



Contents lists available at ScienceDirect

## Molecular Phylogenetics and Evolution

journal homepage: [www.elsevier.com/locate/ympev](http://www.elsevier.com/locate/ympev)

## Short Communication

## Actin 5C, a promising nuclear gene for spider phylogenetics

Cor J. Vink<sup>a,\*</sup>, Marshal Hedin<sup>a</sup>, Melissa R. Bodner<sup>b</sup>, Wayne P. Maddison<sup>c</sup>, Cheryl Y. Hayashi<sup>d</sup>, Jessica E. Garb<sup>d</sup><sup>a</sup> Department of Biology, San Diego State University, San Diego, CA 92182, USA<sup>b</sup> Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4<sup>c</sup> Departments of Zoology and Botany, Centre for Biodiversity Research, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4<sup>d</sup> Department of Biology, University of California, Riverside, CA 92521, USA

## ARTICLE INFO

## Article history:

Received 25 November 2007

Revised 27 February 2008

Accepted 2 March 2008

Available online 13 March 2008

## 1. Introduction

Most molecular based phylogenetic studies on spiders at genus level or deeper have largely relied on datasets generated from mitochondrial genes (e.g., Astrin et al., 2006; Huber et al., 1993; Vink et al., 2002), nuclear ribosomal genes (e.g., Hausdorf, 1999; Hedin and Bond, 2006), or combinations thereof (e.g., Arnedo et al., 2004; Bruvo-Madaric et al., 2005; Hedin and Maddison, 2001; Maddison and Hedin, 2003; Maddison et al., 2007; Murphy et al., 2006; Su et al., 2007). The utility of three nuclear protein-coding genes has been explored for spider phylogenetics: elongation factor-1 $\alpha$  (e.g., Maddison and Hedin, 2003) and Histone 3 (e.g., Arnedo et al., 2004; Maddison and Needham, 2006; Su et al., 2007) at the sub-genus level, and elongation factor-1 $\gamma$  (Ayoub et al., 2007a) at the interfamilial level. However, the number of nuclear markers available to spider phylogeneticists is woeful compared to the suite of nuclear protein-coding genes utilized by their colleagues working on insect phylogenetics (e.g., Banks and Whitfield, 2006; Danforth et al., 2006; Friedlander et al., 1992; Hardy, 2007). For deeper relationships within and among spider families, analyses often depend primarily on the nuclear ribosomal genes, because the mitochondrial genes and Histone 3 are too variable, while the elongation factor genes have been difficult to sequence (Maddison and Hedin, 2003; Maddison and Needham, 2006).

As part of the NSF supported Assembling the Tree of Life: Phylogeny of Spiders project (<http://research.amnh.org/ato1/files/>) an attempt was made to develop new molecular markers for inferring deep phylogenetic relationships in the Araneae. Spider cDNA

libraries were searched for “housekeeping genes” (i.e., those necessary for basic cellular processes), a promising source of new molecular markers. In this paper, we examine the phylogenetic utility of a nuclear protein-coding member of the actin gene family for spider systematics. Actin is the main component of the microfilament system in all eukaryotic cells and is involved in basic cell processes including cellular motility, intracellular transport, muscle contraction and cytokinesis (Van Troys et al., 1999). Actin is also one of the most conserved proteins (Van Troys et al., 1999), a property that facilitates alignment and primer design for new molecular markers. Actin sequences have been used as a phylogenetic marker in mollusks (Donald et al., 2005) but their use in arthropod phylogenetics has been limited to one study on three tick species (da Silva Vaz et al., 2005).

## 2. Methods

## 2.1. cDNA libraries

Sequence data used to construct actin primers came from cDNA libraries that had been constructed from eight spider species spanning the phylogenetic diversity found in the order Araneae; *Euagrus chisoseus* Gertsch, 1939, *Hypochilus thorelli* Marx, 1888, *Plectreurys tristis* Simon, 1893, *Dolomedes tenebrosus* Hentz, 1844, *Paraphidippus aurantius* (Lucas, 1833), *Argiope trifasciata* (Forsskål, 1775), *Uloborus diversus* Marx, 1898, and *Latrodectus hesperus* Chamberlin and Ivie, 1935 (see Gatesy et al., 2001 and Garb and Hayashi, 2005 for cDNA library construction methods). These libraries had been built to facilitate searches for silk coding transcripts and genes (see Ayoub et al., 2007b; Garb et al., 2006; Garb and Hayashi, 2005; Gatesy et al., 2001) and new molecular markers for spider phylogenetics (see Ayoub et al., 2007a). Libraries of approximately 1200 or 1800 recombinant colonies were arrayed

\* Corresponding author. Present address: AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand.

E-mail address: [cor.vink@arachnology.org](mailto:cor.vink@arachnology.org) (C.J. Vink).

and 25–30% of each library was scored for insert size (Beuken et al., 1998). Colonies with inserts  $\geq 500$  bp were sequenced using the universal primers T7 and Sp6. Translated sequences were then compared to the nr protein database in GenBank using BLASTX (Altschul et al., 1997).

Clones containing actin homologs (determined by a BLAST score with an *E* value greater than  $10^{-10}$ ) were found in each library.

Fragments between 500–1300 bp aligning to portions of the full-length coding sequence (1131 bp) and/or parts of the 5'UTR and the 3'UTR were identified from the clones (see Table 1). Sequences were aligned in Sequencher (Gene Codes Corporation) to each other, to actin sequences from the ticks *Boophilus microplus* (Canestrini, 1888), *Haemaphysalis longicornis* Neumann, 1897, *Rhipicephalus appendiculatus* Neumann, 1901, *Ornithodoros moubata* (Murray,

**Table 1**  
Specimen data

Species	Family	Clade	Geographic origin	GenBank Accession Nos.
<i>Phrynus mexicanus</i> Bilimek, 1867	Phryniidae	Phrynoidea	Mexico, Baja California <sup>1</sup>	EU522682
<i>Euagrus josephus</i> Chamberlin, 1924	Dipluridae	Mygalomorphae	Mexico, Baja California Sur, El Triunfo <sup>1</sup>	PCR failed
<i>Atrax robustus</i> O. Pickard-Cambridge, 1877	Hexathelidae	Mygalomorphae	Australia, New South Wales, Scalloway <sup>1</sup>	Fungal contaminant
<i>Antrodiaetus riversi</i> (O. Pickard-Cambridge, 1883)	Antrodiaetidae	Mygalomorphae	USA, California, Sonora Pass <sup>1</sup>	PCR failed
<i>Gradungula sorenseni</i> Forster, 1955	Gradungulidae	Austrochiloidea	New Zealand, Nelson, Moss Reserve <sup>1</sup>	EU522683
<i>Kukulcania utahana</i> (Chamberlin and Ivie, 1935)	Filistatidae	Haplogynae	USA, California, near Amboy <sup>1</sup>	PCR failed
<i>Dysdera crocata</i> C. L. Koch, 1838	Dysderidae	Haplogynae	USA, California, San Diego <sup>1</sup>	Spider contaminant
<i>Pholcus phalangioides</i> (Fuesslin, 1775)	Pholcidae	Haplogynae	USA, California, San Diego <sup>1</sup>	Fungal contaminant
<i>Plectreurus tristis</i> Simon, 1893	Plectreuridae	Haplogynae	USA, Arizona, Sedona <sup>4</sup>	Spider contaminant
<i>Periegops suteri</i> (Urquhart, 1892)	Periegopidae	Haplogynae	New Zealand, Banks Peninsula, Hinewai Reserve <sup>2</sup>	PCR failed
<i>Oecobius navus</i> Blackwall, 1859	Oecobiidae	Eresoidea	USA, California, San Diego <sup>1</sup>	Spider contaminant
<i>Reo eutypos</i> (Chamberlin and Ivie, 1935)	Mimetidae	Palpimanoidea	USA, California, San Diego <sup>1</sup>	Fungal contaminant
<i>Palpimanus gibbulus</i> Dufour, 1820	Palpimanidae	Palpimanoidea	Spain, Andalucia, Bembezar <sup>1</sup>	PCR failed
<i>Cesonia</i> sp.	Gnaphosidae	RTA clade	Mexico, Sonora <sup>3</sup>	EU522700
<i>Lampona cylindrata</i> (L. Koch, 1866)	Lamponidae	RTA clade	New Zealand, Canterbury, Prebbleton <sup>1</sup>	Spider contaminant
<i>Syspira tigrina</i> Simon, 1895	Miturgidae	RTA clade	Mexico, Baja California Sur, Los Barriles <sup>1</sup>	EU522684
<i>Xysticus</i> sp.	Thomisidae	RTA clade	USA, Colorado <sup>3</sup>	EU522701
<i>Acragus</i> sp.	Salticidae	RTA clade	Ecuador, Napo, Estación Biológica Jatun Sacha <sup>3</sup>	EU522702
<i>Cotinus</i> sp.	Salticidae	RTA clade	Ecuador, near El Cisne <sup>3</sup>	EU522703
<i>Evarcha prozysniskii</i> Marusik and Logunov, 1998	Salticidae	RTA clade	Canada, British Columbia, Mission <sup>3</sup>	EU522704
<i>Freya decorata</i> (C.L. Koch, 1846)	Salticidae	RTA clade	Ecuador, Napo, Estación Biológica Jatun Sacha <sup>3</sup>	EU522705
<i>Galianora bryicola</i> Maddison, 2006	Salticidae	RTA clade	Ecuador, Napo <sup>3</sup>	EU522706
<i>Galianora sacha</i> Maddison, 2006	Salticidae	RTA clade	Ecuador, Napo <sup>3</sup>	EU522707
<i>Ghelnia canadensis</i> (Banks, 1897)	Salticidae	RTA clade	USA, North Carolina <sup>3</sup>	EU522708
<i>Goleba lyra</i> Maddison and Zhang 2006	Salticidae	RTA clade	Madagascar, Fianarantsoa <sup>4</sup>	EU522709
<i>Habronattus americanus</i> (Keyserling, 1885)	Salticidae	RTA clade	USA, Nevada, Continental Lake <sup>1</sup>	EU522685
<i>Heliophanus cupreus</i> (Walckenaer, 1802)	Salticidae	RTA clade	Poland, Mieliik <sup>3</sup>	EU522710
<i>Holcolaetis cf. zuluensis</i> Lawrence, 1937	Salticidae	RTA clade	South Africa, Kwazulu-Natal, Lake St. Lucia <sup>3</sup>	EU522711
<i>Hurius cf. vulpinus</i> Simon, 1901	Salticidae	RTA clade	Ecuador, E of Gualaceo <sup>3</sup>	EU522712
<i>Hyllus diardi</i> (Walckenaer, 1837)	Salticidae	RTA clade	Singapore, Lum Chu Kang Mangroves <sup>3</sup>	EU522713
<i>Hyllus treleaveni</i> Peckham and Peckham, 1902	Salticidae	RTA clade	South Africa, Kwazulu-Natal Province, Phinda <sup>3</sup>	EU522714
<i>Lyssomanes viridis</i> (Walckenaer, 1837)	Salticidae	RTA clade	USA, Mississippi, Wall Doxey State Park <sup>3</sup>	EU522715
<i>Pachyballus</i> sp.	Salticidae	RTA clade	South Africa, Kwazulu-Natal, Lake St. Lucia <sup>3</sup>	EU522716
<i>Paraphidippus aurantius</i> (Lucas, 1833)	Salticidae	RTA clade	USA, Arizona <sup>1</sup>	EU522686
<i>Plexippus paykulli</i> (Adouin, 1826)	Salticidae	RTA clade	Singapore, Nee Soon Swamp Forest <sup>3</sup>	EU522717
<i>Portia cf. schultzi</i> Karsch, 1878	Salticidae	RTA clade	Madagascar, Fianarantsoa <sup>4</sup>	EU522718
<i>Salticus scemicus</i> (Clerck, 1757)	Salticidae	RTA clade	Canada, British Columbia, Mission <sup>3</sup>	EU522719
<i>Thrandina parocula</i> Maddison, 2006	Salticidae	RTA clade	Ecuador, Morona Santiago <sup>3</sup>	EU522720
<i>Zorocrates unicolor</i> (Banks, 1901)	Zorocratidae	RTA clade	USA, Arizona, Tucson <sup>1</sup>	Spider contaminant
<i>Stiphidion facetum</i> Simon, 1902	Stiphidiidae	RTA clade	New Zealand, Wellington, Stokes Valley <sup>1</sup>	EU522687
<i>Neolana dalmasi</i> (Marples, 1959)	Amphinectidae	RTA clade	New Zealand, Coromandel, Alderman Island <sup>1</sup>	EU522688
<i>Uliodon cervinus</i> L. Koch, 1873	Zoropsidae	RTA clade	New Zealand, Wellington <sup>1</sup>	Spider contaminant
<i>Dolomedes tenebrosus</i> Hentz, 1844	Pisauridae	RTA clade	USA, Pennsylvania, Feasterville <sup>a</sup>	EU522689
<i>Pirata piraticus</i> (Clerck, 1757)	Lycosidae	RTA clade	USA, California, San Diego <sup>1</sup>	EU522690
<i>Alopecosa kochi</i> (Keyserling, 1877)	Lycosidae	RTA clade	USA, California, Laguna Mountains <sup>1</sup>	EU522691
<i>Schizocosa maxima</i> Dondale and Redner, 1978	Lycosidae	RTA clade	USA, California, Jamul <sup>1</sup>	EU522692
<i>Schizocosa mccooki</i> (Montgomery, 1904)	Lycosidae	RTA clade	USA, California, Laguna Mountains <sup>1</sup>	EU522693
<i>Schizocosa ocreata</i> (Hentz, 1844)	Lycosidae	RTA clade	USA, Mississippi, Sardis Reservoir <sup>1</sup>	EU522694
<i>Homalonychus selenopoides</i> Marx, 1891	Homalonychidae	RTA clade	USA, Arizona, Dome Rock Mountains <sup>1</sup>	EU522695
<i>Waitkera waitakerensis</i> (Chamberlain, 1946)	Uloboridae	Orbiculariae	New Zealand, Coromandel, Mayor Island <sup>1</sup>	EU522696
<i>Cyclosa conica</i> (Pallas, 1772)	Araneidae	Orbiculariae	USA, California, San Luis Obispo County <sup>1</sup>	EU522697
<i>Argiope trifasciata</i> (Forsskål, 1775)	Araneidae	Orbiculariae	USA, Wyoming, Wheatland <sup>1</sup>	EU522698
<i>Eidmannella pallida</i> (Emerton, 1875)	Nesticidae	Orbiculariae	USA, Texas, Ft. Hood <sup>1</sup>	EU522699
cDNA library specimens				
<i>Euagrus chisoseus</i> Gertsch, 1939	Dipluridae	Mygalomorphae	USA, Arizona, Tucson	EU293212–19
<i>Hypochilus thorelli</i> Marx, 1888	Hypochilidae	Paleocribellatae	USA, Tennessee, near Chattanooga	EU293220–24
<i>Plectreurus tristis</i> Simon, 1893	Plectreuridae	Haplogynae	USA, Arizona, Sedona <sup>4</sup>	EU293225, EU293226
<i>Dolomedes tenebrosus</i> Hentz, 1844	Pisauridae	RTA clade	USA, Pennsylvania, Feasterville <sup>a</sup>	EU293227
<i>Paraphidippus aurantius</i> (Lucas, 1833)	Salticidae	RTA clade	USA, Arizona	EU293228–30
<i>Argiope trifasciata</i> (Forsskål, 1775)	Araneidae	Orbiculariae	USA, Wyoming, Wheatland	EU293231
<i>Uloborus diversus</i> Marx, 1898	Uloboridae	Orbiculariae	USA, California, Riverside	EU293232, EU293233
<i>Latrodectus hesperus</i> Chamberlin and Ivie, 1935	Theridiidae	Orbiculariae	USA, California, Riverside	EU293234

Taxonomy follows Platnick (2007). Major spider clade names follow Coddington (2005).

<sup>a</sup>Obtained from Spider Pharm Inc. Voucher specimen localities; <sup>1</sup>Hedin Lab, San Diego State University, <sup>2</sup>American Museum of Natural History, <sup>3</sup>Spencer Entomological Museum (University of British Columbia), <sup>4</sup>California Academy of Sciences.

1877) (GenBank Accession Nos. AY255624, AY254898, AY254899, AY547732), and to partial cDNA sequences from the scorpion *Mesobuthus gibbosus* (Brullé, 1832) (GenBank Accession Nos. BU091989, BU091891). Primers were manually designed in highly conserved regions.

## 2.2. Amplification and sequencing

Initially the primer pair actin-F-254 and actin-R-1009 were designed and trialed (see Table 2). These primers were referred to as actin-F and actin-R respectively in Vink et al. (2005). PCR amplification was performed using *ExTaq* DNA polymerase (Takara) with the following thermocycler profile: 3 min. at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 45–65 °C, 1 min at 72 °C; 5 min. at 72 °C. The use of actin-F-254 and actin-R-1009 resulted in the amplification of multiple PCR products, presumed to represent different actin paralogs. Up to four bands were seen when visualizing the PCR products and re-examination of the cDNA fragments revealed that the sequences encoded five different amino acid sequences.

Most of the cDNA sequences (GenBank Accession Nos. EU293212, EU293213, EU293220–28, EU293231, EU293234) coded for an amino acid sequence that was identical to the amino acid sequence of *Boophilus microplus*, which is believed to be a cytoplasmic actin (da Silva Vaz et al., 2005), and close to actin 5C in *Drosophila melanogaster* Meigen 1830, differing by two amino acids. Hereafter we will refer to this spider actin copy as spider actin 5C. The amino acids encoded by the *Uloborus diversus* cDNA (GenBank Accession Nos. EU293232 and EU293233) differed to spider actin 5C by 5 amino acids. Four of these amino acid differences were also seen in two of the *Paraphidippus aurantius* sequences (GenBank Accession Nos. EU293229 and EU293230). One *Euagrus chisoseus* cDNA sequence (GenBank Accession No. EU293214) differed from spider actin 5C by one amino acid change, which may be an allelic variation. Five of the *E. chisoseus* cDNA sequences (GenBank Accession Nos. EU293215–19) differed from spider actin 5C by ten amino acid changes. Given that *D. melanogaster* actin copies differ from each other by between two and 16 amino acid changes, it would seem reasonable to conclude that we had cDNA sequences representing at least three actin copies for spiders; this, coupled with the four bands seen when visualizing the PCR products, led us to conclude that there appear to be at least four actin copies in some (or all) spiders. This compares with four to seven copies of actin in ticks (Horigane et al., 2007) and six copies in *D. melanogaster* (Crosby et al., 2007).

The primers actin5C-F-229 and actin5C-R-1057 were then designed to specifically amplify actin 5C in spiders (see Table 2). These primers were used in PCR experiments (with conditions as above, with an annealing temperature between 45–65 °C) on a diverse set of high-quality spider genomic DNA (see Table 1). This produced a PCR product approximately 900–1300 bp in length. Although we tried different annealing temperatures (48 °C was optimal) and other primers we had designed, the actin primers did not work on all taxa. Also, multiple bands remained present for some taxa, suggesting some cross-amplification of paralogs, despite our copy-specific primers. Increased annealing tempera-

tures, or other gel purification techniques, might ultimately prove successful in these problem taxa. Fungal contaminants were amplified and sequenced in three cases (see Table 1). An identical sequence (both exon and intron) was amplified in six taxa (see Table 1), which suggested a PCR contaminant. Because we were unable to determine the source of the contamination, these sequences were not included in further analyses.

PCR products were cleaned with Montage PCR filter units (Millipore) and then sequenced in both directions using the PCR primers at the Core Instrumentation Facility (University of California, Riverside) and the Microchemical Core Facility (San Diego State University). The sequences consisted of two exon fragments (384 and 443 bp) flanking an intron of variable length (~80–450 bp); there was no intron in *Phrynus mexicanus* Bilimek, 1867, an outgroup species in the order Amblypygi.

## 2.3. Phylogenetic utility

To investigate the potential utility of actin for resolving phylogeny among spider families, 723 bp of protein-coding (exon) data were compiled for 21 taxa (17 PCR amplified, 4 cDNAs—see Table 1). The intron data are variable and potentially informative at the species level (based on comparison of *Schizochosa* sequences), but the introns are too variable between genera to align easily and were not included in the analyses. A standard heuristic parsimony search (TBR branch swapping, 1000 random addition sequence replicates) in PAUP\* (Swofford, 2002) was used. PAUP\* was also used to calculate bootstrap support (1000 replicates), uncorrected “*p* distances” between taxa and a Chi-square test for base composition bias.

To investigate the potential phylogenetic utility of actin for more recent divergences, 19 additional jumping spider (Salticidae) taxa and two dionychnan outgroups (a thomisid, *Xysticus* sp., and a gnaphosid, *Cesonia* sp.) were sequenced and phylogenetically analyzed. Actin sequences ~1000 bp long were amplified using the primer pair actin5C-F-229 and actin5C-R-1057. PCR was performed using *Taq* (Invitrogen) and the following protocol: 2 min at 95 °C; 35 cycles of 45 s at 95 °C, 45 s at 48 °C, 1 min at 72 °C; 10 min at 72 °C. Double bands were not observed when visualizing the PCR products. Most bands were of one size; however, a few varied in size suggesting either the intron was of variable length between salticid species or different copies were amplified. Samples were sequenced by Macrogen Inc. (ABI 3730 Sequencer). Phylogenetic analysis was done using parsimony (PAUP\* (Swofford, 2002), 1000 random addition sequence TBR searches followed by open ended TBR rearrangements) and Bayesian methods (MrBayes 3.1.2 (GTR invariant-gamma model) Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003).

## 3. Results and discussion

Results for the taxonomic groups of spiders are shown in Table 1 and can be summarized as follows: amplification of genomic actin from taxa belonging to both the RTA Clade and the Orbicularia were moderately to highly successful; amplification of samples from the family Salticidae were moderately successful; however, no actin was successfully amplified from genomic samples of mygalomorphs (despite the use of *Euagrus chisoseus* cDNA for primer design), haplogynes (despite the use of *Plectreurys tristis* cDNA for primer design), eresoids, or palpimanoids.

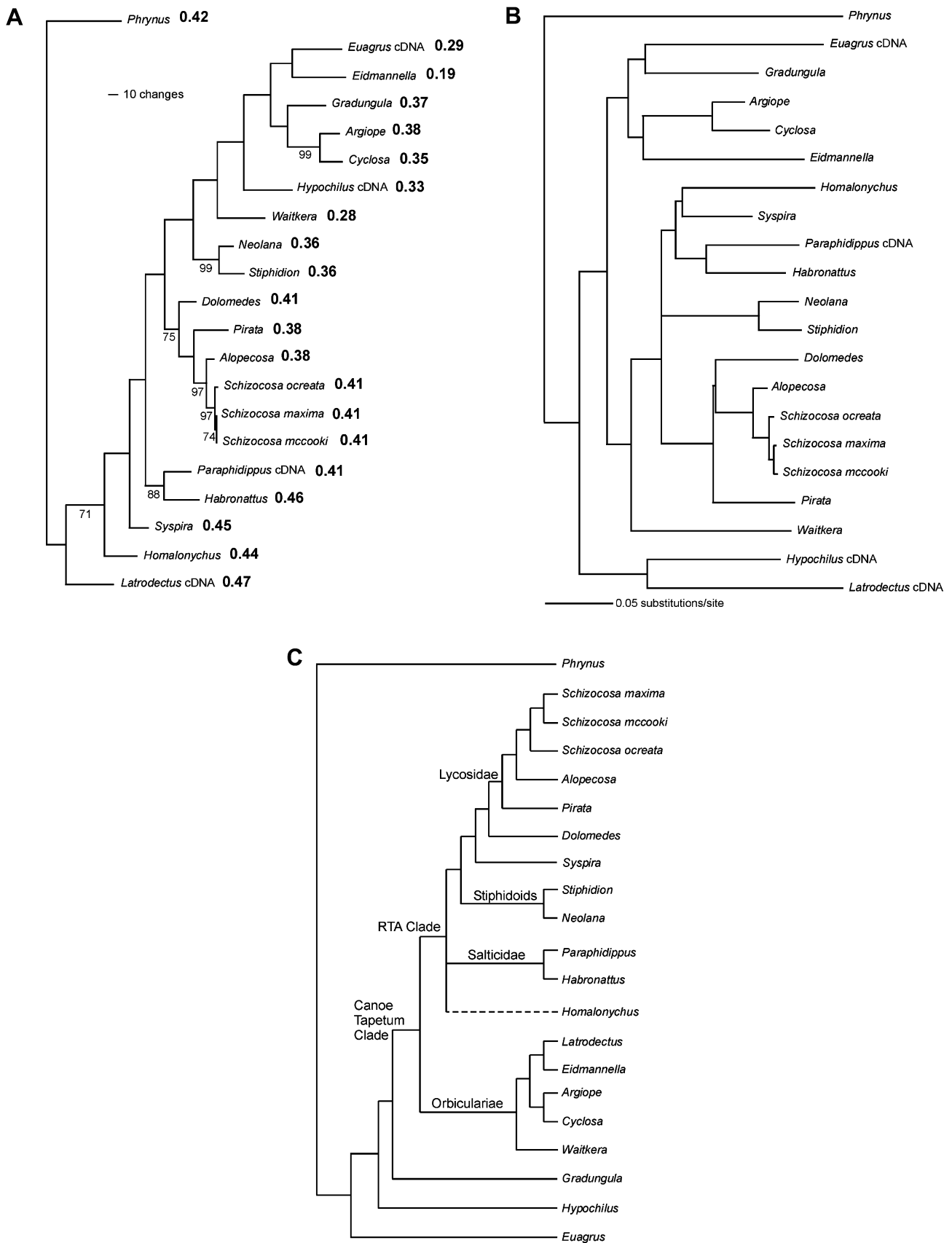
### 3.1. Phylogeny among families

Of 240 parsimony informative exon sites, 211 were third position sites, and the remainder were first position sites. There were

**Table 2**  
Spider specific actin primers

Primer name	Sequence (5'–3')
Actin-F-254	ACNAACTGGGATGATATGGAGAA
Actin-R-1009	CCNCRATCCANACGGARTACTT
Actin5C-F-229	AAGTATCCNATTGACCATGGTATTG
Actin5C-R-1057	TTNGADATCCACATTTGTTGGAA

Primers are named according to direction (F, forward; R, reverse) and position of the 3' clamp on the coding sequence.



**Fig. 1.** (A) One of two most-parsimonious trees for interfamilial analysis,  $L = 1069$ . Values below the branches indicate the bootstrap percentages of 1000 replicates. Values beside taxa are base composition data (proportion of T at third positions) from PAUP\*. (B) Interfamilial phylogenetic results based on neighbor-joining search using LogDet distances, proportion of sites assumed to be invariable = 0.6. (C) Expected topology (based on Coddington, 2005).

no amino acids substitutions observed in the exon matrix, including taxa ranging from the outgroup *Phrynus mexicanus* to derived Orbiculariae. Intergeneric uncorrected “*p* distances” range from ~2% to over 22%, the great majority of distances ranging from 15–20% among spider taxa. A Chi-square test conducted in PAUP\* suggests that base frequencies at all sites are not homogeneous across taxa ( $P = 0.001$ ), and are clearly not homogeneous at third positions ( $P = 0.000$ ; see Fig. 1A); this pattern was further explored below in light of phylogenetic results.

A standard heuristic parsimony search in PAUP\* resulted in two trees, one of which is shown in Fig. 1A. This tree topology includes some “expected” relationships (e.g., monophyletic Salticidae, Lycosidae, Araneidae, Stiphidioids), which were supported by high bootstrap values. There were also several questionable relationships (e.g., basal *Latrodectus*; *Hypochilus*, *Gradungula* and *Euagrus* nested with Orbiculariae), which were not supported by the bootstrap analysis. We suspect that some of these relationships may reflect convergence in base composition in a gene that is highly diverged at the nucleotide level, yet constrained at the amino acid level over highly divergent taxa.

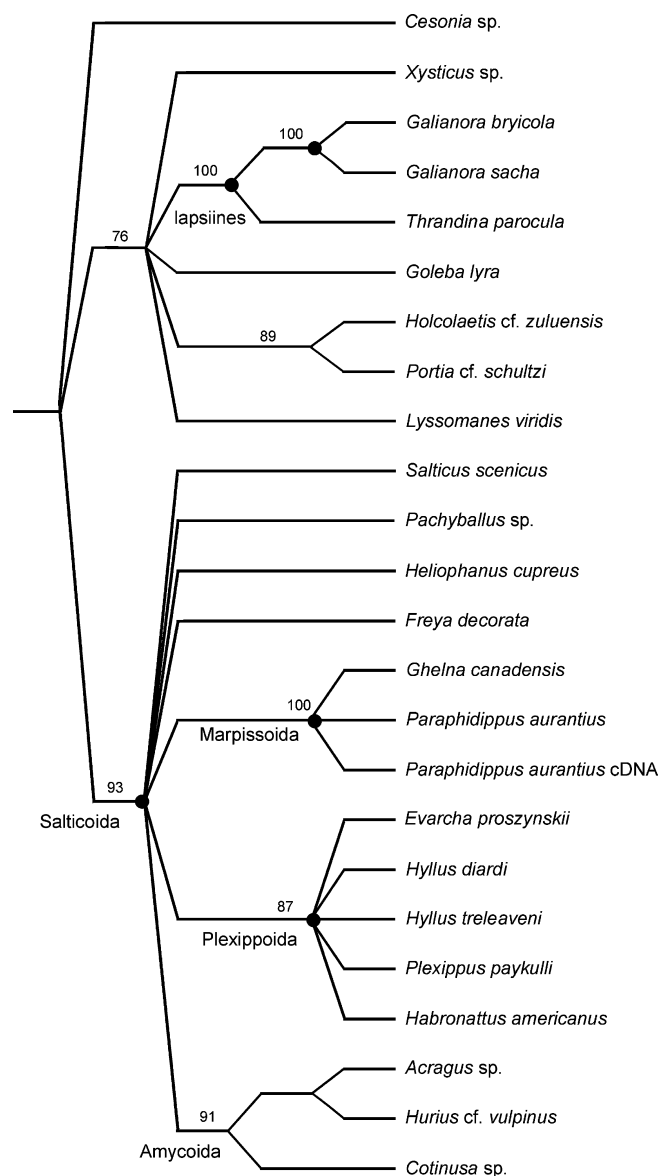
As a preliminary means to gauge the impact of this potential base composition bias, we conducted a distance analysis using Log-Det distances. In such an analysis, there are many possible ways to handle among-site rate variation, objective functions (e.g., minimum evolution, least squares), etc. We conducted a standard analysis that reveals some interesting patterns (Fig. 1B). Recovered clades in a LogDet analysis include Salticidae, Lycosidae, and Stiphidioids, all nested within a monophyletic RTA Clade (presuming that *Homalonychus* is in this clade).

### 3.2. Phylogeny within Salticidae

Both the parsimony and Bayesian analyses resulted in trees largely concordant with previous results from molecular and morphological phylogenetic studies of Salticidae (Fig. 2). In particular, the Bayesian analysis recovered the major clades Salticoida, Marpissoida, Plexippoida, and Amycoida (Maddison and Hedin, 2003; Maddison and Needham, 2006). Outside of the Salticoida, the lapsiines were recovered, as was the expected relationship between *Holcolaetis* and *Portia* (Maddison and Needham, 2006). The one notable discordance with previous results is the weakly-supported inclusion of a non-salticid, *Xysticus*, among the basal salticids. Salticid monophyly is well supported morphologically and molecularly (Maddison and Hedin, 2003; Maddison and Needham, 2006), and so the placement of *Xysticus* here is considered in error and, like some of the questionable relationships among families, may also be a result of convergence in base composition.

Actin 5C, targeted with copy-specific primers, is clearly not readily amplified in all spider taxa. In particular, Mygalomorphae and taxa hypothesized to be near the base of the Araneomorphae (e.g., Haplogynae, Eresoidea, Palpimanoidea) are difficult to amplify (or result in multiple bands) under the “standard” PCR conditions that we have used. Conversely, we have had greater success for most taxa in the RTA Clade and the Orbiculariae, suggesting that there may be something about actin gene family evolution in the entire “Canoe Tapetum Clade” (Coddington, 2005) that facilitates amplification with the primers that we have designed. The preliminary sequence data collected appear to provide “expected” phylogenetic results in some restricted areas of the tree.

Base composition biases and high divergence levels (perhaps tending towards saturation) may limit the utility actin 5C at deeper phylogenetic levels. However, within a family (Salticidae), it shows the ability to recover previously well-established clades much better than mitochondrial genes or Histone 3. This exploratory study indicates that actin 5C is a potentially valuable nuclear protein-coding gene for spider phylogenetic studies. However, the reliabil-



**Fig. 2.** Salticid phylogeny from actin exons, Bayesian analysis. Numbers show posterior probabilities, estimated from last 9 million generations of each of two runs of 10 million generations, with trees sampled every 1000 generations. Spots show clades also recovered in parsimony analysis. Previously recognized clades are named.

ity of PCR amplification of actin remains a challenge for future researchers.

### Acknowledgments

We thank the following people for help providing specimens; Miquel Arnedo, Jason Bond, Sarah Crews, Li Daiqin, Mike Fitzgerald, Charles Griswold, David Maddison, Daniel Palmer, Pierre Paquin, Norm Platnick, Phil Sirvid, Jim Starrett. Thanks to John Gatesy who constructed some of the cDNA libraries. CJV thanks Sean Marshall for informative discussions about this work. This research was funded as part of the NSF supported Assembling the Tree of Life: Phylogeny of Spiders, grant number EAR0228699 to Ward Wheeler, Jonathan Coddington, Gustavo Hormiga, Lorenzo Prendini and Petra Sierwald (<http://research.amnh.org/atol/files/>), by NSF grant DEB0236020 to CYH and by an NSERC (Canada) Discovery Grant to WPM.

## References

- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402.
- Arnedo, M.A., Coddington, J.A., Agnarsson, I., Gillespie, R.G., 2004. From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. *Mol. Phylogenet. Evol.* 31, 225–245.
- Astrin, J.J., Huber, B.A., Misof, B., Klütsch, C.F.C., 2006. Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. *Zoologica Scr.* 35, 441–457.
- Ayoub, N.A., Garb, J.E., Hedin, M., Hayashi, C.Y., 2007a. Utility of the nuclear protein-coding gene, elongation factor-1 gamma (EF-1 $\gamma$ ), for spider systematics, emphasizing family level relationships of tarantulas and their kin (Araneae: Mygalomorphae). *Mol. Phylogenet. Evol.* 42, 394–409.
- Ayoub, N.A., Garb, J.E., Tinghitella, R., Collin, M., Hayashi, C.Y., 2007b. Blueprint for a high-performance biomaterial: Full-length spider dragline silk genes. *PLoS ONE* 2, e514.
- Banks, J.C., Whitfield, J.B., 2006. Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. *Mol. Phylogenet. Evol.* 41, 690–703.
- Beuken, E., Vink, C., Bruggeman, C.A., 1998. One-step procedure for screening recombinant plasmids by size. *BioTechniques* 24, 748–750.
- Bruvo-Madaric, B., Huber, B.A., Steinacher, A., Pass, G., 2005. Phylogeny of pholcid spiders (Araneae: Pholcidae): Combined analysis using morphology and molecules. *Mol. Phylogenet. Evol.* 37, 661–673.
- Coddington, J.A., 2005. Phylogeny and classification of spiders. In: Ubick, D., Paquin, P., Cushing, P.E., Roth, V.D. (Eds.), *Spiders of North America: An Identification Manual*. American Arachnological Society, pp. 18–24.
- Crosby, M.A., Goodman, J.L., Strelets, V.B., Zhang, P., Gelbart, W.M., The FlyBase Consortium, 2007. FlyBase: genomes by the dozen. *Nucleic Acids Res.* 35, D486–D491.
- da Silva Vaz Jr., I., Imamura, S., Nakajima, C., Cardoso, F.C.d., Ferreira, C.A.S., Renard, G., Masuda, A., Ohashi, K., Onuma, M., 2005. Molecular cloning and sequence analysis of cDNAs encoding for *Boophilus microplus*, *Haemaphysalis longicornis* and *Rhipicephalus appendiculatus* actins. *Vet. Parasitol.* 127, 147–155.
- Danforth, B.N., Fang, J., Sipes, S.D., 2006. Analysis of family-level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. *Mol. Phylogenet. Evol.* 39, 358–372.
- Donald, K.M., Kennedy, M., Spencer, H.G., 2005. The phylogeny and taxonomy of austral monodontine topshells (Mollusca: Gastropoda: Trochidae), inferred from DNA sequences. *Mol. Phylogenet. Evol.* 37, 474–483.
- Friedlander, T.P., Regier, J.C., Mitter, C., 1992. Nuclear gene sequences for higher level phylogenetic analysis: 14 promising candidates. *Syst. Biol.* 41, 483–490.
- Garb, J.E., DiMauro, T., Vo, V., Hayashi, C.Y., 2006. Silk genes support the single origin of orb webs. *Science* 312, 1762.
- Garb, J.E., Hayashi, C.Y., 2005. Modular evolution of egg case silk genes across orb-weaving spider superfamilies. *Proc. Natl. Acad. Sci. USA* 102, 11379–11384.
- Gatesy, J., Hayashi, C.Y., Motriuk, D., Woods, J., Lewis, R., 2001. Extreme diversity, conservation, and convergence of spider silk fibroin sequences. *Science* 291, 2603–2605.
- Hardy, N.B., 2007. Phylogenetic utility of dynamin and triose phosphate isomerase. *Syst. Entomol.* 32, 396–403.
- Hausdorf, B., 1999. Molecular phylogeny of araneomorph spiders. *J. Evol. Biol.* 12, 980–985.
- Hedin, M., Bond, J.E., 2006. Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): conflict and agreement with the current system of classification. *Mol. Phylogenet. Evol.* 41, 454–471.
- Hedin, M.C., Maddison, W.P., 2001. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). *Mol. Phylogenet. Evol.* 18, 386–403.
- Horigane, M., Ogihara, K., Nakajima, Y., Honda, H., Taylor, D., 2007. Identification and expression analysis of an actin gene from the soft tick, *Ornithodoros moubata* (Acari: Argasidae). *Arch. Insect Biochem. Physiol.* 64, 186–199.
- Huber, K.C., Haider, T.S., Müller, M.W., Huber, B.A., Schweyen, R.J., 1993. DNA sequence data indicates the polyphyly of the family Ctenidae (Araneae). *J. Arachnol.* 21, 194–201.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Maddison, W.P., Hedin, M.C., 2003. Jumping spider phylogeny (Araneae: Salticidae). *Invert. Syst.* 17, 529–549.
- Maddison, W.P., Needham, K.M., 2006. Lapsiines and hisponines as phylogenetically basal salticid spiders (Araneae: Salticidae). *Zootaxa* 1255, 37–55.
- Maddison, W.P., Zhang, J.X., Bodner, M.R., 2007. A basal phylogenetic placement for the salticid spider *Eupoa*, with descriptions of two new species (Araneae: Salticidae). *Zootaxa* 1432, 23–33.
- Murphy, N.P., Framenau, V.W., Donnellan, S.C., Harvey, M.S., Park, Y.-C., Austin, A.D., 2006. Phylogenetic reconstruction of the wolf spiders (Araneae: Lycosidae) using sequences from the 12S rRNA, 28S rRNA, and NADH1 genes: Implications for classification, biogeography, and the evolution of web building behavior. *Mol. Phylogenet. Evol.* 38, 583–602.
- Platnick, N.I., 2007. The world spider catalog, version 8.0. American Museum of Natural History. Available from: <<http://research.amnh.org/entomology/spiders/catalog/index.html>>.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Su, K.F., Meier, R., Jackson, R.R., Harland, D.P., Li, D., 2007. Convergent evolution of eye ultrastructure and divergent evolution of vision-mediated predatory behaviour in jumping spiders. *J. Evol. Biol.* 20, 1478–1489.
- Swofford, D.L., 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Van Troys, M., Vandekerckhove, J., Ampe, C., 1999. Structural modules in actin-binding proteins: towards a new classification. *Biochim. Biophys. Acta* 1448, 323–348.
- Vink, C.J., Mitchell, A.D., Paterson, A.M., 2002. A preliminary molecular analysis of phylogenetic relationships of Australasian wolf spider genera (Araneae: Lycosidae). *J. Arachnol.* 30, 227–237.
- Vink, C.J., Thomas, S.M., Paquin, P., Hayashi, C.Y., Hedin, M., 2005. The effects of preservatives and temperatures on arachnid DNA. *Invert. Syst.* 19, 99–104.