the systematic and taxonomic status of these populations, other *Mesamphisopus* species, which are more easily identifiable and less likely to be mistaken for *M. abbreviatus* or *M. depressus*, are specifically omitted. (A comprehensive investigation of the phylogenetic relationships within the entire genus is currently under preparation.) Second, in light of the above expectations, the possible evolutionary processes underlying the patterns of genetic differentiation are investigated.

2. Materials and methods

2.1. Sampling

Isopods, assigned to either *M. abbreviatus* or *M. depressus* according to the single available key (Kensley, 2001) and limited species descriptions (Barnard, 1927; Nicholls, 1943), were collected from 15 localities across the Western Cape Province and Eastern Cape Province, South Africa (Fig. 1). This sampling strategy targeted known collection localities of *Mesamphisopus* and attempted to cover the known geographic extent of the above-mentioned, putatively misidentified populations. Individuals were also sampled from additional localities depending on the accessibility of suitable habitat. Collection localities were typically high-altitude, first-order streams, seepage areas, or springs. Collectively, the sampling localities were situated along an ~450-km transect, stretching from the Hottentot's Holland Mountains in the west to the Tsitsikamma Forest in the east and represented seven separate drainage systems (Fig. 1), including the Berg, Steenbras, Palmiet, Breede, Vet and Storms Rivers, and marshlands and lakes around Betty's Bay. The scale of sampling, with populations being collected over such a large area, as well as within close geographical proximity (e.g., three localities were centered around the Steenbras Dam, while two localities, Barrydale and Protea Valley, were within 1 km of each other), afforded the opportunity to examine the extent of genetic differentiation over large and local spatial scales. At each locality, isopods were collected by sifting through leaf litter and sediment using hand nets. Individuals were frozen (−80 °C) or preserved in absolute ethanol upon return

to the laboratory. Voucher specimens[2] from each of the sampled localities were lodged in the collections of the South African Museum, Cape Town.

2.2. Morphometric analyses

Barnard's (1927) syntypic series (*M. abbreviatus*, South African Museum A5173; *M. depressus*, SAM A4185) were examined to determine whether the two species could be distinguished morphometrically. All ($N=21$) undamaged, mature *M. depressus* males (individuals of roughly equal size) and an equal number of randomly chosen, mature *M. abbreviatus* males were digitally photographed and measured following the procedures documented in Gouws et al. (2004). Individuals were determined to be mature by the complete development of the penes and their extension to the ventral midline. As the dissection required for the accurate measurement of pereopod variables was not possible for the type material, only the set of 22 body (cephalon, pereon, pleon, and pleotelson) variables was included in the analysis. Variable details and abbreviations are presented in Gouws et al. (2004).

Morphometric discrimination between the two species was examined by means of a standard discriminant function analysis, using log-transformed (common logarithms) variables. Classification functions were determined for each of the two defined groups, using a jack-knifing procedure. Individuals were then reclassified to groups based on posterior probabilities. All analyses were performed using STATISTICA 6.0 (Statsoft, Inc., 2001).

This analysis was then extended to determine whether individuals sampled from the populations included in the genetic analyses could be identified as either of the two species using morphometric criteria. Five of the largest mature males from each of the populations were measured for the same 22 variables. Due to insufficient numbers of individuals being collected from two localities (and unsuccessful attempts at resampling), only two individuals were included from the Steenbras A population, while no individuals were included from the Betty's Bay B locality. Individuals from each population were classified as *M. depressus* or *M. abbreviatus* based on posterior probabilities using the classification functions determined above. A posterior probability greater than 0.95 was required for an individual to be identified as either species or the particular individual was regarded as unidentified.

[2] Voucher specimens (South African Museum accessions): Betty's Bay A: A44932, A45218, A45219; Betty's Bay B: A45216, A45217; Wemmershoek: A44938, A45232; Steenbras A: A45227; Steenbras B: A45233; Steenbras C: A45235; Kogelberg: A45236; Grabouw: A44931, A44944, A45214, A45215; Greyton: A45239, A45240; Protea Valley: A45237; Barrydale: A45238; Tradouw Pass: A45229; Grootvadersbos: A44934; Riversdale: A44941; Tsitsikamma: A44935, A45154.

2.3. Allozyme electrophoresis

Individuals from each of the 15 populations were prepared for electrophoresis following the procedures documented in Gouws et al. (2004). Gels were also run using identical buffer systems and running conditions (Gouws et al., 2004). The protocols of Shaw and Prasad (1970) were used to stain the sites of enzymatic activity of the allozymes of 12 loci, encoding 10 enzyme systems. Allelic mobilities were scored relative to the most common allele in a reference population (Franschhoek, see Gouws et al., 2004). Numbering of loci and alleles has also been described previously.

Numerical analyses of allozyme data were performed using the BIOSYS 1.7 (Swofford and Selander, 1981) and FSTAT 2.9.3.2 (Goudet, 2001) programs. Observed genotype frequencies were tested for deviation from frequencies expected under Hardy–Weinberg equilibrium, using χ^2 goodness-of-fit tests and exact probabilities. *P*-values were adjusted for table-wide significance using a sequential Bonferroni technique (Rice, 1989). Genetic variability measures were examined as in Gouws et al. (2004). Nei's (1978) unbiased measures of genetic distance (D) and identity (I) were calculated from allele frequencies for pairwise comparisons among populations. Genetic distance values were then used to construct a neighbor-joining tree (Saitou and Nei, 1987), as well as an UPGMA-dendrogram (Sneath and Sokal, 1973), using MEGA2.1 (Kumar et al., 2001). The partitioning of genetic differentiation among populations across the entire sample was examined using Weir and Cockerham's (1984) θ estimates. Estimates of θ were also obtained for individual pairwise comparisons of populations.

2.4. DNA sequencing and sequence data analyses

Although Wetzer (2001) has recommended the use of the 12S and 16S rRNA gene regions for population- and species-level isopod studies, the cytochrome *c* oxidase subunit I (COI) mtDNA gene has recently found increasing use in isopod phylogeographic and phylogenetic studies. This latter protein-coding gene fragment has robustly resolved interspecific relationships and, albeit to a lesser extent, relationships among conspecific populations of isopods (Ketmaier et al., 2003; Rivera et al., 2002; Wares, 2001). To assess whether populations could reasonably be regarded as conspecific across the range of collection localities and to corroborate or refute the broad patterns seen from the allozyme data, a fragment of the COI region was amplified from a single representative individual from each population. An additional four to five individuals were sequenced from four populations (Barrydale, Betty's Bay A, Protea Valley, and Wemmershoek) to determine whether the diversity of haplotypes within single populations would

impede the examination of phylogenetic relationships among populations based on single-sequenced individuals. These populations were selected to include genetically divergent populations, following the allozyme and preliminary sequence data analyses, and to include the two populations in closest geographic proximity (i.e., Barrydale and Protea Valley). A single *M. penicillatus* individual as well as a morphologically distinct, undescribed species (*Mesamphisopus* sp. nov.) were sequenced as out-groups.

Prior to DNA extraction, individuals (10–20 mm) were twice rinsed in distilled water by centrifugation (14,000 rpm for 2 min) to remove debris and epibionts. Total genomic DNA was extracted using a commercial extraction kit (Qiagen DNEasy) or by means of conventional SDS–proteinase K digestion and phenol–chloroform:isoamyl alcohol extraction protocols (Hillis et al., 1996; Sambrook and Russell, 2001).

Polymerase chain reactions were set up in 25 μL volumes, as described in Gouws et al. (2004). The COI region was primarily amplified using the primer pair (LCO1490 and HCO2198) of Folmer et al. (1994). As amplification of certain individuals was problematic, two "internal" primers were designed; COI-intR (5′-G CW CCA AGA ATA GAA GAA GC-3′) and COI-intF (5′-GTT GAA CTG TTT ATC CTC CTT-3′), which amplify ~420 and ~315 bp fragments in combination with LCO1490 and HCO2198, respectively. The thermal cycling regime included an initial denaturing step (94 °C) for 4 min, followed by 33 cycles of denaturing (94 °C, 15 s), annealing (1 min), and extension (72 °C, 1.5 min). A final cycle included annealing for 5 min and extension for 10 min. Annealing was performed at 48 °C for the Folmer et al. (1994) primer pair and at 55 °C for combinations involving the "internal" primers. Purification and cycle sequencing of amplicons and automated sequencing proceeded as described in Gouws et al. (2004). All unique sequences were deposited in GenBank (Accession Nos. AY948290–AY948307).

Sequences were checked for ambiguity against their respective chromatograms, using Sequence Navigator (Applied Biosystems). Due to ambiguity within the first ~15 bases, these characters were trimmed. If further ambiguities were present, the problematic region was reamplified using the respective "internal" — Folmer et al. (1994) primer combination, sequenced, and a consensus sequence was created. As the alignment of the sequences did not require the insertion of gaps (indels), it was done manually. Amino acid translations were examined, using MacClade 4.05 (Maddison and Maddison, 2000), to test for accuracy and functionality of the sequences. No stop codons were detected in translations based on the *Drosophila* mitochondrial code.

Analyses were performed using PAUP*4b10 (Swofford, 2002). Phylogenetic relationships within a reduced data set, including all unique haplotypes, as well as a representative individual from each population, were examined using unweighted parsimony (MP) and maximum likelihood (ML). In both the MP and the ML analyses, heuristic tree searches were performed using the tree-bisection-reconnection branch-swapping algorithm, with a random addition of taxa (100 and 10 replicates in the MP and ML analyses, respectively). Prior to ML analysis, the most appropriate model of sequence evolution was determined and the nucleotide substitution parameters were estimated for this reduced data set using MODELTEST 3.06 (Posada and Crandall, 1998), where the likelihood scores of candidate models were compared using the Akaike Information Criterion (Akaike, 1974). Nodal support was determined by bootstrapping of the data set (Felsenstein, 1985), with 1000 and 100 pseudoreplicates performed for the MP, and ML analyses, respectively. The tree lengths of topologies proposed by the allozyme, MP and ML analyses were determined and compared using MacClade 4.05.

2.5. Isolation by distance

To investigate the relationship between geographic distance and the extent of genetic divergence among populations (isolation by distance: Wright, 1943), the correlations between matrices of log-transformed straight-line geographic distances between collection localities and measures of genetic differentiation among populations or sequence divergences among their representative individuals were examined. Mantel tests (Mantel, 1967) were used, and executed using the Mantel for Windows (version 1.11) program (Cavalcanti, 2000) employing 10,000 randomizations for each comparison. These relationships were explored using the mean genetic distance (D) among populations and measures of differentiation at individual loci. For the latter, Nei's genetic identities (I) were used (Nei, 1978), as D-values for single locus comparisons among populations approach infinity when fixed allelic differences are present. The sequence divergence matrix was comprised of uncorrected sequence divergences among representative individuals. The correlation between sequence divergence among representative individuals and (allozyme) genetic distances among their source populations was also examined.

To account for an uneven sampling strategy with certain areas being more intensively sampled than others, patterns of isolation by distance were also examined among regions, with populations being pooled by mountain range and geographic proximity (populations separated by less than 30 km were considered to belong to the same region). Consequently, seven regions were identified: (i) Wemmershoek; (ii) Hottentot's Holland Mountains (including the Betty's Bay A, Betty's Bay B, Grabouw, Kogelberg, Steenbras A, Steenbras B, and Steenbras C populations); (iii) Greyton; (iv) West

Langeberg Mountains (Barrydale and Protea Valley); (v) East Langeberg Mountains (Grootvadersbos and Tradouw Pass); (vi) Riversdale; and (vii) Tsitsikamma. Inter-region matrices were compiled by determining the mean geographic distances, sequence divergences, and genetic distances (D) among populations belonging to these different regions.

Correlations among distance and sequence divergence matrices compiled for the constituent populations of the Hottentot's Holland Mountains region were also examined to determine whether patterns of isolation by distance were apparent over small geographic scales.

3. Results

3.1. Morphometric analyses

While distinguishing *M. abbreviatus* and *M. depressus* using qualitative, physical characteristics (i.e., setation) was largely equivocal, the syntypes of the two species could be reliably distinguished (Wilks' $\lambda = 0.023$, $F_{(22,19)} = 36.383$, $P < 0.001$) through the discriminant function analysis using the 22-body variables. All variables contributed to the discrimination of the species, with the width of pereonite 3 (Wilks' $\lambda = 0.035$, $P < 0.01$), telson depth (Wilks' $\lambda = 0.032$, $P < 0.05$), and body length (Wilks' $\lambda = 0.032$, $P < 0.05$) being the most significant discriminators within the linear functions. The reclassification of these individuals to groups based on posterior probabilities substantiated the distinction between the species with all individuals ($N = 21$) being correctly reassigned to their respective groups (Table 1). A frequency histogram of scores along the canonical variable (not shown) revealed the substantial difference between the mean canonical scores for each species (6.334 and −6.334 for *M. abbreviatus* and *M. depressus*, respectively).

Using the classification functions determined for the two species, individuals from sampled populations were assigned to groups based on posterior probabilities (Table 1). Most examined individuals were determined to be morphometrically similar to *M. abbreviatus*. In only two populations, Steenbras B and Steenbras C, could all included individuals be classified as *M. depressus*. Both morphotypes were encountered within the Tradouw Pass and Wemmershoek populations, while in certain populations (Barrydale, Betty's Bay A, and Grootvadersbos) single individuals could not be classified.

3.2. Allozyme electrophoresis

Allele frequencies at each locus and genetic variability measures for each population are presented in Appendix A. Although at least 20 individuals from each

Table 1
Assignment of individuals to species based on posterior probabilities calculated from the classification functions[a] determined for the *M. abbreviatus* and *M. depressus* syntypes (South African Museum accession numbers and type localities in parentheses) in the discriminant function analysis using 22 cephalon, pereon, pleon and pleotelson variables

Population	N	Classification		
		M. abbreviatus	*M. depressus*	Unclassified
M. abbreviatus (SAM A5173)	21	21	—	—
M. depressus (SAM A4185)	21	—	21	—
Barrydale	5	4	—	1
Betty's Bay A	5	4	—	1
Grabouw	5	5	—	—
Greyton	5	5	—	—
Grootvadersbos	5	4	—	1
Kogelberg	5	5	—	—
Protea Valley	5	5	—	—
Riversdale	5	5	—	—
Steenbras A	2	2	—	—
Steenbras B	5	—	5	—
Steenbras C	5	—	5	—
Tradouw Pass	5	3	2	—
Tsitsikamma	5	5	—	—
Wemmershoek	5	3	2	—

N, number of individuals included. Cases where posterior probabilities (<0.95) prohibited the assignment of individuals to a species group are referred to as unclassified.

[a] *Mesamphisopus abbreviatus*: Y = 9373.180(BL) − 1511.315(HL) − 1055.443(HD) + 481.325(HW) − 2122.566(P1L) + 839.604(P1D) + 1320.926(P1W) − 1041.157(P3L) − 669.539(P3D) − 6152.109(P3W) − 397.806(P5L) − 465.202(P5D) − 1626.908(P5W) − 2724.097(P7L) − 332.462(P7D) + 4656.551(P7W) − 1360.636(PL4L) + 708.376(PL4D) − 130.131(PL4W) + 298.340(TL) + 1916.953(TD) − 314.259(TW) − 5404.434; *M. depressus*: Y = 8740.905(BL) − 1418.522(HL) − 1173.859(HD) + 301.994(HW) − 2003.568(P1L) + 787.382(P1D) + 1445.613(P1W) − 1074.747(P3L) − 658.538(P3D) − 5135.734(P3W) − 395.618(P5L) − 433.312(P5D) − 1586.701(P5W) − 2541.097(P7L) − 268.888(P7D) + 4323.975(P7W) − 1330.582(PL4L) + 786.825(PL4D) − 48.266(PL4W) + 61.171(TL) + 1601.399(TD) − 190.813(TW) − 4844.632.

population were prepared for electrophoresis, the small volume of homogenate and the rapid and visible loss of enzymatic activity resulted in some differences in the number of individuals of a given population being assayed for each locus. However, the staining intensity of other individuals, the banding patterns predicted from known enzymatic subunit structure, and conformance to Hardy–Weinberg expectations (below) suggested that null alleles were not present at significant frequencies, beyond those cases in which they were fixed (see below). All 12 included loci were found to be polymorphic. The *Lt-1* and *Lt-2* loci, although polymorphic across the entire sample, were monomorphic in individual populations. No loci were found to be polymorphic in all studied populations. From two to 16 alleles were found per locus. Estimates of genetic variability were also found to vary greatly among populations (Appendix A). Although a number of populations were determined to be fixed for null alleles at certain loci, these loci and populations were retained in further numerical analyses with the null alleles coded following the "minimizing" approach discussed by Berrebi et al. (1990) and Machordom et al. (1995). This coding methodology enables mathematical comparisons among taxa with differentially expressed loci resulting from gene duplication (polyploidy) events and subsequent inactivation of loci through "functional diploidization" (Berrebi et al., 1990). Here, it was, however, applied to null alleles apparently fixed at a single locus (*Ldh*) in different populations (Barrydale, Greyton, Kogelberg, Protea Valley, and Riversdale), with null alleles being coded identically in these populations for further analyses. This assumes a common evolutionary inactivation of expression in all populations and has the effect of minimizing genetic differentiation among these populations, while maximizing genetic distance between groups of populations fixed for null alleles and those possessing alternative alleles (Berrebi et al., 1990). Thus, genetic distances between certain populations are likely to be underestimated. The Tsitsikamma population was fixed for a null allele at the *Lt-2* locus. The coding of this single locus does not bias estimates of genetic differentiation among populations.

Significant deviations from Hardy–Weinberg expected genotype frequencies were observed (after Bonferroni correction) at four of 47 individual cases (8.51%) of polymorphism (Appendix A), considering all loci and populations. Although all deviations were due to a deficit of heterozygous individuals, these deviations were not restricted to specific populations or loci and were not considered to result from sampling artifacts. Testing for deviation using exact probabilities showed only the *Idh*-locus in the Tsitsikamma population ($P < 0.01$) to be out of Hardy–Weinberg equilibrium. In none of the populations in which the morphometric analyses revealed the presence of both morphotypes and unclassifiable individuals were there deviations from Hardy–Weinberg expectations, evidence of Wahlund (1928) effects (indicating independent, sympatric gene pools and multiple species), or genetic distinctions underlying the morphometric polymorphism.

Both the neighbor-joining tree and UPGMA dendrogram (Fig. 2), constructed using genetic distances among populations (Table 2), revealed a large genetic distinction between the Tsitsikamma population and the remaining populations. This population was separated from the remainder by a mean genetic distance (D) of 2.020 ± 0.336, primarily due to the occurrence of fixed allele differences at the *Ark-*, *Gpi-*, *Lt-*, and *Mdh*-loci, and significant allele-frequency heterogeneity (all $P < 0.001$ in contingency χ^2 analyses) at all remaining loci.

While little congruence was found among the two topologies, neither topology revealed any distinct patterns relating to geographic locality. In some cases, geographically proximate populations clustered together, for example, Betty's Bay A and Betty's Bay B samples, which were separated by a genetic distance of 0.002. In other cases, geographically proximate populations fell in separate clusters. For example, the Steenbras B and Steenbras C populations grouped together ($D = 0.047$), while the geographically proximate Steenbras A population was placed within a cluster containing the Wemmershoek and Grabouw populations in the neighbour-joining tree and was placed within a larger cluster containing the Grabouw, Grootvadersbos, Tradouw Pass, and Betty's Bay populations in the UPGMA dendrogram. No clear patterns relating to drainage system were found either, as populations from the Palmiet, Steenbras, and Breede River catchments clustered separately throughout both topologies (Fig. 2).

Further, geographically disjunct populations were often characterized by the shared fixation (or occurrence at high frequency) of alleles absent in other, geographically proximate populations. For example, the Wemmershoek and Grabouw populations were fixed for the Ao^{90} allele. This allele was present only at low frequencies in the Steenbras A population and absent from the remaining populations. Simultaneously, examination of allele frequencies within the two sets of geographically most proximate populations revealed substantial fine-scale genetic differentiation. The Steenbras A population was separated from the Steenbras B and Steenbras C populations that were genetically more similar, by a mean D-value of 0.519 ± 0.005. Fixed allelic differences were present, distinguishing the Steenbras A population, with additional, significant (all $P < 0.001$) allele-frequency differences at the *Ao-*, *Gpi-* and *Pgm*-loci between these populations. The Protea Valley and Barrydale populations were separated by a genetic distance (D) of 0.147. This separation was attributable to a fixed allele difference at the *Gpi*-locus and allele frequency differences at the *Idh-*, *Pgm-* (both $P < 0.001$), and *Mdh-2*-loci.

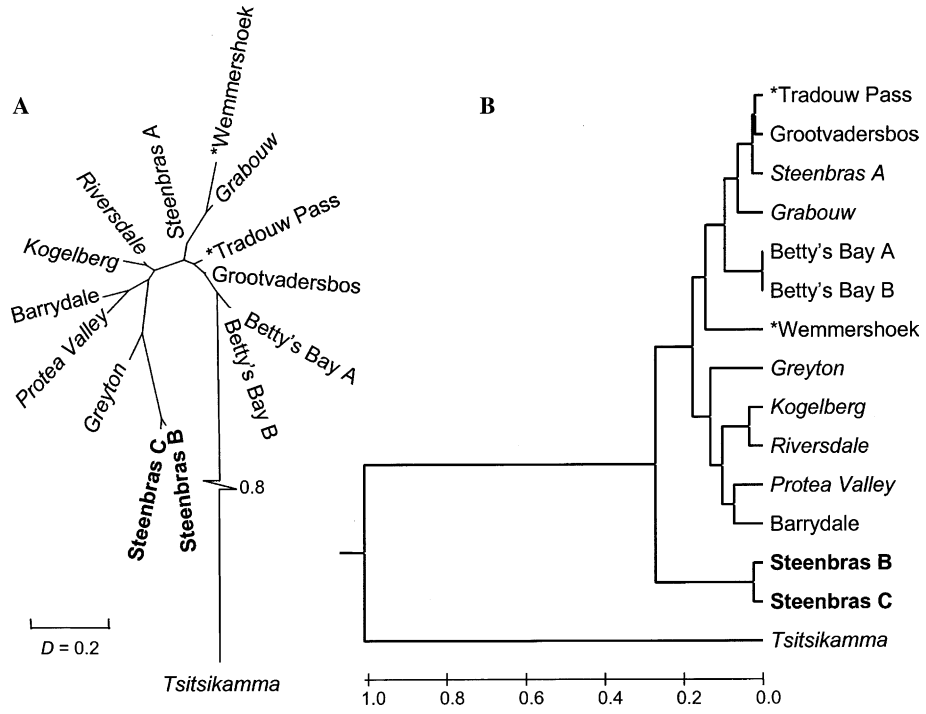

Fig. 2. Unrooted neighbor-joining-tree (Saitou and Nei, 1987) (A) and UPGMA (Sneath and Sokal, 1973) dendrogram (B) constructed from Nei's (1978) unbiased genetic distances obtained in pairwise comparisons of the 15 populations of *Mesamphisopus* studied through allozyme electrophoresis of 12 loci. Populations where all examined individuals were morphometrically similar to *M. depressus* or *M. abbreviatus* are indicated in bold and italicized typeface, respectively. Asterikes indicate populations where both morphotypes were observed, while normal typeface indicates populations where some individuals could not be assigned to either morphotype. The Betty's Bay B population was excluded from the morphometric analyses.

Table 2
Matrix of Nei's (1978) unbiased genetic distance (*D*; above diagonal) obtained in pairwise comparison of populations and Weir and Cockerham's (1984) (θ-estimate; below diagonal) of genetic differentiation among population pairs

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(1) Betty's Bay A	—	0.002	0.410	0.157	0.459	0.433	0.412	0.260	0.507	0.531	0.408	0.168	0.194	0.303	1.773
(2) Betty's Bay B	0.067	—	0.417	0.157	0.428	0.401	0.395	0.261	0.493	0.522	0.398	0.169	0.193	0.287	1.749
(3) Wemmershoek	0.903	0.863	—	0.205	0.723	0.718	0.312	0.152	0.482	0.456	0.475	0.257	0.302	0.394	2.832
(4) Steenbras A	0.667	0.625	0.638	—	0.515	0.522	0.199	0.074	0.318	0.303	0.263	0.042	0.064	0.125	1.801
(5) Steenbras B	0.882	0.838	0.861	0.767	—	0.047	0.525	0.741	0.317	0.477	0.547	0.545	0.635	0.502	2.138
(6) Steenbras C	0.931	0.886	0.904	0.822	0.393	—	0.585	0.715	0.322	0.648	0.625	0.542	0.608	0.559	1.938
(7) Kogelberg	0.964	0.912	0.852	0.678	0.864	0.930	—	0.360	0.259	0.161	0.297	0.274	0.290	0.070	1.762
(8) Grabouw	0.820	0.785	0.648	0.388	0.858	0.885	0.836	—	0.487	0.482	0.390	0.145	0.165	0.258	2.390
(9) Greyton	0.913	0.869	0.834	0.692	0.741	0.819	0.813	0.820	—	0.286	0.280	0.337	0.393	0.238	1.943
(10) Protea Valley	0.859	0.844	0.806	0.653	0.787	0.858	0.680	0.787	0.714	—	0.147	0.319	0.334	0.166	2.244
(11) Barrydale	0.930	0.912	0.888	0.730	0.872	0.921	0.923	0.837	0.822	0.672	—	0.224	0.290	0.201	2.399
(12) Tradouw Pass	0.772	0.708	0.743	0.262	0.816	0.865	0.799	0.614	0.762	0.734	0.775	—	0.040	0.181	1.868
(13) Grootvadersbos	0.748	0.733	0.769	0.322	0.833	0.867	0.809	0.615	0.784	0.736	0.799	0.302	—	0.196	1.696
(14) Riversdale	0.921	0.863	0.863	0.583	0.857	0.913	0.692	0.784	0.788	0.681	0.861	0.725	0.739	—	1.741
(15) Tsitsikamma	0.946	0.940	0.937	0.889	0.921	0.938	0.945	0.919	0.924	0.912	0.947	0.920	0.912	0.941	—

Genetic distances obtained in pairwise comparisons of populations (Table 2) ranged from 0.002 to 2.832. Genetic distances between the Tsitsikamma population and the remaining populations ($1.696 \leqslant D \leqslant 2.832$) were substantially larger than distance values obtained in comparison of the remaining populations ($0.002 \leqslant D \leqslant 0.741$). A mean genetic distance of 0.569 ± 0.606 separated all studied populations.

Estimates of θ across the entire sample were similarly indicative of substantial genetic structuring among populations (Table 2). The overall θ was estimated at 0.848 [95% confidence interval (CI): 0.767–0.934], with estimates at individual loci ranging from 0.577 to 1.000. With the exclusion of the Tsitsikamma population, a geographic outlier, and phylogenetically distinct taxon (see Section 4), θ was estimated at 0.804

(95% CI: 0.693–0.904). Individual locus estimates then ranged from 0.046 to 0.984. With the exception of the pairwise comparison of the two Betty's Bay populations (Table 2; $\theta = 0.067$), substantial differentiation was apparent among all other populations, with θ ranging from 0.262 (the Steenbras A—Tradouw Pass comparison) to 0.964 (Betty's Bay A—Kogelberg).

3.3. Sequence data analyses

A total of 600 bp were aligned for the 32 in-group individuals and two out-group specimens. In populations from which more than one individual was sequenced, few haplotypes were found. A single, unique haplotype was fixed within each of the Barrydale ($N = 6$), Betty's Bay A ($N = 5$), and Wemmershoek ($N = 5$) populations. The Protea Valley population possessed two haplotypes, with the Protea Valley 2 haplotype differing by a single transition from the haplotype present in the remaining four individuals. On this basis, the reduced data set was compiled, including a single representative from each locality. While the haplotype of the Betty's Bay B representative was identical to those sampled in the Betty's Bay A population, the former individual was included as being representative of a different collection locality for the purpose of examining isolation by distance.

Within this reduced data set of 18 taxa, 172 characters were variable and 108 parsimony informative. Of the variable characters, 28 (16.3%) were found in first codon positions, with 15 (8.7%) and 129 (75.0%) occurring in second and third codon positions, respectively. Base frequencies, homogenous across taxa ($\chi^2 = 5.139$, $df = 51$, $P = 1.000$), were adenine and thymine rich (A = 0.224, C = 0.127, G = 0.190, T = 0.459), reflecting a bias documented previously for *Mesamphisopus* (Gouws et al., 2004) and isopods in general (Ketmaier et al., 2003; Wetzer, 2001).

The MP analyses retrieved six equally parsimonious trees of 240 steps (CI = 0.592, RI = 0.672, rescaled CI = 0.398). MODELTEST showed that the general time reversible model (Lanave et al., 1984; Rodríguez et al., 1990) of nucleotide substitution, together with a proportion of invariable sites and a gamma distribution (using four discrete rate categories) of variable sites (GTR + I + Γ), was the most appropriate for the reduced data matrix. The following estimated base frequencies and substitution parameters were subsequently used in the ML analysis: base frequencies: A = 0.247, C = 0.113, G = 0.163, T = 0.477; rate matrix: $R_{A\leftrightarrow C} = 0.222$, $R_{A\leftrightarrow G} = 15.196$, $R_{A\leftrightarrow T} = 1.493$, $R_{C\leftrightarrow G} = 0.523$, $R_{C\leftrightarrow T} = 4.727$, $R_{G\leftrightarrow T} = 1.000$; proportion of invariant sites = 0.427; and a gamma distribution shape parameter (α) of 0.393. The resulting maximum-likelihood topology ($-\ln = 2268.579$) is presented in Fig. 3. Generally, congruent topologies were obtained in the MP and ML analyses. While the sister-taxon relationships of certain individuals were supported in both analyses (e.g., Steenbras A + Kogelberg; Steenbras B + Steenbras C; Wemmershoek + Grabouw; and Protea Valley 1 + Protea Valley 2), other relationships were usually not strongly supported or were retrieved only by one of the analyses. Both analyses, however, supported (with bootstrap $\geqslant 95\%$) the existence of a clade to the exclusion of the distantly related Tsitsikamma representative. Although the two representatives of the populations identified through the morphometric analysis as *M. depressus* (Steenbras B and Steenbras C) grouped together, they were nested among individuals identified morphometrically as *M. abbreviatus*.

Again, no geographic or drainage system patterns were evident from the relationships among representative specimens. Representatives collected from geographically proximate localities were widely separated, lying within different clades (e.g., Steenbras A vs. Steenbras B and Steenbras C), while geographically more distant individuals clustered as sister taxa (e.g., Wemmershoek + Grabouw).

Topologies derived from the analyses of the sequence data were incongruent with those proposed by allozyme data. Indeed, topologies constrained to reflect the in-group relationships proposed by the allozyme neighbor-joining tree and UPGMA dendrogram were, respectively, 29 and 38 steps longer than the topologies retrieved in the analyses of sequence data (MP- and ML-derived topologies both 240 steps, autapomorphic characters excluded). While certain terminal relationships among closely related individuals/populations were consistently retrieved in all analyses, conflicting relationships were suggested for many of the populations/representatives. For example, while the relationship between the geographically proximate Protea Valley and Barrydale populations proposed by the allozyme analysis was not rejected in the analyses of sequence data, a sister-taxon relationship was proposed between the Protea Valley representative and the more distantly collected Tradouw Pass representative, with the Barrydale representative occurring basal to a larger clade including the former individuals.

Uncorrected sequence divergences among in-group individuals and the out-group specimens ranged from 12.00 to 16.50%. Despite the monophyly of the 16 in-group taxa (bootstrap $\geqslant 99\%$ in both MP and ML analyses), the Tsitsikamma representative was less similar to the remaining in-group individuals (distinguished from the remaining in-group specimens by sequence divergences of 15.50–16.50%) than were the out-group individuals (12.00–14.83% divergent). Sequence divergences among these remaining in-group individuals were between 0.00% (the comparison of Betty's Bay A and Betty's Bay B representatives) and 7.83% (the comparison of the Grabouw and Greyton representatives), with a mean of $4.66 \pm 1.87\%$.

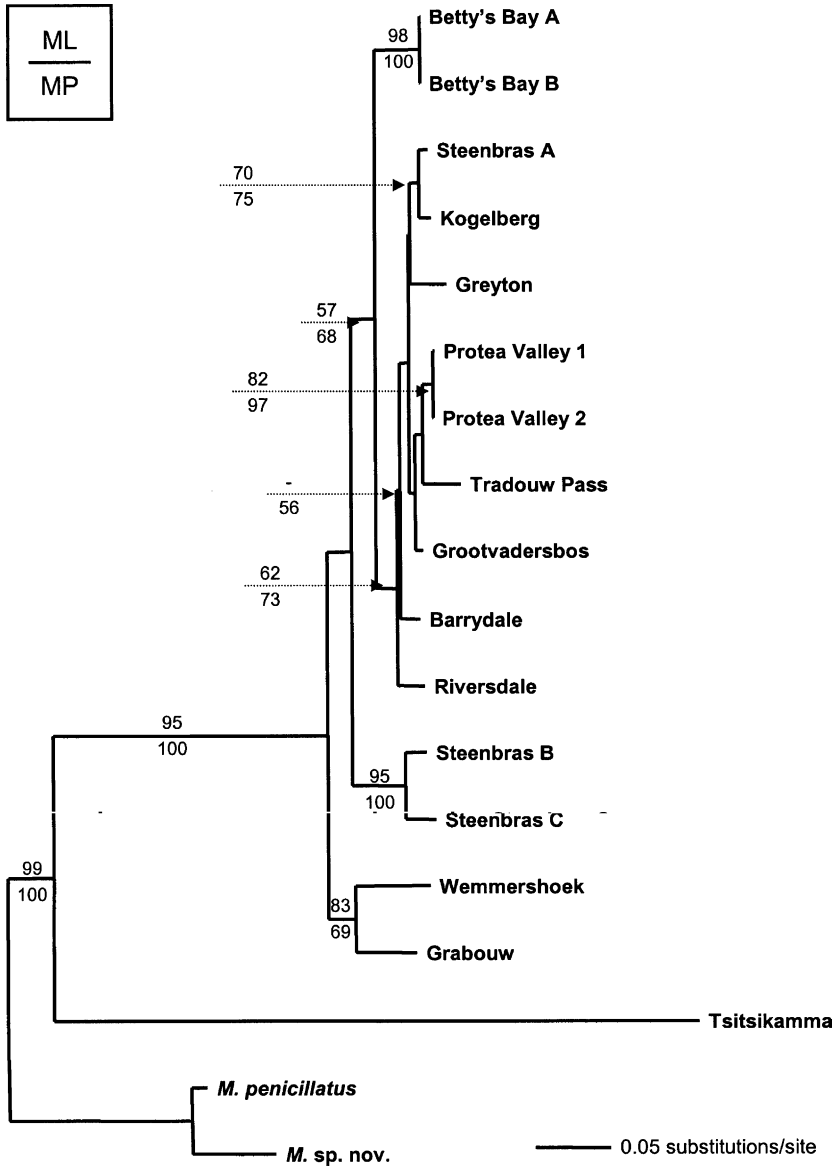

Fig. 3. Maximum likelihood phylogram ($-\ln L = 2268.579$) of the reduced data set of 16 in-group taxa and the two out-group individuals (*Mesamphisopus* sp. nov. and *M. penicillatus*). Analysis included 600-bp COI mtDNA and implemented a GTR + I + Γ (Rodríguez et al., 1990) model of nucleotide evolution. Numbers above the branches represent ML bootstrap support from 100 pseudoreplicates, while numbers below represent bootstrap support from the MP (1000 replicates) analysis. Only bootstrap support >50% is indicated.

3.4. Isolation by distance

When considering individual populations, a significant correlation ($r = 0.779$, $t = 3.561$, $P < 0.05$; Fig. 4A) was found between geographic distance between collection localities and the mean genetic distance (D) among populations, indicating isolation by distance. If comparisons involving the Tsitsikamma population were omitted, this relationship was, however, non-significant ($r = -0.013$, $t = -0.109$, $P = 0.484$). Correlations investigated for individual locus comparisons, using genetic identity (I) as a measure of differentiation, showed no evidence of isolation by distance with one exception; a significant relationship was evident at the *Mdh-2* locus ($r = 0.202$, $t = 1.462$, $P < 0.05$) with the omission of the Tsitsikamma population.

As in the allozyme analysis, a significant correlation was found between sequence divergence and the geographic distance between collection localities (Fig. 4B; $r = 0.468$, $t = 3.249$, $P < 0.01$), but the relationship again became non-significant ($r = 0.131$, $t = 1.249$, $P = 0.112$) upon exclusion of the Tsitsikamma representative.

Considering differentiation among regions, with populations pooled within regions, an identical pattern was observed. There were significant correlations among genetic distance (D) and geographic distance ($r = 0.760$, $t = 2.369$, $P < 0.05$), and sequence divergence and geographic distance ($r = 0.776$, $t = 2.399$, $P < 0.05$), indicating isolation by

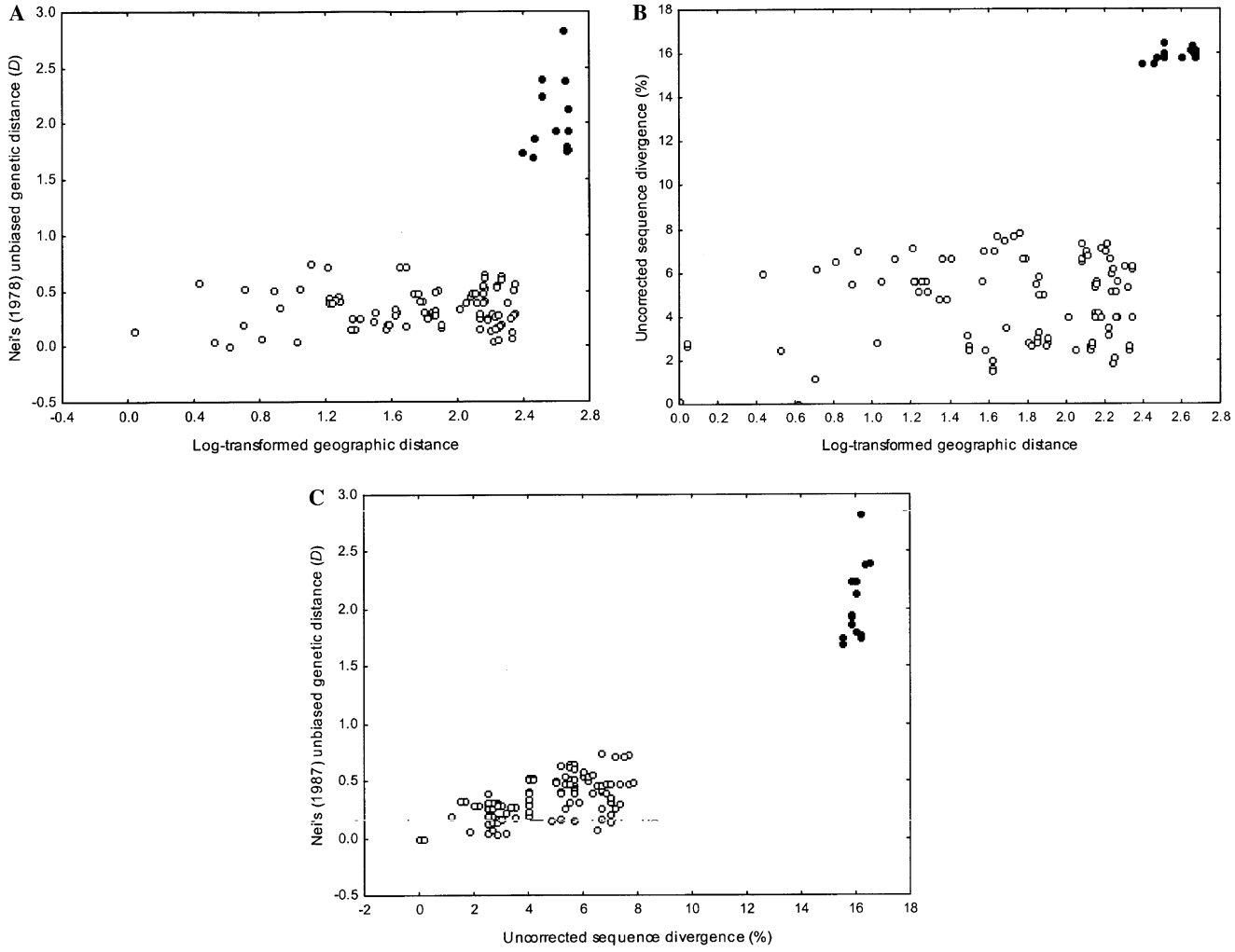

Fig. 4. Scatterplots of (A) Nei's (1978) unbiased genetic distance among populations over the log-transformed geographic distance between their collection localities; (B) uncorrected sequence divergence (calculated from 600 bp of COI mtDNA) among representatives from each population over the log-transformed geographic distances between collection localities; and (C) genetic distance among populations over the uncorrected sequence divergences separating representatives from the same populations. Filled circles represent comparisons involving the Tsitsikamma population/representative, while comparisons between other in-group populations/representatives are indicated by open circles.

distance. Again, these relationships became non-significant upon exclusion of the Tsitsikamma region ($r=0.029$, $t=0.131$, $P=0.412$, and $r=0.224$, $t=1.199$, $P=0.148$, respectively).

Over a smaller geographic scale, there was a significant correlation between sequence divergence and geographic distance ($r=0.456$, $t=2.024$, $P<0.05$) among individual populations of the Hottentot's Holland Mountain region, but not between genetic (D) and geographic distance ($r=0.147$, $t=0.655$, $P=0.189$).

Mantel tests (Mantel, 1967) revealed a significant correlation between genetic distance and sequence divergence in comparisons of distances matrices compiled to examine isolation by distance among individual populations (Fig. 4C; $r=0.922$, $t=3.821$, $P<0.001$) and among regions ($r=0.948$, $t=2.391$, $P<0.01$). These significant relationships held irrespective of the exclusion of the Tsitsikamma population/representative ($r=0.561$, $t=3.642$, $P<0.01$) or region ($r=0.672$, $t=1.810$, $P<0.05$). This correlation was also apparent when considering only the populations from the Hottentot's Holland region ($r=0.609$, $t=2.707$, $P<0.01$).

4. Discussion

While the morphological characters presented in the species descriptions (Barnard, 1927; Nicholls, 1943) and key (Kensley, 2001) appeared to be equivocal for distinguishing *M. abbreviatus* and *M. depressus*, the species appeared to be well separated using morphometric data. *Mesamphisopus depressus* individuals were distinguished from *M. abbreviatus* individuals in a discriminant function analysis, in which the body length, body width (at the third pereonite), and telson depth were among the most important variables of those combined in the

discriminant functions. Nicholls (1943) had suggested differences among these putative species (e.g., the extent of the cervical groove, the shape of the posteroventral margins of the pleura of the pleonites, the setation of the pleopods, and the shape and setation of the uropodal peduncles), mostly through comparisons with *M. capensis*. In subsequent work (Kensley, 2001), these characters were considered to be of little importance for discriminating among the species, if considered at all. These characters need to be reevaluated in these and other species within *Mesamphisopus* and may prove useful in distinguishing the two species.

A subsequent classificatory morphometric analysis determined that individuals of most of the populations sampled were morphometrically identifiable as *M. abbreviatus*. In only two populations (Steenbras B and Steenbras C), were all the examined individuals determined to be morphometrically similar to *M. depressus*. A level of uncertainty is inherent in these diagnoses, as certain individuals could not be assigned to either morphotype. Further, certain populations (Tradouw Pass and Wemmershoek) were shown to possess individuals of both morphotypes, disagreeing with results from the allozyme study (and the sequence data in the case of the Wemmershoek population). The extent or patterns of morphometric differentiation among populations per se were not considered in the present analysis. It is possible, as an artifact of this approach, that intermediate or distinctive morphotypes, which may explain the classification of the Tradouw Pass and Wemmershoek individuals, have not been identified, while the extent of differentiation among populations remains unknown. Further, dimensions of the pereopods and uropods, that may differentiate these populations and the *M. abbreviatus* and *M. depressus* syntypes, were not considered. Given the apparently recent genetic differentiation of the studied populations (see below) and the morphological conservatism of the Phreatoicidea (Wilson and Ho, 1996), it is also probable that morphometric differentiation of populations has not proceeded to the extent that they are recognized as different morphotypes under the current approach.

Both the allozyme and sequence data supported the genetic distinctiveness of the Tsitsikamma population/representative and its exclusion from a clade containing the remaining populations or representatives. Genetic distances between the Tsitsikamma population and the remaining populations were at least double and often an order of magnitude greater than distances among these remaining populations. These values are also substantially larger than those previously found among putative species within *Mesamphisopus* (Gouws et al., 2004). Further, the Tsitsikamma population was distinct from the remainder at all loci examined. Sequence divergences showed the Tsitsikamma representative to be more divergent from the remaining in-group individuals than were the two out-group specimens, both distinct species (*Mesamphisopus* sp. nov. and *M. penicillatus*). In light of this, it must be concluded that the Tsitsikamma population probably represents a phylogenetically distinct, but cryptic species.

The delineation of further species is confounded by the incongruence among topologies generated from the different data sets. Genetic distances and sequence divergences also present a paradox as far as the possible delimitation of species based on distance criteria is concerned. The two sets of divergence estimates were significantly correlated, but the implications of the differentiation suggested by each were different and contrary to expectations, considering the features (uniparental inheritance and fourfold lower effective population size) making mitochondrial DNA a more sensitive marker of population differentiation than nuclear markers (Moritz et al., 1987). Comparisons among the in-group representatives (excluding the Tsitsikamma representative) yielded lower sequence divergences (0.17–7.83%; mean 4.66% ± 1.87) than interspecific values found in other isopod studies (Ketmaier et al., 2003; Rivera et al., 2002; Wares, 2001; Wetzer, 2001). These values were more comparable to intraspecific divergences presented by Wares (2001), Rivera et al. (2002), and Ketmaier et al. (2003). These relatively low sequence divergences and the lack of strong resolution among populations did not strongly suggest the recognition of a species complex among the studied populations. Genetic distances based on allozyme data appeared to be more ambiguous. The genetic distances among the studied populations were more comparable to distances among isopod subspecies (Cobolli Sbordoni et al., 1997; Ketmaier et al., 1999), or interspecific distances within *Mesamphisopus* (Gouws et al., 2004) and other isopod groups (Garthwaite et al., 1992; Ketmaier et al., 1998, 2001; Lessios and Weinberg, 1994; Viglianisi et al., 1992). The greater differentiation revealed by the allozyme data may, however, be an artifact of the specific loci examined or the divergence estimates used (Kalinowski, 2002). Most loci were polymorphic or provided evidence of fixed allelic differences among populations. However, the choice of loci was not explicitly biased in this regard, as loci were included without prior knowledge of polymorphism or variability. The genetic distance measure (Nei, 1978) used may also be vastly inflated or "saturated", as estimates at these individual loci approach infinity when fixed allele differences are present. Nei's (1978) distance measure also appears to be more sensitive than other measures to reductions in population sizes (see below) under modeled conditions (Kalinowski, 2002).

Given the discordance among distances and topologies, only very limited evidence supports the examined populations as being representative of a recently

differentiated species complex: the two populations identified morphometrically as *M. depressus* and collected from near the presumed type locality of *M. depressus* in the Steenbras Valley were consistently retrieved as sister taxa. These formed a phylogenetically distinct lineage and were (with one exception) topologically nested among the remaining populations, all similarly differentiated. These topologies also suggest that *M. abbreviatus* may be a paraphyletic species, should the morphometric identification of these remaining populations be correct and correspond to recognized species boundaries. Molecular studies are revealing an increasing number of species to be paraphyletic or polyphyletic as presently constituted (Funk and Omland, 2003). In some cases, inadequate phylogenetic information and a lack of resolution have revealed gene trees reflecting species-level paraphyly/polyphyly. In other cases, paraphyly and polyphyly may result from taxonomies defining species too narrowly or too broadly (including the failure to recognize cryptic taxa), introgression, or incomplete lineage sorting (Funk and Omland, 2003). Paraphyly resulting from inaccurate taxonomies can be easily rectified. However, in the absence of extended and more intensive sampling, including topotypic specimens, and additional data to adequately resolve the status of *M. abbreviatus*, *M. depressus*, and the above populations and determine the cause of this potential paraphyly, it would be most prudent to consider the populations studied here as potentially conspecific, at least for the sake of further discussion.

Genetic differentiation was evident among nearly all these populations at even the smallest examined spatial scales. A lack of gene flow was inferred from the number of fixed allelic differences, high θ estimates and distance values, and the great disparity in genetic variability estimates, among both local and distant populations. A similar lack of gene flow over small spatial scales has been documented in cave dwelling (Cobolli Sbordoni et al., 1997; Gentile and Sbordoni, 1998; Ketmaier et al., 1998) and intertidal isopods, where differentiation has been observed over less than a few kilometres (Carvalho and Piertney, 1997; Lessios and Weinberg, 1993, 1994; Lessios et al., 1994) or meters (Piertney and Carvalho, 1994, 1995a,b). The patterns observed above appeared to be unrelated to the geographic distances, resulting in non-significant relationships in tests for isolation by distance, and also appeared to bear no immediately apparent relationship to altitude. This is unlike other freshwater isopods, where geography has predicted genetic patterns (Ketmaier et al., 2001), or terrestrial isopods, in which isolation by distance has been demonstrated (Wang and Schreiber, 1999). Patterns of relatedness among populations were also unrelated to drainages, as expected under a nested hierarchy of drainages (Woolschot et al., 1999). The pattern observed, in the analyses of both markers, is best described as a mosaic, with genetic differentiation on local scales being generally equivalent to differentiation over large scales and bearing no relation to geography. Similar patterns of genetic patchiness have been described for other isopods (Bilton et al., 1999; Lessios and Weinberg, 1993, 1994; Piertney and Carvalho, 1995a).

In most of the previous studies (Carvalho and Piertney, 1997; Cobolli Sbordoni et al., 1997; Gentile and Sbordoni, 1998; Ketmaier et al., 1998, 1999; Lessios and Weinberg, 1993, 1994; Lessios et al., 1994; Piertney and Carvalho, 1995a,b), differentiation among isopod populations has been explained by stochastic population crashes (due to habitat instability), leading to local extinction and founder events, or to population bottlenecks. With low gene flow (due to geographic obstacles, the discontinuity of ecologically suitable habitat for dispersal, limited vagility of adults, and the "direct" marsupial development of the young), small effective population sizes, exacerbated by short generation times (Lessios et al., 1994), would lead to the differentiation of populations through mutation, genetic drift, localized selection, and inbreeding. These processes can lead to initial reductions in heterozygosity and variability (Lessios and Weinberg, 1994; Piertney and Carvalho, 1995b) and rapid, drastic, and temporal changes in allele frequencies (Carvalho and Piertney, 1997; Lessios et al., 1994), subsequently observed as spatial genetic differentiation.

Similarly, *Mesamphisopus* has a typically patchy distribution in the high-altitude, slow-flowing portions of catchments in broad mature valleys (Barnard, 1927). Continuous habitats through which dispersal would be possible are lacking, and recent dispersal and gene flow among populations would be, and would have been, unlikely. In addition, generation times are short, with adults being able to breed after 1 year (Barnard, 1927), and dispersal ability among the geographically separated population is poor. While *M. abbreviatus* is known to aestivate over long periods (Barnard, 1927), the highly seasonal nature of the habitats in which *Mesamphisopus* populations occur suggests that populations may be exposed to frequent droughts and may experience population crashes during dry summer months. Repeated population bottlenecks with a sufficient number of individuals surviving through aestivation to enable population survival may provide an explanation for the pattern of differentiation seen among populations. Frequent population crashes and bottlenecks have been invoked as an explanation for the population differentiation observed within paramelitid amphipods (Stewart, 1992), occurring sympatrically with *Mesamphisopus* (Barnard, 1927; personal observation). These bottlenecks would lead to the differentiation of geographically proximate populations, with geographically distant populations potentially remaining less differentiated, resulting in the observed mosaic pattern. Any of the large set of

ancestral alleles could be retained in certain populations and lost in others, resulting in alleles being shared by geographically disjunct populations rather than proximate populations (Cobolli Sbordoni et al., 1997; Lessios and Weinberg, 1993). Under repeated bottlenecks, the patterns revealed by the sequence data may result from stochastic lineage sorting and the random fixation of different haplotypes in these populations. Branch lengths and sequence divergences suggest that the genetic differentiation of most of the in-group representatives was rapid and fairly recent (at least relative to the divergences of the Tsitsikamma lineage and the out-group specimens). Lineage sorting could possibly have proceeded among a large pool of closely related haplotypes, resulting in the mosaic pattern seen. Regardless of the processes involved, the discordances among topologies and among the magnitudes of genetic differentiation suggest that conclusions based on topologies and, particularly, genetic divergences derived from single markers may be spurious and argue for caution in the use of genetic distances for making taxonomic decisions.

Acknowledgments

The study was supported by grants from the National Research Foundation, South Africa, the University of Stellenbosch, and its Department of Botany and Zoology. CapeNature provided the permits under which collections were made. The authors thank the reserve managers (particularly Anita Wheeler, Mark Johns, and Henk Jacobs) for their logistical support. Clive May (Steenbras Catchment Management, Cape Unicity Council) is thanked for his assistance. We also extend our gratitude to Krystal Tolley, who assisted with the drafting of the map, to Savel Daniels, for his invaluable suggestions, and to Fawzia Gordon, André Gouws, Sydwell Hawu, Liz Hoenson, Melanie Marais, Monesi Mvandaba, Nicole Meyer, Jan Schoombee, and Alex Searby, for their assistance in the field. We also thank the South African Museum (Iziko Museums of Cape Town) for the loan of the syntypic material. We also thank the editor (Dr. Gonzalo Giribet) and the two anonymous reviewers, whose input substantially improved the manuscript.

Appendix A

Allele frequencies at the 12 polymorphic loci and genetic diversity measures for the 15 populations of *Mesamphisopus* that were studied. *N* denotes the sample size for each of the populations at the respective locus

Locus		Population														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ao	*N*	5	20	21	23	21	30	20	21	19	25	25	30	30	28	36
100		1.000	1.000	0.000	0.848	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
90		0.000	0.000	1.000	0.152	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ark	*N*	35	33	28	23	25	30	20	48	20	14	11	30	29	28	40
115		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
105		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.000
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.914	1.000	0.000
Gpi	*N*	35	33	28	23	30	30	20	48	20	30	30	27	28	30	40
160		0.000	0.061	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
155		0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145		0.986	0.939	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
140		0.000	0.000	0.000	0.000	0.383	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120		0.000	0.000	0.000	0.391	0.617	0.000	1.000	0.188	0.000	0.967	0.000	0.019	0.107	1.000	0.000
110		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
105		0.000	0.000	0.000	0.348	0.000	0.000	0.000	0.490	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		0.014	0.000	0.982	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
95		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
90		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.444	0.893	0.000	0.000
80		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
70		0.000	0.000	0.018	0.152	0.000	0.000	0.000	0.323	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.537	0.000	0.000	0.000
40		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.775
10		0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.225
Hk	*N*	28	27	28	23	26	30	20	35	18	27	18	30	27	28	40
125		0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		0.964	0.981	0.661	0.935	0.192	0.600	0.975	0.986	0.556	0.000	0.000	0.717	0.963	1.000	1.000
95		0.036	0.000	0.304	0.065	0.731	0.400	0.000	0.014	0.417	1.000	1.000	0.283	0.037	0.000	0.000

(*continued on next page*)

Appendix A (*continued*)

Locus		Population														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
85		0.000	0.000	0.036	0.000	0.077	0.000	0.025	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000
Idh	*N*	7	26	28	7	29	20	20	14	20	29	18	30	26	27	24
170		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.792**
135		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
120		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	0.293	0.806	1.000	0.635	1.000	**0.000**
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.707	0.194	0.000	0.365	0.000	**0.208**
Ldh	*N*	23	33	28	23	30	30	20	34	20	30	30	30	30	30	40
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
80		0.000	0.152	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
70		1.000	0.848	1.000	0.978	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
50		0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Null		0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	0.000	0.000	1.000	0.000
Lt-1	*N*	25	28	25	23	26	30	20	36	20	30	27	30	30	22	37
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
90		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Lt-2	*N*	25	18	25	23	19	30	20	24	17	30	21	30	30	13	35
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
Null		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Mdh-1	*N*	33	31	28	23	30	30	20	37	19	30	30	30	27	30	40
130		0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100		0.000	0.000	0.000	**0.000**	1.000	1.000	0.000	0.000	0.658	0.000	0.000	0.000	0.000	0.000	0.000
80		1.000	1.000	1.000	**0.957**	0.000	0.000	1.000	1.000	0.342	1.000	1.000	1.000	1.000	1.000	0.000
70		0.000	0.000	0.000	**0.043**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*N*	33	33	28	23	30	30	20	40	20	30	30	30	30	30	37
100		1.000	1.000	1.000	0.978	1.000	1.000	0.900	1.000	1.000	0.983	1.000	1.000	0.983	1.000	0.000
40		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
15		0.000	0.000	0.000	0.022	0.000	0.000	0.100	0.000	0.000	0.017	0.000	0.000	0.017	0.000	0.000
Me	*N*	34	33	28	23	30	30	20	27	20	30	30	30	30	30	23
115		0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		1.000	0.985	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90		0.000	0.000	1.000	1.000	0.000	0.000	1.000	0.963	1.000	1.000	1.000	1.000	1.000	1.000	0.000
75		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Pgm	*N*	30	27	28	23	30	30	20	36	20	30	30	30	30	30	40
110		0.000	0.000	0.000	0.000	0.083	0.000	0.000	**0.000**	0.000	0.017	0.000	0.000	0.000	0.000	**0.962**
100		0.000	0.000	0.036	0.087	0.917	1.000	0.000	**0.000**	1.000	0.167	0.000	0.000	0.000	0.000	**0.038**
90		1.000	1.000	0.018	0.826	0.000	0.000	0.000	**0.972**	0.000	0.350	1.000	0.967	0.967	0.700	**0.000**
80		0.000	0.000	0.875	0.087	0.000	0.000	1.000	**0.028**	0.000	0.467	0.000	0.033	0.033	0.000	**0.000**
70		0.000	0.000	0.071	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000	0.000	0.300	**0.000**
A		1.167	1.333	1.500	2.083	1.333	1.083	1.167	1.500	1.250	1.583	1.083	1.333	1.500	1.083	1.250
		± 0.389	± 0.492	± 1.000	± 1.676	± 0.651	± 0.289	± 0.389	± 0.674	± 0.622	± 0.996	± 0.289	± 0.651	± 0.522	± 0.289	± 0.452
H_O		0.008	0.031	0.048	0.138	0.059	0.022	0.021	0.064	0.071	0.087	0.023	0.061	0.070	0.028	0.042
		± 0.021	± 0.059	± 0.104	± 0.237	± 0.118	± 0.077	± 0.058	± 0.151	± 0.179	± 0.193	± 0.080	± 0.129	± 0.108	± 0.096	± 0.115
H_E		0.008	0.037	0.062	0.132	0.089	0.041	0.020	0.077	0.083	0.097	0.027	0.084	0.083	0.036	0.064
		± 0.021	± 0.078	± 0.147	± 0.212	± 0.177	± 0.141	± 0.054	± 0.179	± 0.194	± 0.210	± 0.093	± 0.182	± 0.140	± 0.123	± 0.133
$P_{95\%}$		0.00	16.67	16.67	33.33	25.00	8.33	8.33	16.67	16.67	16.67	8.33	16.67	25.00	8.33	16.67

Alleles are numbered following their mobility relative to an allele present in a reference population. Individual cases of polymorphism showing genotypic deviations from Hardy–Weinberg expectations (as determined by χ^2 test) are indicated in bold font (all $P < 0.001$). Genetic variability measures include: the mean number of alleles per locus (A), the mean observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E), and the percentage of loci that were polymorphic ($P_{95\%}$) using a 95% criterion. Standard deviations are presented below the individual variability estimates. Populations are numbered as in Fig. 1.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723.

Bahri-Sfar, L., Lemaire, C., Ben Hassine, O.K., Bonhomme, F., 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proc. R. Soc. Lond. B. 267, 929–935.

Barnard, K.H., 1914. Contributions to the crustacean fauna of South Africa. 2. Description of a new species of *Phreatoicus* (Isopoda) from South Africa. Ann. S. Afr. Mus. 10, 231–240.

Barnard, K.H., 1927. A study of the freshwater isopodan and amphipodan Crustacea of South Africa. Trans. R. Soc. S. Afr. 14, 139–215.

Barnard, K.H., 1940. Contributions to the crustacean fauna of South Africa. XII. Further additions to the Tanaidacea, Isopoda, and Amphipoda, together with keys for the identification of the hitherto recorded marine and fresh-water species. Ann. S. Afr. Mus. 32, 381–543.

Beck, M.L., Price, J.O., 1981. Genetic variation in the terrestrial isopod, *Armadillidium vulgare*. J. Hered. 72, 15–18.

Berrebi, P., Lévêque, C., Cattaneo-Berrebi, G., Agnèse, J.-F., Guégan, J.-F., Machordom, A., 1990. Diploid and tetraploid African *Barbus* (Osteichthyes, Cyprinidae): on the coding of differential gene expression. Aquat. Liv. Res. 3, 313–323.

Bilton, D.T., Goode, D., Mallet, J., 1999. Genetic differentiation and natural hybridization between two morphological forms of the common woodlouse, *Oniscus asellus* Linnaeus 1758. Heredity 82, 462–469.

Bohonak, A.J., 1999. Dispersal, gene flow, and population structure. Quart. Rev. Biol. 74, 21–45.

Bucklin, A., Smolenack, S.B., Bentley, A.M., Wiebe, P.H., 1997. Gene flow patterns of the euphausiid, *Meganyctiphanes norvegica*, in the NW Atlantic based on mtDNA sequences for cytochrome *b* and cytochrome oxidase I. J. Plankton Res. 19, 1763–1781.

Burton, R.S., Feldman, M.W., 1982. Population genetics of coastal and estuarine invertebrates: does larval behavior influence population structure?. In: Kennedy, V.S. (Ed.), Estuarine Comparisons. Academic Press, New York, pp. 537–551.

Carvalho, G.R., Piertney, S.B., 1997. Interspecific comparisons of genetic population structure in members of the *Jaera albifrons* species complex. J. Mar. Biol. Assoc. UK 77, 77–93.

Cavalcanti, M., 2000. Mantel for Windows: Test for Association between Two Symmetric Distance Matrices with Permutation Iterations (Version 1.11). Setor de Paleovertebrados, Departamento de Geologia e Paleontologia, Museu Nacional do Rio de Janeiro, Rio de Janiero.

Cobolli Sbordoni, M., Ketmaier, V., De Matthaeis, E., Taiti, S., 1997. Biochemical systematics and evolutionary relationships in the *Trichoniscus pusillus* complex (Crustacea, Isopoda. Oniscidea). Heredity 79, 463–472.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Folmer, O.M., Black, M., Hoeh, R., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423.

Garthwaite, R.L., Lawson, R., Taiti, S., 1992. Morphological and genetic relationships among four species of *Armadilloniscus* Uljanin, 1875 (Isopoda: Oniscidea: Scyphacidae). J. Nat. Hist. 26, 327–338.

Gentile, G., Sbordoni, V., 1998. Indirect methods to estimate gene flow in cave and surface populations of *Androniscus dentiger* (Isopoda: Oniscidea). Evolution 52, 432–442.

Goudet, J., 2001. FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices (Version 2.9.3). Institute of Ecology, University of Lausanne, Lausanne, Switzerland.

Gouws, G., Stewart, B.A., Daniels, S.R., 2004. Cryptic species within the freshwater isopod *Mesamphisopus capensis* (Phreatoicidea: Amphisopodidae) in the Western Cape, South Africa: allozyme and 12S rRNA sequence data and morphometric evidence. Biol. J. Linn. Soc. 81, 235–253.

Hillis, D.M., Mable, B.K., Larson, A., Davis, S.K., Zimmer, E.A., 1996. Nucleic acids IV: sequencing and cloning. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, second ed. Sinauer Associates, Sunderland, Massachusetts, pp. 321–381.

Kalinowski, S.T., 2002. Evolutionary and statistical properties of three genetic distances. Mol. Ecol. 11, 1263–1273.

Kensley, B., 2001. Isopoda. In: Day, J.A., Stewart, B.A., De Moor, I.J., Louw, A.E. (Eds.), Guides to the Freshwater Invertebrates of Southern Africa, vol. 4: Crustacea III. Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea, Decapoda. Water Research Commission, Pretoria, pp. 50–74.

Ketmaier, V., Argano, R., Caccone, A., 2003. Phylogeography and molecular rates of subterranean aquatic stenasellid isopods with a peri-Tyrrhenian distribution. Mol. Ecol. 12, 547–555.

Ketmaier, V., Argano, R., Cobolli, M., De Matthaeis, E., 1999. Genetic divergence and evolutionary times: calibrating a protein clock for South-European *Stenasellus* species (Crustacea, Isopoda). Int. J. Speleol. 26, 63–74.

Ketmaier, V., Argano, R., Cobolli, M., De Matthaeis, E., Messana, G., 1998. Cladogenetic events in two relict species of Stenasellidae (Crustacea, Isopoda) from Sardinia Island (Tyrrhenian Sea): a biochemical approach. Vie et Milieu 49, 93–99.

Ketmaier, V., Argano, R., Cobolli, M., De Matthaeis, E., Messana, G., 2001. A systematic and biogeographical study of epi- and hypogean populations of the *Proasellus* species group from Sardinia, central Italy and Jordan: allozyme insights. J. Zool. Syst. Evol. Res. 39, 53–61.

King, J.L., Hanner, R., 1998. Cryptic species in a "living fossil" lineage: taxonomic and phylogenetic relationships within the genus *Lepidurus* (Crustacea: Notostraca) in North America. Mol. Phylogenet. Evol. 10, 23–36.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics 17, 1244–1245.

Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.

Larsen, K., 2001. Morphological and molecular investigation of polymorphism and cryptic species in tanaid crustaceans: implications for tanaid systematics and biodiversity estimates. Zool. J. Linn. Soc. 131, 353–379.

Lessios, H.A., Weinberg, J.R., 1993. Migration, gene flow and reproductive isolation between and within morphotypes of the isopod *Excirolana* in two oceans. Heredity 71, 561–573.

Lessios, H.A., Weinberg, J.R., 1994. Genetic and morphological divergence among morphotypes of the isopod *Excirolana* on the two sides of the Isthmus of Panama. Evolution 48, 530–548.

Lessios, H.A., Weinberg, J.R., Starczak, V.R., 1994. Temporal variation in populations of the marine isopod *Excirolana*: How stable are gene frequencies and morphology? Evolution 48, 549–563.

Machordom, A., Doadrio, I., Berrebi, P., 1995. Phylogeny and evolution of the genus *Barbus* in the Iberian Peninsula as revealed by allozyme electrophoresis. J. Fish Biol. 47, 211–236.

Maddison, D.R., Maddison, W.P., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Meffe, G.K., Vrijenhoek, R.C., 1988. Conservation genetics in the management of desert fishes. Cons. Biol. 2, 157–169.

Meyran, J.-C., Monnerot, M., Taberlet, P., 1997. Taxonomic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 8, 1–10.

Michels, E., Audenaert, E., Ortells, R., De Meester, L., 2003. Population genetic structure of three pond-inhabiting *Daphnia* species on a regional scale (Flanders, Belgium). Freshwater Biol. 48, 1825–1839.

Moritz, C., Dowling, T.E., Brown, W.M., 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18, 269–292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nicholls, G.E., 1943. The Phreatoicoidea. Part I. The Amphisopidae. Pap. Proc. R. Soc. Tasm. 1942, 1–145.

Piertney, S.B., Carvalho, G.R., 1994. Microgeographic genetic differentiation in the intertidal isopod *Jaera albifrons* Leach. I. Spatial distribution of allozyme variation. Proc. R. Soc. Lond. B 256, 195–201.

Piertney, S.B., Carvalho, G.R., 1995a. Microgeographic genetic differentiation in the intertidal isopod *Jaera albifrons* Leach. II. Temporal variation in allele frequencies. J. Exp. Mar. Biol. Ecol. 188, 277–288.

Piertney, S.B., Carvalho, G.R., 1995b. Detection of high levels of genetic relatedness in rock-populations of an intertidal isopod using DNA fingerprinting. J. Mar. Biol. Assoc. UK 75, 967–976.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Rivera, M.A.J., Howarth, F.G., Taiti, S., Roderick, G.K., 2002. Evolution in Hawaiian cave-adapted isopods (Oniscidea: Philosciidae): vicariant speciation or adaptive shifts? Mol. Phylogenet. Evol. 25, 1–9.

Rodríguez, F., Oliver, J.F., Marín, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Rodriguez-Lanetty, M., Hoegh-Guldberg, O., 2002. The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the Western Pacific. Mol. Ecol. 11, 1177–1189.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: a Laboratory. Manual, third ed. Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York.

Sarver, S.K., Silberman, J.D., Walsh, P.J., 1998. Mitochondrial DNA sequence evidence supporting the recognition of two subspecies or species of the Florida spiny lobster *Panulirus argus*. J. Crustacean Biol. 18, 177–186.

Schubart, C.D., Reimer, J., Diesel, R., 1998. Morphological and molecular evidence for a new endemic freshwater crab, *Sesarma ayatum* sp. n., (Grapsidae, Sesarminae) from eastern Jamaica. Zool. Scr. 27, 373–380.

Shaw, C.D., Prasad, R., 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. 4, 297–320.

Sneath, P.H.A., Sokal, R.R., 1973. Numerical Taxonomy: the Principles and Practice of Numerical Classification. W.H. Freeman and Company, San Francisco.

Sponer, R., Roy, M.S., 2002. Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. Evolution 56, 1954–1967.

Statsoft, Inc., 2001. STATISTICA (Data Analysis Software System), Version 6.0. Statsoft, Inc., Tulsa, Oklahoma.

Stewart, B.A., 1992. Morphological and genetic differentiation between allopatric populations of a freshwater amphipod. J. Zool. (Lond.) 228, 287–305.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony, and Other Methods. Version 4.10. David L. Swofford, Illinois Natural History Survey, Illinois.

Swofford, D.L., Selander, R.B., 1981. BIOSYS-1: a Computer Program for the Analyses of Allelic Variation in Population Genetics and Biochemical Systematics. Release 1.7. David L. Swofford, Illinois Natural History Survey, Illinois.

Viglianisi, F., Lombardo, B.M., Caruso, D., 1992. Differenziamento genetico e morfologico in alcune specie siciliane di Isopodi terrestri del genere *Porcellio* e descrizione di tre nuove specie (Crustacea, Isopoda, Oniscoidea). Animalia 19, 235–273.

Wahlund, S., 1928. Zusammersetung von populationen und korrelation-sercheinungen von standpunkt der verebungslehre aus betrachtet. Hereditas 11, 65–106.

Wang, M., Schreiber, A., 1999. Population differentiation of the woodlouse *Oniscus asellus* in central Europe (Isopoda: Oniscoidea). J. Crustacean Biol. 19, 301–312.

Wares, J.P., 2001. Intraspecific variation and geographic isolation in *Idotea balthica* (Isopoda: Valvifera). J. Crustacean Biol. 21, 1007–1013.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Wetzer, R., 2001. Hierarchical analysis of mtDNA variation and the use of mtDNA for isopod (Crustacea: Peracarida: Isopoda) systematics. Contr. Zool. 70, 23–39.

Wilson, G.D.F., Ho, E.L., 1996. *Crenoicus* Nicholls, 1944 (Crustacea, Isopoda, Phreatoicidea): systematics and biology of a new species from New South Wales. Rec. Aust. Mus. 48, 7–32.

Wilson, G.D.F., Keable, S.J., 1999. A new genus of phreatoicidean isopod (Crustacea) from the north Kimberley region, Western Australia. Zool. J. Linn. Soc. 126, 51–79.

Wilson, G.D.F., Keable, S.J., 2001. Systematics of the Phreatoicidea. In: Kensley, B., Brusca, R.C. (Eds.), Isopod Systematics and Evolution. (Crustacean Issues 13). A.A. Balkema, Rotterdam, pp. 175–194.

Wishart, M.J., Hughes, J.M., 2003. Genetic population structure of the net-winged midge, *Elporia barnardi* (Dipitera: Blephariceridae) in streams of the south-western Cape, South Africa: implications for dispersal. Freshwater Biol. 48, 28–38.

Woolschot, L., Hughes, J.M., Bunn, S.E., 1999. Dispersal among populations of *Caridina* sp. (Decapoda: Atyidae) in coastal lowland streams, south-eastern Queensland, Australia. Mar. Freshwater Res. 50, 681–688.

Wright, S., 1943. Isolation by distance. Genetics 28, 114–138.