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Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*

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Abstract

***Aptostichus simus* is a trapdoor spider endemic to the coastal dunes of central and southern California and, on morphological grounds, is recognized as a single species. Mitochondrial DNA 16S rRNA sequences demonstrate that most populations are fixed for the same haplotype and that the population haplotypes from San Diego County, Los Angeles County, Santa Rosa Island, and Monterey County are extremely divergent (6–12%), with estimated separation times ranging from 2 to 6 million years. A statistical cluster analysis of morphological features demonstrates that this genetic divergence is not reflected in anatomical features that might signify ecological differentiation among these lineages. The species status of these divergent populations of *A. simus* depends upon the species concept utilized. If a time-limited genealogical perspective is employed, *A. simus* would be separated at the base into two genetically distinct species. This study suggests that species concepts based on morphological distinctiveness, in spider groups with limited dispersal capabilities, probably underestimate true evolutionary diversity.**

Keywords: Araneae, biogeography, phylogenetics, phylogeography, ribosomal RNA, spider taxonomy

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Introduction

This study examines phylogeography and morphological/ecological divergence in the Californian coastal trapdoor spider species *Aptostichus simus* (Chamberlin 1917) (Araneae: Mygalomorphae: Cyrtaucheniidae). The genus *Aptostichus* comprises ~30 nominal species (Bond 1999) and is placed in the primitive spider infraorder Mygalomorphae. *Aptostichus* species are distinguished by male secondary sexual characteristics, female genitalic, and other somatic features (e.g. size, shape, spination), characteristics typically used to delineate mygalomorph species (e.g. Coyle 1971, 1995; Griswold 1987; Goloboff 1995). However, morphology-based species hypotheses are seldom explicitly tested. *A. simus* is widespread along the coastal dune ecosystem of southern California, having a distribution that extends from Baja Norte northward to Point Conception (Fig. 1). Geographically disjunct populations are also found in the Monterey Bay area. *A. simus* is found in the nontidal dune environment where it builds a heavily silk lined burrow, which is covered with a silken and sand trapdoor. These spiders use their burrow both for shelter and as a vantage point from which to capture prey. The burrows of many adult and juvenile individuals are often clustered suggesting that *A. simus* dispersal capability may be minimal.

Many members of the spider infraorder Araneomorphae are able to disperse great distances across geological barriers by aerial ballooning (Greenstone *et al*. 1987). Spiders 'balloon' by releasing silken threads that are captured by the wind and carry the spiders aloft. Because primitive mygalomorph taxa seldom disperse by ballooning (Coyle 1983; Main 1982), *A. simus* interpopulation gene flow may be limited. Therefore, these spiders may be particularly prone to population divergence and speciation by vicariant

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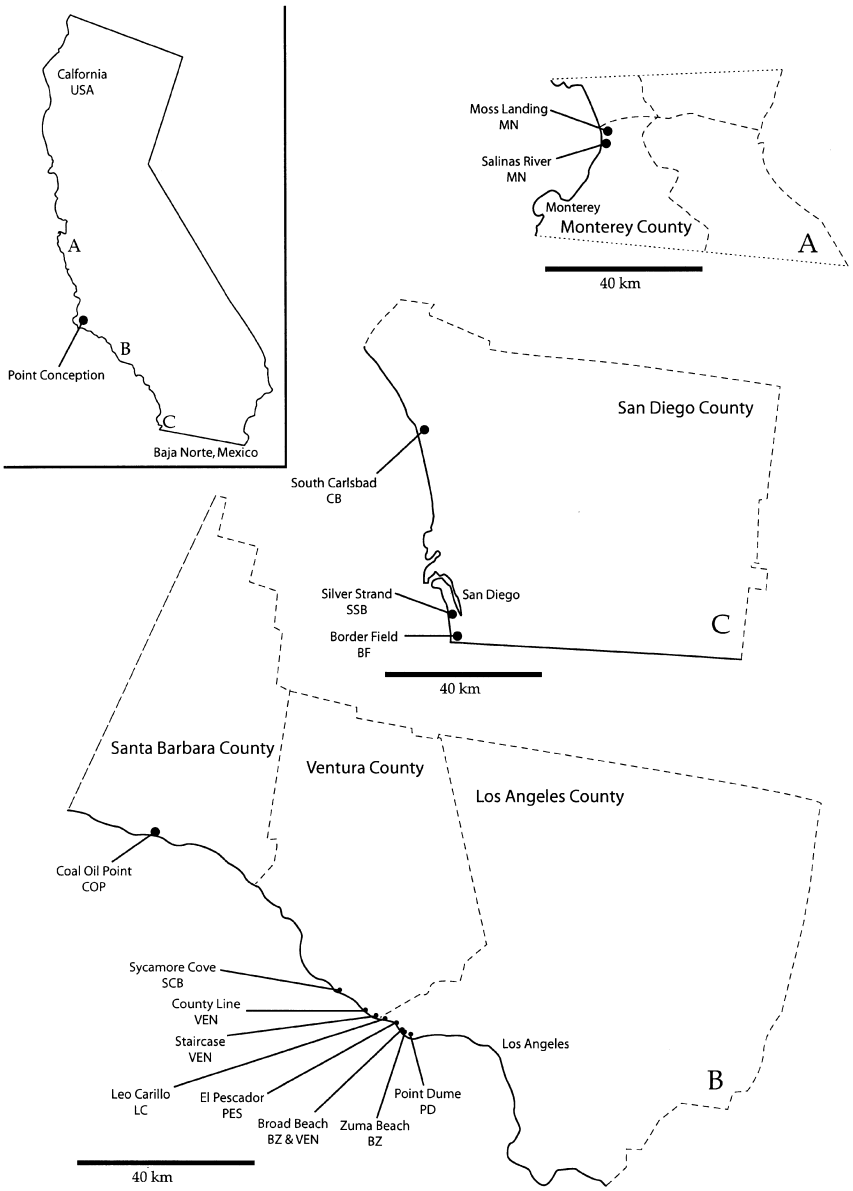

Fig. 1 Distribution map for *Aptostichus simus* populations sampled along the coast of California. Haplotypes recovered at each locality are indicated below locality name.

habitat fragmentation and/or parapatric divergence. The dynamic geological history of this region is well studied (summarized by Yanev 1980) and the dunes are discontinuous because both geological and artificial barriers to dispersal have been imposed.

Objectives and overview

Under the rubric of the traditional morphological species concept often used to delineate spider species (see above) all *A. simus* populations appear to comprise a single 'biological' species and were treated as such by Bond (1999) in his taxonomic revision of the genus. The first objective of this study is to further test the hypothesis that all *A. simus* populations are a single species using morphological data. Once we have assessed the morphological cohesiveness of individuals from multiple *A. simus* populations we will then examine the degree to which these patterns are corroborated at the genetic level. These genetic data are used to assess population divergence, phylogeography, and ecological correlates of divergence in *A. simus*. Additionally, we question the importance of ecological change in *Aptostichus* divergence as these populations are morphologically and, therefore, potentially ecologically interchangeable.

Materials and methods

Population sampling

Specimens were collected along the coastline of southern California from San Diego County northward to Los Angeles County, with two collection sites in Santa Barbara

Table 1 List of haplotypes, localities, and GenBank Accession nos (AF307955 – AF307969) for all of the populations and haplotypes sampled in this study. Los Angeles Basin populations are listed from North to South

Haplotypes (*n*)	Collecting localities	Latitude/ longitude	GenBank Accession no.
	Northern		
MN (4)	CA: Monterey County: Moss Landing State Beach	N 36°48.52′ W 121°47.31′	AF307965
MN (4)	CA: Monterey County: Salinas River State Beach	N 36°47.435′ W 121°47.48′	AF307965
	LA Basin		
COP (7)	CA: Santa Barbara County: Coal Oil Point Preserve	N 34°24.51′ W 119°52.76′	AF307961
SCB (5)	CA: Ventura County: Sycamore Cove Beach	N 34°4.23′ W 119°0.91′	AF307969
VEN (5)	CA: Ventura County: County Line Beach	N 34°3.14′ W 118°57.8′	AF307962
VEN (5)	CA: Ventura County: Staircase Beach	N 34°2.76′ W 118°56.74′	AF307962
LC (5)	CA: Los Angeles County: Leo Carrillo State Beach	N 34°2.63′ W 118°56.32′	AF307964
PES (5)	CA: Los Angeles County: El Pescador State Beach	N 34°2.38′ W 118°53.71′	AF307963
BZ (3) and VEN (2)	CA: Los Angeles County: Broad Beach	N 34°1.99′ W 118°50.95′	AF307960, AF307962
BZ (5)	CA: Los Angeles County: Zuma Beach County Park	N 34°1.32′ W 118°49.9′	AF307960
PD_1 (3) and PD_2 (2)	CA: Los Angeles County: Point Dume State Beach	N 34°0.57′ W 118°48.96′	AF307966, AF307967
	Channel Island		
SRI (5)	CA: Santa Barbara County: Santa Rosa Island	N 34°0.23′ W 120°14.14′	AF307968
	Southern		
CB(3)	CA: San Diego County: South Carlsbad State Beach	N 33°6.23′ W 117°19.16′	AF307959
SSB (3)	CA: San Diego County: Silverstrand State Beach	N 32°37.33′ W 117°8.23′	AF307958
BF_{1-3} (3)	CA: San Diego County: Borderfield State Beach	N 32°32.46′ W 117°7.50′	AF307955 – AF307957

County (Fig. 1, Table 1). Collecting localities were identified from museum records, however, all accessible coastline was checked for suitable habitat. Although *Aptostichus simus* is more widespread on the California Channel Islands, we were only able to obtain specimens from Santa Rosa Island (directly off the coast of Santa Barbara County Fig. 1, not shown). Because of the observed small population sizes and collecting permit constraint at many of the localities, we collected no more than five individuals per population. Voucher specimens corresponding to each unique GenBank accession number have been deposited in the California Academy of Sciences collection.

Morphometric analysis

Morphometric features were evaluated from mature female specimens collected for the molecular study, and additional specimens borrowed from the California Academy of Sciences, San Francisco California and American Museum of Natural History, New York. All measurements are given in millimeters and were made with a Wild M-8 dissecting microscope equipped with an ocular micrometer scale. Quantitative and meristic appendage features are based on left appendages in the retrolateral view using the highest magnification possible and are accurate to 0.03–0.015 mm. These measurements were taken from the mid-proximal point of articulation to the mid-distal point of the article (*sensu* Coyle 1995). All ratios are scaled by a factor of 100. Cluster analyses using distances computed by the unweighted paired group method using arithmetic averages (UPGMA) were performed using the computer program SAS (SAS Institute Inc., Cary, NC). Clusters determined by this analysis were reconstructed in MacClade (Maddison & Maddison 1992) for clearer visualization.

Collection of DNA sequences

Total genomic DNA was extracted from approximately 10–15 mg of leg tissue using the Puregene™ DNA extraction kit. This extraction procedure comprises a lysis step in Tris-EDTA buffer with sodium dodecyl sulfate incubated for 3 h with Proteinase K, a protein precipitation step using potassium acetate, followed by DNA precipitation in isopropanol, and a 70% ethanol wash. DNA was resuspended in Tris-EDTA buffer and diluted 1:100 for subsequent use.

The polymerase chain reaction (PCR) was used to amplify a 3′ region of the 16S rRNA gene of the mitochondrion, initially using the 12S and 16S universal primers 12Sai-5′ 5′ AAACTAGGATTAGATACCCTATTAT 3′ and 16Sbr-3′ 5′-CCGGTCTGAACTCAGATCACGT-3′ (Hillis *et al*. 1997). The primers 12Sai-5′ and 16Sbr-3′ correspond to *Drosophila* mitochondrial genome positions 14 588 and 12 887, respectively. Standard PCR reactions were carried out in 50 µL volumes and run for 35 cycles, each consisting of a 30 s denaturation at 95 °C, 30 s annealing at 50 °C and 45 s (+ 3 s/cycle) extension at 72 °C, with an initial denaturation step of 95 °C for 2.5 min and a final extension step of 72 °C for 10 min.

Amplification products were electrophoresed on a 0.8% agarose gel, excised from the gel and purified using Qiagen QIAquick gel extraction columns. Purified products were sequenced with an ABI PRISM™ 377 automated sequencer using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS. Because these data lacked complex insertions and deletions, alignment was straightforward and could be accomplished by eye. However, the computer program CLUSTALW (Higgins *et al.* 1996) was used to assemble the multiple sequences into a usable format for phylogenetic analysis. Single nucleotide insertion deletions (indels) were scored as present or absent (binary characters) and entered manually into the Nexus file (Maddison *et al.* 1997).

Phylogenetic inference

Standard parsimony. Phylogenetic analyses were performed using PAUP* version 4.0b2 (Swofford 1999) run on a Power Macintosh 6500/275. Because of the relatively small number of terminals (15) included in this analysis the branch and bound search algorithm was used. Measures of branch support for the parsimony (unweighted)-based tree topology are based on decay (Bremer 1988; Donoghue *et al*. 1992) and bootstrap analyses (Felsenstein 1985a). Decay indices (Bremer 1988) were computed using the computer program Autodecay (Eriksson & Wikstrom 1996). Bootstrap values are based on 500 replicates using parsimony and the branch and bound search algorithm in PAUP*. Pairwise proportional divergence values, used in molecular clock calibrations, were computed in PAUP*, standard errors for these values were computed using the computer program MEGA (Kumar *et al*. 1993). Root estimation for parsimony analyses is based on *Aptostichus n.sp.* Bond (1999), a newly described species from San Bernardino County considered to be a member of the Simus species group.

Maximum likelihood. Phylogeny estimation using maximum likelihood (ML) was conducted in PAUP*. The computer program Modeltest (Posada & Crandall 1998) was used to determine the appropriate model of DNA substitution. This program implements a hierarchical, nested, likelihood ratio test (Lrt) for alternatives models of DNA sequence evolution in which $\delta = -2 \log \Lambda$, where δ is approximately χ^2 distributed (Huelsenbeck & Rannala 1997). Using the best fit model of DNA substitution indicated by the Lrt ML heuristic searches were conducted in PAUP* with multiple random addition replicates of taxa followed by tree bisection and reconnection (TBR) branch swapping. Model parameter value estimations were evaluated simultaneously during the course of the analysis. Nucleotide frequencies were based on their empirical values.

TCS Procedure. Standard parsimony, distance and ML methods used to reconstruct interspecific phylogenetic relationships have underlying assumptions that are often violated by intraspecific data sets (see Crandall *et al*. 1994 and Crandall & Templeton 1996 for summary) leading to a lack of phylogenetic resolution. The Templeton, Crandall, Sing Parsimony Algorithm (hereafter referred to as TCS; Templeton *et al*. 1992) is a technique that takes into account the problems associated with reconstructing the relationships of closely related haplotypes/populations. Standard pairwise distances (absolute number of character differences) for TCS phylogeny estimation were computed in PAUP based on the number of nucleotide substitutions and indels. These distances were then used to estimate the probability, P_j, of a parsimonious connection between two haplotypes that differ at j sites and share m sites ($j + m$ = total number of sites surveyed). The estimator P_j is defined as:

$$\hat{P}_j = \prod_{i=1}^{j} (1 - \hat{q}_i)$$

where q_i is the probability of a nonparsimonious connection and is based on m and j (Templeton *et al*. 1992; eqn 8). Connections between haplotypes are justified for $P_j \geq 0.95$. Haplotypes are connected in a network starting with those that differ by $j = 1$ sites until all sites $j > 1$ are incorporated. For connections where $P_j < 0.95$ the estimator $P_j + 1$ was used to evaluate connections that allow one multiple hit (one potential instance of homoplasy). Calculations of P_j and $P_j + 1$ were performed using the computer program ParsProb (Posada & Crandall 2000; P_j) and a program written for the Mathematica package (Wolfram Media Inc., Champaign, IL) by Alan Templeton (Washington University,

St. Louis, MO; $P_j + 1$). The results for the P_j analysis were confirmed using the new computer program TCS: Estimating Gene Genealogies version Alpha 1.01 (Clement *et al.* 2000).

Results

Morphometric analysis

We measured 16 morphological features for 31 specimens, these data are summarized in Table 2. Of these 16, four were combined into ratio values, considered to represent changes in overall structure shape. Figure 2 is the dendogram based on a cluster analysis of pairwise distances using UPGMA for the 12 morphological parameters evaluated. This dendogram shows that the morphological features measured do not differentiate these specimens, either at the population or regional level. Likewise, a principal component analysis of this same data set also failed to differentiate populations as regional clades (not shown).

DNA sequence characteristics

The results presented here are based on 69 sequences comprising 765 base pairs (bp) of the mitochondrial 16S rRNA. From the 15 ingroup population samples, 15 unique mitochondrial DNA (mtDNA) haplotypes were observed (Table 1). Most populations appeared fixed for a unique, or set of unique haplotypes. Table 3 summarizes the degree to which each of these haplotypes differ based the proportion of nucleotide differences (uncorrected 'p'). The average sequence divergence in this data set is 6.9%, with a minimum divergence of 0.1% and a maximum of 12.6%.

The San Diego County Area contains five of the 15 observed haplotypes with an average sequence divergence of 1.1% (range 0.1–2.5%). The three individuals sequenced to represent the Border Field population all carry unique haplotypes. The remaining localities, Silver Strand and South Carlsbad, are fixed for unique haplotypes with the South Carlsbad haplotype (CB) as the most divergent within this area. Eight haplotypes were observed for the populations surveyed within the Los Angeles Basin area (Northern Los Angeles County and southern Ventura County) with an average sequence divergence of 1.7% (range 1.3–6.0%). The Ventura County localities, Staircase and County Line appear fixed for the same haplotype, which is also shared by two of the five individuals examined at Broad Beach. The remaining two haplotypes are those observed for the Santa Rosa Island, Moss Landing, and Salinas River localities in Santa Barbara (Santa Rosa Island) and Monterey Counties. All five individuals surveyed from the Santa Rosa Island locality were fixed for a single, unique haplotype. Likewise, the northern localities in Monterey County appear likewise fixed for a unique haplotype (MN).

Table 2 Morphometric values, means and standard errors, for each of the *Aptostichus simus* haplotypes. CARw = carapace width, CEPHw = cephalic region width, STERNl, w = sternum length and width, LABl, w = labium length and width. All ratio values are multiplied by 100

Haplotype (n)	Carapace len. (mm)	CARw/ CEPHw	STERNl/ STERNw	SIG/ STERNw	LABl/ LABw	Leg1 len. (mm)	Labial cuspules	Endite cuspules	Rastellar spines	Cheliceral dentition	Patella III spines	Tibia III spines
Channel Island (7)	6.30 ± 0.35	149.08 ± 0.96	83.96 ± 0.79	23.67 ± 1.20	69.31 ± 1.29	43.66 ± 0.60	0.14 ± 0.14	~200 ± 0	11.28 ± 0.36	4.43 ± 0.20	17.57 ± 0.43	6.43 ± 0.61
LA Basin (12)	6.59 ± 0.25	148.71 ± 1.07	88.00 ± 0.97	22.48 ± 1.17	70.86 ± 1.49	43.43 ± 0.49	0	~200 ± 0	11.25 ± 0.35	4.08 ± 0.08	22.17 ± 1.17	6.00 ± 0.39
Monterey (2)	5.69 – 6.25	148.00 – 152.81	91.00 – 91.01	20.37 – 27.47	66.67 – 76.39	41.83 – 48.18	0	~200 ± 0	10 – 11	4	15 – 16	2 – 3
San Diego (10)	7.81 ± 0.24	147.26 ± 0.94	89.04 ± 1.38	16.84 ± 1.05	67.51 ± 1.28	45.20 ± 1.28	0	~200 ± 0	13.18 ± 0.42	4.27 ± 0.14	22.18 ± 1.02	5.36 ± 0.58

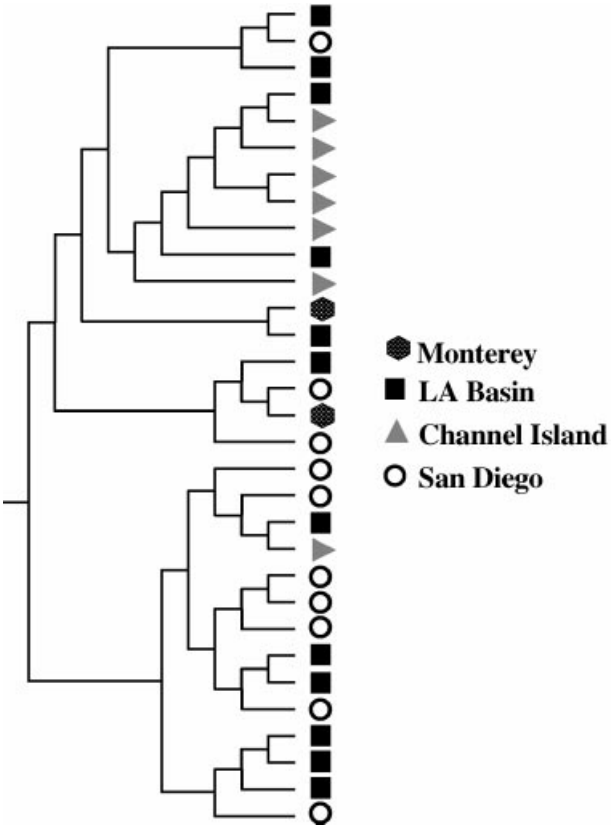

Fig. 2 Dendogram of individuals sampled from each colineal haplotype array evaluated on the basis of a UPGMA cluster analysis.

Phylogenetic inference

Parsimony and maximum likelihood. A branch and bound search in PAUP* resulted in the two equally most parsimonious (MP) trees (170 steps, CI = 0.88, RI = 0.95). Figure 3(A) is the strict consensus of these two trees and is similar to the results obtained by the maximum likelihood (ML) analysis (see below). The two MP trees differ only in their respective resolutions of the LA Basin, group 1 in which one of the trees places the VEN haplotype as basal for this lineage whereas the other tree retains this group only as a polytomy. Bootstrap and decay support (Fig. 3A) is reasonably high for most of the clades in the MP analysis. The branch that unites the two PD haplotypes is the only one that is marginally supported (marginally defined as bootstrap < 75% and decay < 3). The computer program Modeltest indicated by hierarchical nested Lrt that the most suitable ML model for these data is a General Time Reversible (GTR) model (Rodriguez *et al*. 1990) with DNA substitution rates assumed to follow a gamma distribution (GTR + Γ). A ML search using the GTR + Γ model resulted in a single tree (Fig. 3B; –Ln = 1716.55027) with an estimated gamma shape parameter of 0.191033.

Both the parsimony and ML GTR + Γ analyses are identical in their resolution of the major *Aptostichus simus* haplotypes. They unite all of the San Diego County and Los Angeles Basin area haplotypes as distinct, colineal haplotype arrays (see relative branch lengths, Fig. 3B), exclusive of the Santa Rosa Island (SRI) and Monterey County (MN) haplotypes (Fig. 3). Within the LA Basin there are two colineal arrays that roughly comprise a northern (LA_1) and southern (LA_2) basin clade (Fig. 3). However, it is important to note that the southern/central Broad Beach locality is polymorphic for haplotypes (VEN and BZ) found in both northern and southern populations.

Root estimation. We root the haplotype network using *Aptostichus n.sp.* (Bond 1999), a distantly related member of the Simus species group. A Partial 16S rRNA sequence (709 bp) was aligned to the preexisting *A. simus* data set,

Table 3. *Aptostichus simus* haplotype pairwise distances (uncorrected proportional differences) based on 16S rRNA sequences

Hap	SCB	BFB_1	BFB_2	BFB_3	SSB	CB	BZ	COP	VEN	PES	LC	MN	PD_1	PD_2	SRI
BFB_1	—	0.001	0.004	0.005	0.025	0.117	0.122	0.115	0.116	0.118	0.110	0.115	0.113	0.126	0.118
BFB_2		—	0.003	0.004	0.024	0.116	0.120	0.113	0.115	0.116	0.108	0.113	0.112	0.125	0.116
BFB_3			—	0.001	0.021	0.115	0.118	0.111	0.112	0.114	0.106	0.112	0.111	0.122	0.114
SSB				—	0.023	0.116	0.119	0.112	0.114	0.112	0.107	0.113	0.112	0.121	0.115
CB					—	0.117	0.120	0.113	0.115	0.116	0.104	0.115	0.113	0.119	0.116
BZ						—	0.020	0.013	0.015	0.016	0.059	0.005	0.004	0.065	0.019
COP							—	0.007	0.005	0.007	0.065	0.020	0.019	0.067	0.009
VEN								—	0.001	0.003	0.061	0.013	0.012	0.065	0.005
PES									—	0.001	0.060	0.015	0.013	0.064	0.004
LC										—	0.061	0.016	0.015	0.063	0.005
MN											—	0.061	0.060	0.061	0.064
PD_1												—	0.001	0.063	0.019
PD_2													—	0.064	0.017
SRI														—	0.065
SCB															—

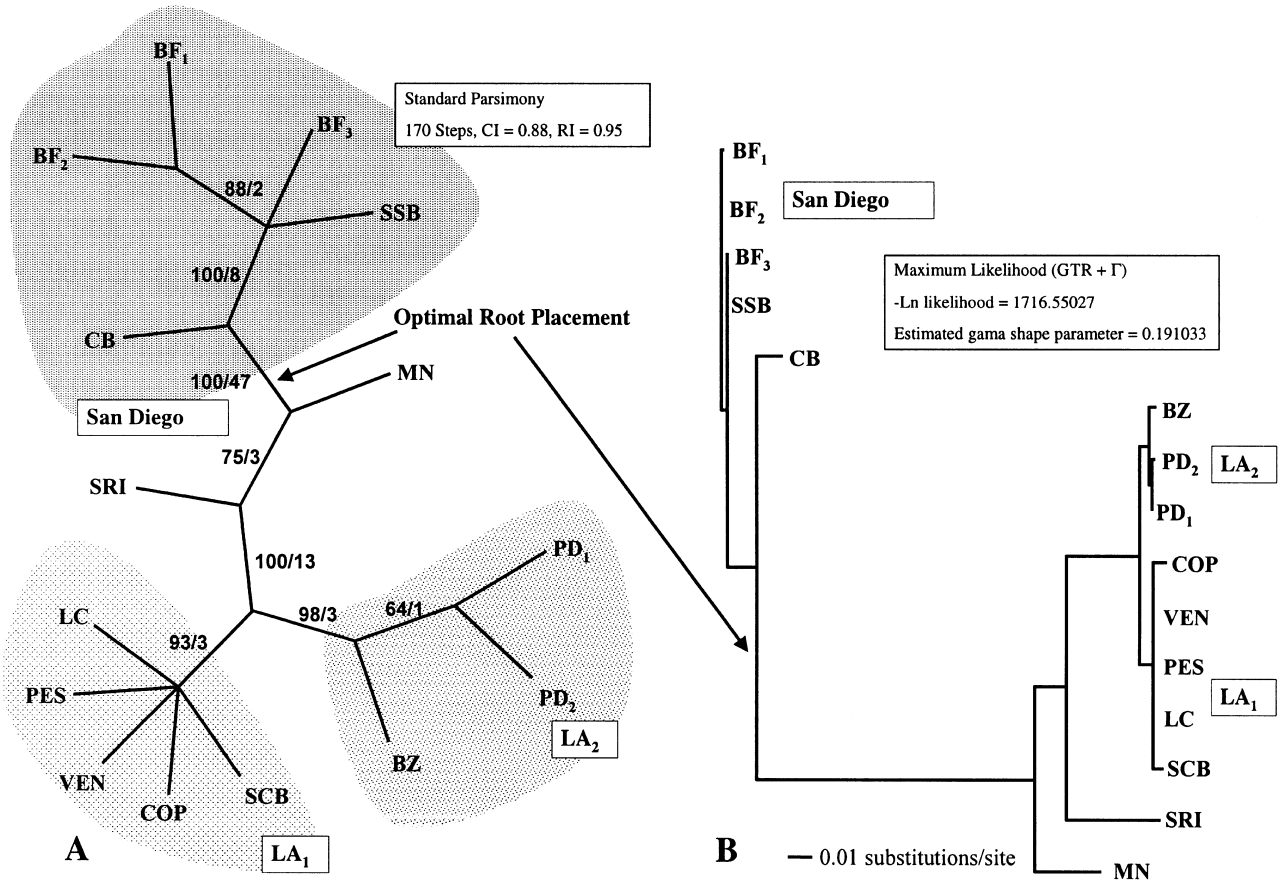

Fig. 3 Haplotype acronyms correspond to Table 1/Fig. 1. LA_1 = northern Los Angeles clade, LA_2 = southern Los Angeles clade. (A) Unrooted strict consensus tree from maximum parsimony analysis. Bootstrap and decay values are indicated at each node (bootstrap/decay). (B) Maximum likelihood phylogeny estimate based on a general time reversible model of nucleotide substitution.

The total data set was then truncated to 709 bp. A branch and bound search in PAUP* under the assumptions of strict parsimony resulted in a single MP tree (303 steps, CI = 0.91, RI = 0.93) that places the root between the San Diego and LA Basin – MN–SRI haplotypes. This root placement suggests that the southernmost haplotypes (i.e. San Diego region) are ancestral within the network (Fig. 3A). Because of high sequence similarity within putative species and the degree of outgroup divergence, intraspecific phylogeny root estimation can be problematic (Crandall & Templeton 1993; Castelloe & Templeton 1994). We, therefore, statistically evaluated alternate root placements between MN and SRI and between SRI and the LA Basin haplotypes. Trees constrained to include the two alternate root placements and the preferred placement were statistically compared using the Templeton Wilcoxon Rank Sum (TWR) test (parsimony only; Templeton 1983; Felsenstein 1985b; Larson 1994, 1998) and the Kishino–Hasegawa (KH) test (parsimony and ML; Kishino & Hasegawa 1989). The optimal root placement indicated by standard parsimony is statistically preferred over the other two suboptimal possibilities in both parsimony and ML analyses ($P < 0.01$ for all analyses).

TCS estimation. The relationships of five haplotypes from the San Diego County area (Fig. 4A) and eight from the LA Basin (Fig. 4B) were resolved using TCS. Network connections where $j < 12$ are justified at $P_j = 0.95$. Connections where $19 > j > 12$ are justified at $P_j + 1 = 0.99$. Although the $P_j + 1$ model allows for a single multiple hit per connection no single variable site was observed to be homoplasious in either network. Haplotypes with the greatest degree of connectivity have the highest probability of being the oldest in the network (Crandall & Templeton 1993). Therefore, for the LA Basin network the PES (El Pescador State Beach) haplotype has the highest probability of being ancestral. Geographically this haplotype is central relative to the others. For the San Diego County area network one of the southernmost Borderfield haplotypes has the highest probability of being ancestral.

Molecular clock hypotheses. To ensure that the rate of molecular evolution has behaved approximately clock-like we implement a likelihood ratio test that compares –Ln values under the assumptions of no molecular clock vs. a molecular clock. The results of this test are marginally not significant [($P > 0.02$); α level of 0.01, Posada & Crandall

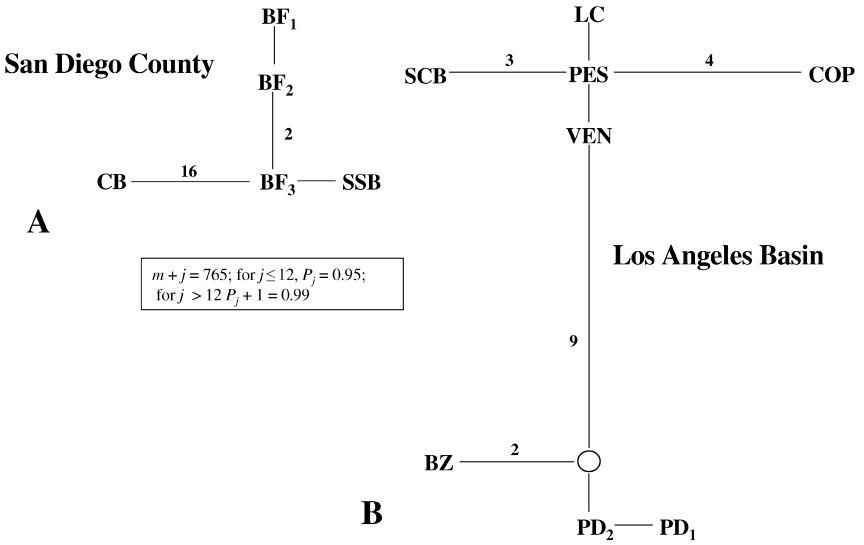

Fig. 4 TCS algorithm estimation of haplotype relationships within the San Diego County and Los Angeles Basin areas. Numbers along branches correspond to branch length.

1998], thus the assumptions of a molecular clock are tentatively appropriate for these data. Because the LA Basin was not above sea level until ~1.5 million years BP (see Yanev 1980) divergence values between the LA Basin populations and their sister lineage on Santa Rosa Island would suggest a rate of nucleotide substitution of approximately 4% per million years. This rate is roughly double that reported by DeSalle *et al*. (1987) for *Drosophila* and used by others (e.g. Hedin 1995, 1997b; Gillespie 1999) as an external calibration rate in spiders. Based on the 4% calibration rate the LA Basin population haplotypes have been separated from the San Diego area haplotypes for 2–3 million years BP and separation between the northern Monterey haplotype and the San Diego area haplotypes is estimated at 2.60–2.75 million years BP. We estimate a comparable separation time of approximately 3 million years BP for SRI and San Diego. Use of the external rate calibration by DeSalle *et al*. (1987) would effectively double all of the above estimated separation times to between 3 and over 6 million years BP. It is important to note that both the biogeographic and external rate calibrations are based on conservative estimates of divergence since proportional differences were not corrected for homoplasy.

Discussion

Population subdivision and divergence

The mtDNA 16S rRNA data show that coastal *Aptostichus simus* populations are geographically subdivided and divergent (i.e. between the four colineal haplotype arrays). The pairwise level of sequence divergence between the four colineal haplotype arrays (Fig. 3) reported ranges from a minimum of ~6% (SRI–MN–LA Basin) to a maximum of over 12% for differences between San Diego County area haplotypes, and all other haplotypes in the network. These values are very high for intraspecific data across such a small geographical area and exceed most of what has been reported in the literature for population level studies (e.g. Vogler *et al*. 1993; Fig. 1). The extremely high level of divergence within the *A. simus* population complex is indicative not only of strong geographical subdivision, but also of long temporal discontinuity. These estimated separation times of 1.5–3.15 million years BP (biogeographic calibration) and 3–6.3 million years BP (external rate calibrations) between *A. simus* populations are consistent with major changes in California coastal topography during the mid Pliocene (Yanev 1980). They are also consistent with California geology because they do not place the San Diego populations as any older than a maximum of 6 million years. This distinction is important because San Diego population localities were not above sea level until *c*. 5 million years BP. These biogeographic and temporal patterns are also corroborated by their similarity to those reported in other Californian groups. For example, the California Newt, *Taricha torosa* is distributed along the coast and inland (Tan & Wake 1995). San Diego *T. torosa* populations are basal, ~5 million years old, and subsequently extended their distribution northward in a pattern similar to *A. simus* with disjunct populations in Orange, Los Angeles, and Monterey Counties.

Microallopatry and dispersal

To this point we have limited the discussion to large scale variation patterns between the major colineal haplotype arrays of San Diego, Santa Rosa Island, Monterey County, and the LA Basin. However, the LA Basin intrahaplotype network (Fig. 4B) is important to consider because therein lies considerable insight into dispersal patterns within the group, an issue integral to the question of genealogical exclusivity across the entire system. Previous population

level study has been done on *A. simus* in the coastal dune habitat of California. Ramirez & Froehlig (1997) examined gene flow and population subdivision in nine *A. simus* populations using allozymes, seven of which are identical to those LA Basin populations included in this study. These populations are distributed in the Los Angeles basin area, extending from Point Dume northward into southern Ventura County (Fig. 1B). They found these populations to be mostly fixed for the 13 loci studied (11 monomorphic, six of the populations fixed for all loci) with an average heterozygosity of 0.006 and concluded that the populations were genetically homogenous and in Hardy–Weinberg equilibrium. Ramirez and Froehlig suggested that the lack of significant interpopulation differentiation could potentially be attributed to one of three factors. The first possibility is that the nine populations comprise a single metapopulation in which significant gene flow has effectively homogenized the individual populations. Because N_m (effective migration) values are low for the two polymorphic loci, they suggested as a second alternative, repeated bottlenecks, or population extinctions, followed by subsequent recolonization by adjacent populations, in which both factors could have minimized population level differences by random lineage sorting. As a third possibility, Ramirez & Froehlig (1997) speculate that selection across a homogenous coastal dune environment could account for the minimal allelic differences (i.e. the enzymatic systems used in the study may not be effectively neutral).

The results presented in this paper are in contrast to those of the allozyme study of Ramirez & Froehlig (1997). Because we use a maternally inherited, haploid marker we would expect the effective population size, N_e, to be approximately one-fourth that of a nuclear marker, rapidly increasing times to coalescence and fixation of unique haplotypes within populations (Neigel & Avise 1986). We therefore, attribute some of the differences between the nuclear and mtDNA data set to differences in N_e. However, the present data make it unlikely that the LA Basin populations constitute a metapopulation. We might expect a mtDNA marker to convey a sex-biased mode of dispersal. This is certainly a possibility in spider groups where long-lived females construct burrows and leave these only when disturbed by some environmental perturbation, whereas sexually mature males wander in search of females. Male dispersal in excess of 2 km over discontinuous habitat is unlikely and such discontinuities in suitable habitat separate the major lineages of *A. simus*. Janowski-Bell (1995) reported dispersal distances of slightly over 1 km for much larger spiders, tarantulas (i.e. spider large enough to fit with radio telemetry packs), within continuous habitat. This is not to say that we have universally excluded male dispersal as a confounding factor. To the contrary, we would argue that male sex-biased dispersal is probably a factor for very close geographical populations like Leo Carillo, El Pescador, and County Line. Although our sampling of five individuals per locality may be marginally sufficient to detect moderate levels of gene flow ($N_m > 1$), it is unlikely that we are able to detect much lower levels (Slatkin 1989; Hedin 1997a).

The fact that Broad Beach, and the more northern Staircase and County Line localities, share haplotypes (VEN), probably signifies retention of ancestral polymorphism within the Broad Beach locality rather than long distance dispersal. The VEN haplotype is most closely related (separated by 1 bp difference) to the PES haplotype (Fig. 4B), which is probably the oldest haplotype in the network, because of it high degree of connectivity to other haplotypes in the network (see Results). Based on our observations of the LA Basin localities in 1998, the Broad Beach–Zuma Beach dune system is the largest and most intact of all the localities and probably harbours the largest *A. simus* populations. Ramirez & Froehlig (1997) also found the Broad Beach locality to be one of only three whose population was heterozygous for one of the allozyme loci examined.

The pattern of mtDNA haplotypes may be, in a restricted sense, due to Ramirez & Froehlig's (1997) second causal factor, repeated extinction and bottlenecks. An apparent lack of correlation between spatial and genetic distance suggests a pattern of habitat fragmentation, or vicariance, resulting in population extinctions followed by exceptionally long temporal separation. For example, the molecular clock calibrations discussed earlier would place the time of separation of the PES and BZ haplotypes at 375 000–750 000 years BP. The extremely dynamic and changing environment along the coast is perhaps responsible for randomly fixing unique haplotypes in small populations and increasing the rate of time to reciprocal monophyly (Tajima 1983; Neigel & Avise 1986; Harrison 1998). In the absence of male-based gene flow, these populations are free to develop along their own unique evolutionary trajectories.

Although the absence of male dispersal in this study may be equivocal over short distances, particularly within the LA Basin, female dispersal is not. As mentioned earlier in the introduction, one common means of long distance dispersal in more advanced, araneomorph, spiders is by ballooning (Coyle 1983). However, these data indirectly suggest that ballooning is not occurring in *A. simus* over long or short distances. Ballooning in mygalomorphs has only been observed in small spiderlings (Coyle 1983; Coyle *et al*. (1985), probably before sex differentiation has occurred. Therefore, both male and female dispersal by ballooning would be detected by the maternally inherited marker used in this study.

Gene exchange and ecology: necessary species' criteria?

The cluster analysis (Fig. 2) based on morphological (and potentially ecological) distinguishing characteristics, demonstrates that the individuals used in this component of the

analysis are indistinguishable as populations or regional clades that correspond to the molecular analysis. Without question this claim would be stronger if males could have been included in the morphometric analysis. Although sufficient museum material was unavailable, we have examined some male specimens from the San Diego, LA Basin and SRI localities and have found them to also be indistinguishable from each other (J. Bond, personal observation, Bond 1999).

Extreme genetic divergence in the absence of comparable morphological divergence is indicative of a cryptic species complex. Hedin (1997b) reports species crypsis in the Southern Appalachian *Nesticus* cave spiders, similar to that in *A. simus*. He points out that the presence of populations that are diagnosable and genetically divergent, yet morphologically cohesive with respect to secondary sexual characteristics, are contrary to Eberhard's hypothesis (Eberhard 1985, 1996) that animal genitalia evolve rapidly and divergently as a result of sexual selection by female choice. That is, at least for some spider groups, divergence in genital morphology may be decoupled from population divergence. The discovery of such a pattern in such disparate groups of spiders (i.e. mygalomorphs vs. Orbiculariae, see Coddington & Levi 1991) may suggest that this phenomena is more widespread than previously thought. It also strongly suggests that a traditional, morphological species concept that hinges on spider genitalic morphology may grossly underestimate the true evolutionary diversity within Araneae.

Like genitalic features, ecology is traditionally thought to be an inherent aspect of speciation (e.g. Mayr 1963). Within *A. simus* it appears that reciprocal monophyly and genetic divergence has occurred in the absence of obvious changes in ecology. We have personally collected *A. simus* in the four major population areas and have observed no interpopulational natural history differences (e.g. burrow construction, burrow placement, substrate composition). Although we think that the arguments for ecological stasis across the *A. simus* range are tenable, they could be viewed as anecdotal. Within the genus *Aptostichus* a number of unrelated species exhibit similar morphologies in similar environments (e.g. desert environments; Bond 1999). Under the assumption that ecology and morphological change are coupled we would expect populations under different selective regimes to exhibit divergent morphologies. As mentioned earlier there is no such morphological divergence in *A. simus* that could be attributed to localized differences in habitat (Fig. 2).

The lack of concomitant change in genes and ecological parameters is not uncommon and has been reported in many recent molecular studies of other taxa (e.g. the echinoderm *Ophiothrix*, Baric & Sturmbauer 1999; freshwater fishes, Schluter 1998). Most compelling, however, are examples that parallel *A. simus* in which extreme divergence at the genetic level has occurred under *identical* constraints of ecological stasis. Ramirez & Beckwitt (1995) studied gene flow and phylogeography of the Californian spider genus *Lutica* along the southern coast. *Lutica*, like *Aptostichus*, is a fossorial, psammophilic spider that builds silk lined burrows in sand dunes and is often found syntopic with *A. simus* across much of its distribution (Ramirez 1995). However, like *Nesticus*, discussed earlier, *Lutica* is a distantly related araneomorph species and thus represents a very disparate taxonomic comparison. Using allozyme data, Ramirez & Beckwitt (1995) found an identical pattern of genealogical exclusivity for southern and northern populations. Although these populations lack distinguishing morphological or ecological features Ramirez & Beckwitt (1995) advocated their 'elevation' to species status.

Conclusion

It is clear from the data reported here and elsewhere (e.g. Ramirez & Beckwitt 1995; Hedin 1997b; Wilcox *et al*. 1997; Baric & Sturmbauer 1999) that morphological and ecological divergence is, and can be, decoupled from genetic divergence. The overriding factor in speciation in some groups may depend upon the constraints of gene flow rather than ecological specialization. In a recent study Peterson *et al*. (1999) demonstrated the 'conservative evolution in ecological niches of 37 sister taxon pairs of birds, mammals, and butterflies isolated on either side of the lowland barrier Isthmus of Tehuantepec' (p. 1266). They used a genetic algorithm to predict the geographical distribution of taxa by 'mirroring' the ecological parameters of its sister species. In all 37 cases the ecological niche of one species predicted the niche and distribution of its sister. On the basis of these results they concluded that speciation is predominantly a vicariance event with ecological differences developing much later. For many groups prespeciational ecological divergence may be simply unimportant.

So, is speciation a foregone conclusion in *Aptostichus simus*? First, as a caveat to this discussion we should acknowledge that the relationships of the *A. simus* populations presented here are based on a mtDNA gene tree that may not necessarily reflect the true species phylogeny. However, the maternal transmission and haploid nature of the mitochondrial genome increases the likelihood of recovering more recent population level history and is more likely to convey a gene tree that is congruent with the species tree (Moore 1997, 1995). On the basis of a time-limited genealogical approach (basal coalescence indicated by the rooted network) to species (not magnitude of divergence) we would designate (conceptually), at the very least, a second new northern coastal (i.e. all populations comprising the LA Basin, SRI and MN haplotypes) species of *Aptostichus*. However, if we retain the genealogical species perspective (*sensu* Baum & Shaw 1995) but allow a time-extended perspective instead, one of two scenarios are possible. Ecological and/or morphological

divergence could happen over time resulting in differences in demographic interchangeability or genetic exchangeability, or secondary contact between populations could occur and genealogical exclusivity is reversed.

From a traditional taxonomist's point of view retaining a time-extended perspective and delineating species solely on the basis of morphology is the most conservative approach. Without the aid of expensive molecular techniques, species identification and classification becomes extremely problematic. However, this approach effectively overlooks potential diversity. The more traditional approach also sets a lower standard for species 'testability'. Baum & Donoghue (1995) demonstrate that character-based approaches to species delineation run the risk of making a time-limited type I error (i.e. designating as a population what in reality is a species). Conversely, time-limited genealogical exclusivity is a falsifiable hypothesis that can be retested at any time.

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References

Baric S, Sturmbauer C (1999) Ecological parallelism and cryptic species in the genus *Ophiothrix* derived from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **11**, 157–162.

Baum DA, Donoghue MJ (1995) Choosing among alternative 'Phylogenetic' species concepts. *Systematic Botany*, **20**, 560–573.

Baum DA, Shaw KL (1995) Genealogical perspectives on the species problem. In: *Experimental and Molecular Approaches to Plant Biosystematics* (eds Hoch PC, Stephenson AG), pp. 289–303. Missouri Botanical Garden, St. Louis.

Bond JE (1999) *Systematics and Evolution of the Trapdoor Spider Genus* Aptostichus *(Araneae: Mygalomorphae: Euctenizidae)*. PhD Thesis. Virginia Polytechnic Institute and State University, Virginia.

Bremer K (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, **42**, 795–803.

Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. *Molecular Phylogenetics and Evolution*, **3**, 102–113.

Chamberlin RV (1917) New spiders of the family Avicularіidae. *Bulletin of the Museum of Comparative Zoology*, **61**, 25–39.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Coddington JA, Levi HW (1991) Systematics and evolution of spiders (Araneae). *Annual Review of Ecology and Systematics*, **22**, 565–592.

Coyle FA (1971) Systematics and natural history of the mygalomorph spider genus *Antrodiaetus* and related genera (Araneae: Antrodiaetidae). *Bulletin of the Museum of Comparative Zoology*, **141**, 269–402.

Coyle FA (1983) Aerial dispersal by mygalomorph spiderlings (Araneae, Mygalomorphae). *Journal of Arachnology*, **11**, 283–286.

Coyle FA (1995) A revision of the funnel web mygalomorph spider subfamily Ischnothelinae (Araneae, Dipluridae). *Bulletin of the American Museum of Natural History*, **226**, 1–133.

Coyle FA, Greenstone MH, Hultsch A, Morgan CE (1985) Ballooning mygalomorphs: estimates of the masses of Sphodros and Ummidia ballooners (Araneae: Atypidae, Ctenizidae). *Journal of Arachnology*, **13**, 291–296.

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134**, 959–969.

Crandall KA, Templeton AR (1996) Applications of intraspecific phylogenetics. In: *New Uses for New Phylogenies* (eds Harvey PH, Brown AJL, Smith JM, Nee S), pp. 81–99. Oxford University Press, New York.

Crandall KA, Templeton AR, Sing CF (1994) Intraspecific phylogenetics: problems and solutions. In: *Models in Phylogeny Reconstruction* (eds Scotland RW, Siebert DJ, Williams DM), pp. 273–298. Clarendon Press, Oxford.

DeSalle R, Freedman T, Prager EM, Wilson AC (1987) Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular Evolution*, **26**, 157–164.

Donoghue MJ, Olmstead RG, Smith JF, Palmer JD (1992) Phylogenetic relationships of Dipscales based on *rbc*L sequences. *Annals of the Missouri Botanical Garden*, **79**, 333–345.

Eberhard WG (1985) *Sexual Selection and Animal Genitalia*. Harvard University Press, Cambridge.

Eberhard WG (1996) *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton.

Eriksson T, Wikstrom N (1996) *Autodecay*. Stockholm University Press, Stockholm.

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

Felsenstein J (1985b) Confidence limits on phylogenies with a molecular clock. *Systematic Zoology*, **34**, 152–161.

Gillespie RG (1999) Comparison of rates of speciation in web-building and non-web-building groups within a Hawaiian spider radiation. *Journal of Arachnology*, **27**, 79–85.

Goloboff PA (1995) A revision of the South American spiders of the family Nemesiidae (Araneae, Mygalomorphae) Part I: Species from Peru, Chile, Argentina, and Uruguay. *Bulletin of the American Museum of Natural History*, **224**, 1–189.

Greenstone MH, Morgan CE, Hultsch AL, Farrow RA, Dowse JE (1987) Ballooning spiders in Missouri, USA and New South Wales, Australia: family landmass distributions. *Journal of Arachnology*, **15**, 163–170.

Griswold CE (1987) The African members of the trap-door spider family Migidae (Araneae: Mygalomorphae) 2: the genus *Poecilomigas* Simon, 1903. *Annals of the Natal Museum*, **28**, 475–497.

Harrison RG (1998) Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. In: *Endless Forms: Species and Speciation* (eds Howard DJ, Berlocher SH), pp. 19–31. Oxford University Press, New York.

Hedin MC (1995) *Speciation and morphological evolution in cave spiders (Araneae: Nesticidae: Nesticus) of the Southern Appalachians*. PhD Thesis. Washington University, St. Louis.

Hedin MC (1997a) Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: Nesticus). *Molecular Biology and Evolution*, **14**, 309–324.

Hedin MC (1997b) Speciational history in a diverse clade of habitat — specialized spiders (Araneae: Nesticidae: Nesticus): Inferences from geographic — based sampling. *Evolution*, **51**, 1929–1945.

Higgins DG, Bleasby AJ, Fuchs R (1996) Clustal V: Improved software for multiple sequence alignment. *Computer Applied Bioscience*, **8**, 189–191.

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1997) Nucleic Acids IV: Sequencing and cloning. In: *Molecular Systematics* (eds Hillis DM, Moritz C, Mable BK), pp. 321–385. Sinauer Associates Inc, Sunderland.

Huelsenbeck JP, Rannala B (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, **276**, 227–232.

Janowski-Bell ME (1995) Analysis of the movements of the Texas Brown Tarantula, *Aphonopelma hentzi* (Girard) (Araneae: Theraphosidae) using radio telemetry. *American Arachnology*, **52**, 2.

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution*, **29**, 170–179.

Kumar S, Tamura K, Nei M (1993) *MEGA: Molecular Evolutionary Genetics Analysis*. Pennsylvania State University Press, University Park.

Larson A (1994) The comparison of morphological and molecular data in phylogenetic systematics. In: *Molecular Ecology and Evolution: Approaches and Applications* (eds Schierwater B, Streit B, Wagner GP, DeSalle R), pp. 371–390. Birkhauser, Basel.

Larson A (1998) The comparison of morphological and molecular data in phylogenetic systematics. In: *Molecular Approaches to Ecology and Evolution* (eds DeSalle R, Schierwater B), pp. 275–296. Birkhauser, Basel.

Maddison WP, Maddison DR (1992) *Macclade*. Sinauer Associates Inc, Sunderland.

Maddison DR, Swofford DL, Maddison WP (1997) NEXUS: An extensible file format for systematic information. *Systematic Biology*, **46**, 590–621.

Maine BY (1982) Adaptations to arid habitats by mygalomorph spiders. In: *Evolution of the Flora and Fauna of Arid Australia* (eds Barker WR, Greensdale PJM), pp. 273–284. SA Peacock Publishing Co., Frewville.

Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge.

Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718–726.

Moore WS (1997) Mitochondrial-gene trees versus nuclear-gene trees, a reply to Hoelzer. *Evolution*, **51**, 627–629.

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory* (eds Karlin S, Nevo E), pp. 515–534. Academic Press, New York.

Peterson AT, Soberón J, Sánchez-Cordero V (1999) Conservation of ecological niches in evolutionary time. *Science*, **285**, 1265–1267.

Posada D, Crandall KA (1998) Modeltest: Testing the model of DNA substitution. *Bioinformatics.*, **14**, 817–818.

Posada D, Crandall KA (2000) *ParsProb version 1.1*. Provo, Utah.

Ramirez MG (1995) Natural history of the spider genus *Lutica* (Araneae, Zodariidae). *Journal of Arachnology*, **23**, 111–117.

Ramirez MG, Beckwitt RD (1995) Phylogeny and historical biogeography of the spider genus *Lutica* (Araneae, Zodariidae). *Journal of Arachnology*, **23**, 177–193.

Ramirez MG, Froehlig JL (1997) Minimal genetic variation in a coastal dune arthropod: the trapdoor spider *Aptostichus simus* (Cyrtuacheniidae). *Conservation Biology*, **11**, 256–259.

Rodríguez FJ, Oliver JL, Marin A, Medina JR (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, **142**, 485–501.

Schluter D (1998) Ecological causes of speciation. In: *Endless Forms: Species and Speciation* (eds Howard DJ, Berlocher SH), pp. 114–129. Oxford University Press, New York.

Slatkin M (1989) Detecting small amounts of gene flow from the phylogeny of alleles. *Genetics*, **121**, 609–612.

Swofford DL (1999) *PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0b4a*. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Tan A, Wake DB (1995) MtDNA phylogeography of the Californian Newt, *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution*, **4**, 383–394.

Templeton AR (1983) Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *International Journal of Organic Evolution*, **37**, 221–244.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. III. Cladogram estimation. *Genetics*, **132**, 619–633.

Vogler AP, DeSalle R, Assmann T, Knisley CB, Schultz TD (1993) Molecular population genetics of the endangered tiger beetle *Cicindela dorsalis* (Coleoptera: Cicindelidae). *Annals of the Entomological Society of America*, **86**, 142–152.

Wilcox TP, Hugg L, Zeh JA, Zeh DW (1997) Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of neotropical pseudoscorpions. *Molecular Phylogenetics and Evolution*, **7**, 208–216.

Yanev KP (1980) Biogeography and distribution of three parapatric salamander species in coastal and borderland California. In: *The California Islands: Proceedings of a Multidisciplinary Symposium* (ed. Power DM), pp. 531–550. Santa Barbara Museum of Natural History Press, Santa Barbara.

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