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PRIMARY RESEARCH PAPER

From genetic structure to wetland conservation: a freshwater isopod *Paramphisopus palustris* (Phreatoicidea: Amphisopidae) from the Swan Coastal Plain, Western Australia

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Abstract The freshwater isopod *Paramphisopus palustris* is ubiquitous and abundant in the groundwater-fed wetlands of the Swan Coastal Plain around Perth, Western Australia. Taxonomically, an additional variety (*P. palustris fairbridgei*) and species (*P. montanus*) are recognized from geographically outlying localities. Here a 486 bp fragment of cytochrome *c* oxidase subunit I (COI) mtDNA was sequenced in 68 individuals from 23 localities in order to evaluate the accepted taxonomy, to examine the evolutionary history of the species, and to identify lineages to prioritize conservation of wetlands already substantially modified. MtDNA showed individual populations to be largely distinct and differentiated. The 41 unique haplotypes formed seven independent, geographically defined networks. Phylogenetic analysis retrieved corresponding subclades, with three well-supported larger clades occurring (1) north of the Swan River, (2) south of the Swan River, and (3) in an area further south. A clear pattern of isolation by distance was detected suggesting an ancient serial founder event, with the pattern possibly persisting in the face of limited gene flow through priority effects. The possibility of incipient speciation, the monophyly of the recognized subspecies and the paraphyly of *P. palustris* with respect to *P. montanus*, suggest that the current taxonomy is invalid and requires re-examination. Divergences suggest a mid- to early Pliocene divergence of the major clades, with early Pliocene divergences among subclades probably driven by documented intense arid periods. Lineages are present in wetlands in geologically younger environments suggesting in situ survival and persistence. Seven Evolutionarily Significant Units were identified for the conservation of *Paramphisopus*, two of which are not currently represented in conservation reserves. With increased water demand and the negative impact of surrounding land-use, the current study provides a first phylogeographic assessment of conservation priorities for wetlands of the Swan Coastal Plain.

Keywords COI mtDNA · Dune systems · Groundwater · Isolation by distance · Persistent founder event · *P. montanus* · *P. palustris* var. *fairbridgei*

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Introduction

The ancient isopodan suborder Phreatoicidea is represented in the freshwaters of Western Australia by eight endemic genera (Wilson & Keable, 2002). These genera, mostly included within the families Hypsimetopidae and Amphisopidae senso stricto (see Wilson & Edgecombe, 2003), are phylogenetically distinct and are an important component of Western Australia's relictual and diverse Gondwanan fauna (Wilson & Keable, 1999, 2002; Wilson & Edgecombe, 2003). While overall phreatoicidean diversity is greatest in Australia, the generic diversity in Western Australia is nonetheless remarkable when considering the region's aridity and rivals that of the wetter southeast (Wilson & Keable, 2002; Wilson & Edgecombe, 2003). By contrast, species diversity is low in Western Australia. Six of these genera are presently monotypic and have narrow distributions (see Nicholls & Milner, 1923; Knott & Halse, 1999; Wilson & Keable, 1999, 2002). Only *Amphisopus* and *Paramphisopus*, both restricted to the southwestern portion of the state, contain multiple recognized species (Nicholls, 1943).

Paramphisopus palustris (Glauert, 1924) was described from specimens collected in Dog Swamp/Smith's Lake, in northern Perth (31°55′57″ S, 115°51′02″ E). The species was originally placed within the genus *Phreatoicus* (Glauert, 1924; Nicholls, 1924). Subsequent taxonomic treatments (Nicholls, 1926; Sheppard, 1927) included the species in *Amphisopus* and *Phreatomerus*, before Nicholls (1943) established the genus *Paramphisopus* for its reception. Early collections revealed *P. palustris* to be present in numerous shallow, coastal swamps and lakes in the immediate vicinity of Perth. The species was believed to be absent from the adjacent Darling Ranges and restricted to the low-lying areas by the persistence of surface waters during the summer months (Glauert, 1924; Nicholls, 1924). Subsequent collections extended both the geographical and altitudinal range of *Paramphisopus*. Specimens were found in swamps and ditches in the Pinjarra (32°38′ S, 115°52′ E) area further south, while specimens from further east, towards York (31°53′ S, 116°46′ E), inhabited a temporary lake at the summit of the Darling Range (Nicholls, 1926, 1943). *Paramphisopus palustris* has since been recorded and is routinely found in the majority of wetlands of the Swan Coastal Plain (SCP) (e.g., Balla & Davis, 1993; Growns et al., 1993a).

The SCP is a narrow, low-lying belt of alluvial and aeolian sands with a north-south extent of some 550 km, and is confined by the Indian Ocean coastline to the west and the Darling Ranges to the east (Balla & Davis, 1993). Many seasonal and permanent wetlands are found along the contact boundaries of two major dune systems (the Bassendean and Spearwood Dune systems) and in inter-dune depressions. These shallow wetlands are largely groundwater-fed and are the surface expressions of a superficial, unconfined and heterogenous aquifer (Balla & Davis, 1993; Chessman et al., 2002). Some wetlands are also centred on two major (the Gnangara and Jandakot Mounds) and a number of smaller elevated, water-table mounds (Balla & Davis, 1993). These wetlands are of undoubted biological significance, as areas of primary production and in maintaining ecosystem function, and conservation importance, e.g., the RAMSAR status of Thomson's and Forrestdale Lakes. Despite this, and the city's reliance on the wetlands and groundwater resource for its domestic and industrial needs (Majer, 1979a; Balla & Davis, 1993; Davis et al., 1993), more than 70% of the original 10,000 basins and wetlands have already been lost (Davis & Rosich, 1993a; Davis et al., 1993; Chessman et al., 2002). The remaining wetlands and their biota remain under threat by virtue of their current urban and agricultural setting. Routine physico-chemical investigations and, particularly, invertebrate community structure surveys have been recognized as a management strategy to monitor wetlands for the maintenance of water quality and ecosystem health (Davis & Rosich, 1993a, b; Mitchell et al., 2003). However, an understanding and an accurate assessment of the biodiversity present within the wetlands are integral to such invertebrate monitoring programmes. This is particularly pertinent in situations where the presence and distribution of rare species may be used to evaluate wetland integrity, define conservation priorities or be of importance in the definition of reserve networks, or where

species diversity is used to consider conservation value (see Balla & Davis, 1993; Davis & Rosich, 1993a, b).

Paramphisopus palustris may present a case where historical taxonomic treatments have misrepresented the true diversity of a SCP wetland invertebrate. Early authors (Glauert, 1924; Nicholls, 1924) did not record any variation among individuals from various localities around Perth, beyond noting differences in coloration, which may have been environmentally induced (Glauert, 1924). Nicholls (1926) did suggest subsequently that differences between specimens from the two outlying localities and the typical form found around Perth could be of varietal significance. He later regarded the Pinjarra specimens as a variety, *P. palustris* var. *fairbridgei* Nicholls, 1943 and recognized those from the Darling Range as a new species, *P. montanus* Nicholls, 1943. The differences between these forms are in most cases subtle (see Nicholls, 1943), and he conceded that the resemblances between *P. montanus* and *P. palustris* were close. The more notable differences (differences in body length, brood size, and pigmentation) may be attributed to the acquisition of a cryptozoic habit by *P. montanus*, impelled by the seasonal desiccation of its habitat (Nicholls, 1943). Within *P. palustris*, Nicholls (1943) recognized that morphological variation occurred among geographically defined population groups and suggested that further varietal designations may be warranted. Unfortunately, he provided no discussion of the features that differed among these populations. Consequently, it is difficult to determine the significance of these differences, or to evaluate the importance of the characters used in his taxonomic designations. Regardless, the presence of cryptic species, as suggested by Wilson & Keable (2002), multiple varieties or even diverse conspecific lineages within *P. palustris s. l.* could have significant implications for the management of the wetlands of the SCP.

Thus, the aims of the present study were to investigate the phylogeographic structure of *Paramphisopus*, sampled from across its distribution and including collections made from the type localities of *P. p. fairbridgei* and *P. montanus*. An extensive sampling regime, and the combination of phylogeographic and phylogenetic analyses of the mtDNA sequence data provides a rigorous assessment of the validity of the present taxonomy, and allows for the identification of cryptic diversity within *Paramphisopus*, including unique lineages for consideration in prioritizing wetland conservation. Simultaneously, aspects of the evolutionary processes resulting in the establishment and differentiation of unique lineages may be illuminated.

Materials and methods

Collection

Isopods were collected using hand-nets or dip-nets from 23 localities (Table 1; Fig. 1) situated on the SCP, Western Australia. While most samples were taken from groundwater-fed wetlands, some small streams, drains and surface springs were also sampled. The north-south extent of sampling encompassed 135 km, with Perth located centrally, and the Swan River bisecting this transect. While sampling from the type locality of *P. p. fairbridgei* (Fairbridge Farm, Pinjarra: Nicholls, 1943) was unsuccessful, two localities (the Dandalup River and Buchanan Drains, Pinjarra) in close proximity to the type locality were included. A collection made from the type locality of *P. montanus* (''The Lakes''), approximately 50 km east of Perth, was also included. Samples were also taken from groundwater in a cave, associated with the karstic cave system of the Yanchep National Park, located on Lot 51 in the Yanchep area. Voucher specimens from each of the sampling localities were placed in 75% ethanol and are housed in the reference collection of the Centre of Excellence in Natural Resource Management, The University of Western Australia, Albany. Specimens to be used for DNA analyses were placed in absolute ethanol and kept frozen until needed.

MtDNA-sequencing and sequence data analyses

Total genomic DNA was extracted from multiple individuals from each population. Individuals were removed from ethanol and kept overnight

Table 1 Sampling localities of *Paramphisopus* populations included in the study and putative species or variety identifications, based on recorded distributions (Nicholls, 1943)

Locality		Putative identification	Co-ordinates (WGD84, Geodetic)		*N*
			S	E	
(1)	Lake **Bambun**	*P. palustris*	31°25′41.90″	115°53′28.58″	3
(2)	**Lot 51** cave, Yanchep area	*P. palustris*	31°34′35.80″	115°42′04.10″	3
(3)	**Mariginiup** Lake	*P. palustris*	31°43′18.61″	115°49′10.97″	3
(4)	**Jandabup** Lake	*P. palustris*	31°44′48.31″	115°50′47.94″	3
(5)	Lake **Joondalup**	*P. palustris*	31°45′40.68″	115°47′50.31″	3
(6)	**Edgecombe** Springs	*P. palustris*	31°47′38.28″	115°59′45.54″	3
(7)	Lake **Goollelal**	*P. palustris*	31°48′38.39″	115°48′59.19″	3
(8)	Big **Carine** Swamp	*P. palustris*	31°51′15.34″	115°47′06.57″	3
(9)	**Careniup** Swamp	*P. palustris*	31°51′40.11″	115°47′35.64″	3
(10)	Lake **Gwelup**	*P. palustris*	31°52′43.13″	115°47′40.73″	3
(11)	Lake **Monger**	*P. palustris*	31°55′34.69″	115°49′24.94″	3
(12)	The **Lakes**	*P. montanus*	31°52′33.75″	116°19′23.63″	3
(13)	**Yelgup** Brook	*P. palustris*	32°01′01.51″	115°58′56.96″	2
(14)	**Yule** Brook, Canning River	*P. palustris*	32°02′00.34″	115°57′22.74″	3
(15)	Swamp, **Roe** Highway	*P. palustris*	32°03′09.69″	115°56′26.21″	3
(16)	**North** Lake	*P. palustris*	32°04′39.10″	115°49′20.50″	3
(17)	**Bibra** Lake	*P. palustris*	32°05′46.30″	115°49′12.50″	3
(18)	**Kogolup** Lake	*P. palustris*	32°08′15.63″	115°49′57.75″	3
(19)	**Spearwood**, Market Garden Swamp	*P. palustris*	32°06′53.40″	115°46′48.46″	3
(20)	**Balannup** Lake	*P. palustris*	32°06′53.70″	115°56′43.80″	3
(21)	**Forrestdale** Lake	*P. palustris*	32°09′37.73″	115°55′49.29″	3
(22)	**Dandalup** River, Patterson Road, Pinjarra	*P. p.* var. *fairbridgei*	32°35′43.80″	115°52′43.50″	3
(23)	**Buchanan** Drain canal, Moores Road, Pinjarra	*P. p.* var. *fairbridgei*	32°37′18.42″	115°50′35.40″	3

The number of individuals (*N*) sequenced from each locality is indicated. Localities/populations are referred to in the text by names indicated in bold font

in 500 µl 1× STE (10 mM Tris, 1 mM EDTA, 0.1 M NaCl, pH 8.0) at 4°C to re-hydrate. These were then rinsed in an additional 500 µl STE by centrifugation (13,000 r min^{-1} for 2 min). DNA was extracted using proteinase K digestion and a modified Chelex-100 protocol (Walsh et al., 1991). Individuals were dissected and soft tissue was transferred into a micro-centrifuge tube containing 250 µl of a 5% solution of beaded Chelex-100 resin (Sigma-Aldrich). To prevent downstream PCR inhibition, care was taken to minimize the transfer of chitinous and cuticular exoskeletal material. Proteinase K (0.1–0.2 mg ml^{-1}) was added to the sample, and incubated at 55°C for 2 h. Following digestion, samples were incubated further at 95°C for 10 min and centrifuged (13,000 r min^{-1} for 2 min). The supernatant was transferred to a final tube containing 125 µl 5% Chelex-100 and centrifuged. Alternatively, a commercially available extraction kit (DNeasy Tissue Kit, Qiagen) was used as directed.

A fragment of the protein-coding mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by means of PCR from 2 to 3 individuals from each population (Table 1). PCRs were initially set up as in Gouws et al. (2005), using the invertebrate-specific COI primer set of Folmer et al. (1994). As the amplification and sequencing of certain individuals were unsuccessful, a set of *Paramphisopus*-specific COI primers was designed using Amplicon b09 (Jarman, 2004). These primers (Para-COI-F: 5′-TGA GCT GGT GTA GTA GG-3′, and Para-COI-R: 5′-GGG TCA AAG AAT GAA GTG T-3′) amplify a fragment of approximately 560 bp. PCRs were performed as before, with the concentration of the primers halved and annealing performed at 56°C. PCR-products were visualized on a UV-transilluminator following electrophoresis in 1.5% agarose gels stained with SYBR-Safe (Molecular Probes, Invitrogen). PCR products were purified using Montáge PCR filter units (Millipore). Cycle sequencing reactions were conducted by a com-

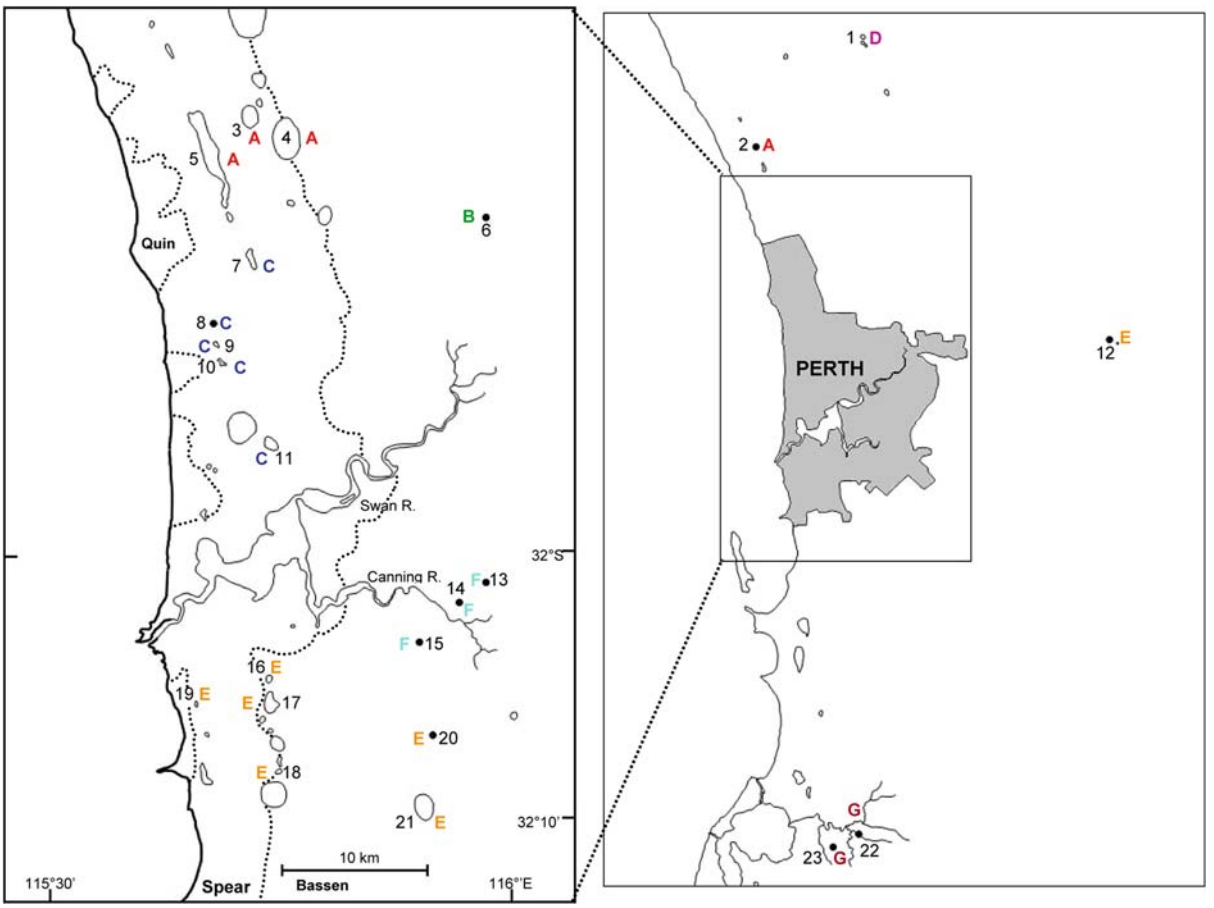

Fig. 1 Sampling localities of the 23 populations of *Paramphisopus* included in the present study. Localities are numbered as in Table 1. Stippled lines represent the approximate positions of the contact boundaries between the Quindalup (Quin), Spearwood (Spear) and Bassendean (Bassen) Dune Systems. Letters next to the locality numbers indicate respective phylogroup membership (see Figs. 2 and 3)

mercial sequencing facility (Macrogen Inc., Seoul) and samples were analysed on an ABI 3730XL automated sequencer. Samples were sequenced in one direction. Sequences were checked against their respective chromatograms for ambiguity and misreads, using Chromas LITE 2.0 (Technelysium Pty Ltd). In cases where ambiguity was encountered, the individual was re-sequenced using the reverse primer and a consensus sequence created. The sequence of a southern African species, *Mesamphisopus penicillatus* was retrieved from GenBank (AY948306) and a single *Amphisopus lintoni* (Nicholls, 1924) individual sequenced (EF203063) for use as outgroup taxa in the phylogenetic analyses.

Sequences were aligned manually and trimmed to equal length. Unique and shared haplotypes were identified using Collapse 1.2 (Posada, 2004), ignoring missing data. Haplotype diversities (i.e., the probability that two sampled haplotypes in the sample are different) and nucleotide diversities (i.e., the probability that two sampled homologous nucleotides are different, calculated as the average number of nucleotide differences per site among sequences in the sample) were determined for each population and the total sample using Arlequin 3.01 (Excoffier et al., 2005). Genetic differentiation among individual populations was examined using exact tests of population differentiation and by means of estimates of Φ_{ST}, thus considering the frequency of haplotypes as well as the divergence among them. Tamura's (1992) model of nucleotide evolution was employed for the latter, this model being the most similar of those available in Arle-

quin to the model revealed by Modeltest 3.7 (Posada & Crandall, 1998) to be most appropriate for the complete in-group data set—a Kimura (1981) model with unequal base frequencies. The α-shape parameter (0.137) of the gamma-distribution of variable sites was determined with ModelTest. An AMOVA (Excoffier et al., 1992) was also performed to examine the partitioning of variation within and among individual populations and among *a priori* defined groups, with permutation tests (1,000 permutations) conducted to determine significance of Φ-estimates.

To investigate possible isolation by distance (IBD: Wright, 1943), the correlation between the matrix of sequence divergences among all sequenced individuals and the matrix of geographic distances between their collection localities was examined by means of a Mantel (1967) test. Sequence divergences were corrected according to the nucleotide substitution model determined to be most appropriate for the entire in-group data set (above), while the geographic distance matrix included straight-line geographic distances between collection localities. The Mantel test was conducted using MANTEL for Windows 1.18 (Cavalcanti, 2005), with 10,000 permutations employed to determine significance.

To account for the possible presence of ancestral haplotypes and the non-bifurcating or reticulate nature of genealogical relationships at the population level, a network approach was used in addition to the phylogenetic analyses of the relationships among haplotypes. For the former, TCS1.21 (Clement et al., 2000) was used to construct a minimum-spanning network of in-group haplotypes with 95%-plausible parsimony links. Phylogenetic relationships were examined using a reduced data set, in which only unique haplotypes and outgroup taxa were included. Using PAUP*4b10 (Swofford, 2002), phylogenies were constructed using unweighted parsimony (MP) and maximum-likelihood (ML). In the parsimony analysis, a heuristic search was employed to find the most parsimonious tree(s). TBR branch-swapping of a starting tree, constructed using multiple replicates (1,000) of random sequence additions, was employed. Prior to the ML analysis, Modeltest was used to determine the optimal model of nucleotide substitution for the data set. The Akaike Information Criterion (AIC: Akaike, 1974) was used to compare the likelihoods of competing models. Optimal model parameters were implemented, and a heuristic search employed (with 100 random taxon addition iterations) to find the most likely topology. Nodal support was determined by non-parametric bootstrapping of the data set (Felsenstein, 1985) in both MP and ML analyses, where, respectively, 1,000 pseudo-replicates with 100 random taxon addition iterations and 100 pseudo-replicates with 10 random taxon addition iterations were used.

Results

Following alignment and trimming, 486 nucleotide characters were available for analyses. About 41 unique haplotypes were identified among the 68 sequenced in-group individuals (Table 2). Representatives of each unique haplotype have been deposited in GenBank (EF203022–EF203062). Only 13 haplotypes were found in more than a single individual and, of these, only five were found to be shared among localities. Where haplotypes were shared among localities, these localities were either adjacent or in close geographic proximity (see Table 2; Fig. 1). Overall haplotype diversity (0.975 ± 0.009 S.D.) was high, with diversities at individual populations ranging from 0 to 1.000 ± 0.272. Nucleotide diversity was greatest in the Roe population (0.011 ± 0.009), while the overall nucleotide diversity was 0.056 ± 0.023.

Substantial genetic differentiation in terms of Φ_{ST} was observed among most populations (see Electronic supplementary material), with the exception of Carine, Careniup and Monger. Haplotype 9 was shared among these populations and fixed in Careniup and Monger. Individual Φ_{ST}-estimates among the remaining populations ranged between –0.004 and 1.000 (mean $\Phi_{ST} = 0.881 \pm 0.231$). Although exact tests revealed no populations to be significantly differentiated ($0.094 \leq P \leq 1.000$; matrix not shown), a subsequent AMOVA showed overall significant differentiation among all 23 populations ($\Phi_{ST} = 0.956$, $P < 0.001$). More variation was partitioned among groups (97.07%) when 22

Table 2 Distribution of the 41 COI mtDNA haplotypes among the 23 *Paramphisopus* localities sampled

Population	Haplotypes																																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
(1) Bambun																	1	1	1																						
(2) Lot 51																													1					1	1						
(3) Mariginiup											1	1	1																												
(4) Jandabup																													1	1	1										
(5) Joondalup																										1	1	1													
(6) Edgecombe																																				3					
(7) Goollelal										1																						1	1								
(8) Big Carine									2	1																															
(9) Careniup									3																																
(10) Gwelup			2	1																																				
(11) Monger									3																																
(12) Lakes																																				3					
(13) Yelgup	2																																								
(14) Yule																																							3		
(15) Roe	1						1	1																																	
(16) North																							1	1	1																
(17) Bibra																				1	1	1																			
(18) Kogolup														1	1	1																									
(19) Spearwood																							2															1			
(20) Balannup		3																																							
(21) Forrestdale					2	1																																			
(22) Dandalup																																									3
(23) Buchanan																																							3		

groups (combining the Careniup and Monger populations, and regarding each of the remaining populations as an independent ''group'') were defined ($\Phi_{CT} = 0.971$, $P < 0.01$). Only 4.39% of variation was found within individual populations.

Mantel tests revealed a highly significant correlation ($r = 0.557$, $t = 15.591$, $P < 0.001$) among matrices of sequence divergences, corrected according to the nucleotide substitution model most appropriate to the entire in-group data set (K81uf + Γ: A = 0.308, C = 0.192, G = 0.168, T = 0.333; $R_{A-G} = R_{C-T} = 23.259$, $R_{A-C} = R_{G-T} = 1.000$ and $R_{A-T} = R_{C-G} = 0.308$; and $\alpha = 0.137$) among all sequenced individuals, and the geographic distances between their sampling localities. Thus, there is significant evidence for a clear pattern of isolation by distance, notwithstanding the sharing of a few haplotypes among localities.

The in-group haplotypes were joined in seven separate parsimony networks, among which 95%-plausible links could not be established (Fig. 2). Haplotypes from a central group of northern localities (Jandabup, Joondalup, Lot 51 and Mariginiup) formed Network A. The single haplotype (36, Network B) fixed in the Edgecombe population was not connected to any other network. Network C linked haplotypes from lakes occurring immediately north of the Swan River (Careniup, Carine, Goollelal, Gwelup and Monger). Network D comprised the three haplotypes from the northern-most sampling locality (Bambun). Network E included haplotypes from a group of wetlands in close geographic proximity immediately south of the Swan River (Bibra, Kogolup, North and Spearwood), from lakes further east (Balannup and Forrestdale) and the haplotype (37) from the type locality of

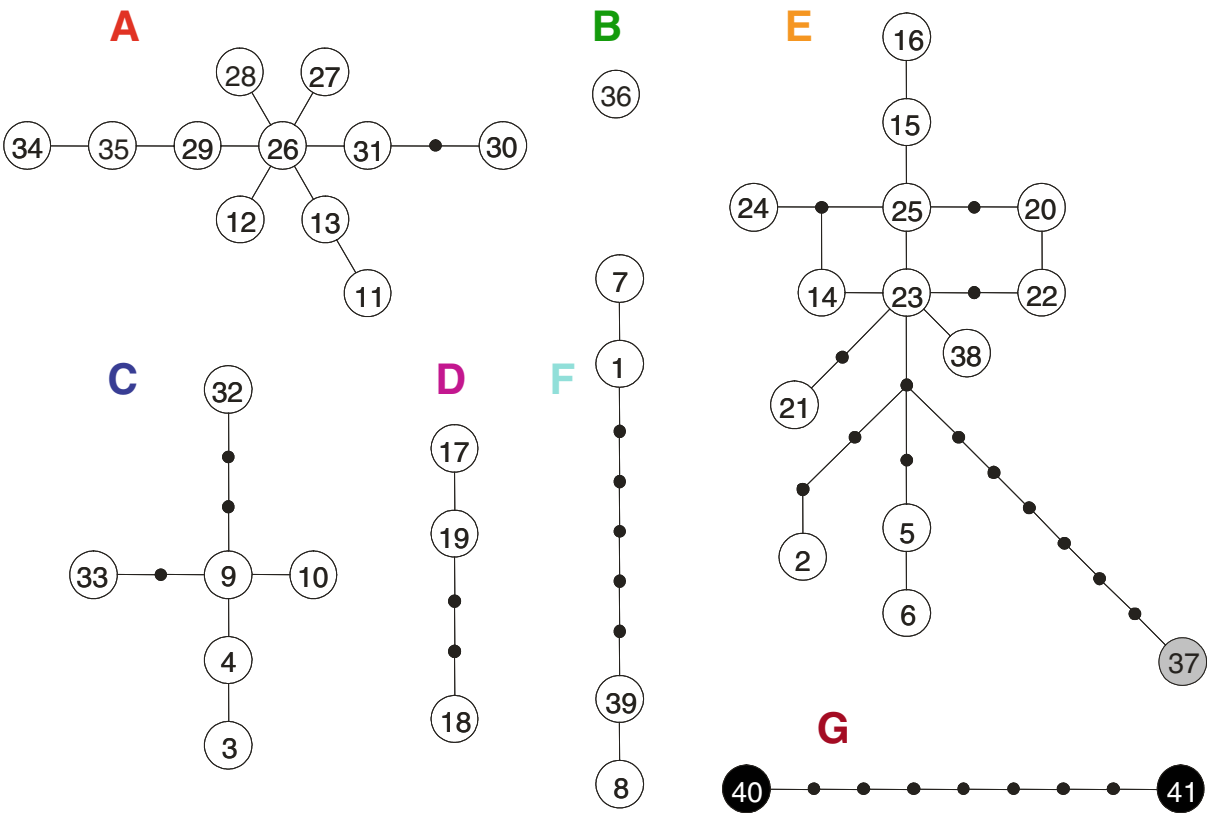

Fig. 2 The seven independent 95% parsimonious minimum-spanning networks depicting genealogical relationships among the 41 in-group COI mtDNA haplotypes. Haplotypes are numbered as in Table 2. Filled circles indicate haplotypes belonging to populations identified as *P. montanus* (grey) and *P. palustris* var. *fairbridgei* (black). Intermediate haplotypes are indicated by smaller filled circles

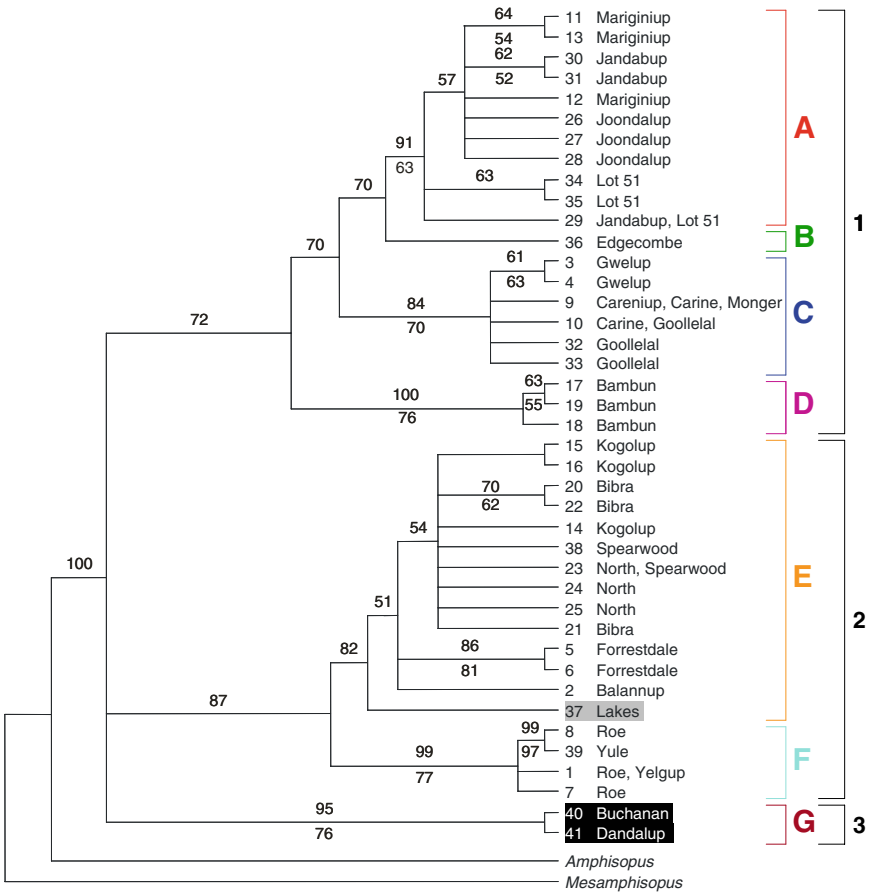

Fig. 3 Strict consensus of the 256 equally parsimonious trees obtained in the parsimony analysis of relationships among 41 in-group and outgroup (*Amphisopus* and *Mesamphisopus*) COI mtDNA haplotypes. Haplotypes are numbered as in Table 2. Sampling localities of haplotypes are presented to the right of terminals. Text blocked in grey and black indicate haplotypes belonging to individuals tentatively identified as *P. montanus* and *P. palustris* var. *fairbridgei*, respectively. Major identified clades are identified numerically and subclades within these, corresponding to networks identified in Fig. 2, labelled alphabetically. Nodal (non-parametric bootstrap) support from the MP and ML analyses are presented above and below branches, respectively. Bootstrap support <50% is not indicated

P. montanus. Network F contained haplotypes from a group of localities (Roe, Yelgup and Yule) occurring south-east of Perth in the vicinity of the Canning River. The two haplotypes (40 and 41) from the Pinjarra area comprised Network G. As none of the networks were particularly extensive, in terms of geographic coverage or the number of haplotypes included, nested clade analyses (Templeton et al., 1995) were not conducted.

For the examination of evolutionary relationships among haplotypes, the reduced data set provided 190 variable characters, of which 99 were parsimony informative. The parsimony analysis retrieved 256 equally parsimonious trees (228 steps; CI = 0.597, RI = 0.886, Rescaled CI = 0.529). The strict consensus of these (Fig. 3) revealed three major well-supported (bootstrap ≥72%) clades, corresponding to haplotypes sampled north of the Swan River (1), south of the Swan River (2), and from the extreme south (3). The basal relationships among these clades were unresolved. Further supported (bootstrap ≥70%), geographically defined subclades could be discerned within Clades 1 and 2, corresponding to the networks identified earlier (and are labelled correspondingly in Fig. 3). ML-analyses proceeded implementing the parameters of the Hasegawa et al. (1985) model (HKY + I + Γ) revealed by

Modeltest to be the most appropriate for the reduced data set: base frequencies of A = 0.3038, C = 0.1541, G = 0.1305 and T = 0.4116; Ti/Tv = 7.788; I = 0.536; and α = 0.973. The resulting ML-phylogram (–lnL = 2108.757, not shown) retrieved most individual clades and subclades with high support (Fig. 3), but failed to resolve relationships among these. As no contradictory basal relationships proposed by ML were supported by bootstrapping (see Fig. 3), more credence is given to the relationships proposed by parsimony in subsequent discussion.

Model-corrected (ML) sequence divergences among haplotypes within each of the identified subclades ranged from 0.19 to 3.08%. Sequence divergences of 2.12–5.96% separated the subclades of Clade 1, while those separating the two subclades of Clade 2 ranged from 4.85 to 8.42%. Haplotypes belonging to the three major clades were 7.71–14.45% divergent, with mean sequence divergences of 10.86% (±0.89), 9.31% (±0.72) and 10.15% (±0.67) separating Clades 1 and 2, Clades 1 and 3, and Clades 2 and 3, respectively.

Discussion

In summary, the data analysed revealed that: (1) few COI mtDNA haplotypes are shared among *Paramphisopus* populations; (2) overall mtDNA differentiation among populations was significant; (3) this differentiation distinguishes and indicates the independence of individual populations, rather than groups of populations; (4) a clear pattern of isolation by distance was evident, and (5) both population-level genealogical and phylogenetic relationships of haplotypes indicate the existence of unique and discrete lineages that are geographically defined.

Taxonomic implications

The results of the above analyses may have taxonomic implications for *Paramphisopus* and mtDNA evidence strongly urges a further re-examination of the specific status of existing taxa (*P. montanus*, *P. palustris* var. *fairbridgei*) and of individual *P. palustris* lineages. Although apparently possessing a distinct ecology and being morphologically distinguishable (Nicholls, 1943), the Lakes population (*P. montanus*) was no more extensively differentiated from the remaining *P. palustris* populations than these were from each other. The phylogenetic analyses revealed the Lakes haplotype to be nested deeply within a clade containing minimally divergent *P. palustris* haplotypes. Network VI revealed the association of the *P. montanus* haplotype with several *P. palustris* haplotypes, while other *P. palustris* haplotypes could not be linked to this particular network with 95% confidence. Although the only documented *P. montanus* population was included and this taxa's monophyly cannot be demonstrated, the above results would suggest either that *P. montanus* and the morphologically variable *P. palustris* are synonymous or that *P. palustris* is currently paraphyletic.

Haplotypes from individuals collected near the type locality of *Paramphisopus palustris* var. *fairbridgei* were retrieved as a major well-supported clade. This clade and the two other well-supported and geographically defined clades were separated with sequence divergences of ca. 10%. Divergences of similar magnitude have been considered indicative of a cryptic species complex (see McGaughran et al., 2006) or of recently speciated sibling species (see Rivera et al., 2002). These divergences, and those among the individual subclades, remain lower than interspecific COI-divergences found in several freshwater isopods (Ketmaier et al., 2003; Keable & Wilson, 2006), including phreatoicidean isopods such as *Mesamphisopus* (Gouws et al., 2005) and *Amphisopus* (Gouws, unpublished data). Being more comparable to large intraspecific values (Verovnik et al., 2004), these divergences may in fact reflect incipient species. Regardless, the taxonomic status of many populations requires further investigation. This includes the varietal status of *P. palustris* var. *fairbridgei*. The retention of this variety as subspecies or species suggests that further designations, corresponding to the monophyletic clades or subclades, could be made. Their recognition would resolve the apparent paraphyly of *P. palustris*. The possibility of additional, geographically circumscribed varieties had been suggested earlier by Nicholls (1943), who had made specific mention of populations from the

Guildford area. Indeed, haplotypes from individuals sampled from this area (Roe, Yelgup and Yule) formed one of the monophyletic and well-supported clades (Subclade F).

Evolutionary history and wetland conservation

The significant pattern of isolation by distance is interesting and suggests a pattern of serial founder events (see Ramachandran et al., 2005), with populations potentially having been established by migrants from nearby populations, each forming a genetic sub-sample of the parental population on which drift can act. That this is ancient, rather than a recent pattern maintained by current migration-drift processes, is evident from the independence of individual populations and the geographic fidelity of the larger phylogroups. Contrary to the expectation of reduced diversity in recently founded populations, the high nucleotide and haplotype diversities are suggestive of well-established populations that are large, relatively old and demographically stable. It is clear that gene flow is largely lacking and there is very little evidence for migration among wetlands. Many unique haplotypes are encountered in single populations, few haplotypes are shared (none more extensively than among proximate populations) and populations are highly differentiated with large Φ_{ST}-values. Generally, distributions and patterns of localized genetic or morphological differentiation suggest that phreatoicideans possess only limited capacity for dispersal, even over small spatial scales (e.g., Wilson & Ho, 1996; Gouws et al., 2005). There is however some circumstantial evidence for chance long-distance dispersal events. The geographic separation and association of the Lakes haplotype (37) with Subclade E is a potential example. In light of this, albeit limited, dispersal, it is plausible that the isolation-by-distance signature of such a ''persistent founder event'' (De Gelas & De Meester, 2005) and the fidelity of the current phylogeographic pattern may be preserved by priority effects (Gómez et al., 2002; De Gelas & De Meester, 2005; De Meester et al., 2002). These, including the numerical and adaptive advantage of the resident population and its domination of resources, prevent the successful establishment of newer migrants at these localities or regions. What is remarkable about the current pattern of isolation by distance is the fact that it is apparent over a small spatial scale (~100 km). Such patterns have been observed in other isopods (Wang & Schreiber, 1999a, b; Gouws et al., 2005), but typically on a larger regional or ''sub''-continental scale and often without evidence of phylogeographic structure. In other organisms, such patterns have been documented on regional (e.g., Gómez et al., 2002), continental (e.g., De Gelas and De Meester, 2005) or global scales (e.g., Ramachandran et al., 2005).

From the extent of divergence among the major geographically defined clades and subclades, it becomes clear that these are products of considerable evolutionary history. Indeed, the tentative application of a molecular clock calibrated for isopods (Ketmaier et al., 2003) reveals the three major lineages to have diverged in the early to mid-Pliocene, 4.34–3.44 million years ago (Mya). This timeframe argues for a significant influence of increasing aridity and the decreasing extent of surface waters, initiated in the late Tertiary and established by the mid-Miocene (Unmack, 2001; Hopper & Gioia, 2004). These phenomena have been implicated in the contemporaneous allopatric divergence of major lineages of other freshwater Crustacea in Western Australia (Gouws et al., 2006). The divergence of lineages within each of the major clades appears to coincide with further periods of intense aridity in the Late Pliocene, as reflected by regional palynology and palaeodrainages (Clarke, 1994; Dodson & Macphail, 2004). Here, the separation of the two southern lineages (Subclades E and F) was estimated as occurring around 2.75 Mya, while the most northerly lineage (Subclade D) diverged from the remaining northern lineages 2.08 Mya. The divergence of the latter (Subclades A + B and C) occurred in the Early Pleistocene (1.16 Mya). Given the geological history of the dune systems of the SCP, the persistence of lineages of these ages is remarkable. While the eastern Bassendean Dune system is of similar Late Pliocene–Early Pleistocene age, the western Spearwood Dune system, on which many of the examined wetlands are situated, appears younger

than the lineages inhabiting them (Bastian, 1996). The subunits of this system, many representing successive Pleistocene interglacial sea-level highs, are derived from denuded Late Pleistocene limestone and the youngest units are of Holocene age (Bastian, 1996; Semeniuk, 1997). Thus, lineages have survived Quaternary dune-building, significant deposition over Recent times (see Churchill, 1959) and both Pleistocene and Holocene transgressions (see Semeniuk, 1997). Despite the superimposition of many of the present wetlands on these dune systems during the Late Pleistocene and Holocene, it appears likely that the major lineages were largely confined to multiple refugia more or less within the present distribution of each phylogroup during these periods of deposition and groundwater and fluvial modification (Semeniuk, 1997). Thus the geographic fidelity of the old phylogroups and pattern of isolation by distance were preserved. Pertinently, the initial establishment of populations and subsequent processes have contributed to a phreatoicidean isopod fauna that is largely unique and independent in individual wetlands.

Disregarding the current unresolved taxonomy, seven lineages (Subclades A–G) are regarded as Evolutionarily Significant Units for consideration of the conservation status and conservation requirements of *Paramphisopus*. Although nuclear differentiation is yet to be investigated (see Moritz, 1995), their delineation is consistent with the concept's central philosophy: the recognition of evolutionarily unique lineages representing historically isolated and independently evolving populations (Moritz, 1995, 1999). Similarly, many individual populations may be considered as management units, being demographically and genetically independent and possessing phenotypic and adaptive variation (e.g., the cryptozoic features of the ''*P. montanus*'' population) (Moritz, 1999). The possibility of priority effects maintaining the pattern of isolation be distance implies that there is indeed adaptive variation worth conserving. The desired goal of the conservation of habitat, ecosystem function and maximum species diversity in these wetlands, by preserving a mosaic of wetland types (Balla & Davis, 1993; Davis & Rosich, 1993b), can be extended to include diversity within species. The conservation of these lineages must thus be considered in terms of their representation within conservation reserves, current wetland management, use and impacts.

Representatives of Subclades A, D and E appear to be well-represented in conservation reserves. Lake Bambun is included in a conservation area with restricted access (Majer, 1979b; Arnold, 1990). Lakes Jandabup and Joondalup are managed as conservation reserves due to their considerable biological significance (Balla & Davis, 1993; Growns et al., 1993b; Mitchell et al., 2003). However, conservation and recreation demands are in conflict in Joondalup, while Jandabup is only partially included in the reserve, with activities on surrounding private land posing a threat (see Arnold, 1990; Growns et al., 1993b). Forrestdale Lake and Thomsons Lake (presumably also possessing individuals belonging to Subclade E due to its location) are both conservation reserves (Balla & Davis, 1993; Growns et al., 1993b). Here, the additional conservation of Lakes Kogolup and Balannup—privately owned, relatively unaltered, well-vegetated wetlands, with high faunal diversity (Arnold, 1990; Balla & Davis, 1993; Davis & Rosich, 1993a; Schmidt et al., 1993)—would enable further conservation of the diversity within Subclade E. To a lesser extent, Subclade F is protected by inclusion of a population in the Yule Brook Reserve, used by the University of Western Australia as a teaching resource (Arnold, 1990). While the only representative population of Subclade B was taken from privately owned land, mound springs (the present included) in the area are routinely monitored, indicating some concern for the fauna and its effective management.

The lack of wetlands containing representatives of Subclade C explicitly designated as conservation reserves (see Arnold, 1990) is of some concern; wetlands in this heavily urbanized area generally being managed for recreation (Majer, 1979a, b; Arnold, 1990). The sampling localities of Subclade G/Clade 3 are in modified environments and not included in reserves. Extirpation of local populations in this area has long been known (Nicholls, 1943).

With *Paramphisopus* occurring in nearly all surveyed wetlands (Growns et al., 1993a), appar-

ently unaffected by eutrophic conditions and cyanobacterial blooms (Balla & Davis, 1993; Cheal et al., 1993; Davis & Rosich, 1993b), the most significant threats relate to groundwater abstraction and future water demand. These surpass the negative impacts of intense surrounding urban and rural land-use, vegetation clearing and altered hydrology documented by Arnold (1990), Growns et al. (1993b) and Schmidt et al. (1993). Populations survive in seasonally desiccated wetlands by aestivation (Nicholls, 1926), facilitated by the water table's proximity to the soil surface (Balla & Davis, 1993). Water abstraction from the Gnangara and Jandakot Mounds has already decreased water levels, and is at maximum capacity on the Jandakot Mound (Majer, 1979a; Balla & Davis, 1993). Increased abstraction will impact negatively on these populations, particularly those of the discrete wetlands of the Gnangara Mound (Balla & Davis, 1993), groundwater populations (e.g., Lot 51 cave) and the fauna of the sensitive tumulus springs (Mitchell et al., 2003). Increased water demand will also necessitate future dam sites earmarked for systems such as the Dandalup (Majer, 1979a), altering other habitats.

Many SCP wetlands contain unique taxa—approximately a quarter of surveyed taxa are known from a single wetland only and more than half occur in fewer than 5% of wetlands (Balla & Davis, 1993; Davis & Rosich, 1993b; Growns et al., 1993a), unique phytoplankton assemblages (Schmidt & Rosich, 1993) and are largely unique physico-chemically (Balla & Davies, 1993). Conservation of wetlands can be justified on these grounds alone. However, studies, such as the present, can aid in prioritizing conservation efforts. Future phylogeographic research can provide a body of evidence, especially when considering taxa unique to the SCP wetlands. Ideally, to accurately identify immediate conservation concerns, a comprehensive approach should consider taxa more sensitive to threats such as eutrophication and those requiring permanent water. A variety of life histories and dispersal capabilities should also be considered, as wetlands may contain independent populations, while more vagile taxa form metapopulations inhabiting several wetlands (see Balla & Davis, 1993).

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