Biogeography in the Death Valley region: evidence from springsnails (Hydrobiidae: *Tryonia*)

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Allozyme and mitochondrial DNA variation were analysed to examine evolution of the nine species of springsnails (genus *Tryonia*) living in the Death Valley system (Owens and Amargosa basins) of southeastern California and southwestern Nevada. Both allozyme and mtDNA evidence indicate that this highly endemic fauna is non-monophyletic. Species from the upper Amargosa basin comprise a clade most closely related to snails living in the Colorado basin. Snails from the lower Amargosa basin (Death Valley trough) reflect a complex evolutionary history and two of these species are more closely related to an estuarine species from western California than to other snails of the region. These results indicate a commonality of pattern with the well-studied Death Valley pupfishes (*Cyprinodon*), which also are non-monophyletic and include species that are most closely related to Colorado basin congeners. These biogeographic patterns are interpreted within the context of a recently proposed model for the early history of the lower Colorado River.

ADDITIONAL KEY WORDS:—Colorado River – phylogenetic relationships – evolution – mitochondrial DNA – allozymes – Gastropoda – pupfish – *Cyprinodon*.

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INTRODUCTION

The 'Death Valley system' (Miller, 1943) of southeast California and southwest Nevada is composed of the now isolated Amargosa, Mohave, and Owens basins, which were integrated, although not necessarily contemporaneously (Brown & Rosen, 1995), via a series of overflowing lakes that terminated in Lake Manly in Death Valley during the late Pleistocene pluvial period. Basinal aquatic habitats in this region are profoundly isolated by inhospitable deserts and imposing mountain ranges and harbour a diverse endemic biota (Sada, Britten & Brussard, 1995), the history of which has been the subject of considerable biogeographic speculation (e.g. Hubbs & Miller, 1948; Taylor, 1985; Hershler & Pratt, 1990). These inquiries generally have focused on biotic dispersal and the extent to which regional biogeography 'fits' pluvial drainage of the Death Valley system.

Recent studies of mitochondrial DNA and allozyme variation (Echelle & Dowling, 1992; Echelle & Echelle, 1993) among pupfishes (*Cyprinodon*) permitted construction of the first phylogenetic hypotheses relevant to regional biogeography. Echelle & Echelle (1993) concluded that pupfish of the Death Valley system are monophyletic, but their data are open to alternative interpretations. In this paper we analysed allozyme and mtDNA variation to examine evolution of a second biotic element (springsnails) and to further our understanding of aquatic biogeography of the Death Valley region.

Aquatic snails of the genus *Tryonia* (Caenogastropoda: Hydrobiidae) are distributed from the Pecos River drainage to the California coast and from the northern Great Basin to an indeterminate southern limit in Mexico. Two additional species live in peninsular Florida (Hershler & Thompson, 1987). In the West, *Tryonia* parallel pupfish (Miller, 1981) in their extensive local endemism, with most of the 19 described species restricted to single springs or drainage systems. These small, gill-breathing, benthic snails are obligately aquatic, have direct development, disperse only within their habitat and are highly sensitive to terrestrial barriers. Their evolution is thus assumed to reflect hydrographic history (Taylor & Bright, 1987).

In the Death Valley system *Tryonia* is represented by nine species, all but one of which are endemic (Hershler & Sada, 1987; Hershler, 1989). These snails typically live in thermal, often highly mineralized springs where they often are sympatric with pupfish. Within the Death Valley system both *Tryonia* and pupfish are confined to the upper Owens basin (both groups are absent in lower portions comprising Indian Wells, Searles, and Panamint Valleys) and the Amargosa basin, with most of the taxa concentrated in the latter drainage. Four species of *Tryonia* are restricted to the upper drainage of the Amargosa River, an intermittent stream which extends from headwater springs near Beatty, Nevada south through the Amargosa Valley and across the California state line where it abruptly turns west and north to enter the southern end of Death Valley. Within this area three species are endemic to the Ash Meadows spring oasis (*T. angulata*, *T. elata*, *T. ericae*) whereas a fourth, *T.*

variegata, lives there and also along the Amargosa Valley and in the southern end of Death Valley (Saratoga Spring). An additional four species are endemic to the lower portion of the Amargosa basin, which consists of the Death Valley trough. Two species are locally endemic in central Death Valley (*T. robusta*, *T. salina*), while another two species are restricted to the northern end of this valley (*T. margae*, *T. rowlandsi*). *Tryonia protea* lives in the upper Owens River basin and the Colorado and Bonneville basins (Taylor, 1985: fig. 35). This species is parthenogenetic, whereas other *Tryonia* are sexual. Within the Death Valley system *Tryonia* species generally are distributed allopatrically although two species are sympatric in several springs in Ash Meadows and northern Death Valley.

There has been no analysis of phylogenetic relationships among species of *Tryonia*. Competing scenarios have been proposed for evolutionary development of the Death Valley fauna. Taylor (1985:317) argued generally that aquatic mollusks on either side of Death Valley are strongly dissimilar, and suggested that *Tryonia* from the Amargosa basin are allied with *T. clathrata* (from southern Nevada), and not with *T. protea*, which is found in the western portion of the Death Valley system. Hershler (1989:229–231) later suggested that *Tryonia* species from the Amargosa basin may be monophyletic, and that *T. protea* may be closely allied to at least one of these species.

Our purpose was to construct a molecular based phylogenetic hypothesis for *Tryonia* species of the Death Valley system. We have not fully addressed monophyly of this fauna as we only analysed three of the 13 extra-limital species. Instead we tested hypotheses of relationships among components of this fauna and with all congeners from geographically proximal areas. We compared and contrasted this phylogeny with that proposed for regional pupfish and proposed a scenario for the evolution of this fauna in relation to hydrographic history.

MATERIAL AND METHODS

Specimens

We analysed one or more populations of all of the recognized *Tryonia* species in the Death Valley system. Inasmuch as phylogenetic relationships among species of *Tryonia* are not known, we selected three congeners as outgroups. *Tryonia imitator*, an estuarine species from coastal California (Kellogg, 1985), was included to test the hypothesis that the Death Valley fauna is most closely related to taxa from west of this region. *Tryonia clathrata* from southern Nevada was included as the most proximate representative east of the Death Valley system. This snail is found along the course of the pluvial White River, a now fragmented drainage which was tributary to the Colorado River during the late Pleistocene (Hubbs & Miller, 1948; DiGiuseppi & Bartley, 1991) and which still maintains such a relationship at its lower end (Moapa Valley). *Tryonia brevissima*, profoundly isolated from western *Tryonia* in peninsular Florida, was used to root all trees. Representative shells of these species are in Figure 1.

Taxa and localities sampled are listed in Table 1 and locations of sample sites are shown in Figures 2 and 3. Additional details of sampling localities are available from the first author. Live-collected snails were sorted in the field, placed in tubes

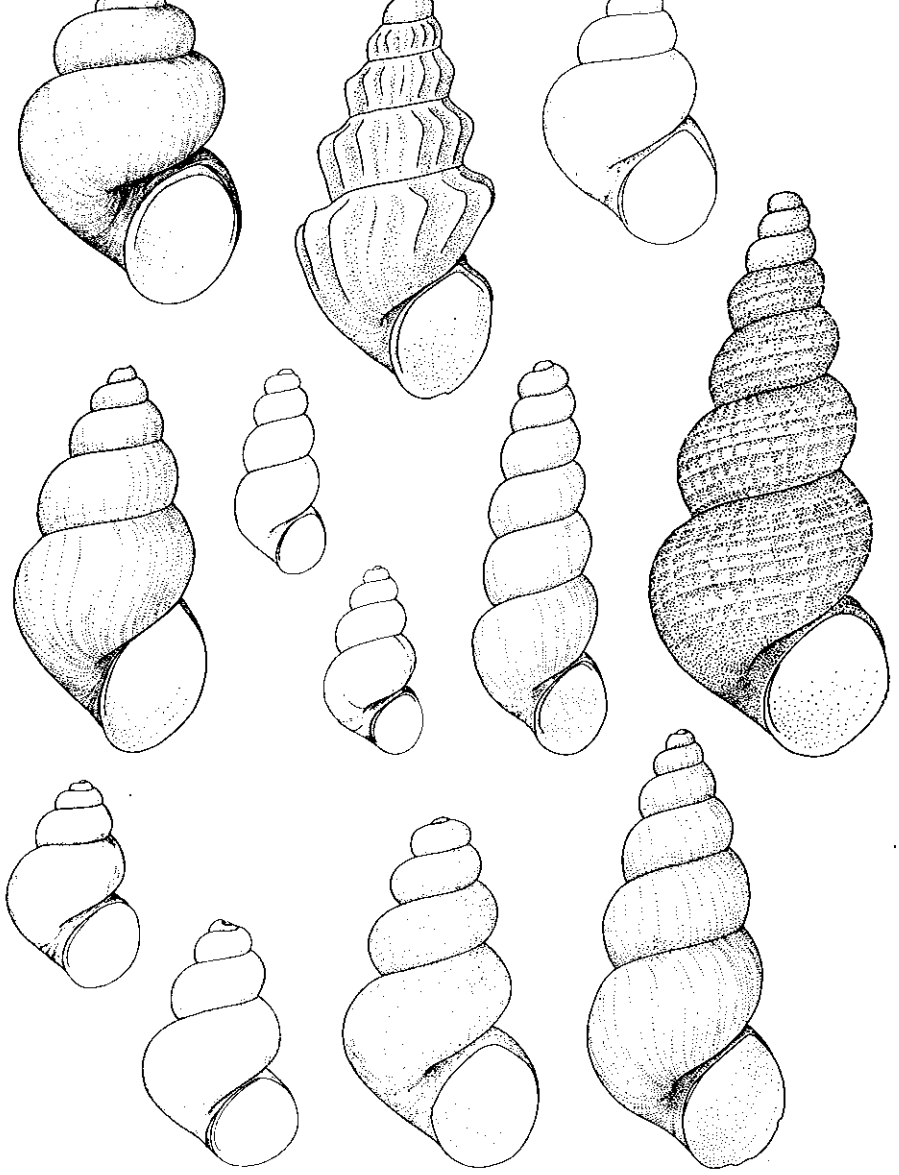

Figure 1. Camera lucida drawings of shells of *Tryonia* species. Top row (left to right), *T. brevissima*, *T. clathrata*, *T. imitator*; middle row, *T. angulata*, *T. elata*, *T. ericae*, *T. margae*, *T. protea*; bottom row *T. robusta*, *T. rowlandsi*, *T. salina*, *T. variegata*. The shell of *T. brevissima* is 3.0 mm tall and 9 mm wide; other shells are illustrated at the same scale.

filled with tris-buffer (pH 7.4), and flash-frozen in a portable liquid nitrogen cannister. Voucher material for these samples is reposited in the Recent mollusk collection of the National Museum of Natural History (USNM).

Species	Population	Location
T. angulata	B	Big Spring, Ash Meadows, Nye Co., NV
	C	Crystal Pool, Ash Meadows, Nye Co., NV
	F	Fairbanks Spring, Ash Meadows, Nye Co., NV
T. brevissima	–	Lake Panasoffkee (south end), Sumter Co., FL
T. clathrata	A	Ash Spring, Pahranagat Valley, Lincoln Co., NV
	M	Warm Springs, Moapa Valley, Clark Co., NV
T. elata	Pa	Spring (A), Point of Rocks, Ash Meadows, Nye Co., NV
	Pb	Spring (B), Point of Rocks, Ash Meadows, Nye Co., NV
T. ericae	C	Spring north of Collins Ranch, Ash Meadows, Nye Co., NV
	N	North Scruggs Spring, Ash Meadows, Nye Co., NV
T. imitator	M	Moro Cojo Slough, Moss Landing, Monterey Co., CA
	S	Penasquitos Lagoon, San Diego Co., CA
T. margae	G1	Grapevine Springs (cool spring), Death Valley, Inyo Co., CA
	G2	Grapevine Springs (lower warm spring), Death Valley, Inyo Co., CA
	G3	Grapevine Springs (upper warm spring), Death Valley, Inyo Co., CA
T. protea	O	'Oasis Spring', Salt Creek, Salton Trough, Riverside Co., CA
	Hu	Hunters Spring, Salt Creek, Salton Trough, Riverside Co., CA
	Ht	Hot Creek, Long Valley, Mono Co., CA
	W	Whitmore Hot Springs, Long Valley, Mono Co., CA
T. robusta	N	Nevares Springs, Death Valley, Inyo Co., CA
	T	Travertine Springs, Death Valley, Inyo Co., CA
T. rowlanasi	G2	Grapevine Springs (lower warm spring), Death Valley, Inyo Co., CA
	G3	Grapevine Springs (upper warm spring), Death Valley, Inyo Co., CA
T. salina	–	spring, Cottonball Marsh, Death Valley, Inyo Co., CA
T. variegata	D	Devils Hole, Ash Meadows, Nye Co., NV
	F	Five Springs, Ash Meadows, Nye Co., NV
	N	North Scruggs Spring, Ash Meadows, Nye Co., NV
	Pc	spring (C), Point of Rocks, Ash Meadows, Nye Co., NV
	Sa	Saratoga Spring, Amargosa River drainage, San Bernardino Co., CA
	Sh	Shoshone Spring, Amargosa River drainage, Inyo Co., CA

Protein electrophoresis

Whole snails were homogenized in 20 µl grinding buffer following methods of Mulvey & Vrijenhoek (1981) and Mulvey, Newman & Woodruff (1988). Individual snails were used except for the very small taxa, *T. elata* and *T. ericae*, in which three to six specimens were pooled for each gel run. Because population samples did not yield data for every locus, samples also were pooled to represent the allozymes present for these species. This was especially necessary for *Icd*, which was only detected in the freshest material. In no case where it was possible to compare occurrence of allozymes among contributing populations were any differences noted, therefore pooling of data was considered appropriate. The following combinations of buffer and enzymes were used with 12% horizontal starch gels: Tris-citrate, pH 8.0 (Selander *et al.*, 1971) for glucose phosphate isomerase (*Gpi*, 5.3.7.9), 6-phosphogluconate dehydrogenase (*Pgd*, 1.1.1.44), and NADP-dependent malate dehydrogenase (*Me*, 1.1.1.40); lithium hydroxide, pH 8.1 (Selander *et al.*, 1971) for mannose phosphate isomerase (*Mpi*, 5.3.1.8) and purine nucleoside phosphorylase (*Nsp*, 2.4.2.1); Tris-citrate-EDTA, pH 7.1 (Ayala *et al.*, 1972) for isocitrate dehydrogenase (*Icd*, 1.1.1.42); and Tris-borate-EDTA, pH 8.0 (Selander *et al.*, 1971) for phosphoglucomutase (*Pgm*, 5.4.2.2), aspartate amino transferase (*Aat*), and esterase (*Est*, 3.1.1._) with 4-methylumbellyliferyl acetate as substrate. Gels were stained

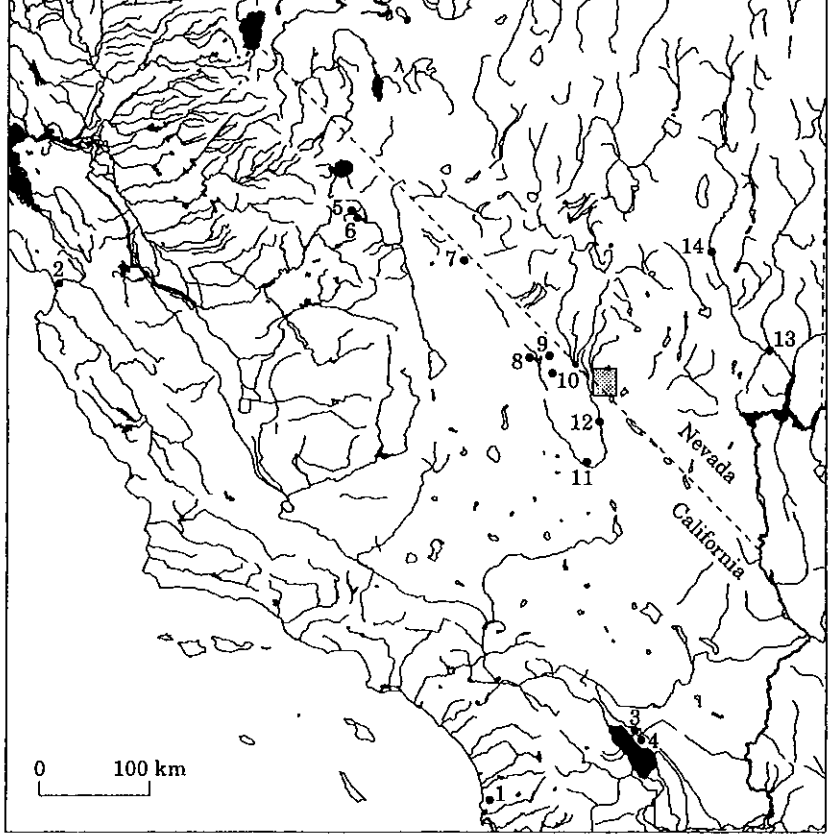

Figure 2. Map of southern California and Nevada showing sampling localities. 1 = Penasquito Lagoon (*T. imitator*), 2 = Moro Cojo Lagoon (*T. imitator*), 3, 4 = Hunter, Oasis Springs (*T. protea*); 5, 6 = Hot Creek, Whitmore Hot Spring (*T. protea*); 7 = Grapevine Springs (several sites; *T. margae*, *T. rowlandsi*), 8 = Cottonball Marsh (*T. salina*); 9, 10 = Nevares, Travertine Springs (*T. robusta*); 11, 12 = Saratoga, Shoshone Springs (*T. variegata*); 13, 14 = Moapa National Wildlife Refuge, Ash Spring (*T. clathrata*). The shaded box indicates location of Ash Meadows (see Fig. 3). The sampling locality of *T. brevissima* in peninsular Florida is not shown.

using methods outlined in Selander *et al.* (1971) and Richardson, Baverstock & Adams (1986). Specimens from several populations were run concurrently on all gels to facilitate comparison of electrophoretic mobilities. Two specimens of *T. protea* (from Whitmore Hot Springs) also were included on all runs as reference for allozyme mobilities. The *T. protea* allozyme for each locus was arbitrarily designated 100 and designations for other allozymes represent mobilities relative to this reference. Nine loci were consistently resolved for most populations. Additional loci were resolved for the larger species, but could not be assayed for the smaller species due to the limited sample size.

DNA sequences

Genomic DNA was isolated from whole snails using the chelex extraction method of Walsh, Metzger & Higuchi (1991). A 710 base pair segment of mitochondria

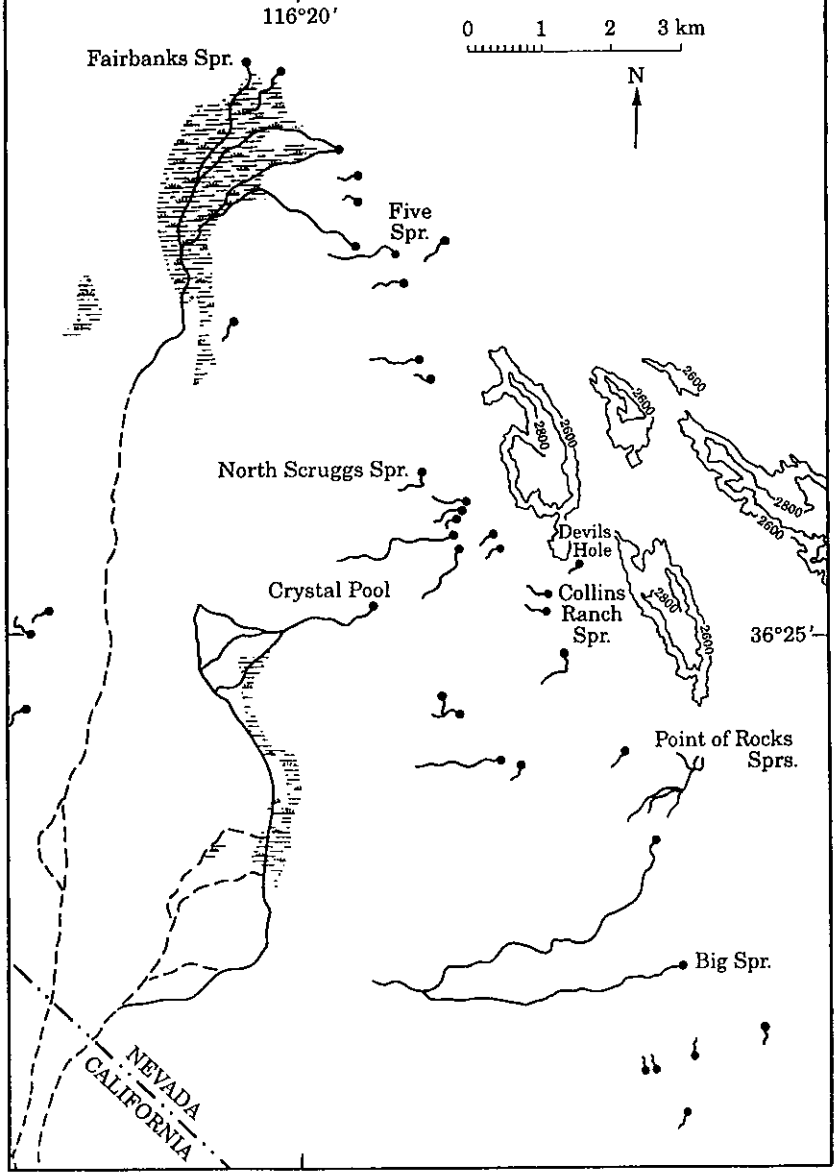

Figure 3. Map of Ash Meadows showing sampling localities. Modified from Ash Meadows Quadrangle (USGS 15 minute series). Species sampled were *T. angulata* (Big Spring, Crystal Pool, Fairbanks Spring), *T. elata* (Point of Rocks Springs), *T. ericae* (North Scruggs Spring, Spring north of Collins Ranch), and *T. variegata* (Devils Hole, Five Springs, North Scruggs Spring, Point of Rocks Springs).

cytochrome-*c* oxidase subunit I (COI) gene was amplified via polymerase chain reaction (PCR) using primers CO1L1490 (5′ GGT CAA CAA ATC ATA AAG ATA TTG G3′) and COIH2198 (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA3′) (Folmer *et al.*, 1994). For 50 µl reactions we used 2 µl (100–150 ng/µl) DNA, each dNTP at 250 µM, each primer at 0.5 µM, 1 unit Taq polymerase (Promega,

for 30 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min. Amplification products were checked for size by electrophoresis on 1.5% agarose gels. Double-stranded DNA products were purified with Microcon-100 filters. Purified products were used as template for automated sequencing following Applied Biosystems protocol. Chromatograms were edited with Sequencher. COI is a protein coding gene and therefore there was no difficulty in terms of alignment. Sequences were aligned with Sequencher and checked by eye.

Sequences do not vary within populations of *Tryonia* (Hershler, Liu & Mulvey 1999) and thus we analysed only one specimen per population. In order to assess conspecific variation, specimens were sequenced from two different populations of each of three relatively widespread species (*T. imitator*, *T. protea*, *T. variegata*). Because every nucleotide position does not have the same phylogenetic information, a thorough analysis of base composition and substitution patterns was performed prior to phylogenetic analysis (Ritland & Eckenwalder, 1992). All nucleotide sequences were deposited in GenBank (Accession Number AF061764–AF061778).

Data analysis

Allozyme data were analysed using BIOSYS-1 (Swofford & Selander, 1981) to summarize proportion of polymorphic loci, mean heterozygosity, and Nei (1978) genetic distance. For loci with more than two alleles, rare alleles were pooled prior to this analysis. Genetic distances were clustered using UPGMA.

The COI sequence data were subjected to phylogenetic analysis using PAUP 3.1 (Swofford, 1993). Because we found no evidence of saturation or base pair composition bias, all characters were weighed equally. Maximum parsimony with the branch and bound option was used to search for the shortest trees. Uninformative characters were ignored and zero length branches were collapsed. Bootstrapping (Felsenstein, 1985) with 500 iterations was used to estimate the reliability of branches on the shortest trees.

RESULTS

Allozyme frequencies are tabulated in the Appendix and population genetic characteristics are summarized in Table 2. The percent of polymorphic loci ranged from zero in *T. elata*, *T. protea*, and *T. variegata* (sample from Five Springs) to 55% in samples of *T. clathrata* from Moapa and Pahranagat Valleys, and *T. angulata* from Big Spring. Direct count heterozygosity values ranged from 0.00 in samples of *T. protea* and *T. variegata* (Five Springs) to 0.191 in *T. variegata* from a spring at Point of Rocks. The four populations of parthenogenetic *T. protea* were identically monomorphic. Levels of allozyme polymorphism and heterozygosity are comparable to those reported in other surveys of hydrobiid snails from desert springs (Hamlin, 1996; Ponder *et al.*, 1996). The low levels of genetic diversity documented in these studies apparently reflect founder effects and isolation typical of hydrobiid snail populations living in these habitats.

An UPGMA dendrogram derived from the allozyme data is shown in Figure 4.

NALL = mean number of alleles per locus, P = percent of loci polymorphic, and H = mean direct count heterozygosity. Standard errors are in parentheses. Pooling of several individuals for resolution of some loci in *T. elata* and *T. ericae* prevented calculation of N and H for these samples

Species	Population	N	NALL	P	H
T. angulata	B	27.2(2.8)	2.0(0.3)	55.6	0.106(0.047)
	C	35.7(2.3)	1.7(0.3)	44.4	0.076(0.034)
	F	33.6(3.2)	1.8(0.4)	44.4	0.157(0.091)
T. brevissima	..	10.6(0.9)	1.1(0.1)	11.1	0.009(0.009)
T. clathrata	A	33.3(1.8)	1.7(0.2)	55.6	0.076(0.035)
	M	29.7(2.2)	1.6(0.2)	55.6	0.084(0.036)
T. elata	(pooled)	..	1.0(0)	0.00	..
T. ericae	(pooled)	–	1.3(0.2)	22.2	..
T. imitator	M	32.3(0.2)	1.1(0.1)	11.1	0.10(0.10)
	S	21.3(2.7)	1.4(0.2)	44.4	0.52(0.024)
T. margae	G1	25.6(2.9)	1.3(0.2)	33.3	0.80(0.048)
	G2	22.8(1.6)	1.2(0.1)	22.2	0.084(0.062)
	G3	25.1(2.2)	1.2(0.1)	22.2	0.102(0.068)
T. protea	O	18.8(1.5)	1.0(0)	0.00	0.00
	Hu	22.8(1.8)	1.0(0)	0.00	0.00
	Hc	27.0(1.8)	1.0(0)	0.00	0.00
	W	50.0(0)	1.0(0)	0.00	0.00
T. robusta	N	21.3 (3.5)	2.1(0.5)	44.4	0.061(0.027)
	T	19.3(3.2)	1.7(0.3)	44.4	0.078(0.056)
T. rowlandsi	G2	25.7(2.9)	1.2(0.1)	22.2	0.019(0.013)
	G3	24.2(3.3)	1.2(0.1)	22.2	0.013(0.009)
T. salina	–	28.2(0.09)	1.2(0.1)	22.2	0.008(0.005)
T. variegata	D	27.8(1.7)	1.4(0.2)	44.4	0.022(0.010)
	F	26.6(1.7)	1.0(0)	0.00	0.00
	N	34.3(1.3)	1.3(0.2)	22.2	0.015(0.010)
	P	25.1(1.7)	1.4(0.2)	44.4	0.191(0.096)
	Sh	34.4(1.9)	1.3(0.2)	33.3	0.018(0.012)
	Sa	32.6(1.8)	1.1(0.1)	11.1	0.003(0.003)

The western species of *Tryonia* formed a cluster well-differentiated from *T. brevissima* of Florida. This outgroup was fixed for one allele (*Mpi*-122) not seen in any of the western species. The nine species from the Death Valley system did not form a distinct cluster, owing to placement of other western species, *T. clathrata* and *T. imitator*. Fauna of the upper Amargosa basin (*T. angulata*, *T. elata*, *T. ericae*, *T. variegata*) formed a distinct cluster, and were more similar to *T. protea* than to any of the species in Death Valley (lower Amargosa basin). The two minute *Tryonia* from Ash Meadows, *T. elata* and *T. ericae*, shared one fixed, unique allele (*Pgd*-105). *Tryonia* from the lower Amargosa basin (*T. margae*, *T. robusta*, *T. rowlandsi*, *T. salina*) did not form a distinct cluster. *Tryonia salina* and *T. robusta* were especially divergent and outside the large cluster which included extralimital species (*T. clathrata*, *T. imitator*) and other species from Death Valley (*T. rowlandsi* and *T. margae*). *Tryonia salina* was the only western species to have autapomorphic alleles (*Aat*-84; *Pgm*-118). The dendrogram shows *Tryonia robusta* as the most divergent species of the Death Valley system relative to other western *Tryonia*.

Most species from the Death Valley system were well-differentiated from one another, and conspecific samples for *T. clathrata*, *T. imitator*, *T. margae*, *T. protea*, *T.*

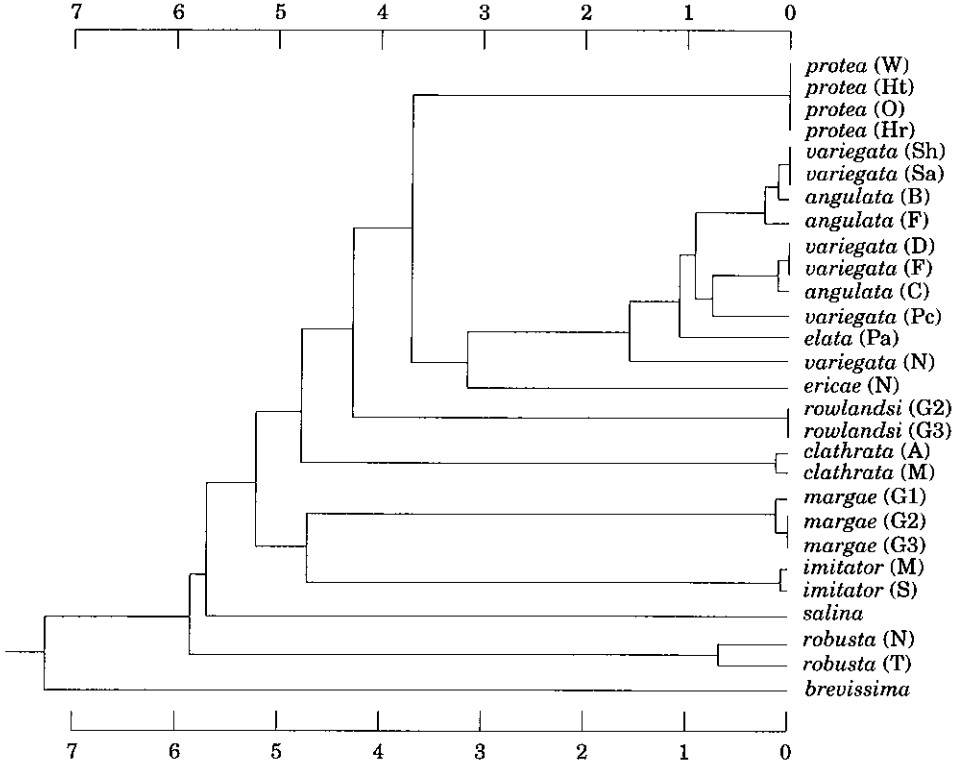

Figure 4. UPGMA dendrogram summarizing Nei's genetic distances (from allozymes) among 28 samples of *Tryonia*. Cophenetic correlation = 0.913. Locality abbreviations are from Table 1.

robusta, and *T. rowlandsi* formed clusters that excluded other species. Population samples of these species were weakly differentiated with the exception of two samples of *T. robusta*, which were five times more divergent (D = 0.09) than any other set of conspecific populations. This level of allozyme differentiation approaches that observed between species from Ash Meadows (e.g. *T. elata* and the *T. angulata*/*T. variegata* complex; $D \geq 0.105$). Woodruff *et al.* (1988) suggested that for mollusks, $D < 0.10$ is typical of conspecific comparisons and $D > 0.50$ for interspecific comparisons.

Genetic distances (Fig. 4) suggest that the taxonomy of *Tryonia angulata* and *T. variegata* is confused, and that more diversity is present than suggested by earlier morphology-based work (Hershler & Sada, 1987). These 'species' were not clearly differentiated from one another as conspecific samples did not form distinct clusters. Two populations of *T. variegata* from the lower Amargosa basin formed a cluster, which in turn clustered with two populations of *T. angulata*. While two of the populations of *T. variegata* from Ash Meadows (Devils Hole and Five Springs) were closely similar (D = 0.00012), this cluster was most similar to a population of *T. angulata* and not to the other populations of *T. variegata* from this area (Point of

(Shoshone, Saratoga Springs) were substantially differentiated from Ash Meadows populations and, although evaluation of allopatric populations always is problematic, probably warrant separate specific status.

Direct sequencing of PCR products yielded an aligned matrix of 600 bp from the mitochondrial COI gene. This sequence corresponds to positions 1566–2165 in the homologous *Drosophila yakuba* mtDNA sequence (Clary & Wolstenholme, 1985). Of the 600 bp sequence, 135 were variable and 82 were phylogenetically informative. Sequence divergence (uncorrected p-distance; Swofford *et al.* 1996) among samples is shown in Table 3. Cladistic analysis of the sequence data yielded four equally parsimonious trees of 211 steps (CI = 0.73, RI = 0.69). A strict consensus tree is shown in Figure 5. Topology of these trees varied with respect to placement of *T. rowlandsi,* which was positioned as in the consensus tree or alternatively as sister to the clade comprising *T. protea, T. margae, T. salina,* and *T. imitator.* In addition, trees varied in terms of whether *T. margae* and *T. salina* were paraphyletic (as in the consensus tree) or formed a clade, and in relative positions of *T. ericae, T. elata,* and *T. variegata* within the clade formed by these three species.

The consensus tree indicates that the species of *Tryonia* from the Death Valley system are not monophyletic owing to in-group placement of *T. clathrata* and *T. imitator.* In concordance with the allozyme dendrogram, species from the upper Amargosa basin formed a clade, to which *T. clathrata* (Colorado basin) is sister. As suggested by the dendrogram, taxa from lower Amargosa basin (*T. margae, T. robusta, T. rowlandsi, T. salina*) are non-monophyletic. Two of the Death Valley species, *T. margae* and *T. salina,* together with *T. imitator* from the California coast, formed a clade sister to *T. protea. Tryonia robusta* does not appear closely related to other Death Valley species and occupies a basal position on the tree.

Sequence divergence between the two samples of *T. imitator* and *T. protea* was low (*T. protea,* p = 0%; *T. imitator,* p = 0.17%), however, the two samples of *T. variegata* that were sequenced (Devils Hole and Saratoga Springs) were much more divergent (p = 5.2%). Furthermore, the sample of *T. variegata* from Saratoga Spring was much more similar to *T. angulata* (p = 0.67%) than to *T. variegata* from Devils Hole. This is consistent with the allozyme data and suggests that *T. variegata* is in need of revision.

DISCUSSION

Allozyme and mtDNA data presented herein indicate that the nine *Tryonia* species of the Death Valley system are non-monophyletic. The pupfish fauna in the region was originally regarded as monophyletic (Miller, 1946, 1948, 1950), but more recently it has been suggested that the Owens Valley pupfish, *C. radiosus,* represents a second lineage (Miller, 1981; Minckley, Hendrickson & Bond, 1986). Mitochondrial DNA evidence (Echelle & Dowling, 1992: figs 3–5) revealed *C. radiosus* as more closely related to *C. macularius,* from the lower Colorado basin, than to the clade comprised of the three congeneric species from the Amargosa basin whereas allozyme evidence was equivocal (Echelle & Echelle 1993: figs 2, 3). These data also suggested that the Amargosa basin clade was most closely related to species from endorheic drainage of northern Mexico (closely allied to Rio Grande drainage), which conflicts with a presumed derivation from Colorado basin progenitors (Miller, 1981) and is

TABLE 3. Sequence divergence (p) among pairwise comparison of populations of *Tryonia* species based on mtDNA COI

	angulata (B)	*brevissima*	*clathrata* (M)	*elata*	*ericae*	*imitator* (M)	*imitator* (S)	*margae* (G3)	*protea* (O)	*protea* (W)	*robusta* (T)	*rowlandsi* (G2)	*salina*	*variega* (D)
angulata (B)	–													
brevissima	0.0998	–												
clathrata (M)	0.0416	0.0965	–											
elata	0.0416	0.1165	0.0499	–										
ericae (N)	0.0549	0.1098	0.0499	0.0233	–									
imitator (M)	0.0433	0.1032	0.0449	0.0549	0.0483	–								
imitator (S)	0.0416	0.1048	0.0433	0.0532	0.0466	0.0017	–							
margae (G3)	0.0483	0.1115	0.0549	0.0599	0.0516	0.0216	0.0200	–						
protea (O)	0.0632	0.1165	0.0632	0.0666	0.0649	0.0632	0.0483	0.0616	–					
protea (W)	0.0632	0.1165	0.0632	0.0666	0.0649	0.0632	0.0483	0.0616	0.0000	–				
robusta (T)	0.0915	0.0932	0.0965	0.1048	0.0948	0.0998	0.0998	0.0982	0.1148	0.1148	–			
rowlandsi (G2)	0.0383	0.1032	0.0433	0.0516	0.0433	0.0449	0.0433	0.0449	0.0616	0.0616	0.0740	–		
salina	0.0416	0.1032	0.0466	0.0532	0.0499	0.0183	0.0166	0.0266	0.0549	0.0549	0.1048	0.0466	–	
variegata (D)	0.0483	0.1231	0.0599	0.0366	0.0300	0.0582	0.0566	0.0582	0.0716	0.0716	0.1048	0.0566	0.0566	–
variegata (Sh)	0.0067	0.0982	0.0499	0.0499	0.0300	0.0433	0.0416	0.0516	0.0666	0.0483	0.0932	0.0416	0.0499	0.0516

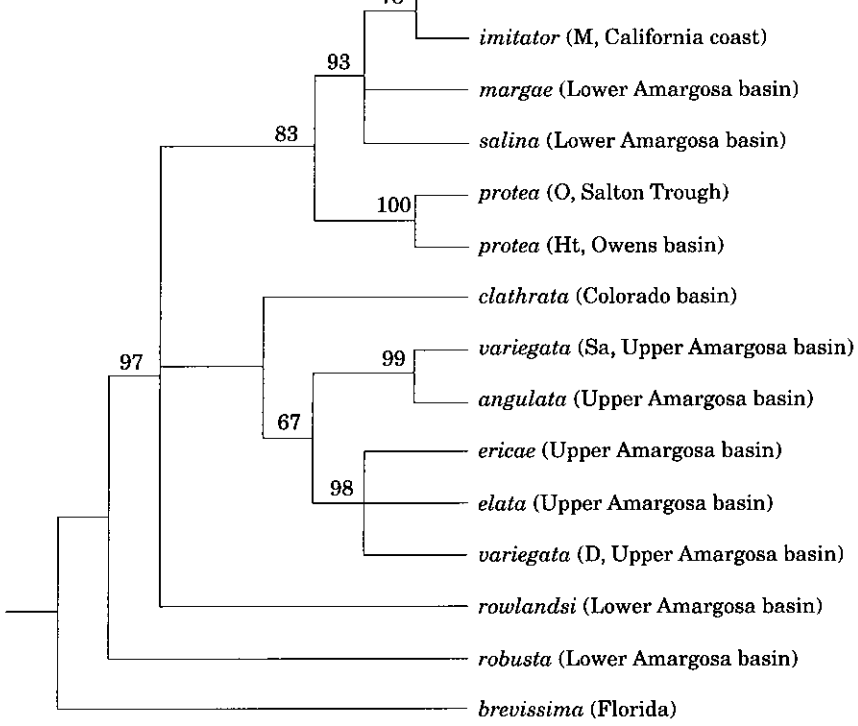

Figure 5. Strict consensus tree based on maximum parsimony analysis of mtCOI sequence data. Numbers are bootstrap percentages for well-supported clades (500 replicates).

enigmatic within the context of regional hydrographic history. Although Echelle & Echelle (1993) concluded that the pupfish fauna as a whole is monophyletic, but has been subjected to introgression by a divergent lineage, a diphyletic origin is an equally if not more tenable hypothesis based on these data.

Whereas the three species of pupfish of the Amargosa basin form a monophyletic unit, only the *Tryonia* species from the upper segment of this drainage comprise a clade, which is most closely related to snails found in the Colorado basin. (One of these Colorado basin snails, *T. protea*, also occurs in the western portion of the Death Valley system.) The four species found in the lower segment of the Amargosa basin reflect a complex evolutionary history.

Phylogenetic evidence thus suggests that both *Tryonia* and *Cyprinodon* from the Death Valley system are non-monophyletic, and that elements of both faunas are closely related to species from the lower Colorado basin. Elements of the snail fauna also suggest a close relationship with a coastal estuarine species, paralleling a scenario proposed for pupfish based largely on similarities between stressful desert waters inhabited by these animals and marginal marine habitats (Smith, 1981). We suggest that these shared biogeographic patterns can be explained within the context of a recently proposed model for early history of the lower Colorado River (Howard, 1996).

Colorado basin often has been conjectured. Several geologists (e.g. Blackwelder, 1993; Hale, 1984) postulated southerly overflow from the Mohave component of the pluvial Death Valley system to the Colorado River, but there is no convincing physical evidence for this (Metzger, Loeltz & Irelna, 1973; Brown & Rosen, 1995). Hubbs & Miller (1948:101) suggested that pluvial transfer of fishes from Las Vegas Valley (Colorado basin) to the Amargosa basin may have occurred by stream captures resulting from migration of low, alluvial divides. Hershler & Pratt (1990: fig. 9) suggested, in the absence of physical evidence, that the Amargosa River early integrated with the White River (Colorado basin) to the east. Our data favour Howard's (1996) geology-based hypothesis that an older connection (permitting transfer of snails and pupfishes) was provided by an integrated Amargosa-Colorado paleoriver prior to incision of Grand Canyon and development of the modern course of the lower Colorado River less than 5 Ma (Elston & Young, 1991). The early Amargosa drainage to the south presumably was disrupted upon Pliocene diversion of the river into the opening Death Valley trough (Howard, 1996).

The fossil record confirms the early presence of snails and pupfish in the Death Valley region as required by the above hypothesis. A minimum date for regional pupfish is provided by fossils from the northern portion of Death Valley (Miller, 1945) which are tentatively considered of late Miocene age (McAllister in Miller, 1981). Although fossil *Tryonia* in the Death Valley system all are Pleistocene (Taylor, 1986; Roth & Reynolds, 1990), the Pliocene Copper Canyon Formation contains unidentified high-spired hydrobiids (Drewes, 1963:35) that also may belong to the genus. Lacustrine strata in the Whipple Mountains (just south of the Death Valley system) of Miocene age (K.K. Beratan, letter to first author, 24 August 1988) contain fossils that also appear to be *Tryonia* (Hershler unpublished).

Mitochondrial DNA sequences indicate that *T. margae* and *T. salina* from Death Valley, together with *T. protea*, are most closely related to estuarine *T. imitator* from coastal California; allozyme data also support a close relationship between *T. margae* and *T. imitator*. (Neither fossil nor living pupfish have been found west of the Death Valley system; Miller, 1981.) These snail relationships also can be tied to early faunal exchange via the Colorado paleoriver, which was hypothesized to have drained into the coastal Los Angeles basin during the Miocene prior to its re-routing into the proto-Gulf of California by movement along the San Andreas fault system (Howard, 1996). Support for this hypothesis is provided by fossils identified as *Tryonia imitator* from fluviatile and lacustrine deposits of the Miocene Mint Canyon Formation north of Los Angeles (Kew, 1924; Oakenshott, 1958), as these strata were deposited westward of the Chocolate Mountains (Ehlig, Ehlert & Crowe, 1975; Ehlert, 1982) in the vicinity of the Colorado paleodelta (Howard, 1996).

Alternatively, or in addition, snail transfer between California coastal waters and the Death Valley system may have been effected by westward drainage from the Owens Valley area into San Joaquin Valley prior to an increasing rate of uplift of the Sierra Nevada (and development of a drainage divide) during the Pliocene (Huber, 1981). This would have provided integration with coastal drainages (within the modern range of *T. imitator*) as the San Joaquin Valley then contained a large marine embayment (Bartow, 1991). *Tryonia* are recorded from nonmarine Plio-Pleistocene deposits of the Tulare Formation in the San Joaquin Valley (i.e. as *Calipyrgula*; Pilsbry, 1935; Woodring, Stewart & Richards, 1940), confirming their early presence in this region.

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APPENDIX

Allele frequencies at nine polymorphic loci in populations of *Tryonia* species. See Material and Methods for locus abbreviations

	Population													
	angulata			*brevissima clathrata*			*elata*	*ericae*	*imitator*		*margae*			*protea*
	B	C	F	–	A	M	Pa	N	M	S	G1	G2	G3	O
Aat														
(*N*)	14	24	13	10	33	34	11	01	33	27	04	14	22	07
120	·	·	–	1.00	1.00	1.00	–	–	–	–	–	–	–	–
114	–	0.10	–	–	–	–	–	–	–	–	–	–	–	–
109	–	–	–	–	–	–	–	–	–	0.28	–	·	·	–
100	1.00	0.90	1.00	–	–	–	1.00	1.00	1.00	0.72	1.00	1.00	1.00	1.00
84	–	–	–	–	–	–	–	·	·	·	–	–	–	–
Icd														
(*N*)	19	28	31	09	33	25	01	02	33	10	28	23	23	19
100	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	–	–	–	1.00
80	–	–	–	–	–	0.02	–	–	–	–	1.00	1.00	1.00	–
Est														
(*N*)	35	28	44	12	36	36	33	35	32	12	30	27	34	21
120	0.01	·	0.09	0.96	–	–	–	–	1.00	0.96	–	–	–	–
100	0.01	–	0.19	0.04	0.01	0.18	–	0.03	–	0.04	–	–	–	1.00
75	0.98	1.00	0.72	–	0.99	0.82	1.00	0.97	–	–	1.00	1.00	1.00	·
69	–	–	–	–	–	–	–	–	–	–	–	·	·	–
53	–	–	–	–	–	–	–	·	·	·	·	–	–	–
Me														
(*N*)	36	40	44	09	43	29	06	04	32	20	23	23	22	21
223	·	·	–	1.00	–	–	–	–	–	–	–	–	–	–
195	1.00	1.00	1.00	–	–	–	1.00	–	–	–	–	–	·	
100	–	–	–	–	1.00	1.00	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mpi														
(*N*)	25	40	37	11	36	23	34	10	33	38	33	21	14	20
122	–	–	–	1.00	–	–	–	–	–	–	–	–	–	–
105	–	–	–	–	1.00	0.83	–	–	0.96	1.00	–	–	–	·
102	–	–	–	–	–	0.17	–	–	–	–	–	–	·	–
100	1.00	1.00	1.00	–	–	–	1.00	1.00	0.04	·	0.97	1.00	1.00	1.00
91	–	–	–	–	–	·	·	·	·	–	0.03	–	–	–
80	–	–	·	·	–	–	–	–	–	–	–	–	–	–
Nsp														
(*N*)	24	40	38	17	30	36	26	26	32	20	26	28	28	20
120	0.04	–	–	–	0.87	1.00	–	–	·	·	·	·	–	–
112	0.90	0.92	0.94	–	0.10	–	1.00	1.00	·	·	·	·	–	–
106	0.06	0.05	0.06	·	·	·	–	–	–	–	–	–	–	–
100	–	0.03	-	1.00	0.03	–	–	–	1.00	0.93	0.65	0.41	0.38	1.00
89	·	–	–	–	–	–	–	–	–	–	0.35	0.59	0.62	·
80	–	–	–	–	–	–	–	–	–	0.07	–	·	·	–
Pgm														
(*N*)	34	41	36	09	23	29	12	15	32	21	26	25	20	19
118	–	–	·	·	·	–	–	–	–	–	–	–	–	–
114	0.04	·	0.01	1.00	0.13	0.05	–	–	1.00	0.98	1.00	1.00	1.00	·
100	0.96	1.00	0.99	–	0.87	0.95	1.00	1.00	–	0.02	–	–	·	1.00
85	–	–	–	–	–	–	–	–	–	–	–	·	–	–
61	–	–	–	–	–	–	–	-	·	·	·	·	–	–
Pgd														
(*N*)	22	40	26	09	30	18	06	18	32	22	27	17	30	21
128	0.04	·	·	–	0.12	–	–	–	–	–	–	–	–	·
111	0.25	0.96	0.08	–	0.88	0.64	–	–	–	–	1.00	1.00	1.00	–
105	–	–	–	–	–	–	1.00	1.00	–	·	·	·	–	–

continued

	Population													
	angulata			*brevissima clathrata*			*elata*	*ericae*	*imitator*		*margae*			*protea*
	B	C	F	–	A	M	Pa	N	M	S	G1	G2	G3	O
100	0.71	0.04	0.39	1.00	–	0.36	–	–	–	–	–	–	–	1.00
84	–	–	0.48	–	–	–	–	–	1.00	1.00	–	–	–	–
64	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–
Gpi														
(N)	36	40	38	09	36	37	38	33	32	22	33	27	33	21
225	–	–	–	–	–	–	–	–	–	–	–	–	–	–
204	–	–	–	–	–	–	–	–	–	–	–	–	–	–
174	–	–	–	–	–	–	–	0.01	–	–	–	–	–	–
130	0.03	0.21	–	1.00	–	–	–	0.97	–	–	–	–	–	–
100	0.90	0.78	1.00	–	0.99	1.00	1.00	0.02	1.00	1.00	0.80	0.85	0.71	1.00
75	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–
37	–	–	–	–	–	–	–	–	–	–	–	–	–	–
32	0.07	0.01	–	–	–	–	–	–	–	–	0.20	0.15	0.29	–

	Population													
	protea			*robusta*		*rowlandsi*		*salina*	*variegata*					
	Hu	Ht	W	N	T	G2	G3	–	D	F	N	P	Sa	Sh
Aat														
(N)	09	18	50	14	19	06	05	22	34	22	35	18	28	33
120	–	–	–	–	–	–	–	–	–	–	–	–	–	–
114	–	–	–	–	–	–	–	–	–	–	–	–	–	–
109	–	–	–	–	–	–	–	–	–	–	–	–	–	–
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00	1.00	1.00	1.00	1.00	1.00
84	–	–	–	–	–	–	–	1.00	–	–	–	–	–	–
Icd														
(N)	22	18	50	18	13	26	26	30	23	29	36	17	22	25
100	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.68	1.00	1.00
80	–	–	–	0.06	–	–	–	–	0.02	–	–	0.32	–	–
Est														
(N)	25	31	50	30	29	32	27	30	25	31	33	21	36	35
120	–	–	–	0.02	0.90	–	–	–	–	–	–	–	–	–
100	1.00	1.00	1.00	0.10	0.07	–	–	1.00	–	–	–	–	–	–
75		–	–	0.03	–	1.00	1.00	–	1.00	1.00	1.00	1.00	1.00	1.00
69	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–
53	–	–	–	0.83	0.03	–	–	–	–	–	–	–	–	–
Me														
(N)	24	30	50	08	07	24	17	29	27	24	32	28	33	37
223	–	–	–	–	–	1.00	1.00	–	0.02	–	–	–	0.02	–
195		–	–	1.00	1.00	–	–	1.00	0.98	1.00	1.00	1.00	0.98	0.99
100	1.00	1.00	1.00	–	–	–	–	–	–	–	–	–	–	0.01
Mpi														
(N)	24	30	50	19	14	27	30	29	22	21	29	29	40	42
122	–	–	–	–	–	–	–	–	–	–	–	–	–	–
105	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–
102	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–
100	1.00	1.00	1.00	0.26	–	1.00	1.00	0.98	0.96	1.00	1.00	1.00	1.00	0.99
91	–	–	–	0.66	0.96	–	–	–	–	–	–	–	–	0.01
80	–	–	–	0.08	0.03	–	–	–	–	–	–	–	–	–
Nsp														
(N)	29	30	50	34	31	20	18	29	25	19	29	28	30	37
120	–	–	–	–	–	–	–	–	–	–	–	–	–	–
112	–	–	–	1.00	1.00	–	–	–	1.00	1.00	–	0.55	1.00	1.00

	Population													
	protea			*robusta*		*rowlandsi*		*salina*	*variegata*					
	Hu	Ht	W	N	T	G2	G3	–	D	F	N	P	Sa	Sh
106	–	–	–	–	–	–	–	–	–	–	–	–	–	–
100	1.00	1.00	1.00	–	–	0.05	–	1.00	–	–	1.00	0.45	–	–
89	–	–	–	–	–	0.95	1.00	–	–	–	–	–	–	–
80	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pgm														
(N)	24	30	50	29	23	27	25	30	36	31	39	28	36	26
118	–	–	–	–	–	–	–	1.00	–	–	–	–	–	–
114	–	–	–	–	–	–	–	–	–	–	–	–	–	–
100	1.00	1.00	1.00	–	–	0.96	0.96	–	1.00	1.00	1.00	1.00	1.00	1.00
85	–	–	–	1.00	0.97	0.04	0.04	–	–	–	–	–	–	–
61	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–
Pgd														
(N)	24	26	50	07	07	33	40	26	25	30	37	29	32	37
128	–	–	–	–	–	–	–	–	–	–	–	0.66	–	–
111	–	–	–	1.00	1.00	–	–	–	1.00	1.00	–	0.34	–	–
105	–	–	–	–	–	–	–	–	–	–	–	–	–	–
100	1.00	1.00	1.00	–	–	–	–	–	–	–	0.97	–	1.00	0.95
84	–	–	–	–	–	–	–	0.02	–	–	0.03	–	–	0.05
64	–	–	–	–	–	1.00	1.00	0.98	–	–	–	–	–	–
Gpi														
(N)	24	30	50	33	31	36	30	29	33	32	39	28	36	38
225	–	–	–	0.06	0.24	–	–	–	–	–	–	–	–	–
204	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–
174	–	–	–	0.89	0.71	–	0.25	–	–	–	–	–	–	–
130	–	–	–	–	–	1.00	0.75	–	–	–	0.01	0.09	–	–
100	1.00	1.00	1.00	–	–	–	–	1.00	0.99	1.00	0.96	0.91	1.00	1.00
75	–	–	–	0.03	0.05	–	–	–	–	–	–	–	–	–
37	–	–	–	–	–	–	–	–	–	–	–	–	–	–
32	–	–	–	–	–	–	–	–	0.01	–	0.03	–	–	–