Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 36 (2005) 42–57
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Speciation history of the North American funnel web spiders, *Agelenopsis* (Araneae: Agelenidae): Phylogenetic inferences at the population–species interface

Nadia A. Ayoub [a,*], Susan E. Riechert [a], Randall L. Small [b]

[a] *Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, TN 37996, USA*
[b] *Department of Botany, 437 Hesler Biology Building, University of Tennessee, Knoxville, TN 37996, USA*

Received 24 July 2004; revised 11 March 2005
Available online 22 April 2005

Abstract

Intra- and interspecific relationships of 12 out of 13 described species as well as a potential new species in the spider genus *Agelenopsis* (Araneae: Agelenidae) were analyzed using sequence data from two mitochondrial genes, cytochrome oxidase I (COI) and 16S ribosomal RNA. Approximately half of the species examined formed well-supported monophyletic groups, whereas the rest of the species were part of well-supported monophyletic species groups. Rather than viewing cases where species were not identified as being monophyletic as poor taxonomy, these cases more likely represent recent speciation and offer insights into the process of speciation. The clade with the lowest levels of interspecific sequence divergence was found in eastern North America, whereas western species displayed much higher levels of interspecific divergence. These patterns appear to extend below the species level as well, with southwestern species exhibiting the highest levels of intraspecific sequence divergence and geographic structuring. The relationship between *Agelenopsis* and *Barronopsis*, a genus once considered a sub-genus of *Agelenopsis*, was also examined. The two genera are reciprocally monophyletic but more generic level sampling is needed to confirm an apparent sister relationship between the two.

Keywords: Mitochondrial DNA; *Barronopsis*; Bayesian inference; Data partitions; Cytochrome oxidase I; 16S ribosomal RNA; Agelenidae

1. Introduction

One of the major objectives of evolutionary biology is to understand the process of speciation. Interspecific phylogenies offer a record of speciation events and can be used in combination with geographical or ecological data to indirectly explore the causes of speciation within a particular group (Barraclough and Nee, 2001). When intraspecific data are also available, we have the added insight of being able to explore speciation across a continuum of population divergence to species divergence (Avise, 1994, 2000; Templeton, 2001). Application of population sampling to interspecific phylogenetic studies is essential for addressing a number of questions about speciation. One of the most basic of such questions is whether or not taxonomic species actually represent evolutionary units (i.e., monophyletic groups; Morando et al., 2003). Population based sampling is also important in identifying geographic zones of hybridization between closely related species and in determining the geographic context of speciation. For instance, did speciation occur through the split of one ancestral species into two allopatric units or did a daughter species arise in a small subset of the geographic range of a parent species. Applying population sampling to an entire group of

* Corresponding author. Present address: Department of Biology, University of California, Riverside, CA 92521, USA. Fax: +1 951 827 4286.
E-mail address: nadiaa@ucr.edu (N.A. Ayoub).

1055-7903/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.03.017

species, such as a genus, also allows us to determine if patterns seen at the intraspecific level translate into patterns at the interspecific level, such as whether levels of population divergence correspond to speciation rates. The use of molecular data has become increasingly important in exploring this "population–species" interface (Templeton, 2001) because other character systems (i.e., morphological) often lack intraspecific variability (Avise, 1994; Avise et al., 1987).

Many groups of spiders display negligible intraspecific morphological variation. Even spider genera typically contain little morphological variation and species are described on the basis of genitalic characters of male palpal organs involved in sperm transfer and female epigyna (Eberhard, 1983). Variable morphological characters, though sufficient to describe species, are often too few in number to create phylogenies. The advent of molecular methods has thus become increasingly useful in phylogenetic and phylogeographic studies of spiders (e.g., Bond et al., 2001; Croucher et al., 2004; Garb et al., 2004; Gillespie, 2004; Hedin, 1997, 2001; Hedin and Maddison, 2001a,b; Hedin and Wood, 2002; Huber et al., 1993; Johannesen et al., 2002; Masta, 2000a; Piel and Nutt, 1997, 2000; Smith and Bond, 2003; Tan et al., 1999; Zehothofer and Sturmbauer, 1998). However, only a few studies have attempted population sampling for most members of a genus (e.g., Hedin, 1997, 2001), making additional phylogenetic studies at the population–species interface necessary to identify general patterns of speciation in spiders.

An ideal group of spiders in which to examine patterns of speciation is a genus of funnel web spiders, *Agelenopsis* Geibel (Araneae: Agelenidae). *Agelenopsis* is widely distributed in North America and generally abundant where found (Chamberlin and Ivie, 1941; personal observations by NAA and SER). The dispersal ability of these spiders is thought to be restricted because they are not known to disperse aerially through ballooning. Agelenids have not been collected during the course of ballooning studies of spiders (e.g., Bishop, 1990; Weyman et al., 1995). We, further, have personally never observed *Agelenopsis* species to disperse via ballooning. Despite this likely restricted mode of dispersal, most species are widely distributed (Paison, 1997; Platnick, 2005). This combination of large ranges and restricted dispersal may lead to high levels of geographic differentiation within species, since organisms with limited dispersal tend to retain historical patterns of divergence (Avise, 1994). Additionally, because *Agelenopsis* is distributed throughout North America, a phylogenetic analysis of the genus should offer important insights into the biogeography of the continent, such as whether speciation rates relate to specific geographic regions. Only a few morphological characters found in the male and female genitalia distinguish the 13 described species (Chamberlin and Ivie, 1941), precluding phylogenetic analysis based solely on morphology. Thus, molecular analysis is critical to clarifying the species and population relationships within this group.

The only attempt at inferring historical relationships among *Agelenopsis* species was based on a crude branching diagram of a few genitalic characters discussed in an unpublished Master's thesis (Paison, 1997). However, questions have surfaced from morphological analysis of the genus. One interesting hypothesis proposed by Paison (1997) was that one of the described species, *A. aleenae*, is a rare first generation hybrid between two other species, *A. aperta* and *A. spatula*. This hypothesis was based on the observation that the male genitalia of *A. aleenae* are intermediate between the other two species and that the single *A. aleenae* individual recorded at the time was found in a region where the ranges of *A. aperta* and *A. spatula* overlap. Another question concerns the removal of two species from *Agelenopsis* to a new sub-genus, *Barronopsis*, by Chamberlin and Ivie (1941). Lehtinen (1967) later elevated *Barronopsis* to generic level but the monophyly of these two genera has not been assessed. Currently, seven species of *Barronopsis* have been described (Platnick, 2005).

The main objective of this paper is to uncover within and among species relationships of *Agelenopsis* to explore patterns of speciation. We use sequence data from the mitochondrial genome to assess monophyly of described species with special attention to the status of *A. aleenae*, the putative hybrid species. Because mitochondrial DNA is typically maternally inherited in animals, *A. aleenae* should exhibit identical mitochondrial sequence to the maternal species, either *A. aperta* or *A. spatula*, if it is a first generation hybrid. In addition to assessing monophyly of described species, we examine geographic patterns of variation at both the inter- and intraspecific levels. Finally, we assess whether *Barronopsis* is monophyletic, and if so, what its relationship is to *Agelenopsis*.

2. Methods

2.1. Sampling

Taxon sampling for this study included 12 of the 13 described *Agelenopsis* species (Table 1). We also collected two *Agelenopsis* individuals that could not be identified to any previously described species and probably constitute a new species, which we will refer to as "*A. sp. nov.*" throughout this paper. Most specimens were collected live in the field but specimens of *A. potteri* and *A. oregonensis* from Oregon, California, and Canada were sent to us preserved in 95 or 100% ethanol (see Table 2 for list of sampling localities). We included multiple individuals of each species from multiple populations when possible.

Table 1
Haplotypes used in this study, with localities from which they were found

Species	COI haplotypes	GenBank Accession No. Top = COI Bottom = 16S	Locality
A. aleenae Chamberlin and Ivie	aleenae1	AY770786 AY770755	KS1 (3)
	aleenae2	AY770791 AY770752	TX1 (5)
	aleenae3	AY770788	TX1 (1)
	aleenae4	AY770827	TX1 (1)
	aleenae5	AY770825 AY770751	TX1 (3)
	aleenae6	AY770819	TX1 (2)
	aleenae7	AY770820	TX1 (2)
	aleenae8	AY770821	TX1 (2)
	aleenae9	AY770822	TX1 (1)
	aleenae10	AY770823	TX1 (4)
	aleenae11	AY770824	TX1 (1)
	aleenae12	AY770787 AY770756	NM3 (4)
	aleenae13	AY770788 AY770754	NM1 (2)
	aleenae14	AY770789	NM1 (3)
	aleenae15	AY770790	NM2 (1)
	aleenae16	AY770818	NM4 (2)
A. aperta Gertsch	aperta-G	AY770778 AY770758	TX5 (3); NM6 (5); NM7 (3); NM8 (15); NM9 (10); NM10 (6); AZ1 (15); AZ2 (5); UT2 (14); UT3 (6); CA4 (7)
	aperta-WE	AY770780 AY770762	CA3 (6)
	aperta-II	AY770782 AY770761	CO4 (7); CO5 (7); CO6 (8)
	aperta-S	AY770781 AY770760	CO6 (2)
	aperta-U	AY770783 AY770759	TX6 (9)
	aperta-WP	AY770779 AY770763	CA2 (10)
	aperta-T	AY770785	OK (8)
	aperta-R	AY770784	TX1 (25); TX6 (12)
A. emertoni Chamberlin and Ivie	emertoni1	AY770805 AY770767	OK (1); TX2 (1)
	emertoni2	AY770806 AY770765	TN2 (1)
A. kastoni Chamberlin and Ivie	kastoni1	AY770800	TN2 (1)
	kastoni2	AY770807 AY770766	TN2 (1)
A. longistylus Banks	longistylus1	AY770814 AY770770	NM2 (4)
	longistylus2	AY770828	NM5 (1)
A. naevia Walckenaer	naevia1	AY770801	TN2 (2)
	naevia2	AY770809 AY770769	TX2 (2)
A. oklahoma Gertsch	oklahoma1	AY770803 AY770749	OK (1); KS1 (1); TX3 (1)
	oklahoma2	AY770804 AY770750	CO1 (1)
	oklahoma3	AY770808	CO3 (4)
A. oregonensis Chamberlin and Ivie	oregonensis1	AY770792 AY770774	CA1 (2)

Table 1 (*continued*)

Species	COI haplotypes	GenBank Accession No. Top = COI Bottom = 16S	Locality
A. pensylvanica C. Koch	pensylvanica1	AY770799 AY770768	OK (1); TN1 (3); KS2 (1); CO2 (1)
A. potteri Blackwell	potteri1	AY770793 AY770764	BC (1)
	potteri2	AY770794	MB (1)
A. spatula Chamberlin and Ivie	spatula1	AY770802 AY770753	OK (1); TX4 (6)
A. utahana Chamberlin and Ivie	utahana1	AY770811 AY770772	NH2 (1)
	utahana2	AY770812 AY770773	NH3 (1)
	utahana3	AY770813	NH1 (1)
	utahana4	AY770829 AY770775	UT1 (1)
	utahana5	AY770795 AY770771	UT1 (3)
A. sp. nov.[a]	A. sp. nov.	AY770817 AY770757	TX5 (2)
Barronopsis texana Gertsch	B. texana1	AY770796 AY770747	TN1 (2)
	B. texana2	AY770815	FL1 (2)
	B. texana3	AY770816	TX1 (1)
Barronopsis sp.[a]	*Barronopsis* sp.	AY770810 AY770748	FL2 (1)
Hololena sp.[a]	*Hololena*	AY770797 AY770777	CO7 (1)
Novalena sp.[a]	*Novalena*	AY770798 AY770776	CA5 (1)

Refer to Table 2 for locality information. Numbers in parentheses indicate number of individuals from that locality exhibiting the COI haplotype. 16S sequences came from one of these individuals.

[a] Specimens that could not be identified to species.

To evaluate the hypothesis that *A. aleenae* is a first generation hybrid of *A. aperta* and *A. spatula* we collected *A. aperta*, *A. spatula*, and *A. aleenae* throughout their ranges (Fig. 1). Complete collections of *A. aperta* are described in Ayoub and Riechert (2004) and we include a subset of those data here. We collected 15–90 *Agelenopsis* individuals from each of nine sites throughout the known range of *A. spatula* (based on data from Paison, 1997). Although *A. aleenae* had previously been known from only a single collection locality in New Mexico (Chamberlin and Ivie, 1941; Platnick, 2005), four of the sites that we sampled within the range of *A. spatula* yielded *A. aleenae* individuals and only two of the sites yielded *A. spatula* (Table 1). We also found *A. aleenae* at two sites outside of the known range of *A. spatula*—one in central Texas (TX1 on Fig. 2, Table 2) and one in southeastern New Mexico (NM1). *Agelenopsis aleenae* and *A. spatula* were never found in the same sampling site, but *A. aperta* was found in conjunction with *A. aleenae* at site TX1 and with *A. spatula* at site OK (Table 1).

We also sampled two species of *Barronopsis*: *B. texana* and one that we could not identify. When Chamberlin and Ivie (1941) described *Barronopsis* they only placed two species in the subgenus. Subsequently, five additional species of *Barronopsis* have been described. Each species is described on the basis of micromorphological characters of the male genitalia. Roth (1954) revised the subgenus but did not provide descriptions of females and no other work has since described North American *Barronopsis* females. We thus could not identify the female, *Barronopsis* sp. (see Table 1), to species but included it in our analyses to assess monophyly of *Barronopsis*.

We included specimens of two other North American agelenid genera to use as outgroups, *Hololena* Chamberlin and Gertsch and *Novalena* Chamberlin and Ivie. Lehtinen (1967) and Roth and Brame (1972) classified all North American agelenid genera plus Agelena into the subfamily, Ageleninae. This classification suggests a close relationship among North American genera and thus other North American agelenid genera would be appropriate outgroup candidates in phylogenetic analysis of *Agelenopsis* and *Barronopsis*. In addition, molecular data support *Novalena* and *Hololena* being part of a

Table 2
Sampling localities of agelenids used in this study

Site	Town or park	Latitude °N, Longitude °W
NH1	Lincoln, NH	44.05, 71.67
NH2	White Mtns, NH	44.2, 71.3
NH3	Monadnock SP, NH	42.83, 72.06
TN1	Knoxville, TN	35.99, 83.9
TN2	Loudon, TN	35.73, 84.31
KS1	Meade SP, KS	37.17, 100.45
KS2	Ellsworth, KS	38.76, 98.22
OK	Foss Lake State Park, OK	35.5, 99.17
TX1	Hye, TX	30.27, 98.34
TX2	Dallas/Fort Worth, TX	32.77, 96.78
TX3	Lake Arrowhead SP, TX	33.75, 98.37
TX4	Caprock Canyons SP, TX	34.4, 101.1
TX5	Balmorhea,TX	30.97, 103.72
TX6	San Angelo, TX	31.53, 100.54
TX7	Palo Duro Canyon SP, TX	34.96, 101.67
NM1	Hobbs, NM	32.70, 103.13
NM2	Cimarron Canyon, NM	36.51, 104.91
NM3	Clines Corners, NM	35.01, 105.67
NM4	Valmora, NM	35.8, 105.88
NM5	Galisteo, NM	35.4, 105.9
NM6	Albuquerque, NM	35.08, 106.65
NM7	Nogal Mt, NM	33.48, 105.8
NM8	Carrizozo, NM	33.63, 105.87
NM9	Clovis, NM	34.39, 103.28
NM10	Brantley Lake SP, NM	32.57, 103.38
NM11	Deming, NM	32.27, 107.75
CO1	Trinidad, CO	37.17, 104.51
CO2	Barber Ponds SP, CO	40.17, 105.01
CO3	Colorado River State Park, CO	39.05, 108.55
CO4	Colorado City, CO	37.94, 104.85
CO5	Avondale, CO	38.22, 104.39
CO6	Sedalia, CO	39.43, 104.95
CO7	Colorado Springs, CO	38.83, 104.82
AZ1	South Western Research Station, AZ	31.91, 109.37
AZ2	Dead Horse Ranch State Park, AZ	34.6, 112.1
UT1	Wasatch Mountains SP, UT	40.55, 111.49
UT2	Springdale, UT	37.18, 112.99
UT3	St. George	37.10, 113.58
CA1	8.3 mi west of Del Loma, CA	40.78, 123.45
CA2	Near Don Pedro Reservoir, CA	37.82, 120.3
CA3	Newport Beach, CA	33.57, 117.84
CA4	San Diego, CA	32.73, 117.15
CA5	Yosemite National Park, CA	37.85, 119.57
MB	Winnipeg, MB, Canada	49.9, 97.11
BC	Victoria, BC, Canada	48.41, 123.35
FL1	Silver Springs, FL	29.21, 82.05
FL2	Salt Springs, FL	29.35, 81.73

clade that is sister to a clade containing *Agelenopsis* and *Barronopsis* (personal communication with J. Spagna and R. Gillespie).

Spiders collected as juveniles were reared in our laboratory to maturity, at which time species determination was made using genitalic characters. Legs were removed and stored at −80 °C until DNA extraction; the remainder of each specimen was preserved in 75% ethanol, and voucher specimens were deposited in the Arachnid Collection at the California Academy, San Francisco.

2.2. DNA extraction and sequencing

We extracted total genomic DNA from frozen spider legs using a modified CTAB protocol and standard phenol–chloroform extraction (Sambrook et al., 1987; Shahjahan et al., 1995). We sequenced two mitochondrial genes for this study: (1) the protein coding gene, cytochrome oxidase I (COI) and (2) the ribosomal RNA gene, 16S. To assess within species variation, we initially sequenced COI from multiple individuals and multiple populations of each species where possible (see Table 1). We further sequenced 16S from a subset of these individuals to obtain a better understanding of among species relationships.

Sequencing of COI involved PCR-amplifying a 700 base pair portion of COI with the primers LCO1490 (Folmer et al., 1994) and C1-N-2191 (Simon et al., 1994) and then direct sequencing with C1-N-2191. Sequencing of 16S involved amplifying a 500 base pair portion using the primers 16S_A and 16S_B2 (Tan et al., 1999) and then direct sequencing with both primers. Amplifications and sequencing followed the procedures outlined in Ayoub and Riechert (2004). The only modification was a 55 °C annealing temperature for amplifying 16S.

COI sequences contained no internal length variation and were unambiguously aligned by eye. The 16S sequences included length variation among taxa and these sequences were aligned using the program ClustalW (Higgins et al., 1994) with default gap opening and gap extension costs. We then compared this alignment to an hypothetical secondary structure of 16S, which we determined by visually comparing the agelenid sequences to the proposed secondary structures of multiple spider taxa (Hedin and Maddison, 2001b; Masta, 2000b; Smith and Bond, 2003).

2.3. Phylogenetic analyses

2.3.1. Model fit

To determine which model of evolution best fit the sequence data for Bayesian and likelihood analyses described below, we used MODELTEST v3.06 (Posada and Crandall, 1998) to carry out the likelihood ratio test of progressively more complex models of evolution. We evaluated the model of evolution for each gene separately, and for the two genes combined. For use in Bayesian analysis we further evaluated the model of evolution for additional data partitions, each codon position in COI, and paired and unpaired sites in 16S.

2.3.2. All haplotypes (COI)

We initially evaluated within and among species relationships of COI haplotypes by performing maximum parsimony (MP) and maximum likelihood (ML) analyses on all observed COI haplotypes using PAUP* V4.0b10 (Swofford, 2002). Searches were heuristic with

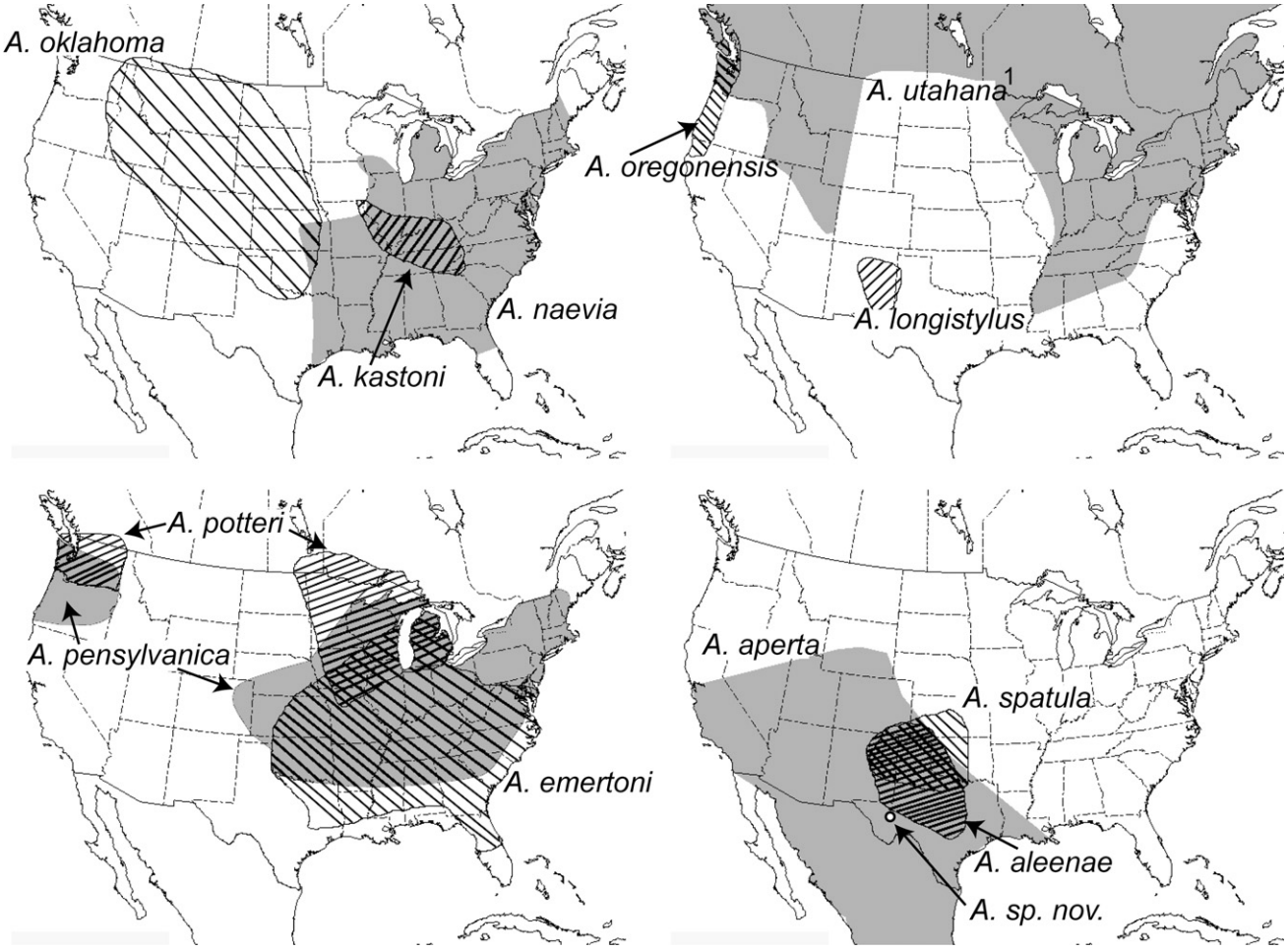

Fig. 1. Ranges of *Agelenopsis* species sampled for this study. Ranges are based on recorded collection localities and our collections. Species do not necessarily use all types of habitat within the defined area. [1]*A. utahana's* range extends throughout Canada and into Alaska.

100 replications of random stepwise addition sequences with tree bisection reconnection (TBR) branch swapping. We used similar search settings for a parsimony bootstrap analysis but with 10 replications of random stepwise addition and 1000 bootstrap pseudo replicates.

Additionally, we tested for monophyly of species by searching for ML trees with each species' haplotypes constrained to be monophyletic. We carried out the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) in PAUP with RELL distribution and 1000 bootstrap replicates to see if the constrained ML tree was significantly less likely than the unconstrained ML tree.

2.3.3. Subset haplotypes (COI and 16S)

To further assess interspecific relationships we evaluated relationships among a subset of individuals from the COI data and the corresponding 16S haplotypes using both MP and ML searches. Gaps were treated as missing data. We used PAUP* to search for MP and ML trees for each gene separately. We conducted heuristic searches as was done for all COI haplotypes and used the same search settings for parsimony bootstrap analyses with 1000 pseudo replications.

2.3.4. Combined data

We evaluated congruence of COI and 16S data partitions in the context of parsimony with the incongruence length difference (ILD) test (Farris et al., 1994) carried out in PAUP* with 100 replicates. Before beginning the permutations of the two data partitions we excluded invariant characters as suggested by Cunningham (1997). The results of the ILD test indicated that the COI and 16S data sets were homogeneous ($P = 0.81$), thus, we combined the two data sets. We searched for MP and ML trees for the combined data using the same settings described above.

2.3.5. Bayesian

The ML searches conducted for the 16S + COI data were based on a model of evolution calculated for the two genes combined. However, such a model of evolution may not adequately describe the differences in evolution between the two genes. Thus, we employed a Bayesian framework to explore the likelihood space of the two genes combined. We used MRBAYES v3.0 (Huelsenbeck and Ronquist, 2001), which is the only currently available phylogenetic software program

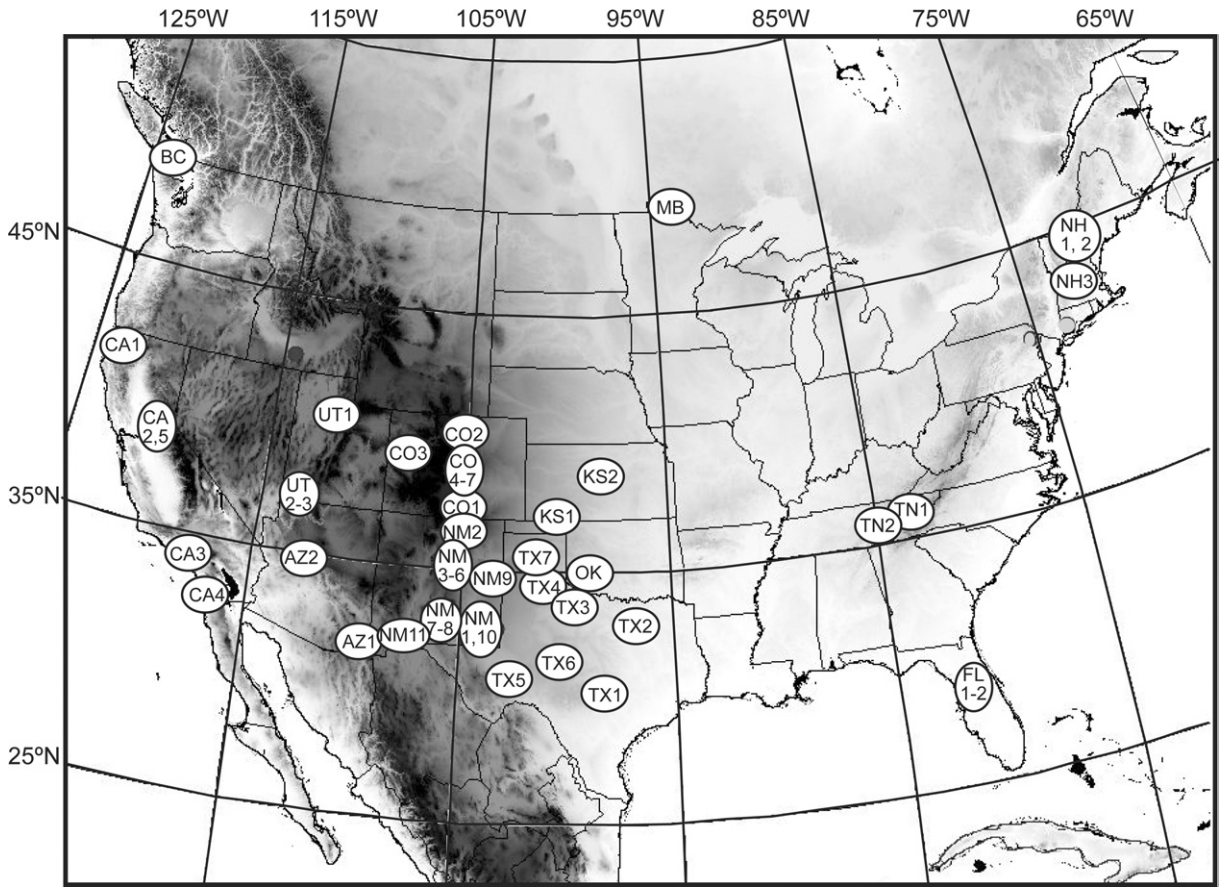

Fig. 2. Collection localities of agelenids sampled for this study. Refer to Table 2 for locality information.

that will allow different likelihood models to be applied to different data partitions within the same analysis.

We searched for trees using three progressively more complex models of evolution. In the first, we did not partition the data and used the model of evolution calculated by MODELTEST for 16S and COI combined (no partition). In the second, we partitioned the data into two sets, COI and 16S (partition by gene). And in the third we partitioned the data into five sets, by codon position (1st, 2nd, and 3rd) for COI and by paired (stems) versus unpaired (loops) characters for 16S (5-way partition). For the latter two analyses we input the model of evolution calculated by MODELTEST for each data partition. We allowed MRBAYES to calculate optimal parameter values (the rate of change between bases, the proportion of invariant sites, and the gamma shape parameter) for each tree generated during the sampling procedure.

For each of the three models we ran multiple independent Bayesian analyses each starting from a different random tree as suggested by Huelsenbeck et al. (2002). To adequately search all of the likelihood space, we employed a Metropolis-coupled, Markov chain, Monte Carlo algorithm with four differently heated chains, using the default settings in MRBAYES. For the no partition and partition by gene analyses we ran three independent searches for 10-million generations (every 500th tree saved). We found that likelihood scores reached a plateau by 1000 generations. However, initial Bayesian analyses run for 1-million generations produced variable posterior probabilities among independent runs for some nodes, indicating that the sampling method had not yet reached stationarity (although topology was the same among all runs). Thus, we discarded the first 10% (1-million generations) of trees from each run as "burn-in" to ensure that we were sampling from a valid posterior distribution.

The Bayesian analysis with five data partitions consisted of three independent searches for 1-million generations (every 100th tree saved). Likelihood scores reached a plateau by 1000 generations, but we discarded the first 10% (100,000 generations) of trees as "burn-in." The posterior probabilities for each of the three runs were similar, indicating that the MCMC procedure had reached stationarity. However, to make sure that a much longer sampling time did not change the topology or posterior probabilities we ran two more independent searches for 10-million generations. These runs were identical in topology and gave similar posterior probabilities to the runs that lasted 1-million generations. For each partition type, we computed the 50% majority rule

consensus tree for each run using PAUP* to give us the posterior probabilities for each node and considered posterior probabilities of 95% or greater to be significantly resolved.

Adding complexity by partitioning the data as described above may increase the realism of the model but may not significantly improve the phylogenetic results over a simpler model. Currently, however, MODELTEST cannot evaluate improvements in a model that increases complexity by partitioning the data. Thus, we used an approach similar to Castoe et al. (2004) to determine if our partitioned models improved the likelihood and posterior probabilities of trees. In addition to comparing post-burn-in likelihood scores, we examined the plots of generation versus likelihood scores to see which model had the fastest rate of convergence. We tested if increased complexity offered significant improvements in posterior probabilities using the Wilcoxon sign-rank test.

3. Results

3.1. Sequence characteristics

3.1.1. COI

We obtained 635 bp of COI sequence data for 177 *Agelenopsis* individuals (including 95 of 474 *A. aperta* individuals from Ayoub and Riechert, 2004), six *Barronopsis* individuals, and one each *Hololena* and *Novalena* individuals. This sequencing resulted in 47 unique COI haplotypes in *Agelenopsis* (including 8 of 51 *A. aperta* haplotypes from Ayoub and Riechert, 2004) and four haplotypes in *Barronopsis*. Among all agelenid haplotypes there were 192 variable characters of which 139 were parsimony informative. Among the *Agelenopsis* haplotypes 153 characters were variable of which 111 were parsimony informative. Third codon positions accounted for most of the variation in COI sequences with 90% of variable sites among *Agelenopsis* sequences located at third positions, 9% at first positions, and 1% at second positions. No indels or premature stop codons were observed.

3.1.2. 16S

We obtained 446 aligned bp (434–438 bp for individual sequences) of 16S sequence data for the 27 *Agelenopsis*, two *Barronopsis*, and one each *Hololena* and *Novalena* individuals sequenced. Gaps required for sequence alignment were never more than two bases long and alignment was unambiguous. Our proposed secondary structure conforms well to other proposed spider 16S secondary structures (Hedin and Maddison, 2001b; Masta, 2000b; Smith and Bond, 2003) and to *Drosophila yakuba* (Gutell and Fox, 1988) secondary structure. Like other spiders, a hyper-variable region is located between positions 216 and 280 of our aligned gene sequences and approximately 40% of the total number of parsimony informative characters is concentrated in this 65 bp region. Among all agelenids sequenced, 86 sites were variable, of which 61 were parsimony informative. Within *Agelenopsis*, only 51 sites were variable and 33 parsimony informative. Splitting 16S into paired (i.e., stems) and unpaired (i.e., loops) sites, unpaired sites accounted for 80% of the variable sites within *Agelenopsis*.

3.2. Phylogenetic analyses

3.2.1. COI

The results of the likelihood ratio test as carried out in MODELTEST chose the best fit model of evolution for COI sequences to be that of Tamura and Nei (1993) including a proportion of invariant sites and a gamma shape correction for among-site rate variation (TrN + I + G; see Table 3 for details of parameters). The strict consensus of 867 MP trees was similar in topology to the ML tree, with the only differences being in relationships among *A. aperta* haplotypes (Fig. 3). Monophyly of *Agelenopsis* and *Barronopsis* received 74 and 100% parsimony bootstrap support, respectively. The following species' haplotypes formed well-supported monophyletic groups: *A. aperta*, *A. oklahoma*, *A. longi-*

Table 3
Estimates of model parameters for each data partition obtained using the likelihood ratio test carried out in MODELTEST (Posada and Crandall, 1998)

	Base frequencies				Rate matrix						I	G
	A	C	G	T	A<>C	A<>G	A<>T	C<>G	C<>T	G<>T		
COI + 16S	0.46	0.19	0.11	0.29	4.39	23.79	5.51	3.57	71.84	1	0.68	2.16
COI	0.25	0.10	0.19	0.46	1	8.56	1	1	19.16	1	0.65	2.49
16S	0.40	0.11	0.12	0.43	21.48	14.75	14.06	0.00	166.52	1	0.55	0.46
3rd position	0.28	0.02	0.14	0.56	1	32.8	1	1	82.61	1	NA	3.61
Unpaired	0.46	0.12	0.06	0.36	1	2	1	1	10.81	1	NA	0.17
					Transition/transversion ratio							
1st position	0.25	0.25	0.25	0.25	3.15							
2nd position	0.13	0.26	0.17	0.44	NA							
Paired	0.31	0.14	0.16	0.39	15.40							

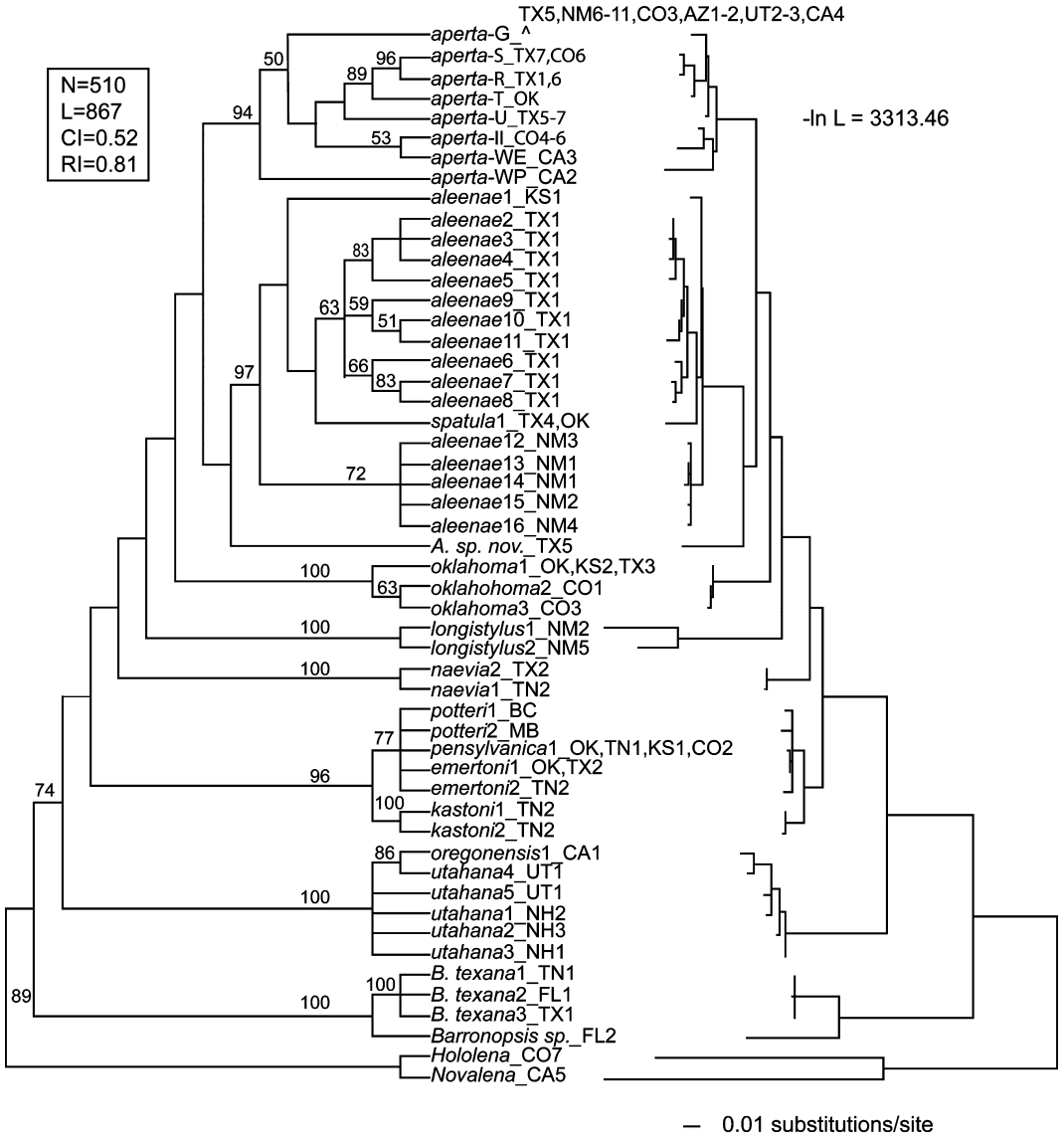

Fig. 3. Strict consensus of MP trees (left) and the ML tree (right) for all COI haplotypes sequenced in this study. Following each haplotype name is a list of collection localities from which that haplotype was found. Refer to Fig. 1 and Table 2 for locality information and Table 1 for numbers of individuals for each collection locality exhibiting a haplotype. *N*, number of MP trees; *L*, length; CI, consistency index; and RI, retention index.

stylus, *A. naevia*, and *A. kastoni*. Each of these species received at least 94% bootstrap support. Other *Agelenopsis* species did not form well-supported monophyletic groups and of these, *A. aleenae*, *A. utahana*, *A emertoni*, and *A. potteri* are paraphyletic with respect to their sister species according to MP and ML trees. However, constraining each species to be monophyletic did not produce a significantly less likely tree ($\delta = 6.25$, $P = 0.31$).

3.2.2. 16S

Based on MP and ML analyses of all COI haplotypes we chose representatives of each species to sequence 16S. The results of the likelihood ratio test of the best fit model of evolution for the 16S sequences was the General Time Reversible model (Rodriquez et al., 1990) including a proportion of invariant sites and a gamma shape correction for among-site rate variation (GTR + I + G; see Table 3 for details of parameters). Relationships among *Agelenopsis* haplotypes according to both MP and ML analyses were not well resolved, reflecting the paucity of variable characters within the genus (trees not shown). Most of the variable characters were concentrated in separating a basal *A. utahana*/*A. oregonensis* species group from the rest of the *Agelenopses* (12 out of 33 parsimony informative characters). There were no conflicts between the ML tree and the strict consensus of 76,189 MP trees except that the ML tree was more resolved.

3.2.3. Combined data

There were only a few differences between the topologies of the strict consensus of MP COI and 16S trees. Specifically, 16S placed *A. longistylus* as the first species

to branch off after the basal *A. utahana*/*A. oregonesis* group followed by *A. oklahoma*. In contrast, the COI tree placed *A. longistylus* sister to an *A. oklahoma*/*A. spatula*/*A. aleenae*/*A. aperta*/*A. sp. nov.* group. However, these differences were not supported by greater than 50% parsimony bootstrap support for either gene. The only other difference was that COI indicated paraphyly of *A. utahana* with respect to *A. oregonensis* and the 16S data indicated monophyly of *A. utahana*.

The combined COI plus 16S parsimony analysis produced 60 MP trees of 627 steps. The strict consensus of these trees had a similar topology to the COI tree alone but with greater bootstrap support for some nodes (tree not shown; bootstrap values in Table 4). Specifically, support for monophyly of *Agelenopsis* plus *Barronopsis* increased from 89 to 100%; monophyly of *Agelenopsis* increased from 74 to 100%; monophyly of all *Agelenopses* excluding *A. utahana* and *A. oregonensis* increased from <50 to 85%; and monophyly of *A. aperta*, *A. spatula*, *A. aleenae* plus *A. sp. nov.* increased from <50 to 55%.

The major differences between the MP trees from the combined analysis versus the COI data alone were in the placement of *A. longistylus* and *A. naevia* but these placements were not well-supported by either analysis. Thus, neither gene provided a clear assignment of these two species. Additionally, the COI data indicated paraphyly of *A. utahana* while the combined data indicated monophyly of *A. utahana*.

The ML tree of the combined data (Fig. 4) was similar to the strict consensus of MP trees. The main difference was that the ML tree placed *A. naevia* as basal to all *Agelenopses* except *A. uahana* and *A. oregonensis* while the MP trees placed *A. naevia* sister to the group composed of *A. kastoni*, *A. potteri*, *A. pensylvanica*, and *A. emertoni*.

3.2.4. Bayesian

The Bayesian trees resulting from the three types of data partitions differed slightly in topology, and posterior probabilities tended to increase with increasing complexity of the model (Table 4). The increased complexity offered nearly significant improvements in posterior probability (no partition—partition by gene: $z = 1.6$, $P = 0.054$; partition by gene—5-way partition: $z = 1.46$, $P = 0.072$). However, these differences were mostly for nodes that did not receive 95% or greater posterior probability in any analysis. Only four nodes improved from less than 95% to greater than 95% posterior probability when increasing complexity from the no partition model to one of the partitioned models (bipartition #s 6, 11—greater than 95% in both partition types; and #s 22,

Table 4
Posterior probabilities and parsimony bootstrap support for bipartitions found in ML tree (Fig. 4)

Bipartition	No partition	Partition by gene	5-way partition	Parsimony bootstrap support
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	85
6	88	99	96	60
7	46	59	55	44
8[a]	NA (65)	NA (77)	NA (54)	NA (38)
9	48	NA (44)	67	33
10	99	97	98	92
11	91	95	99	84
12	44	43	60	25
13	70	69	69	38
14	21	NA (44)	NA (67)	38
15	100	100	100	100
16	47	NA (47)	66	55
17	100	100	100	98
18	52	47	72	39
19	97	98	97	82
20	83	87	NA (83)	55
21	NA (46)	NA (75)	72	NA (33)
22	51	NA (75)	96	47
23	98	96	100	98
24	90	70	99	69
25	62	94	76	50
26	100	99	99	90
27	100	100	100	99

If a bipartition from the ML tree was not found in the Bayesian or MP trees then it is marked with NA and the support for the alternate topology is shown in parentheses.

[a] This bipartion found only in ML tree. All other analyses placed *A. naevia* sister to *A. kastoni–A. emertoni*.

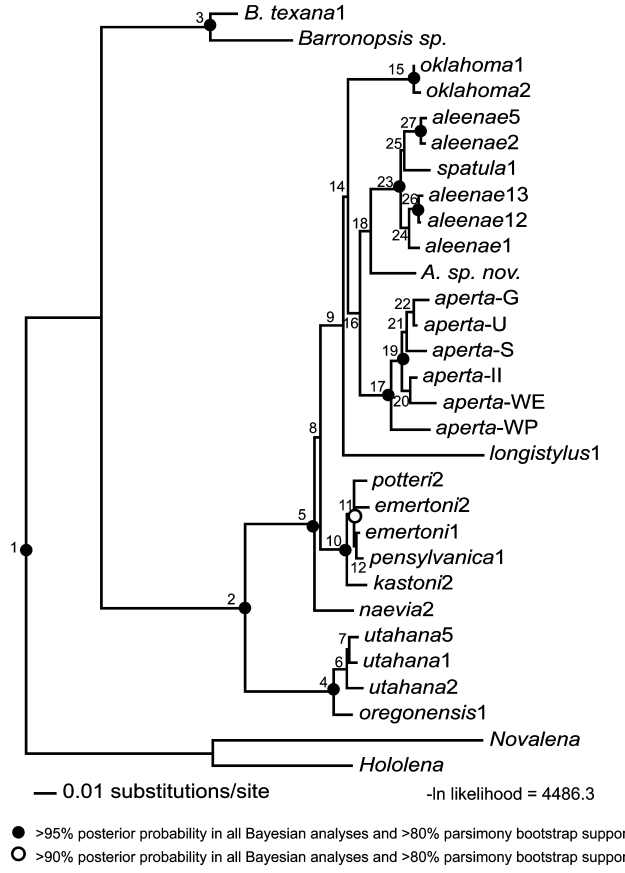

Fig. 4. ML tree of 16S and COI data combined. Refer to Table 4 for Bayesian posterior probabilities and parsimony bootstrap support values for each of the bipartitions numbered in this figure.

24—greater than 95% in 5-way partition; see Fig. 4, Table 4). Additionally, in only one case was a difference in topology among Bayesian trees supported by greater than 95% posterior probability. This was for the sister relationship between the *A. aperta* haplotypes G and U (bipartition No. 22 in Fig. 4, Table 4), which received 96% posterior probability in the 5-way partition analysis and was not recovered in the partition by gene analysis.

Likelihood scores plateaued by 1000 generations for each model. However, the 5-way partition analysis appeared to converge on a valid posterior distribution faster than the other analyses. After 1-million generations of the 5-way data partition analysis posterior probabilities were nearly identical among three independent runs. In contrast, three independent runs of the other two types of data partitions produced variable posterior probabilities after 1-million generations. Surprisingly, though, the post burn-in range of likelihood scores for the partition by gene analysis was higher than the range for the other two types of analyses (range −ln likelihood: no partition 4600–4540, partition by gene 4540–4490, and 5-way partition 4600–4530). In sum, partitioning the data by gene seems to offer meaningful improvements in phylogenetic resolution and likelihood scores. Further partitioning did not significantly improve posterior probabilities or likelihood scores but did improve convergence time.

There were only a few differences between the Bayesian trees and the ML tree. Most notably, in each Bayesian tree *A. naevia* was sister to the group composed of *A. kastoni*, *A. potteri*, *A. pensylvanica*, and *A. emertoni*. However, differences in topology between Bayesian trees and the ML tree were not supported by greater than 95% posterior probability (Table 4).

4. Discussion

This paper presents the first phylogenetic hypothesis for the prominent agelenid genus, *Agelenopsis*. Although species relationships are not completely resolved by the data presented here, inferences can be made concerning the relationship between *Agelenopsis* and *Barronopsis*; species monophyly, including the status of *A. aleenae* (the putative first generation hybrid); and geographic patterns of variation at both the intra- and interspecific level.

Our results support reciprocal monophyly of *Agelenopsis sensu stricto* and *Barronopsis*. Our findings are consistent with Chamberlin and Ivie's (1941) description of *Barronopsis* as a sub-genus of *Agelenopsis* and with Lehtinen's (1967) classification of *Barronopsis* as a separate genus. More generic level sampling is needed to verify if *Barronopsis* is in fact sister to *Agelenopsis* as appears to be the case from our phylogenetic results.

4.1. Species monophyly

Our phylogenetic results strongly supported monophyly of some *Agelenopsis* species but not others. However, we could not reject the hypothesis that each species' mtDNA haplotypes formed monophyletic groups. Thus, we do not view instances of paraphyly in MP or ML trees as representing poor taxonomy. Instead we view these species to be part of closely related species groups that offer insights into the process of speciation.

Examples of species for which we had sufficient geographic sampling to evaluate monophyly and for which monophyly was well-supported are *A. aperta*, *A. naevia*, *A. oklahoma*, and *A. longistylus*. *Agelenopsis kastoni* is probably also monophyletic but the two haplotypes observed came from a single population and, thus, we cannot reject the possibility that other populations of *A. kastoni* would prove to be more closely related to some other *Agelenopsis* species.

We also discovered two specimens that probably represent a new species, which we have referred to as "*A. sp. nov.*" in this paper. We were unable to identify these specimens to any previously described *Agelenopsis* species. In addition, the haplotype exhibited by these two individuals is fairly divergent from other *Agelenopsis*

species (approximately 5% uncorrected COI + 16S sequence divergence between *A. sp. nov.* and the most similar species, *A. aleenae*).

Other described *Agelenopsis* species were not found to represent monophyletic groups in MP and ML trees but were part of well-supported monophyletic species groups. One group of closely related species is composed of *A. aleenae* and *A. spatula*. With the mitochondrial data presented here we were able to reject the hypothesis that *A. aleenae* is simply a first generation hybrid between *A. aperta* and *A. spatula*. If *A. aleenae* was a first generation hybrid then its mtDNA should be identical to the maternal species. Our phylogenetic results show, however, that *A. aleenae* exhibited multiple haplotypes that were distinct both from *A. aperta* haplotypes and from the single *A. spatula* haplotype observed. These results do not preclude the possibility that hybridization has ever occurred between any of these species, but they do provide evidence against the hypothesis that the *A. aleenae* morphology strictly results from recent hybridization. Instead, the mitochondrial data supports *A. aleenae* being a distinct species and *A. spatula* being a daughter species of *A. aleenae* produced by peripatric speciation. Phylogenetic analyses show four geographically localized lineages within the *A. aleenae*/*A. spatula* clade (Figs. 3 and 5): a New Mexico *aleenae*, a central Texas *aleenae*, a Kansas *aleenae*, and a north Texas and Oklahoma *spatula*. These data support at least four allopatric divergences from a common ancestor that probably resembled *A. aleenae* with development of the *A. spatula* morphology in a subset of the ancestral range of *A. aleenae*. This peripatric speciation mode, which leads to paraphyletic species, has been increasingly documented (Harrison, 1998). In one of the few spider molecular analyses to consider intra- as well as interspecific sequence variation, Hedin (1997) also concluded that peripatric speciation was the most likely explanation for paraphyly of two *Nesticus* species.

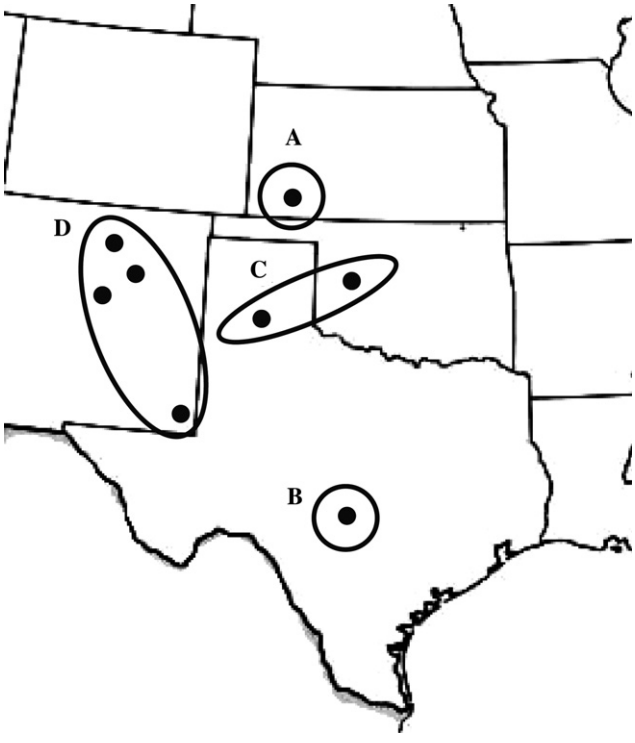

Fig. 5. Geographic distribution of lineages within the *A. aleenae*/*A. spatula* species group (see Fig. 3). (A) Kansas *A. aleenae* (site KS1); (B) Central Texas *A. aleenae* (site TX1); (C) *A. spatula* (sites OK and TX4); and (D) New Mexico *A. aleenae* (sites NM1, 2, 3, and 4).

Agelenopsis utahana and *A. oregonensis* form another group of closely related species. In contrast to the *aleenae*/*spatula* group, the *utahana*/*oregonensis* group does not form geographically distinct lineages, even though samples of *A. utahana* came from such distant locations as Utah and New England (Fig. 1). An informative geographic pattern observed is that one of the western COI haplotypes of *A. utahana* is more closely related to the *A. oregonensis* haplotype than to other *A. utahana* haplotypes (Fig. 3). This pattern is suggestive of peripatric speciation with *A. oregonensis* formed from a once isolated western population of *A. utahana*. However, the region of overlap of *A. oregonensis* and *A. utahana* (Fig. 2) corresponds to an area of the Pacific Northwest where hybridization is common for many different animal taxa and is considered to be an important area of secondary contact between previously isolated biotas (Remington, 1968). Hybridization could thus potentially play a role in causing the close relationship of one of the western *A. utahana* COI haplotypes and the *A. oregonensis* haplotype.

A third species group is composed of *A. potteri*, *A. pensylvanica*, and *A. emertoni*. Although males of these species are easy to distinguish based on genitalic characters, the molecular divergence among haplotypes exhibited by these three species is quite small (average uncorrected sequence divergence 0.8% for COI + 16S). Although the low levels of sequence divergence suggest recent speciation, the ranges of these three species widely overlap (Fig. 2). An important question, then, is what allows coexistence of such closely related species. Perhaps the overlap in ranges is maintained by strong divergent sexual selection (i.e., Eberhard's (1985) theory). Differences in habitat-use could also allow ranges to overlap. For instance, in Tennessee we collected *A. penslyvanica* from bushes in a suburban neighborhood and *A. emertoni* from leaf litter in a hardwood forest.

An alternative hypothesis that would explain the widely overlapping ranges and close relationship among haplotypes of *A. potteri*, *A. pensylvanica*, and *A. emertoni* is that these species actually represent a single interbreeding polymorphic species. Traditional ideas about spider genitalia would make this hypothesis seem unlikely; spider genitalia were thought to form a lock-and-key mechanism, such that any switch in male genitalic morphology would immediately lead to reproductive

isolation (Eberhard, 1985). However, the lock-and-key mechanism is apparently not important for *Agelenopsis*; according to Gerring (1953) any male structure can mechanically inseminate any female structure. Even though the lock-and-key hypothesis is becoming more commonly disputed (see Eberhard, 1985; Eberhard et al., 1998), most spider taxonomists still consider genitalia to be monomorphic within species and Eberhard et al. (1998) found little variation in genitalic characters within six spider species. We are aware of only two documented cases of polymorphic spider genitalia (Huber and González, 2001; Kaston, 1970) and a few cases of polymorphic insect genitalia (Hausmann, 1999; Kunze, 1959; Mound et al., 1998; Ulrich, 1988). Thus, it seems unlikely that a polymorphism in spider genitalia could be maintained over the entirety of the combined ranges of *A. potteri*, *A. pensylvanica*, and *A. emertoni*. Regardless, detailed experiments on the biology and ecology of this species group are needed to determine what factors lead to morphological differentiation with little associated mitochondrial differentiation. We are currently engaged in pheromone and courtship analyses to help elucidate this issue.

4.2. Geographic patterns of variation

We found geographic variation in levels of inter- and intraspecific sequence divergence. In particular, a clade of species with very low interspecific divergence, *A. kastoni*, *A. emertoni*, *A. pensylvancia*, and *A. potteri*, was found in eastern North America. In contrast, western species such as *A. longistylus*, *A. aperta*, *A. aleenae*, *A. spatula*, and *A. oklahoma* displayed much higher levels of interspecific sequence divergence (Figs. 3 and 4). This pattern contrasts with patterns found in other North American animal genera. For instance, molecular phylogenetic data for *Cincindela* beetles (Vogler et al., 1998) and *Enallagma* damselflies (McPeek and Brown, 2000) both show evidence for low sequence divergence within clades found in western North America. These studies indicate recent speciation events in western North America consistent with Cracraft's (1985) lithospheric complexity hypothesis. According to this hypothesis, geographic regions that are undergoing topographic change, such as mountain building in western North America, will cause geographic separation among populations and promote speciation. Our findings, on the other hand, are not consistent with the lithospheric complexity hypothesis, suggesting that geographic isolation alone cannot explain speciation rates within *Agelenopsis*. Instead, as discussed above, sexual selection or habitat differentiation may play a more important role in promoting the apparent recent speciation of some of the eastern species.

Geographic variation in levels of sequence divergence appears to extend below the species boundary. For instance, the western species *A. longistylus* exhibits almost 9% uncorrected COI sequence divergence between populations found only 152 km apart, whereas the eastern species, *A. naevia*, exhibits 0.2% COI sequence divergence between populations found 1230 km apart and *A. pensylvanica* shows no sequence variation across 1895 km. Other species exhibiting fairly deep molecular divergence and geographic structuring are the southwestern species, *A. aperta* and *A. aleenae*, with maximum levels of COI sequence divergence of 6.3 and 3.4%, respectively. The only other species to approach this level of intraspecific divergence is *A. utahana* with maximum COI sequence divergence of 2.5% among haplotypes found 3300 km apart. Why intraspecific divergence would be higher in species found in southwestern United States is not immediately clear. However, Ayoub and Riechert (2004) concluded that primary geographic structuring of *A. aperta* haplotypes corresponded to splits along mountain ranges. In the case of *Agelenopsis*, then, the topographic complexity of the southwestern United States seems to have caused population level differentiation rather than speciation as predicted by Cracraft's (1985) lithospheric complexity hypothesis.

The very low level of divergence exhibited by haplotypes found 1000–4000 km apart suggests that recent range expansions may be common for many *Agelenopsis* species. For instance, *A. utahana* is found throughout northern United States, Canada, and even Alaska. Most of this area was under ice sheets during the last glacial maximum, which means that *A. utahana* must have dispersed across most of its range over the last 10,000 years. Even within *A. aperta*, which shows high sequence divergence overall, the haplotype "G" is fixed throughout a large portion of *A. aperta's* range (see Figs. 1 and 3 for distribution of "G"). Ayoub and Riechert (2004) concluded that the widespread fixation of "G" was best explained by recent range expansion. Recent range expansions are consistent with Noonan's (1988, 1990) prediction that many modern insect ranges are the result of post-Pleistocene range expansions.

Additionally, the widespread geographic distribution of single haplotypes indicates that *Agelenopsis* species have better dispersal abilities than predicted by their dispersal mode (ground dispersing rather than aerial dispersing). Phylogeographic studies of other non-aerial dispersing spider species have found deep molecular divergence and population monophyly on fairly small geographic scales [i.e., *Nesitcus* species (Hedin, 1997); *Habronattus pugilis* (Masta, 2000a); *Apostichus simus* (Bond et al., 2001); *Hypochilus* species (Hedin, 2001); and *Hypochilus thorelli* (Hedin and Wood, 2002)], consistent with limited dispersal abilities. Our results for *A. aperta*, *A. aleenae*, and *A. longistylus* are similar to these studies in that they show geographic structure and fairly high levels of intraspecific molecular divergence,

but other *Agelenopsis* species do not. Complete generic level sampling of each of the study systems mentioned might reveal a similar pattern with some species having strong geographic structure and others not. However, the two studies that did include most species within a genus found similar patterns of strong geographic structure for most species (Hedin, 1997, 2001). At the moment, there is only one phylogeographic study of an aerial dispersing species, *Stegodyphus dumicola*, which showed mtDNA haplotypes found in multiple sites 150 km apart (Johannesen et al., 2002). Although the geographic scope of this study was limited, the widespread distribution of many different haplotypes probably reflects the better dispersal ability of these spiders compared to ground dispersing spiders.

Unequal mutation rates among species could also contribute to the differing levels of observed geographic structure. For instance, *A. longistylus* appears to form a very long branch (see Fig. 3). Possibly a higher mutation rate in *A. aperta*, *A. aleenae* and *A. longistylus* allows for a stronger signature of geographic structure to be detected within these species. However, we feel that a variable mutation rate is not sufficient to negate the overall pattern of geographic variation in genetic relatedness.

4.3. Higher level relationships

Aside from the well-supported species groups, relationships among *Agelenopsis* species are not completely resolved by the data presented here. However, a few relationships are worth discussing. First, the *A. oregonensis*/*A. utahana* clade is sister to the remainder of *Agelenopsis* species. A well-supported sister relationship also occurs between *A. kastoni* and the *A. pensylvanica*/*A. emertoni*/*A. potteri* species group. All analyses consistently placed *A. sp. nov.* sister to the *A. aleenae*/*A. spatula* group even though this relationship is never supported by greater than 50% bootstrap probability. Similarly, *A. aperta* is consistently sister to the *A. aleenae*/*A. spatula*/*A. sp. nov.* group but this relationship only receives greater than 50% bootstrap support in the combined MP analysis. Among the remainder of the *Agelenopsis* species, the positions of *A. longistylus*, *A. naevia*, and *A. oklahoma* are ambiguous.

Although the relationships noted above among *A. aperta*, *A. aleenae*, *A. spatula*, and *A. sp. nov.* haplotypes are not well-supported by mtDNA, the male genitalia have similarities that support monophyly of this group. As we mentioned in the introduction, *A. aleenae* has intermediate genitalia between *A. spatula* and *A. aperta*. The tip of the embolus for *A. aleenae* is spatulate, like *A. spatula*, but also twists, like *A. aperta*. Similarly, *A. sp. nov.* is intermediate between *A. aleenae* and *A. aperta*. The new species has a twisted embolus like *A. aleenae* and *A. aperta* and the tip of the embolus is broader than *A. aperta's* but does not have the distinct spatulate shape of *A. aleenae*.

5. Conclusions

Approximately half of the *Agelenopsis* species form well-supported monophyletic groups, whereas half are part of well-supported species groups. Rather than viewing species groups as problematic taxonomy we view them as excellent areas for further research on the process of speciation, as they are probably either on the cusp of speciating or have recently speciated. We also feel that these groups present an excellent opportunity to better understand the role of genitalic variation and divergence in the process of speciation. We also found lower levels of sequence divergence and geographic structuring than expected for a non-aerial dispersing spider group, suggesting that dispersal ability of these spiders is better than previously thought, and that recent range expansions have played an important role in modern geographic distributions. Higher level relationships were not completely resolved by the data presented here but represent an important first step in discerning deep evolutionary relationships within *Agelenopsis*.

Acknowledgments

We thank Joseph Spagna for sending leg tissue of *A. oregonensis* individuals from northern California and for sending us *A. potteri* and *A. oregonesis* specimens that had been given to him by R.G. Bennett. Marius Pfieffer sent us specimens of *A. naevia* and *A. emertoni* from Dallas/Fort Worth, TX. All molecular work was done in the lab of Gary McCracken at the University of Tennessee. Funding for this project came from NSF grants to S.E.R., an NSF graduate fellowship to N.A.A., and research grants to N.A.A. from the Department of Ecology and Evolutionary Biology at the University of Tennessee. This manuscript was improved by the comments of Florencia Campón, Andrew Crawford, Charles Winder, and an anonymous reviewer.

References

Avise, J.C., 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J., Reeb, C., Saunders, N., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489–522.

Ayoub, N.A., Riechert, S.E., 2004. Molecular evidence for Pleistocene glacial cycles driving diversification of a North American desert spider, *Agelenopsis aperta*. Mol. Ecol. 13, 3453–3465.

Barraclough, T.G., Nee, S., 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16, 391–399.

Bishop, L., 1990. Meteorological aspects of spider ballooning. Environ. Entomol. 19, 1381–1387.

Bond, J.E., Hedin, M.C., Ramirez, M.G., Opells, B.D., 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. Mol. Ecol. 10, 899–910.

Castoe, T.A., Doan, T.M., Parkinson, C.L., 2004. Data partitions and complex models in Bayesian analysis: the Phylogeny of gymnophthalmid lizards. Syst. Biol. 53, 448–469.

Chamberlin, R.V., Ivie, W., 1941. North American Agelenidae of the genera *Agelenopsis*, *Calilena*, *Ritalena* and *Tortolena*. Ann. Entomol. Soc. Am. 34, 585–628.

Cracraft, J., 1985. Biological diversification and its causes. Ann. Mo. Bot. Gdn. 72, 794–822.

Croucher, P.J.P., Oxford, G.S., Searle, J.B., 2004. Mitochondrial differentiation, introgression and phylogeny of species in the *Tegenaria atrica* group (Araneae: Agelenidae). Biol. J. Linn. Soc. 81, 79–89.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined?. Mol. Biol. Evol. 14, 733–740.

Eberhard, W.G., 1983. Why are genitalia good species characters. In: Eberhard, W.G., Lubin, Y.D., Robinson, B.C. (Eds.), Proceedings of the Ninth International Congress of Arachnology. Panama. Smithsonian Institution Press, Washington, DC, pp. 53–59.

Eberhard, W.G., 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge, MA.

Eberhard, W.G., Huber, B.A., Rodriguez, R.L., Briceno, R.D., Salas, I., Rodriguez, V., 1998. One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. Evolution 52, 415–431.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

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

Garb, J.E., González, A., Gillespie, R.G., 2004. The black widow genus *Latrodectus* (Araneae: Theridiidae): phylogeny, biogeography, and invasion history. Mol. Phylogenet. Evol. 31, 1127–1142.

Gerring, R.L., 1953. Structure and function of the genitalia in some American agelenid spiders. Smithsonian Miscellaneous Collections, vol. 121, Smithsonian Institution, Washington, DC, p. 84.

Gillespie, R., 2004. Community assembly through adaptive radiation in Hawaiian spiders. Science 303, 356–359.

Gutell, R.R., Fox, G.E., 1988. A compilation of large subunit RNA sequences presented in structural format. Nucleic Acids Res. 16, R175–R313.

Harrison, R.G., 1998. Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York, pp. 19–31.

Hausmann, A., 1999. Falsification of an entomological rule: polymorphic genitalia in Geometrid moths. Spixiana 22, 83–90.

Hedin, M.C., 1997. Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic-based sampling. Evolution 51, 1929–1945.

Hedin, M.C., 2001. Molecular insights in species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). Mol. Phylogenet. Evol. 18, 238–251.

Hedin, M.C., Maddison, W.P., 2001a. Phylogenetic utility and evidence for multiple copies of elongation Factor-1alpha in the spider genus *Habronattus* (Araneae: Salticidae). Mol. Biol. Evol. 18, 1512–1521.

Hedin, M.C., Maddison, W.P., 2001b. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). Mol. Phylogenet. Evol. 18, 386–403.

Hedin, M.C., Wood, D.A., 2002. Genealogical exclusivity in geographically proximate populations of *Hypochilus thorelli* Marx (Araneae, Hypochilidae) on the Cumberland Plateau of North America. Mol. Ecol. 11, 1975–1988.

Higgins, D., Thompson, J., Gibson, T., Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Huber, K.C., Haider, T.S., Manfred, M.W., Huber, B.A., Schweyen, R.J., Barth, F.G., 1993. DNA sequence data indicates the polyphyly of the family Ctenidae (Araneae). J. Arachnol. 21, 194–201.

Huber, B.A., González, A.P., 2001. Female genital dimorphism in a spider (Araneae: Pholcidae). J. Zool. Lond. 255, 301–304.

Huelsenbeck, J., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huelsenbeck, J., Larget, B., Miller, R., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–687.

Johannesen, J., Hennig, A., Dommermuth, B., Schneider, J.M., 2002. Mitochondrial DNA distributions indicate colony propagation by single matri-lines in the social spider *Stegodyphus dumicola* (Eresidae). Biol. J. Linn. Soc. 76, 591–600.

Kaston, B.J., 1970. Comparative biology of American black widow spiders. San Diego Soc. Nat. Hist. Trans. 16, 33–82.

Kunze, L., 1959. Die funktionsanatomischen Grundlagen der Kopulation der Zwergkikaden, untersucht en Euscelis plebejus (Fall.) und einigen Typhlocybinen. Dtsch. Entomol. Z. 6, 322–387.

Lehtinen, P.T., 1967. Classification of the cribellate spiders and some allied families, with notes on the evolution of the suborder Araneomorpha. Ann. Zool. Fenn. 4, 199–468.

Masta, S., 2000a. Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations?. Evolution 54, 1699–1711.

Masta, S., 2000b. Mitochondrial sequence evolution in spiders: intraspecific variation in tRNAs lacking the TgammaC arm. Mol. Biol. Evol. 17, 1091–1100.

McPeek, M.A., Brown, J.M., 2000. Building a regional species pool: diversification of the *Enallagma* damselflies in eastern North America. Ecology 81, 904–920.

Morando, M., Avila, L.J., Sites, J.W., 2003. Sampling strategy for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. Syst. Biol. 52, 159–185.

Mound, L.A., Crespi, B.J., Tucker, A., 1998. Polymorphism and kleptoparasitism in thrips (Thysanoptera: Phalaeothripidae) from woody galls on *Casuarina* trees. Aust. J. Entomol. 37, 8–16.

Noonan, G.R., 1988. Biogeography of North American and Mexican insects, and a critique of vicariance biogeography. Syst. Zool. 37, 366–384.

Noonan, G.R., 1990. Biogeographical patterns of North American *Harpalus* Latreille (Insecta: Coleoptera: Carabidae). J. Biogeogr. 17, 583–614.

Paison, T., 1997. A biogeographic review of the spider genus *Agelenopsis* (Araneae: Agelenidae). Masters Thesis, University of Tennessee, Knoxville, TN.

Piel, W.H., Nutt, K.J., 1997. *Kaira* is a likely sister group to *Metepeira*, and *Zygiella* is an araneid (Araneae: Araneidae): evidence from mitochondrial DNA. J. Arachnol. 25, 262–268.

Piel, W.H., Nutt, K.J., 2000. One species or several? Discordant patterns of geographic variation between allozymes and mtDNA sequences among spiders in the genus *Metepeira* (Araneae: Araneidae). Mol. Phylogenet. Evol. 15, 414–418.

Platnick, N.I., 2005. The world spider catalog, version 5.5. American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog/index.html.

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

Remington, C.L., 1968. Suture-zones of hybrid interaction between recently joined biotas. Evol. Biol. 2, 321–428.

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

Roth, V.D., 1954. Review of the spider subgenus *Barronopsis* (Arachnida, Agelenidae). Am. Museum Novitiates 1678, 1–7.

Roth, V.D., Brame, P.L., 1972. Nearctic genera of the spider family Agelenidae (Arachnida: Araneida). Am. Musuem Novitates 2505, 1–52.

Sambrook, J.E., Fritsch, F., Maniatis, T., 1987. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York.

Shahjahan, R., Hughes, K., Leopold, R., DeVault, J., 1995. Lower incubation temperature increases yield of insect genomic DNA isolated by the CTAB method. Biotechniques 19, 333–334.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Simon, C., Frati, R., Bechenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Smith, S.D., Bond, J.E., 2003. An analysis of the secondary structure of the mitochondrial large subunit rRNA gene (16S) in spiders and its implications for phylogenetic reconstruction. J. Arachnol. 31, 44–54.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis using Parsimony (* and Other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Tan, A.M., Gillespie, R.G., Oxford, G.S., 1999. Paraphyly of the *Enoplognatha* group (Araneae, Theridiidae) based on DNA sequences. J. Arachnol. 27, 481–488.

Templeton, A.R., 2001. Using phylogeographic analyses of gene trees to test species status and processes. Mol. Ecol. 10, 779–791.

Ulrich, H., 1988. Das Hypopygium von *Microphor holosericus* (Meigen) (Diptera, Empidoidae). Bonn. Zool. Beitr. 39, 179–219.

Vogler, A.P., Welsh, A., Barraclough, T.G., 1998. Molecular phylogeny of the Cicindela maritima (Coleoptera: Cicindelidae) group indicates fast radiation in western North America. Ann. Entomol. Soc. Am. 91, 185–194.

Weyman, G.S., Jepson, G.S., Sunderland, K.D., 1995. Do seasonal changes in numbers of aerially dispersing spiders reflect population density on the ground or variation in ballooning motivation?. Oecologia 101, 487–493.

Zehothofer, K., Sturmbauer, C., 1998. Phylogenetic relationships of central European wolf spiders (Araneae: Lycosidae) inferred from 12S ribosomal DNA sequences. Mol. Phylogenet. Evol. 10, 391–398.