

Barcoding generalist predators by polymerase chain reaction: carabids and spiders

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Abstract

Identification of arthropod predators is challenging when closely related species are found at a given locality. Identification of the immature stages is especially problematic, because distinguishing morphological features are difficult to use or have not been described. We used polymerase chain reaction (PCR) to distinguish closely related carabids and spiders, and to match eggs and larvae (or nymphs) with identified adult parents. Within the Carabidae, we amplified species-specific mitochondrial cytochrome oxidase I (COI) fragments for three species each in the genera *Poecilus* and *Harpalus*, and two each in *Chlaenius* and *Bembidion*. Within the Araneae, we amplified species-specific COI fragments for two *Hibana* species (Anyphaenidae), *Pardosa milvina* and *Rabidosa rabida* (Lycosidae), *Frontinella communis* and *Grammonota texana* (Linyphiidae), and *Cheiracanthium inclusum* (Miturgidae). We are able to correctly identify all immature stages tested — eggs, larvae (or nymphs) and pupae — by comparison of the amplified fragments with those of the adults. Using COI markers as species identifiers is a tenet of the Barcode of Life initiative, an international consortium to provide a molecular identifier for every animal species.

Keywords: Araneae, Barcode of Life, Carabidae, Cytochrome Oxidase I, Immature predators, PCR

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Introduction

Generalist predators play a major role in biological control of agricultural pests (Symondson *et al.* 2002). Nevertheless determining their significance in any given situation is technically challenging. Detecting arthropod predation is inherently difficult, since it involves small, secretive animals, many of which have sucking mouthparts and, hence, no formed prey parts in their guts (Greenstone & Morgan 1989). Until recently, most gut analysis has been by serological

methods, with monoclonal antibody-based assays representing the state of the art (Greenstone 1996; Symondson *et al.* 1999; Harwood *et al.* 2004). Within the past 5 years, however, polymerase chain reaction (PCR; Ehrlich 1989) amplification of prey DNA has begun to displace monoclonal antibody technology as the state of the art, due to the ease of designing primers and disseminating the technology to other practitioners, who can have their own primer sets developed for a fraction of the time and cost of a monoclonal antibody (Zaidi *et al.* 1999; Chen *et al.* 2000; Hoogendoorn & Heimpel 2001). Advances in assay technology are increasing the versatility and rapidity of this approach (Harper *et al.* 2005).

Although agro-ecosystems are usually simpler than less-managed systems, they can be surprisingly complex (Greenstone & Sunderland 1999), often hosting groups of closely related arthropod predators that may be difficult to distinguish morphologically. Identification of the immature stages is even more problematic than for the adults,

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because they lack genitalic characters, and other distinguishing morphological features are difficult to find or have not been described.

An alternative to morphological characters is molecular markers, many of which have been well characterized and are abundant in animal tissues. Appropriate markers for our purposes are DNA sequences, which remain constant throughout the life of an animal.

Using PCR, DNA can be amplified from even the very smallest arthropods. Since the sensitivity of PCR depends upon the number of copies of the target sequence in the sample, multicopy sequences are preferred. Multiple copies of a given DNA sequence are found throughout the genome, variously arrayed among chromosomes in the nucleus or within cellular organelles. Both within and among individual animals and populations, these sequences may be identical or variable to greater or lesser degree; they also display varying rates of evolution (Hillis & Dixon 1991; Elder & Turner 1995; Hoy 2003). Mitochondrial sequences (mtDNA) are present as hundreds or thousands of copies per cell (Hoy 2003); a catalogue of conserved primers (Simon *et al.* 1994) makes it a simple matter to 'fish out' specific mtDNA regions for sequencing to support specific primer design. mtDNAs usually evolve faster than nuclear DNA because mitochondria lack a proofreading mechanism to correct errors in DNA synthesis (Hoy 2003). These high rates of evolution make them particularly well suited for distinguishing closely related and rapidly evolving populations.

One particular mtDNA sequence, cytochrome oxidase I (COI), has been proposed as a universal barcode for all animal species (see <http://www.barcodinglife.com>), and has already been used as an identifier for numerous insect taxa (Zaidi *et al.* 1999; Wells & Sperling 2001; Wells *et al.* 2001; Agustí *et al.* 2003; Ashfaq *et al.* 2004; Hogg & Hebert 2004; Hufbauer *et al.* 2004; Sheppard *et al.* 2004; Harper *et al.* in press). Here we show that partial COI sequences reliably distinguish a number of common agro-ecosystem carabids and spiders, including groups of congeners, and enable unambiguous assignment of immature carabids and spiders to species.

Materials and methods

Predators

The carabid *Poecilus cupreus* (L.) from populations in Braunschweig and Sagerheide, Germany, was reared on pupae of *Calliphora vomitoria* (L.) as described earlier (Heimbach 1992); unfed adults were utilized for sequencing. *Poecilus chalcites* (Say) from Champaign, Illinois, USA, were fed on grass seed and artificial diet (Lundgren *et al.* 2005). The spiders *Hibana arunda* Platnick, *Hibana futilis* Banks, *Cheiracanthium inclusum* (Hentz) and *Grammonota texana*

Banks were collected at the research farm of the USDA-ARS, Kika de la Garza Subtropical Agricultural Research Center, in Weslaco, Texas, USA, and maintained as laboratory colonies (R.S.P., unpublished). Briefly, spiders were placed into 15 × 90 mm Petri dishes that contained a piece of cotton dental wick soaked in distilled water to provide moisture and were subsequently fed eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). All spiders were kept in environmental chambers at 25 ± 1 °C and 60 ± 20% RH at a 15:9 photoperiod. The spiders were starved for 24 h before being frozen for sequencing.

Additional carabids and spiders were collected from crop fields in Braunschweig, Champaign, Weslaco, or at the USDA-ARS Beltsville Agricultural Research Center in Beltsville, Maryland, USA; *Rabidosia rabida* (Walckenaer) and *Achaearanea tepidariorum* (C.L. Koch), spiders known from other crop field situations (Young *et al.* 1989; Young & Lockley 1994), were collected at nearby sites (Table 1). *Lebia grandis* Hentz (GenBank Accession no. AY574580) and *Coleomegilla maculata* (De Geer) (AY615732) were collected from potato fields infested with *Leptinotarsa decemlineata* (Say). *Lebia grandis* adults were housed in 2-L ventilated plastic containers (18 × 13 × 10 cm deep) in growth chambers under a 16:8 L : D photoperiod with relative humidity 50 ± 10% and temperature 25 ± 1 °C for the 8-h scotophase ramped over 5 h to 30 °C, held for 6 h in the middle of the photophase, and ramped down the final 5 h of photophase, and fed 1.5–3 third-instar *L. decemlineata* reared on foliage of *Solanum tuberosum* (L.) every 2 days. *Coleomegilla maculata* were maintained under a 16:8 L : D photoperiod with relative humidity 50 ± 10% at 24 ± 1 °C in 6 oz. ice cream cups (Sweetheart Cup Co.). First and second instar larvae were fed *H. zea* eggs and bee pollen substitute (BPS; Betterbee, Inc.) and eggs of *L. decemlineata* reared on foliage of *S. tuberosum*; older larvae and adults were fed eggs of *L. decemlineata* and BPS.

To provide context and broaden taxon sampling, we added sequences of additional taxa that had been previously deposited in GenBank by other investigators (Table 2). In order to evaluate the utility of barcoding for matching immatures and adults, we collected carabid and spider eggs from the laboratory colonies and hatched and raised the immature stages. Eggs were also collected from field-caught females of the carabid *Bembidion nigriceps* (Kirby) kept individually on soil in the laboratory, and allowed to develop into larvae. Additionally, spiderlings were collected directly into 95% EtOH from webs of wild-caught *A. tepidariorum* females, egg cases of wild-caught *Pardosa milvina* (Hentz) females, and the abdomen of a wild-caught *R. rabida* female.

DNA procedures

Animals were partially homogenized in 0.5 or 1.5 mL mortar-and-pestle centrifuge tubes in 100–400 µL of high-

Table 1 Collecting localities for carabids and spiders sequenced in this study

Species	Locality	Habitat
Carabidae		
<i>Agonum octopunctatum</i>	Champaign, IL, USA	Crop field
<i>Agonum palustre</i>	Champaign, IL, USA	Crop field
<i>Agonum punctiforme</i>	Champaign, IL, USA	Crop field
<i>Amara sinuosa</i>	Champaign, IL, USA	Crop field
<i>Anisodactylus sanctaecrusis</i>	Champaign, IL, USA	Crop field
<i>Bembidion nigriceps</i>	Champaign, IL, USA	Crop field
<i>Bembidion rapidum</i>	Champaign, IL, USA	Crop field
<i>Chlaenius nemoralis</i>	Champaign, IL, USA	Crop field
<i>Chlaenius pusillus</i>	Champaign, IL, USA	Crop field
<i>Chlaenius tricolor</i>	Champaign, IL, USA	Crop field
<i>Clivina bipustulata</i>	Champaign, IL, USA	Crop field
<i>Cyclotrachelus seximpressus</i>	Champaign, IL, USA	Crop field
<i>Harpalus herbivagus</i>	Champaign, IL, USA	Crop field
<i>Harpalus indianis</i>	Champaign, IL, USA	Crop field
<i>Harpalus indigens</i>	Champaign, IL, USA	Crop field
<i>Harpalus pennsylvanicus</i>	Champaign, IL, USA	Crop field
<i>Lebia grandis</i>	Beltsville, MD, USA,	Crop field
<i>Poecilus chalcites</i>	Champaign, IL, USA	Crop field
<i>Poecilus cupreus</i>	Braunschweig and Sagerheide, Germany	Crop field
<i>Poecilus lucublandus</i>	Champaign, IL, USA	Crop field
<i>Pterostichus melanarius</i>	Champaign, IL, USA	Crop field
<i>Zabrus tenbrioides</i>	Braunschweig, Germany	Crop field
Araneae		
<i>Achaearanea tepidariorum</i>	Ellicott City, MD, USA	Suburban garage
<i>Cheiracanthium inclusum</i>	Weslaco, TX, USA	Crop field
<i>Frontinella communis</i>	Ellicott City, MD, USA	Deciduous forest remnant
<i>Grammonota texana</i>	Weslaco, TX, USA	Crop field
<i>Hibana arunda</i>	Weslaco, TX, USA	Crop field
<i>Hibana futilis</i>	Weslaco, TX, USA	Crop field
<i>Pardosa milvina</i>	Beltsville, MD; Champaign, IL, USA	Crop field
<i>Rabidosa rabida</i>	Beltsville, MD, USA	Laboratory

salt extraction buffer (Aljanabi & Martinez 1997) supplemented with 2% SDS and 400 µg/mL proteinase K and digested at 65 °C overnight. DNA precipitates were resuspended in 50–600 µL (depending upon the size of the specimen) of sterile diH₂O or 0.1 × TE pH 8.0. Preliminary PCRs (30 µL) for nucleotide sequencing of COI utilized primers C1-J-1751 and C1-N-2191 (Simon *et al.* 1994) per Promega's protocol with modifications: the addition of 0.1 µL of 20 mg/mL RNaseA (Gibco BRL), and 1% polyvinylpyrrolidone (Fisher Scientific) and 0.2% bovine serum albumin (Sigma) to the PCR cocktail (Xin *et al.* 2003) after the primers and before the *Taq* polymerase; and reduced annealing temperatures (37–40 °C) and elevated Mg⁺⁺ concentration (to 3.75 mM) to allow for imperfect matches. Initial denaturation was for 3 min at 94.5 °C, followed by 40 cycles of 45 s at 94.5 °C, 1 min at 37 °C, and 2 min at 72 °C; 5 min at 72 °C completed the program. The success of the reaction was checked by electrophoresis of 6 µL of the PCR/stop reaction in 1.5% agarose in 0.5 × TAE. The remainder of the reaction was loaded and the fragments

for sequencing were excised from 1.5% NuSieve agarose (Cambrex Bio Science Rockland Inc.) run in 1 × TAE modified to have a final EDTA concentration of 0.1 mM. Sequencing was by BigDye Terminator version 3.1 kits on an ABI 3100 sequencer (Applied Biosystems). Editing, alignments and primer design were performed with LASERGENE (DNASStar).

Primer sequences and annealing temperatures (60 s) for the species-specific PCRs are given in Table 3. In order to resolve some nearly identical sequences, we performed restriction length polymorphism (RFLP) analysis of amplified fragments using *Xba*I and *Bpm*I (New England Biolabs) digestions for 6 h at 37 °C according to the manufacturer's protocol. Total digest volume was 20 µL.

Phylogenetic analysis

Nucleotide characters were equally weighted and analysed by maximum parsimony (MP) in PAUP 4.0b10 (Swofford 2002), with *C. maculata* (Coccinellidae) and *Hypochilus thorelli* (Hypochilidae) as outgroups for the carabids and spiders,

Table 2 GenBank Accession nos for additional carabid and spider sequences

Species	Number
Carabidae	
<i>Pterostichus mutus</i>	AY165644
<i>Carabus albrecthi</i>	AB095260
<i>Carabus japonicus</i>	AB095262
<i>Ceroglossus darwini</i>	AB109857
<i>Scaphinotus viridis</i>	AB109806
<i>Scaphinotus bilobus</i>	AB109808
<i>Cychrus koiwayai</i>	AB109848
<i>Cychrus caraboides</i>	AB109837
<i>Nebria sahlbergii</i>	AB109837
<i>Nebria haida</i>	AF095197
Araneae	
<i>Artoria separata</i>	AY059993
<i>Artoria flavimanus</i>	AY059992
<i>Anoteropsis okatainae</i>	AY059984
<i>Anoteropsis canescens</i>	AY059970
<i>Xysticus</i> sp.	AY297423
<i>Salticus scenicus</i>	AY297416
<i>Poultonella alboimmaculata</i>	AY32800
<i>Tutelina hartii</i>	AY328013
<i>Sassacus</i> sp.	AY328010
<i>Hibana</i> sp.	AY297422
<i>Cheiracanthium</i> sp.	AY297421
<i>Castianeira</i> sp.	AY297419
<i>Anelosimus</i> sp.	AY231055
<i>Theridion</i> cf. <i>frondeum</i>	AY231061
<i>Latrodectus mirabilis</i>	AY383076
<i>Argyrodes trigonum</i>	AY231048
<i>Euryopsis funebris</i>	AY231042
<i>Coelotes atropos</i>	AY138838
<i>Nephila maculata</i>	AY052597
<i>Nephila maculata</i>	AY052591
<i>Tegenaria saeva</i>	AY138830
<i>Nephila clavata</i>	AY052586
<i>Tidarren sisyphoides</i>	AY231067
<i>Erigone atra</i>	AY383537
<i>Neriere radiata</i>	AY078696
<i>Pityohyphantes costatus</i>	AY078695
<i>Frontinella communis</i>	AY078692
<i>Argiope argentata</i>	AY231021
<i>Lepthyphantes minutus</i>	AY078689
<i>Castianeira</i> sp.	AY297419
<i>Hypochilus thorelli</i>	AY102048

respectively. Most parsimonious trees (MPT) were inferred by heuristic MP searches using 1000-replicate searches implementing the random stepwise-addition and tree-bisection–reconnection (TBR) options. Assignments of carabids to subfamily, and spiders to family, follow Lawrence & Newton (1995) and Platnick (1997), respectively.

Results

COI sequences derived in this research were deposited in GenBank (nos AY574578, DQ05982–816 and DQ063219–

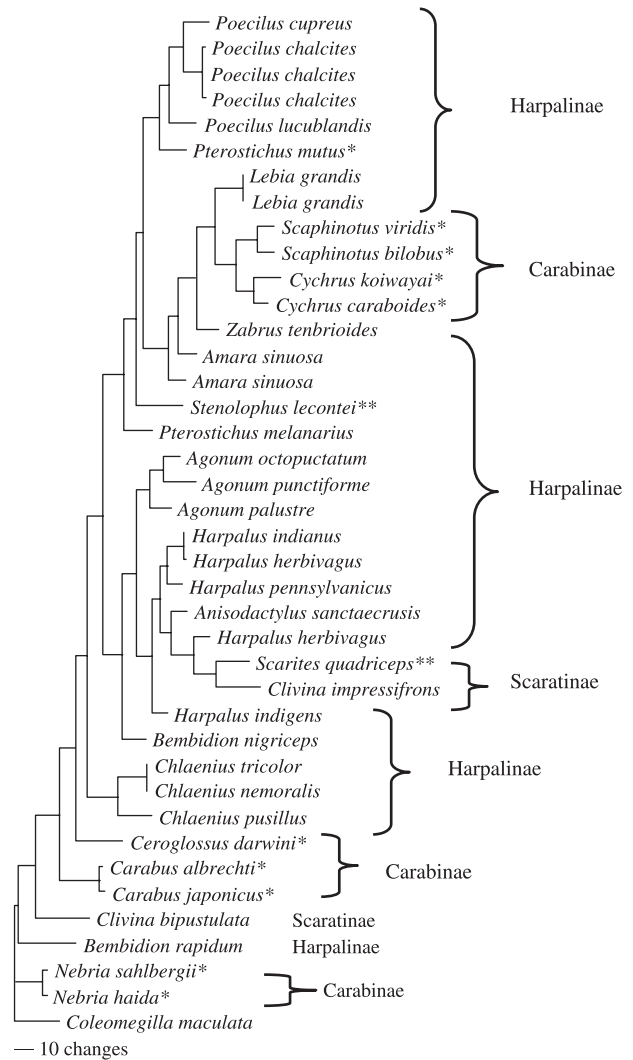


Fig. 1 One of six most parsimonious trees for Carabidae. The tree is a phylogram (branch lengths proportional to number of character state changes). Species marked by an asterisk identify previously published GenBank sequences; those marked by two asterisks are represented by both GenBank and original sequences. Subfamily assignments from Lawrence & Newton (1995).

223 for carabids; DQ029215–40 and DQ072280 for spiders); alignments are shown in Appendices 1 and 2 (for previously published sequences in GenBank that did not use the same forward primer, the starting number refers to the position in the authors' published sequences). The most parsimonious trees (Figs 1 and 2) constructed with these sequences show that conspecifics and congeners tend to cluster as expected: three of the four carabid species for which we have multiple sequences clustered, as did six of the 10 carabid genera for which we have more than one species sequence (Fig. 1); and all four of the spider species for which we have multiple sequences (*Pardosa milvina* not shown), and both spider genera for which we have more than one species sequence, clustered (Fig. 2).

Table 3 Specific primer sequences

Genus	Species	T_a	Primer sequence	Size
Carabidae				
<i>Bembidion</i>	<i>nigriceps</i>	59	F: ATTAATGAGAAGATTAGTAGAAAGTGA R: ACTGGTAAAGATAGTAATAATAATAAAGCA	266
<i>Bembidion</i>	<i>rapidum</i>	59	F: TATTTTGTAGTCTTCATTTAGCAGGTGTA R: CCGACAGATCAAACAAATAGAGGT	128
<i>Chlaenius</i>	<i>nemoralis</i>	63	F: TGAACAGTGTACCTCCTTTATCTT R: ATCTCCTCCTCCTGCTGGGT	305
<i>Chlaenius</i>	<i>pusilus</i>	63	F: GTTTTTGACTTCTTCCTCCTTCTCT R: TTGATGAAACTCCTGCTAAATGA	180
<i>Harpalus</i>	<i>pennsylvanicus</i>	63	F: TTTTTTTTTTTTGTAGAAGTATAGTGAAAGAGGGG R: TCCTACTGATCAAACAAATAATGG	236
<i>Harpalus</i>	<i>herbivagus</i>	63	F: AAGAGGAGCTGGGACCG R: TCCTACTGATCAAACAAATAATGG	210
<i>Harpalus</i>	<i>indigenus</i>	63	F: AGTTTTTGATTACTTCCGCC R: TCCTACTGATCAAACAAATAATGG	266
<i>Poecilus</i>	<i>cupreus</i>	60	F: TCTTTTAAATGAGAAGTATGGTAGAA R: TGCTAATACAGGTAATGAAAGAAG	276
<i>Poecilus</i>	<i>chalcites</i>	60	F: GTGGAGCAGGGACAGGTT R: TGCTAATACAGGTAATGAAAGAAG	250
<i>Poecilus</i>	<i>lucublandis</i>	60	F: TGAACAGTCTACCCACCCC R: TGCTAATACAGGTAATGAAAGAAG	233
<i>Pterostichus</i>	<i>melanarius</i>	60	F: GTGGAGCTGGTACTGGATG R: GATGATACTCCGGCTAAATGTA	111
Araneae				
<i>Frontinella</i>	<i>communis</i>	60	F: GGTGGAGCTGGGTGAA R: AATCAAATAAATGTTGAAATAAAATA	417
<i>Grammonota</i>	<i>texana</i>	60	F: TCTAGTATGGATGAAATGGGAGTG R: TAATACAGATCAAACAAATAAAGGAAC	209
<i>Hibana</i>	<i>futilus</i>	59	F: ATTTGAGATTTTGATTGTTACCACCAT R: TAAAACAGATCAAACAAATAAAGGAA	272
<i>Hibana</i>	<i>arunda</i>	59	F: GAGTTGGAGCGGTTGG R: CTGCTAAAACAGGTAAAGATAACA	249
<i>Cheiracanthium</i>	<i>inclusum</i>	59	F: AGATTTTGACTTTTACCTCCTTC R: GAAAAAATAGCAAATCAACAGA	146
<i>Pardosa</i>	<i>milvina</i>	59	F: TCTTTCTTTTGTGATTACTACCTCCTTC R: AAAAAACCAAATAAATGCTGAAAT	417
<i>Rabidosia</i>	<i>rabida</i>	59	F: GGCTTCTAGAGTAGGTATATAGGGAGTT R: ATACAGGTAAAGAAAGCAATAATAAACAG	209

T_a , annealing temperature. Size, size of amplicon in bp.

To test whether we could distinguish closely related species, we ran multiplex PCRs with the species-specific primers in Table 3 against members of the same subfamily (carabids) or family (spiders). We also included in these reactions species that have similar-looking immatures, or similar sequences (by inspection), regardless of traditional taxonomic assignment.

Among the carabids, we are able to distinguish congeners of *Bembidion*, *Chlaenius*, *Poecilus* and *Pterostichus* by size differences in the COI fragments alone (Figs 3 and 4). However, *Anisodactylus sanctacrusis* and *Harpalus pennsylvanicus* had indistinguishable fragment sizes. In order to resolve these

species, we digested the COI PCR products individually with the restriction enzymes *Xba*I and *Bmp*I. These enzymes did not have sites in the PCR product of *H. herbivagus* but did produce different-sized fragments in *A. sanctacrusis* (*Bmp*I, 148 and 88 bp), *H. pennsylvanicus* (*Xba*I, 166 and 170 bp), and *Harpalus indigenus* (*Bmp*I, 178 and 88 bp) (Fig. 5).

Among the spider species tested, COI fragment size differences alone were sufficient to distinguish the two *Hibana* species from one another and from *Cheiracanthium inclusum*, which has similar-looking spiderlings, and also to distinguish members of two other families, Lycosidae (*P. milvina* and *R. rabida*), and Linyphiidae (*Grammonota texana* and

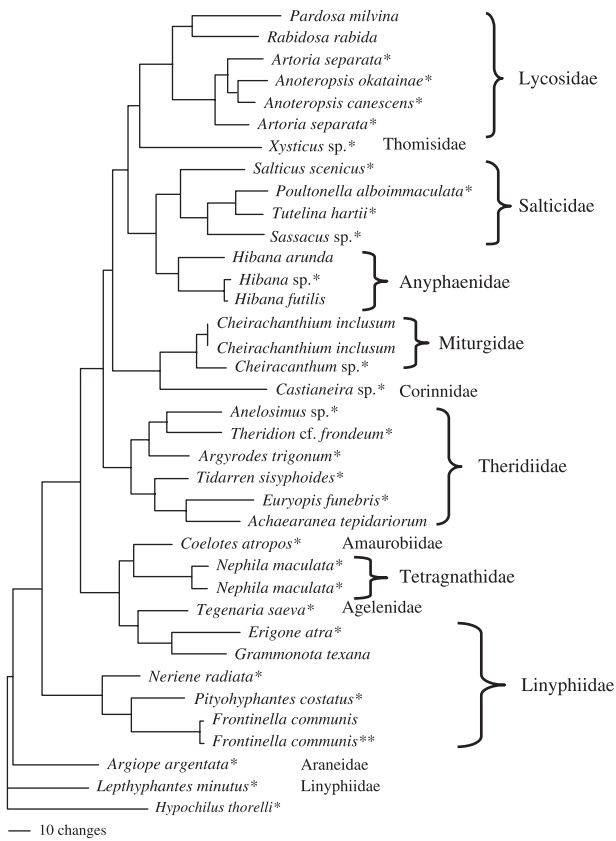


Fig. 2 Most parsimonious tree for Araneae; notation as in Fig. 1. Family assignments from Platnick (1997).

Frontinella communis) (Figs 6–8). *Achaearanea tepidariorum*, a theridiid that was assayed with the linyphiids because of sequence similarity, was amplified by the linyphiid primer pair, but produced a band 20 bp smaller than that for *G. texana*. This difference is sufficient to distinguish them, but they could be further differentiated by RFLP analysis using *Bse*RI, for which there is a restriction site on the *G. texana* fragment but not that of *A. tepidariorum* (data not shown).

As expected, the immatures of all species tested had identical species-specific sequences to the adults. Among the carabids we had immatures for *Poecilus cupreus*, *Poecilus chalcites*, and *Bembidion nigriceps* (Figs 3 and 4), and among the spiders, for the two *Hibana* species, *C. inclusum*, *P. milvina*, *R. rabida*, and *G. texana* (Figs 6–8). With few exceptions most specimens of feeding stages had been starved before preservation, been fed on plant material, or were collected as hatchlings before there was opportunity to feed (see Materials and methods). In the few instances where this was not the case, agreement in sequence and band size between adults and immatures of a species confirms that the results represent predator rather than prey DNA.

Discussion

The terminal clades on our gene trees generally correspond to sequences extracted from specimens identified on the basis of morphological characters as coming from populations of the same species, or from populations of congeneric species, which is necessary if COI sequences are to enable

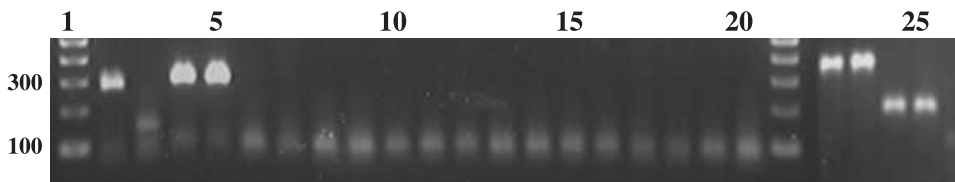


Fig. 3 Multiplex PCR to separate species of *Bembidion* and *Chlaenius*. Lanes 1 and 21, 100 bp ladder (Invitrogen). Lane 2, *Bembidion nigriceps* adult. Lane 3, *Bembidion rapidum* adult. Lanes 4 and 5, *Bembidion nigriceps* larvae. Lane 6, *Stenolophus lecontei* adult. Lane 7, *Scarites quadriceps* adult. Lane 8, *Clivina impressifrons* adult. Lane 9, *Agonum palustre* larva. Lane 10, *Agonum punctiforme* adult. Lanes 11 and 12, *Chlaenius nemoralis* adults. Lanes 13 and 14, *Chlaenius pusillus* adults. Lanes 15 and 16, *Harpalus herbivagus* adults. Lanes 17 and 18, *Harpalus pennsylvanicus* adults. Lane 19, *Harpalus indigenus* adult. Lane 20, no-DNA control. Lane 22 and 23, *C. nemoralis* adults. Lanes 24 and 25, *C. pusillus* adults. Lane 26, *Clivina impressifrons* adult.

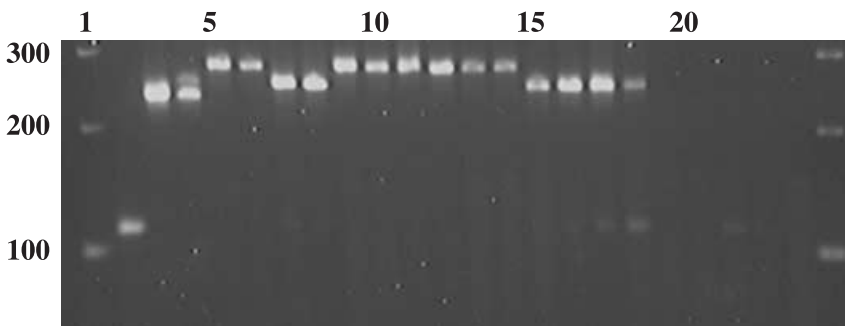


Fig. 4 Multiplex PCR to separate species of *Poecilus* and *Pterostichus*. Lanes 1 and 24, 100 bp ladder. Lane 2, *Pterostichus melanarius* adult. Lanes 3 and 4, *Poecilus lucublandus* adults. Lanes 5 and 6, *Poecilus cupreus* adults. Lanes 7 and 8, *Poecilus chalcites* adults. Lanes 9 and 10, *P. cupreus* pupae. Lanes 11 and 12, *P. cupreus* larvae. Lanes 13 and 14, *P. cupreus* eggs. Lanes 15 and 16, *P. chalcites* larvae. Lanes 17 and 18, *P. chalcites* eggs. Lanes 19 and 20, *Harpalus herbivagus* adults. Lanes 21 and 22, *Harpalus pennsylvanicus* adults. Lane 23, no-DNA control.

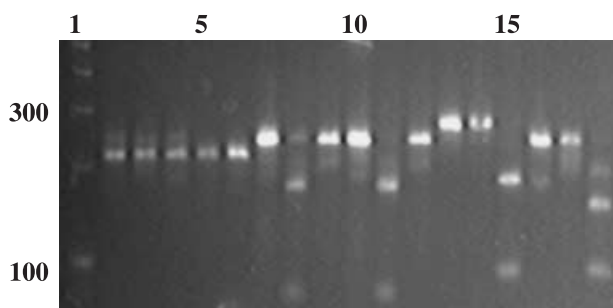


Fig. 5 Multiplex PCR to separate *Harpalus* and *Anisodactylus* species. Lane 1, 100 bp ladder. Lane 2, *Harpalus herbivagus* adult. Lane 3, *H. herbivagus* adult digested with *Xba*I. Lane 4, *H. herbivagus* adult digested with *Bpm*I. Lanes 5 and 6, *H. herbivagus* adults. Lanes 7 and 10, *Harpalus pennsylvanicus* adults. Lane 8 and 11, *H. pennsylvanicus* adults digested with *Xba*I. Lanes 9 and 12, *H. pennsylvanicus* adults digested with *Bmp*I. Lane 13, *H. indigens* adult. Lane 14, *Harpalus indigens* adult digested with *Xba*I. Lane 15, *H. indigens* adult digested with *Bmp*I. Lane 16, *Anisodactylus sanctaecrucis* adult. Lane 17, *A. sanctaecrucis* adult digested with *Xba*I. Lane 18, *A. sanctaecrucis* adult digested with *Bmp*I.

the correct placement of an unknown specimen (Paquin & Hein 2004). Our limited data therefore suggest that these short COI fragments will be useful for barcoding carabids and spiders, although other sequences may prove more

useful depending on the evolutionary histories of taxa to be distinguished (e.g. Hedin 1997a, b; Hedin & Maddison 2001a; Hormiga *et al.* 2003).

Phylogenetic reconstruction is not a goal of this study, nor should it be expected that the relatively short (439 bp) partial sequence we used, of a rapidly evolving region such as COI, would necessarily provide accurate deep phylogenetic resolution. Indeed it is clear by inspection of Figs 1 and 2 that COI is unable to address deeper phylogenetic questions such as the composition of carabid subfamilies or of spider families. We wish to emphasize that COI barcoding is a diagnostic tool, specifically, an identifier for animal species; it cannot be expected to serve double duty as a character for deeper phylogenetic reconstruction. Indeed, no single gene can be expected to resolve phylogenies at all levels of interest (Maddison *et al.* 1999). Rather, molecular phylogenetic inference should be based on several sets of more slowly evolving sequences (Hedin & Maddison 2001b; Ober 2002; Arnedo *et al.* 2004), informed by sets of morphological characters.

Molecular barcodes for predators have a number of potential uses. With the increase of international trade and tourism, rates of movement and establishment of exotic species are also increasing. Although we tend to think of invasive species as being primarily pests, natural enemies may also invade, posing the possibility of disrupting ecosystem

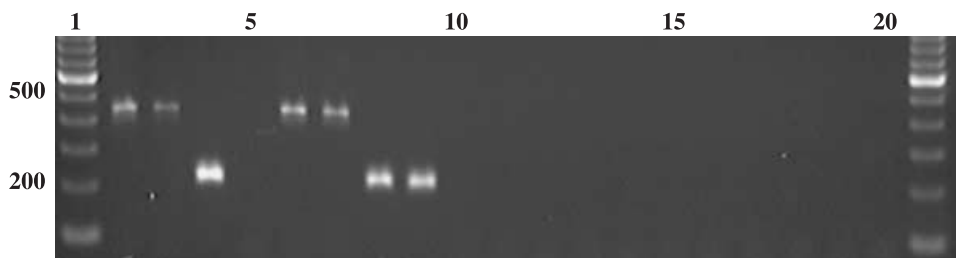


Fig. 6 Multiplex PCR to identify lycosid spiders. Lanes 1 and 21, 100 bp ladder. Lanes 2 and 3, *Pardosa milvina* male and female. Lane 4, *Rabidosia rabida* female. Lane 5, *Cheiracanthium inclusum* male. Lanes 6 and 7, *P. milvina* spiderlings. Lanes 8 and 9, *R. rabida* spiderlings. Lanes 10 and 11, *C. inclusum* spiderlings. Lanes 12 and 13, *Achaearanea tepidariorum* spiderlings. Lanes 14 and 15, *Grammonota texana* spiderlings. Lanes 16 and 17, *Hibana futilis* spiderlings. Lanes 18 and 19, *Hibana arunda* spiderlings. Lane 20, no-DNA control.

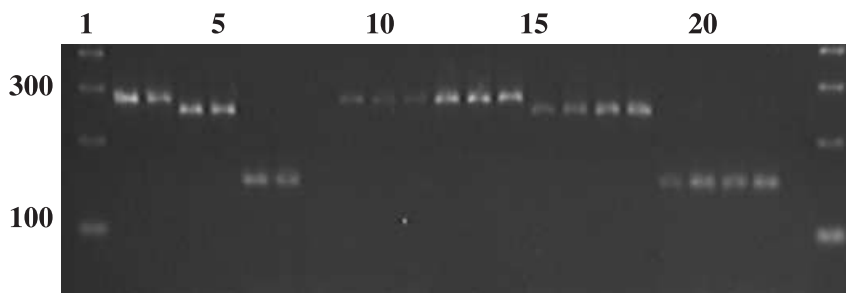


Fig. 7 Multiplex PCR to identify *Hibana* and *Cheiracanthium* species. Lanes 1 and 24, 100 bp ladder. Lanes 2 and 3, *Hibana futilis* males. Lanes 4 and 5, *Hibana arunda* male and female. Lanes 6 and 7, *Cheiracanthium inclusum* males. Lane 8, *Pardosa milvina* male. Lanes 9 and 10, *H. futilis* eggs. Lanes 11–14, *H. futilis* spiderlings. Lanes 15 and 16, *H. arunda* eggs. Lanes 17 and 18, *H. arunda* spiderlings. Lanes 19 and 20, *C. inclusum* eggs. Lanes 21 and 22, *C. inclusum* spiderlings. Lane 23, no-DNA control.

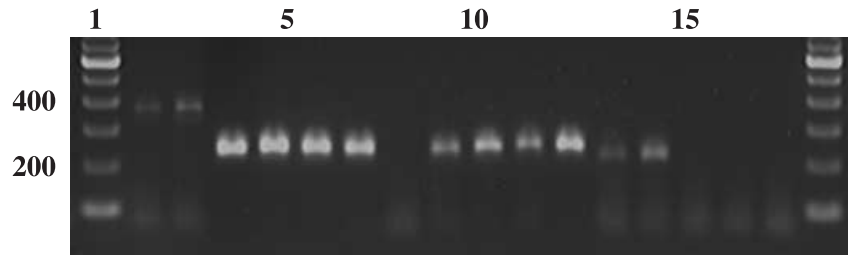


Fig. 8 Multiplex PCR to identify linyphiid spiders. Lanes 1 and 18, 100 bp ladder. Lanes 2 and 3, *Frontinella communis* females. Lanes 4 and 5, *Grammonota texana* females. Lanes 6 and 7, *G. texana* males. Lane 8, *Cheiracanthium inclusum* male. Lanes 9 and 10, *G. texana* eggs. Lanes 11 and 12, *G. texana* spiderlings. Lanes 13 and 14, *Achaearanea tepidariorum* spiderlings. Lanes 15 and 16, *C. inclusum* spiderlings. Lane 17, no-DNA control.

processes (e.g. Elliott *et al.* 1996). As with *Poecilus chalcites* and *Poecilus cupreus* in the present study, many important agroecosystem predators belong to genera that are Holarctic or even cosmopolitan in distribution, and barcoding may enable discovery and tracking of invading predator species.

Predator barcoding may also lead to discovery of previously unknown sequences, thence through careful morphological studies to new previously undescribed species, thereby helping to build up a more accurate picture of biodiversity (e.g. Hogg & Hebert 2004). For agroecosystem ecologists, the most important application for barcoding will be specific identification of predators collected in the field. Inability to accurately identify the myriad predators in agroecosystems has led to an under-appreciation of their role in suppressing insect pest populations. Since arthropods that are predators as adults are often also predators as immatures, our inability to identify immatures has produced a major gap in our knowledge of predator-prey dynamics. This is all the more troubling because densities of immatures may rival and even exceed those of adults in the field. Although this seems intuitively obvious, it is difficult to demonstrate because quantitative sampling methods have not been much used, and because the immatures are in some cases more difficult to sample than the adults. Most published 'censuses' of epigeal carabids and spiders, for example, have been by pitfall trapping, which is not quantitative since captures vary with weather, activity, and the differential catchability of species and stages (Topping & Sunderland 1992; Greenstone 1998) and whereas immature spiders tend to be found on the surface along with the adults, larval carabids may be largely fossorial (Nordhuis *et al.* 2001), with significant portions of the population found below 10 or even 20 cm (Andersen 1997; Thomas 2002).

Nevertheless some quantitative data sets do exist. In a study of predators of corn rootworm (*Diabrotica undecimpunctata howardi* Barber) eggs, Brust & House (1990), using soil cores taken to a depth of 15 cm, found carabid and staphylinid larval populations to be similar to those of adults (by inspection of their Fig. 2) across the season and under different cropping regimes. Holland *et al.* (2004), using a

motorized suction sampler to census cereal aphid predators in wheat, found that larvae represented between 19% and 64%, and 34% to 76%, of all carabids and staphylinids, respectively, collected over four summer sampling dates. In a suction sampling study of cotton interplanted with lucerne, Godfrey & Leigh (1994) found that nymphal heteropteran predators of the genera *Geocoris*, *Orius*, and *Nabis*, often equaled or exceeded those of adults in a variety of treatments.

For this test of the feasibility of barcoding arthropod predators, we deliberately chose species that are numerically important in cropping systems throughout North America. Two of those that we sequenced, *Stenolophus lecontei* and *Scarites quadriceps*, had identical sequences to those already published in GenBank. The two widely separated *Pardosa milvina* populations we sampled from Beltsville and Champaign also had identical sequences, as did the two German *P. cupreus* populations. On the other hand, some species had more than one COI haplotype in GenBank or among species we sampled and sequenced (see Figs 1 and 2). In order for DNA barcoding to be proven useful as a diagnostic tool, we shall have to sample and sequence across the geographical range of a number of carabid and spider species to determine whether within-species haplotype variation allows unambiguous identification and differentiation of species (Sperling 2004).

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Appendix 1

Alignments for carabid sequences. Sequences beginning with place numbers > 1 are from GenBank accessions derived utilizing different forward primers

	10	20	30	40	50	60	70	80	90								
1	TCGAATAAATAATATAAGTTT	TTTGACTACTTCC	CCCTTCTTTA	ACTCTCTTTT	TAATGAGAAGTATGGT	FAGAAAGTGGAGCAGGAACAGG				<i>Poecilus cupreus</i>							
1	T.....	T.....	C.....	A.....	G.....	<i>Poecilus chalcites</i>							
1	T.....	T.....	C.....	A.....	G.....	<i>Poecilus chalcites</i>							
1	T.....	T.....	C.....	A.....	G.....	<i>Poecilus chalcites</i>							
1	T.....	T.....	T.A.....	A.....	A.....	G.....	T.....	<i>Poecilus lucublandis</i>							
219	C.....	T.....	T.....	A.....	A.....	T.....	C.....	T.....	<i>Pterostichus mutus</i>						
1	C.....	A.....	GT.A.....	A.....	A.....	C.....	A.....	AC.A.....	T.....	A.....	T.....	<i>Lebia grandis</i>				
1	C.....	A.....	GT.A.....	G.....	A.....	C.....	A.....	AC.A.....	T.....	A.....	T.....	<i>Lebia grandis</i>				
235	A.....	T.GT.A.....	T.....	A.....	A.....	G.....	A.....	AT.A.....	G.....	A.....	T.....	T.....	<i>Scaphinotus viridis</i>			
235	A.....	GT.....	T.A.....	A.....	A.....	A.....	T.A.....	G.....	A.....	A.....	C.....	A.....	T.....	T.....	<i>Scaphinotus bilobus</i>	
235	A.....	A.....	A.....	T.....	G.....	A.....	C.....	AC.T.....	A.....	T.....	A.....	A.....	G.....	C.....	T.....	T.....	<i>Cychrus koivayai</i>
235	A.....	C.....	A.....	C.....	C.....	C.....	AC.T.....	T.....	A.....	G.....	A.....	T.....	T.....	T.....	T.....	<i>Cychrus caraboides</i>
1	T.....	T.A.....	T.....	GC.T.....	CT.A.....	A.....	A.....	A.....	T.....	A.....	T.....	T.....	T.....	T.....	<i>Zabrus tenebrioides</i>
1	A.....	T.....	A.....	A.....	A.....	A.....	A.....	A.....	T.....	T.....	T.....	T.....	T.....	T.....	<i>Amara sinuosa</i>
1	T.....	A.....	A.....	T.....	<i>Amara sinuosa</i>
1	T.....	G.....	G.....	A.....	CC.T.....	C.....	AT.A.....	A.....	A.....	T.....	T.....	T.....	T.....	T.....	<i>Stenolophus lecontei</i>
1	T.....	T.....	T.....	A.....	A.....	T.....	T.....	T.....	T.....	T.....	T.....	<i>Pterostichus melanarius</i>
1	C.....	T.....	T.....	A.....	G.....	AT.A.....	A.....	A.....	T.....	T.....	T.....	T.....	<i>Agonum octopunctatum</i>
1	T.....	T.....	T.....	A.....	A.....	C.....	A.....	A.....	G.....	T.....	T.....	C.....	<i>Agonum punctiforme mix</i>
1	T.....	T.....	A.....	A.....	CC.A.....	G.....	T.....	G.....	<i>Agonum palustre</i>
1	T.....	T.....	T.....	GC.....	A.....	A.....	A.....	A.....	T.....	G.....	C.....	<i>Harpalus indianus</i>
1	T.....	T.....	T.....	GC.....	A.....	A.....	A.....	A.....	T.....	G.....	C.....	<i>Harpalus herbivagus</i>
1	T.....	T.....	T.....	C.....	A.....	G.....	A.....	G.....	T.....	G.....	T.....	<i>Harpalus pennsylvanicus</i>
1	T.....	T.A.....	T.....	A.....	C.....	A.....	G.....	T.....	T.....	T.....	T.....	T.....	<i>Anisodactylus sanctaecrucis</i>
1	T.....	T.G.....	T.....	A.....	CC.....	A.....	A.....	A.....	A.....	T.....	G.....	T.....	T.....	<i>Harpalus herbivagus</i>
1	TT.A.....	A.....	C.T.....	A.....	AC.A.....	A.....	G.....	T.....	T.....	<i>Scarites quadriceps</i>
1	C.....	A.....	TT.A.....	T.....	T.A.....	A.....	G.....	A.....	A.....	T.....	G.....	<i>Clivina impressifrons</i>
1	T.....	G.....	T.A.....	A.....	A.....	T.....	T.....	T.....	<i>Harpalus indigenus</i>
1	T.....	G.....	A.....	T.A.....	AT.A.....	T.....	T.....	<i>Bembidion nigriceps</i>
1	A.....	TT.A.....	T.....	A.....	T.A.T.....	G.....	A.....	TTC.T.A.....	T.....	A.....	T.....	T.....	T.....	T.....	<i>Chlaenius tricolor</i>
1	A.....	TT.A.....	T.....	A.....	T.A.T.....	R.....	G.....	A.....	TTC.T.A.....	T.....	A.....	T.....	T.....	T.....	<i>Chlaenius nemoralis</i>
1	T.....	T.....	T.....	C.....	GC.....	A.....	T.....	T.A.....	T.....	A.....	G.....	T.....	T.....	<i>Chlaenius pusillus</i>
235	A.....	T.....	T.....	CT.AC.....	A.....	C.....	A.....	T.....	<i>Ceroglossus darvini</i>
235	A.....	T.....	G.....	T.....	G.....	CC.....	A.....	T.....	A.....	A.....	G.....	AA.....	G.....	<i>Carabus albrecti</i>
235	G.....	A.....	T.....	G.....	T.....	G.....	CC.....	A.....	T.....	A.....	A.....	G.....	AA.....	G.....	<i>Carabus japonicus</i>
1	A.....	T.....	T.....	T.....	CC.....	C.....	C.....	A.....	A.....	A.....	T.....	A.....	G.....	C.....	<i>Clivina bipustulata</i>
1	A.....	T.....	T.....	T.....	A.....	T.A.....	A.....	TTCC.....	A.....	T.....	A.....	G.....	<i>Bembidion rapidum</i>
34	C.....	T.....	T.A.....	T.....	C.....	A.....	TTCA.....	A.....	<i>Nebria sahlbergii</i>
34	T.....	T.A.....	T.....	A.....	TTCA.....	A.....	A.....	<i>Nebria haida</i>
1	<i>Coleomegilla maculata</i>

Appendix 1 Continued

	100	110	120	130	140	150	160	170	180	
91	ATGAACAGTTTATCCTCCCTATCTCTGGTATGCCCCATGCTGGAGCATCAGTTGATTTAGCTATPTTTAGTPTACATTTAGCAGGAGT									<i>Poecilus cupreus</i>
91	T.....TT.....A...T.....A.....A.....T....									<i>Poecilus chalcites</i>
91	T.....C.....TT.....A...T.....A.....A.....T....									<i>Poecilus chalcites</i>
91	T.....C.....TT.....A...T.....A.....A.....T....									<i>Poecilus chalcites</i>
91C.C.A.....A.....T.....A.....A.....T....									<i>Poecilus lucublandis</i>
309	T.....C...T...A...G...T.....T.....A.....T....									<i>Pterostichus mutus</i>
91T.C.C.C.A.T.A.A.A...A...A.....A.....C.T.....T..A									<i>Lebia grandis</i>
91T.C.C.C.A.T.A.A.A...A...A.....A.....C.T.....T..A									<i>Lebia grandis</i>
325T.A.C.C.A.T.....AA.A...T..AGA...G..A...C.T.A.....TA									<i>Scaphinotus viridis</i>
325T.....A.T.....A.....AGA..T.....A...C.T.A.....A									<i>Scaphinotus bilobus</i>
325	T...T.G.C...T.A.A.A...A...AGA...G.G.A...A.....A.....T....									<i>Cychrus koivayai</i>
325T.G.C.A...T.A.A.A...AGA.G.G...A...A.....A.....T..A									<i>Cychrus caraboides</i>
91	T.....G.C.C...TT...AAA.....A.....A.....C.T.....A									<i>Zabrus tenebrioides</i>
91TT.....A.A...T...A...T...A.....C.....A									<i>Amara sinuosa</i>
91C.....A.A.A...T.....G.T...A.....T..A									<i>Amara sinuosa</i>
91C...T.T...A.A.....AG...T.T.....A.....TA									<i>Stenolophus lecontei</i>
91C.C...TT...A.A.....T.....G.....AC.....C....									<i>Pterostichus melanarius</i>
91C.A.T...A.A.A...T...A...T.T.....A...C.T.....T....									<i>Agonum octopunctatum</i>
91A.T...A.A...C.....C...T.....A.....C.T.....T....									<i>Agonum punctiforme mix</i>
91C.A...TT...A...A.....T.....T.....A.....T..GA									<i>Agonum palustre</i>
91A...TT...A...A...T..AGA...T...A.....T..A									<i>Harpalus indianus</i>
91A...TT...A...A...T..AGA...T...A.....T..A									<i>Harpalus herbivagus</i>
91C.A...TT...A.A.A...T..AGA...T...A...A.....T..A									<i>Harpalus pennsylvanicus</i>
91	T.....C...TT...A.....A...GA...T.....A.....A.....T....									<i>Anisodactylus sanctaecrusis</i>
91C.A.....A.....GA..T.T.....C.....C.....									<i>Harpalus herbivagus</i>
91	T...T.A.C...T...A.A...T..AGA..T.T...A.....C.T.....G..									<i>Scarites quadriceps</i>
91	T.....A.C.....C.....AGA...T.T...A.....C.....T....									<i>Clivina impressifrons</i>
91C...TT...A...A.....AGA...T...A.....A.....T....									<i>Harpalus indigenus</i>
91C...TT...A...A...A...AGA...T.....C.....CC.....C....									<i>Bembidion nigriceps</i>
91G.C...TT...A.....AGA...T.T.A...C...A.....C....									<i>Chlaenius tricolor</i>
91G.C...TT...A.....AGA...T.T.A...C...A.....C....									<i>Chlaenius nemoralis</i>
91	T.....G.....TT...G.AA...T..AGA...T...A.....C.T.....									<i>Chlaenius pusillus</i>
325C.C...TT...A.A.A...T..AGA..T.T.T.A...A.....C.....A									<i>Ceroglossus darwini</i>
325A.C.C.....A.A...A.CAGA..T.....A...C.....A.....A									<i>Carabus albrechti</i>
325A.C.C.....A.A...A.CAGA..T.....A...C.....A.....A									<i>Carabus japonicus</i>
91	G.....A...T.....A.C.....AGA...T.A...C...A.....A...C.....									<i>Clivina bipustulata</i>
91	...G.C...C.C.A.T.A...TC.....AGA...C.C.C.CC.....C.T.....T..									<i>Bembidion rapidum</i>
124A.C...T...A.A.A...T..AGA...T.A...C.....C.....T....									<i>Nebria sahlbergii</i>
124A.C...TT...A.A.A...T..AGA...T.A...C.....C.....T....									<i>Nebria haida</i>
25T...C...AA.C.CC.T...AA...T.T.T.A...TA...CNA.....A									<i>Coleomegilla maculata</i>

Appendix 1 Continued

	370	380	390	400	410	420	430	
361	AAATACCTCTTTTPTTGGATCCAGCAGGAGGAGGAGATCCTATTTTATATCAACATTTATTTTGATTPTTTGGACATCCA							<i>Poecilus cupreus</i>
361T.....T.....T.....A.....							<i>Poecilus chalcites</i>
361T.....T.....T.....							<i>Poecilus chalcites</i>
361T.....T.....T.....							<i>Poecilus chalcites</i>
361T.....G..T.....T.....R..Y..							<i>Poecilus lucublandis</i>
579T..A.....C.....T..G.....							<i>Pterostichus mutus</i>
361T.....C..T.....G.....C.....T							<i>Lebia grandis</i>
361T.....Y..T.....G.....Y.....T							<i>Lebia grandis</i>
595C..A.....C.....T							<i>Scaphinotus viridis</i>
595T.....A.....C.....T							<i>Scaphinotus bilobus</i>
595T.....T..T..G.....C.....C.....T..C..							<i>Cychnus koivayai</i>
595T.....C..T..T.....C..A.....T..C..							<i>Cychnus caraboides</i>
361T.....C.....T.....C.....T.....T							<i>Zabrus tenebrioides</i>
361T..C.....T.....A.....C.....T.....T							<i>Amara sinuosa</i>
361T..A.....C..T..T.....T.....G.....T							<i>Amara sinuosa</i>
361A.....C.....C.....G.....A.....T.....							<i>Stenolophus lecontei</i>
361T..A.....C.....T.....C..G.....C.....							<i>Pterostichus melanarius</i>
361A..A.....C.....T.....A.....C..T..C.....							<i>Agonum octopunctatum</i>
361A..A.....C..C..G.....C..A.....C..T.....							<i>Agonum punctiforme</i>
361T.....C.....C.....G.....C..A.....C..T..C.....T							<i>Agonum palustre</i>
361T.....T..T.....C..A.....T.....							<i>Harpalus indianus</i>
361T.....T..T.....A.....T.....							<i>Harpalus herbivagus</i>
361T..A.....C..T.....C.....							<i>Harpalus pennsylvanicus</i>
361T.....C.....T.....C.....C..T.....T.....T							<i>Anisodactylus sanctaecrusis</i>
361T..A.....C.....C.....T.....C.....T							<i>Harpalus herbivagus</i>
361T..C.....T..T.....C..T.....C.....							<i>Scarites quadriceps</i>
361T.....T..T.....T..C.....C.....T							<i>Clivina impressifrons</i>
361T..A.....C.....T.....G.....T.....							<i>Harpalus indigens</i>
361T..C.....C.....T.....C..A.....C.....C.....T.....							<i>Bembidion nigriceps</i>
361A..A..C.....C.....C.....C.....							<i>Chlaenius tricolor</i>
361A..A..C.....C.....C.....C.....Y.....							<i>Chlaenius nemoralis</i>
361T..A.....T.....C.....C.....T.....							<i>Chlaenius pusillus</i>
595T.....T.....A.....C..T..C.....T.....T							<i>Ceroglossus darwini</i>
595T..A.....C.....G.....A.....C..T..C.....T							<i>Carabus albrechti</i>
595T..A.....C.....G.....A.....C..T.....T							<i>Carabus japonicus</i>
361T..A..C.....T.....G..G.....C..T.....T.....							<i>Clivina bipustulata</i>
361T..A.....C.....T..G.....C..A.....C..T..C.....T..C..							<i>Bembidion rapidum</i>
394A..A.....C..T.....C.....C.....C.....C.....							<i>Nebria sahlbergii</i>
394A..A.....C..T.....C.....C.....C.....C.....							<i>Nebria haida</i>
295A.....C..TATG.....T..C.....C.....C.....T..C..T							<i>Coleomegilla maculata</i>

Appendix 2

Alignments for spider sequences. Sequences beginning with place numbers > 1 are from GenBank Accessions derived utilizing different forward primers

	10	20	30	40	50	60	70	80	90	
1	TCGAATAAATAATCTTTTC	TTTTTGATTACTACCTCCTTC	TTTATTTTATTATCTATATCT	TATCTTCTATGGTGGAGATAGGGGT	TGGTCTGG					<i>Pardosa milvina</i>
1	. . . T T G A T A	<i>Rabidosia rabida</i>
13 T A . A G . G . T A A . T A . A	<i>Artoria separata</i>
1	----- T A . G T . . G A . A . A A G	<i>Anoteropsis okatainae</i>
24 A T A T A A A	<i>Anoteropsis canescens</i>
17 G T A G T A . A . A A . A . A	<i>Artoria flavimanus</i>
24 T . A G T G T A A . A . A T	<i>Xysticus</i> sp.
1	----- T T G T . A . CA A A . A . A	<i>Salticus scenicus</i>
24 T . AAGA T T T CT . A A . A . A	<i>Poultonella alboimmaculata</i>
24 T . AAGA T T T A A . CT A . G	<i>Tutelina hartii</i>
24 T . AAGA T TC T A . CT	<i>Sassacus</i> sp.
1 T . AAGC T A A T A . CA A A . G	<i>Hibana arunda</i>
1 T . AAG T A A T A . CA A A . G	<i>Hibana arunda</i>
1 T . GAGA GT A A T T YT . A A	<i>Hibana fulilis</i>
24 T . GAGA T A . A T T CT . A A A	<i>Hibana</i> sp.
1 G T . AAGA C . TT T A A A	<i>Cheiracanthium inclusum</i> A
1 G T . AAGA C . TT T R . A A A	<i>Cheiracanthium inclusum</i>
24 G T . AAGA C . TT T G A . A . A	<i>Cheiracanthium</i> sp.
24 T . GAG C . TT A T T . G A A A	<i>Castianeira</i> sp.
34	A T . AAGA G T G . A . G T T A . A . A A . A . A	<i>Anelosimus</i> sp.
34 T . AAG T C T T A . T . A A . A . AT . A	<i>Theridion</i> cf. <i>frondeum</i>
31 T . AAG G GT A G T T A . A . A	<i>Argyrodes trigonum</i>
34 T . GAG T C T T A A A	<i>Tidarren sisyphoides</i>
34 T . GAGA T A A . A T T A . A . A A . G	<i>Euryopsis funebris</i>
1 T . GAGA T C A T T . G A . A . A A . A . A	<i>Achaearanea tepidariorum</i>
1	----- T A G . T T T T . A T . A . A	<i>Coelotes atropos</i>
232 T . AAGA G T A T T . A . A A . A T . A	<i>Nephila maculata</i>
232 C T . AAGA T C A T T . A . A A . A	<i>Nephila maculata</i>
1	----- GT A G T A . T . AG T A	<i>Tegenaria saeva</i>
61 T . AAGA T A A T C AG A . AT	<i>Erigone atra</i>
1 G T . GAGA GT . G G . G T T AG AT . A	<i>Grammonota texana</i>
1 CT . AAGA T A T T T . A . CT . A T G	<i>Neriere radiata</i>
1 T . AAG T A A . G T T . A A . A A	<i>Pityohyphantes costatus</i>
238 G T . AAG G T C G T A . ATCA . A	<i>Frontinella communis</i>
1 G T . AAG G T C G T A . ATCA . A	<i>Frontinella communis</i>
34	A G T . AAGA T C C CT . AT . G A A . A . A	<i>Argiope argentata</i>
238 T . G T . AAG C . T C CC . T . A T T . AAGA . A . A . A A . A	<i>Lepthyphantes minutus</i>
1 T . AAGA T G GC . C . G G . TAC . G A . AT . AAAT AG T . A . AA . G	<i>Hypochilus thorelli</i>

Appendix 2 Continued

	100	110	120	130	140	150	160	170	180	
91	ATGAACTGTTTATCCTCCCTTAGCATCTACAGTGGACATATAGGAAGTCAATAGATTTGCTATTTTTCCTTCATTAGCTGGGGC									<i>Pardosa milvina</i>
91	T.....G..T...G..A..T.....G...T.....G.....									<i>Rabidosa rabida</i>
103	G....G.....A...T...G.A.A..T.....T.....A.....T.A.....A..T..									<i>Artoria separata</i>
85G..C.....G..T...G.A.A..T.....T.....A.....T.A.....G..T..									<i>Anoteropsis okatainae</i>
114A.....T...G.A.A..T.....T.....A.....T.G.....A..T..									<i>Anoteropsis canescens</i>
107G.....C...T...G.A.A..T.....G...T.....A.....AT.A.....A..T..									<i>Artoria flavimanus</i>
114	T.....T.....GTT.G.....A..T..G.....C...A..T..									<i>Xysticus</i> sp.
39	T....S..G.....A...TT...T...AT..G..A..TG.G.....T.A.....G..T..									<i>Salticus scenicus</i>
114	T....C..A.....A...T..A.TT..A...AT..G..A..GG.....A.....T.A.....T..									<i>Poultionella alboimmaculata</i>
114	T.....A..C..A..A...T..A.TT..A...C.AT...A..G.....A...C...T.A...G...T..									<i>Tutelina hartii</i>
114	G.....G...A..A...T...GTGA...Y...AT...A..GG.T.....T.A...G..C....									<i>Sassacus</i> sp.
91	T..G...A.....A..A...T...G...T.....T..AG.TG.....T.A.....T..									<i>Hibana arunda</i>
91	T..G...A.....A..A...T...G...T...G..T..AG.TG.....T.A.....T..									<i>Hibana arunda</i>
91	T.....A.....G.....AG.T.....AT.A.....T..									<i>Hibana futilis</i>
114	T.....A.....G.....G.....AG.T.....C.....AT.A.....T..									<i>Hibana</i> sp.
91	T.....A.....T...AGTTA.A...GCT.....TG.T.....T.A.....T.T									<i>Cheiracanthium inclusum</i>
91	T.....A.....T.R..AGTTA.A...GCT.....TG.T.....T.A.....T.T									<i>Cheiracanthium inclusum</i>
114	T.....T...AGTTA.A...GCT.....TG.C.....T.A.....T.T									<i>Cheiracanthium</i> sp.
114	G....G.....A..A...C..AGT.A.A..G..GCT...G.GG.G.....A.....T.G...G.....									<i>Castianeira</i> sp.
124GT.T...TTG.AA...TC.....TG.T.....A..A..									<i>Anelosimus</i> sp.
124A...T.T..ATT..AA...GG...A..TG.....A.....									<i>Theridion</i> cf. <i>frondeum</i>
121C.....T.T...TT..AA..T..GCT..T..A..GG.T.....C.....T.G.....T..									<i>Argyroides trigonum</i>
124A..A.....A...T.T...TT..A...T..GGT...A..TG.T.....A..C...T.A.....									<i>Tidarren sisypoides</i>
124A.....T...TT..A...G..GCT..T.A...TG.T...C..A.....T.A.....A..A..									<i>Euryopis funebris</i>
91	G.....A...A...C..T...TT..A...T..CGGT..T..A...G.....A.....T.G.....									<i>Achaearanea tepidariorum</i>
66	...G..G.....A..A..G..T...GCA.A..T..GCT...A..TG.T.....T.A.....									<i>Coelotes atropos</i>
322A.....A...T...TT..AA..T..GCT..G..G..TG.....T.A.....G..T..									<i>Nephila maculata</i>
322A.....A...T...TT..AA..T..GCT..G..A..TG.....A.....G..T..									<i>Nephila maculata</i>
66	G.....A.....C...G..T...T.TA...T...T.T..T.A..TG.T.....T.A.....									<i>Tegenaria saeva</i>
151AA.....C...T...TT..AG..T..TCT..T...TG.T.....A.....T.A..C.....T..									<i>Erigone atra</i>
91	T.....A.....C.T..T...TT..AA..G...TCT..G...GG.....T.A...G..A.....									<i>Grammonota texana</i>
91	G.....G.....T.T...TT..AA..T...TCG...A..T.....C...T.A.....A..									<i>Neriere radiata</i>
91	T.....T.T...ATT..AA...TCT..GG.A..TG.T.....T.A.....A.....									<i>Pityohyphantes costatus</i>
328	G.....G.....T.T...TT..AA..G..TC...G..A..TG.T.....T.A...G.....									<i>Frontinella communis</i>
91	G.....G...Y...T.T...TT..AA..G..TC...G..A..TG.T.....G.....T.A...G.....									<i>Frontinella communis</i>
124	T....A..A.....TGG.TTG.AA...GCT...A...G.T.....T.A...G..G....									<i>Argiope argentata</i>
328	G....G.....C...C.T..T...TT..AG..G..GCT...A..TG.....A..A..									<i>Lepthyphantes minutus</i>
90	G..G..GA...C..C...AG.GGA.TTA...CG..GC...GGT...TG...A.G.....AT.A.....G..T..									<i>Hypochilus thorelli</i>

Appendix 2 Continued

	190	200	210	220	230	240	250	260	270	
181	TTCTTCTATTATAGGAGCTGTTAAATTTTATTTCTACTATTATTAAATATACGAATATTAGGAATATCTATAGAGAAAGTTCCCTCTTTTGT									<i>Pardosa milvina</i>
181G..G.....				T.....			A..G.....		<i>Rabidosa rabida</i>
193T..AA.....	A.....			TT.....	T..A.....	A..A.....	AT.G.....		<i>Artoria separata</i>
175T..AA.....	A.....			TT..A.....	A..A.....	A..GA.....	T.A.....		<i>Anoteropsis okatainae</i>
204T..AA.....	A.....			TT..A.....	GA.A.....	A..A.....	T.A.....		<i>Anoteropsis canescens</i>
197T..A.....	A.....			TT.....		T.T.....	T.A.....		<i>Artoria flavimanus</i>
204A.....	G...A.....			GG.TG.T..G.....		A..G..G.....			<i>Xysticus</i> sp.
129A.....	G..G..A.....	AG.....		GTCTG.....		T..G.....	A.....		<i>Salticus scenicus</i>
204	A...A.....	A.....	A.....		TC.A.....		AC...T..GA.....	T.A.....		<i>Poultonella alboimmaculata</i>
204	A...A.....	G...A.....	G.....		TC.A.G.....	AT.....	T..GA.....	T.A.....		<i>Tutelina hartii</i>
204A.....	A.....	G.....		TTC.AG.A.....	AT...T..A.....	GT.A.....			<i>Sassacus</i> sp.
181A.....	A.....	A.....		TTCTG.T..T..AGA.....	A..A.....	T.A.....			<i>Hibana arunda</i>
181G...A.....	A.....	A.....		TTCTG.T..T..AGA.....	A..A.....	T.A.....			<i>Hibana arunda</i>
181A.....	A.....	A.....		TTCTG.T..T..GGA..G..A.....		T.A.....			<i>Hibana futilis</i>
204A.....	G...A.....	A.....		TTCTG.T..T..GGA..G..A.....		T.A.....			<i>Hibana</i> sp.
181GA.....				T.G..A.T.....	A..A..G..A.....	T.A.....			<i>Cheiracanthium inclusum</i>
181R..GA.....				T.G..A.T.....	A..A..G..A.....	T.A.....			<i>Cheiracanthium inclusum</i>
204G..AA.....				T.G..TA.T.....	A..A..G.....	T.A.....			<i>Cheiracanthium</i> sp.
204	C..C..A.....	G..T..G..A.....	A.....		G..GG.T..G..GAGA.....	G...A.....				<i>Castianeira</i> sp.
214G..AA.....	A..T.A.....			TTCT..T..G..AGA.....	A..T.A.....				<i>Anelosimus</i> sp.
214A..A.....	G..T.A.....			TC..AT.....	AGA.....	T..T.A.....			<i>Theridion</i> cf. <i>frondeum</i>
211A..A.....	A.....	T.A.....		TTCT.....	AGAT.....	A..A..T..T.A.....			<i>Argyrodes trigonum</i>
214A.....	G...A.....	G..T.A.....	G..TTCT..AT.....	GAGA.....	A..G..A.....	T.A.....			<i>Tidarren sisypoides</i>
214A.....	A.....	A..G..AT.A.....		TTCT..T..T..AGAT.....	A..GA..A..T.A.....				<i>Euryopsis funebris</i>
181T..G..A.....	G..AC.C.....	G..TCT..AT..G..GAGA.....			A..G.....	T.A.....			<i>Achaearanea tepidariorum</i>
156T.....	A.....	T.....		GTC..AT.....	GAG..T.T..A..G..A.....	T.A.....			<i>Coelotes atropos</i>
412A.....	G...A.....	A..A..T.A.....	G..TCG..AT..T.....			T.A.....			<i>Nephila maculata</i>
412A.....	G...A.....	A..A..T.A.....	G..TC..AT.....			G...T.A.....			<i>Nephila maculata</i>
156	A.....	A.....	G..T.G.....	TCT..A.....	A.....	A.....	T.G.....			<i>Tegenaria saeva</i>
241G...A.....	A..T.A.....	G..TGGG..AT.....		A.....	A.....	T.A.....			<i>Erigone atra</i>
181A.....	A.....	A.....		GG..AT..T..A.....	A.....	T.A.....			<i>Grammonota texana</i>
181A.....	G...A.....	A..T.A.....	GCT..AT..G.C.....	A.....	T..T.A.....				<i>Neriere radiata</i>
181G...A.....	C..A..T.A.....			GC..ATAT..TAG.....	G..A..G..GT..CT.A.....				<i>Pityohyphantes costatus</i>
418A.....	A.....	T.A.....		GG..AT..TG.T.....	G..T..A..T.....				<i>Frontinella communis</i>
181A.....	G...A.....	T.A.....		GG..AT..TG.T.....	G..T..A..T.....				<i>Frontinella communis</i>
214T..AA.....	A.....			GT.T..AT.....	A..A..G.....	T.A.....			<i>Argiope argentata</i>
418G..AA.....	A..C.....			GG.CAT..AG.....	T..A..GA.....	T.A.....			<i>Lepthyphantes minutus</i>
180G..GA.....	A.....	G..T..AAG..GG.CAG.....		A.....	G...T.A.....				<i>Hypochilus thorelli</i>

Appendix 2 Continued

	280	290	300	310	320	330	340	350	360	
271	TTGATCAGTATTAAATTACTGCAGTATTATTATTACTTTCTTTTACCTGTATTAGCAGGGGCTATTACCATATTATTGACAGATCGAAATTT									<i>Pardosa milvina</i>
271	G.....G.....T..T.....G.....T.....T.....A..G.....									<i>Rabidosa rabida</i>
283T..T.....TA.T.....T.A.....T.....T.....									<i>Artoria separata</i>
265G..T..T.....TA.T.....G..A.....T.....T.....A.....									<i>Anoteropsis okatainae</i>
294T..T.....T..T.....GT.A..A.....T.....T.....A.....									<i>Anoteropsis canescens</i>
287	A.....T..T.....TA.T.....T.A..A.....T.....T.....A.....G..A.....									<i>Artoria flavimanus</i>
294T..T.....A..TA.T..C.TC.TT.A..C.T..T.....A.....A.....C.T..T..G....									<i>Xysticus</i> sp.
219	A..G.....TG.....TA.TC.T..G..T.A...G.....T.....T.....T.....T..C.....									<i>Salticus scenicus</i>
294	A.....A.G.....G..T.....G.....A..T.....T.....T.....C.A.T.....									<i>Poultonella alboimmaculata</i>
294	A.....A.A.....T.....T.....A..T.....T..T.....T.....A..T.....									<i>Tutelina hartii</i>
294	G...T..A.....T.A..A.....T..A...T.....A..T.....									<i>Sassacus</i> sp.
271T.....G..A.T.....GT.A.....T.....T.....T.....A..T.....T.....									<i>Hibana arunda</i>
271T.....G..A.T.....A.....T.....T.....T.....A..T.....T.....									<i>Hibana arunda</i>
271T..T.....G..G..T.....T.A.....A.....T..G...A..T.....									<i>Hibana futilis</i>
294T..T.....G..G..T.....T.A.....A.....T..G...A..T.....									<i>Hibana</i> sp.
271	A.....T..T.....A..T.....G.....A.....T..T.....T.....A..T.....									<i>Cheiracanthium inclusum</i>
271	A.....T..T.....A..T.....Y.G.....A.....T..T.....T.....A..T.....									<i>Cheiracanthium inclusum</i>
294	A.....T..T.....TA.T..C..C..T.G.....A...G..T..T.....T..G.....T.....									<i>Cheiracanthium</i> sp.
294T..T.....T...G...G.....G.....T.....T.....A..G...T.....									<i>Castianeira</i> sp.
304G..G...C...GT.A.....T.....A.....A..T.....									<i>Anelosimus</i> sp.
304	A.....T.....T.....GT.A..A.....T.....T..T.....T.....A..T.....									<i>Theridion</i> cf. <i>frondeum</i>
301T.....G..T..T.....T.A..C.T..T.....T.....T.....A..T.....T.....									<i>Argyrodes trigonum</i>
304	A.....T.....T.....T.A.....A.....T.....T.....T.....									<i>Tidarren sisypoides</i>
304	A.....G.....T.CT.....C..T.A..A...A..T...G..A.....T.....A.....									<i>Euryopsis funebris</i>
271	A.....TC.....T..GC.....T.A..A...A.....T.....T.....A..T.....									<i>Achaearanea tepidariorum</i>
246T.....TT.....T.A.....G..T.....A...G..A..T..									<i>Coelotes atropos</i>
502	A.....T.....C...C.T.....A...A.....T..T..A..C..A...G..A..T.....									<i>Nephila maculata</i>
502	A.....T.....G.....T...C.T.....A...A.....T..T..A...A...A..T.....									<i>Nephila maculata</i>
246	G...T..T.....TA.T.....G..T.A.....T.....A..G..G..A..T..									<i>Tegenaria saeva</i>
331	A..G..T..T.....A..T..C.G.....T.A.....GC.T...A...C..T..GC.T..A..T.....									<i>Erigone atra</i>
271T.....C..A..T..T.....T.A.....A...G..T..T.....T..C.T..A..T.....									<i>Grammonota texana</i>
271T.....A..T..C.T.....A.....T.....A.....A..C..A..T..T.....									<i>Neriere radiata</i>
271TA.T.....T..CC.T.....T.G.....T.....T.....T..C...A..T.....									<i>Pityohyphantes costatus</i>
508	A.....T.....A..T..TC.T..C.TT.A..C.....T.....T.....T...G...T.....									<i>Frontinella communis</i>
271	A.....T.....A..T..TC.T..C.YT.A..C.....T.....T.....T...G...T.....									<i>Frontinella communis</i>
304T..T..G...A..T..TC.T.....T.A.....G.....T..A...T..GC.T..A..T.....									<i>Argiope argentata</i>
508T.....G..T..T..G...T.A.....T...G..A..A...A...GC.T..T.....									<i>Leptyphantes minutus</i>
270	G...T..TC.....G...GC.TC.C...T.A.....G..T..G..T.....T...C.GC.T..T.....									<i>Hypochilus thorelli</i>

Appendix 2 Continued

	370	380	390	400	410	420	430	
361	TAATACTTCTTTTTTTGATCCTGCTGGAGGAGGAGATCCTATTTTATTTCAGCATTATTTTGGTTTTTTGGTCATCCA							<i>Pardosa milvina</i>
361A.A.T.G.....G.....A.....A.....A.....T							<i>Rabidosa rabida</i>
373A.....G.T.....A.....A.....G.....A.....T							<i>Artoria separata</i>
355C.....A.....A.....A.....A.....T							<i>Anoteropsis okatainae</i>
384G.....A.....A.....A.....T							<i>Anoteropsis canescens</i>
377G.....A.....A.....A.....							<i>Artoria flavimanus</i>
384C.....A.G.....A.....A.....A.....T							<i>Xysticus</i> sp.
309A.....G.....T.....G.....A.....A.....G							<i>Salticus scenicus</i>
384C.....G.....G.....A.....A.....A.....A.....T							<i>Poultonella alboimmaculata</i>
384C.....G.....G.....A.....A.....A.....A.....T							<i>Tutelina hartii</i>
384A.....T.T.....A.....A.....A.....T							<i>Sassacus</i> sp.
361A.....C.....A.....G.....A.....A.....A.....							<i>Hibana arunda</i>
361A.....C.....A.....G.....A.....A.....A.....							<i>Hibana arunda</i>
361A.G.T.G.T.....A.....A.....A.....T							<i>Hibana futilis</i>
384A.G.T.G.T.....A.....A.....A.....T							<i>Hibana</i> sp.
361T.....A.....A.....A.....T							<i>Cheiracanthium inclusum</i>
361T.....A.....A.....A.....Y.T							<i>Cheiracanthium inclusum</i>
384A.....A.....A.....A.....A.....T							<i>Cheiracanthium</i> sp.
384	C.....A.A.....G.G.....G.....G.C.A.....G.....A.....T							<i>Castianeira</i> sp.
394A.....A.....A.....A.....A.....G.....T							<i>Anelosimus</i> sp.
394A.....A.G.G.G.....G.....A.....A.....T							<i>Theridion</i> cf. <i>frondeum</i>
391A.....A.....A.....A.....G.....T							<i>Argyrodes trigonum</i>
394G.....G.T.....A.....A.....A.....G							<i>Tidarren sisypoides</i>
394A.....T.....A.....G.....A.....							<i>Euryopsis funebris</i>
361A.....A.....T.....G.....C.A.....T							<i>Achaearanea tepidariorum</i>
325T.....							<i>Coelotes atropos</i>
592C.....T.....							<i>Nephila maculata</i>
325T.....							<i>Nephila maculata</i>
421C.....T.....G.G.T.....G.G.....A.....A.....G.C...							<i>Tegenaria saeva</i>
361AT.G.G.....G.....G.C.....C.A.....A.....T							<i>Erigone atra</i>
361C.....C.....A.....A.....A.....A.....T							<i>Grammonota texana</i>
361A.A.....G.....A.....A.....A.....T							<i>Neriere radiata</i>
598C.....G.G.....G.....A.....							<i>Pityohyphantes costatus</i>
361C.....G.....G.....A.....A.....							<i>Frontinella communis</i>
394	C.....A.....T.....T.T.....A.....A.....A.....							<i>Frontinella communis</i>
598A.....C.G.A.G.....G.....G.....A.....							<i>Argiope argentata</i>
360CT.A.....C.....C.....A.G.G.....A.....G.....C.G.....A.....C.....T							<i>Lepthyphantes minutus</i>
								<i>Hypochilus thorelli</i>