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The black widow spider genus *Latrodectus* (Araneae: Theridiidae): phylogeny, biogeography, and invasion history

Jessica E. Garb,[a,*] Alda González,[b] and Rosemary G. Gillespie[a]

[a] *Department of Environmental Science, Policy and Management, University of California, Berkeley, 201 Wellman Hall, Berkeley, CA 94720-3112, USA*

[b] *Centro de Estudios Parasitologicos y de Vectores (CEPAVE), Facultad de Ciencias Naturales y Museo, 1900 La Plata, Argentina*

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Abstract

The spider genus *Latrodectus* includes the widely known black widows, notorious because of the extreme potency of their neurotoxic venom. The genus has a worldwide distribution and comprises 30 currently recognized species, the phylogenetic relationships of which were previously unknown. Several members of the genus are synanthropic, and are increasingly being detected in new localities, an occurrence attributed to human mediated movement. In particular, the nearly cosmopolitan range of the brown widow, *Latrodectus geometricus*, is a suspected consequence of human transport. Although the taxonomy of the genus has been examined repeatedly, the recognition of taxa within *Latrodectus* has long been considered problematic due to the difficulty associated with identifying morphological features exhibiting discrete geographic boundaries. This paper presents, to our knowledge, the first phylogenetic hypothesis for the *Latrodectus* genus and is generated from DNA sequences of the mitochondrial gene cytochrome *c* oxidase subunit I. We recover two well-supported reciprocally monophyletic clades within the genus: (1) the *geometricus* clade, consisting of *Latrodectus rhodesiensis* from Africa, and its is sister species, the cosmopolitan *L. geometricus*, and (2) the *mactans* clade containing all other *Latrodectus* species sampled, including taxa occurring in Africa, the Middle East, Iberian Peninsula, Australia, New Zealand, and North and South America. Recovery of the *geometricus* and *mactans* clades is consistent with previous designations of species groups within the genus based on female genitalic morphology. All *L. geometricus* sampled, consisting of specimens from Africa, Argentina, North America, and Hawaii, were recovered as a strongly supported monophyletic group with minimal amounts of genetic divergence, corroborating the hypothesis that human transport has recently expanded the range of this species.
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1. Introduction

The widow spider genus *Latrodectus* Walckenaer, 1805 (Araneae: Theridiidae) has a worldwide distribution, occurring across multiple continents and oceanic islands. *Latrodectus* includes a suite of species commonly referred to as black widow spiders (e.g., the North American *L. mactans* (Fabricius, 1775) and *L. tredecimguttatus* (Rossi, 1790) in Europe), frequently recognized by their red abdominal "hour-glass" mark, as well as the Australian red-back spider (*Latrodectus hasselti* Thorell, 1870) and the cosmopolitan brown widow (*L. geometricus* C.L. Koch, 1841). Members of the genus are notorious due to the highly potent neurotoxin α-latrotoxin contained in their venom, which triggers massive neurotransmitter release upon injection in vertebrates (Orlova et al., 2000). *Latrodectus* spiders are generalist predators known to feed on insects, crustaceans, other arachnids, and on small vertebrates including lizards, geckos, and mice (Forster, 1995; Hódar and Sánchez-Piñero, 2002), and this broad diet may in part explain the presence of a vertebrate specific toxin in their venom. Several *Latrodectus* species are synanthropic, i.e., associated with human habitats, often found in urban areas around houses, garden sheds, and barns (Muller, 1993; Smithers, 1944), as well as in agricultural areas (Costello and Daane, 1998; Muller, 1993). Because of their affiliation with modified landscapes and possession of α-latrotoxin, members of the *Latrodectus* genus are among the few spiders that cause medically significant bites. In humans, *Latrodectus* bites

* Corresponding author. Fax: 1-510-642-7428.
E-mail address: garb@nature.berkeley.edu (J.E. Garb).

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most commonly result in severe muscle pain, cramps, and nausea but are only occasionally fatal (Maretic, 1983; Muller, 1993). The Australian red-back spider, *L. hasselti*, is also well known for its sexual cannibalism, as females often consume males during copulation following the stereotyped self-sacrifice "somersault" behavior performed by the male (Andrade, 1996, 1998; Andrade and Banta, 2001, 2002; Forster, 1992).

Interest in *Latrodectus* spiders, probably due to their medical importance, has generated a substantial amount of effort towards their taxonomy (Ábalos and Báez, 1967; Cambridge, 1902a,b; Chamberlin and Ivie, 1935; Dahl, 1902; Kaston, 1970; Knoflach and van Harten, 2002; Levi, 1959, 1983; Levy and Amitai, 1983; Lotz, 1994; McCrone and Levi, 1964; Melic, 2000; Smithers, 1944). Thirty species of *Latrodectus* are currently recognized (Platnick, 2003). However, the taxonomy of the genus has experienced a chaotic history, reflecting the great difficulty associated with recognizing discrete morphological boundaries between members of the genus. In early studies, slight variation in characters such as somatic coloration, presence and shape of abdominal patterns, and setae length and abundance, were used in diagnosing species (Cambridge, 1902a,b; Dahl, 1902; Smithers, 1944). More recently, Levi (1959) examined a large series of specimens across wide geographic ranges and found that much of the variation in these characters is continuous. Emphasizing the value of genital morphology rather than setae and coloration for species recognition, he consolidated the 22 species recognized at the time into six (Levi, 1959). Several of the previously recognized species were synonymized with *L. mactans*, creating a nearly cosmopolitan species spanning North and South America, Africa, Asia, and Australia. Subsequent revisions, particularly in South America (Ábalos and Báez, 1967), Africa (Lotz, 1994), and the Iberian Peninsula (Melic, 2000) resurrected some of the formerly synonymized species and described several new species (Ábalos and Báez, 1967; Lotz, 1998; Mackay, 1972; Melic, 2000). However, the genus has not been revised on a worldwide scale since Levi (1959). While the taxonomic delimitations of species in the genus have been examined repeatedly, phylogenetic relationships among members of *Latrodectus* remain unstudied. The considerable confusion regarding the taxonomic definitions and uncertainty of relationships within *Latrodectus* highlights the need for a rigorous assessment of the phylogenetic relationships within and between these species.

Although *Latrodectus* is worldwide in its distribution, human transport has undoubtedly widened the range of some species in the genus as a consequence of their affinity for disturbed habitats. Certain members of the genus are increasingly being detected in new and distantly separated localities. For example, it appears certain that several species have recently been introduced to Hawaii (Pinter, 1980), Japan (Ono, 1995), Southern California (J. Kempf, pers. comm.), Australia (Raven and Gallon, 1987), and to New Zealand (Forster, 1992; Raven and Gallon, 1987), where they have been intercepted in association with imported goods arriving from different countries at post-border quarantine facilities (Reed and Newland, 2002). Establishing the extent to which some species have spread as a consequence of human movement is especially difficult in cases where a species is described subsequent to range extension. In particular, the brown widow, *L. geometricus* has an extremely widespread distribution (see Fig. 1). While it is clear that *L. geometricus* has been recently introduced to Hawaii (Pinter, 1980), Japan (Ono, 1995), Australia (Forster and Forster, 1999; Raven and Gallon, 1987), and Southern California (J. Kempf, pers. comm.), it is uncertain what portion of its remaining distribution (including Africa, parts of North America, and South America) constitutes its native range, as it was documented in both South America and Africa at the time of its description in 1841. Given the difficulty of recognizing taxa within *Latrodectus*, the repeated introduction of these spiders to new localities further emphasizes the need to determine the biogeographic distribution of the genus in a phylogenetic context, particularly as such a framework may be utilized to identify invasion pathways.

An increasing number of systematic studies of spiders are incorporating molecular sequence data to investigate phylogenetic relationships among lineages within this tremendously diverse order (e.g., Arnedo et al., 2001; Bond et al., 2001; Garb, 1999; Gillespie et al., 1997; Hausdorf, 1999; Hedin, 1997a,b, 2001; Hedin and Maddison, 2001a,b; Hormiga et al., 2003; Masta, 2000; Piel and Nutt, 2000; Zehethofer and Sturmbauer, 1998). Molecular characters have proven particularly valuable for clarifying relationships among spiders when homologous morphological characters are difficult to identify, as may be the case when independent adoption of similar ecological roles leads to morphological convergence (Gillespie et al., 1997), or when organisms appear similar as a consequence of morphological stasis (Bond et al., 2001; Bond and Sierwald, 2002; Hedin, 2001). Moreover, the assumption that certain molecular characters evolve in a roughly clock-like manner, particularly on a "local" scale, permits the estimation of temporal phenomena. Thus, molecular characters may be used to estimate the relative age of clades regardless of the amount of morphological variation they exhibit.

In *Latrodectus*, the high level of intraspecific morphological variation and indistinct taxonomic boundaries make the use of molecular character data to determine phylogenetic relationships particularly appropriate. The ease with which mitochondrial gene sequences are gathered, coupled with a substantial understanding of the processes governing their evolution and utility in clarifying phylogenetic relationships among lineages of spiders makes them a practical

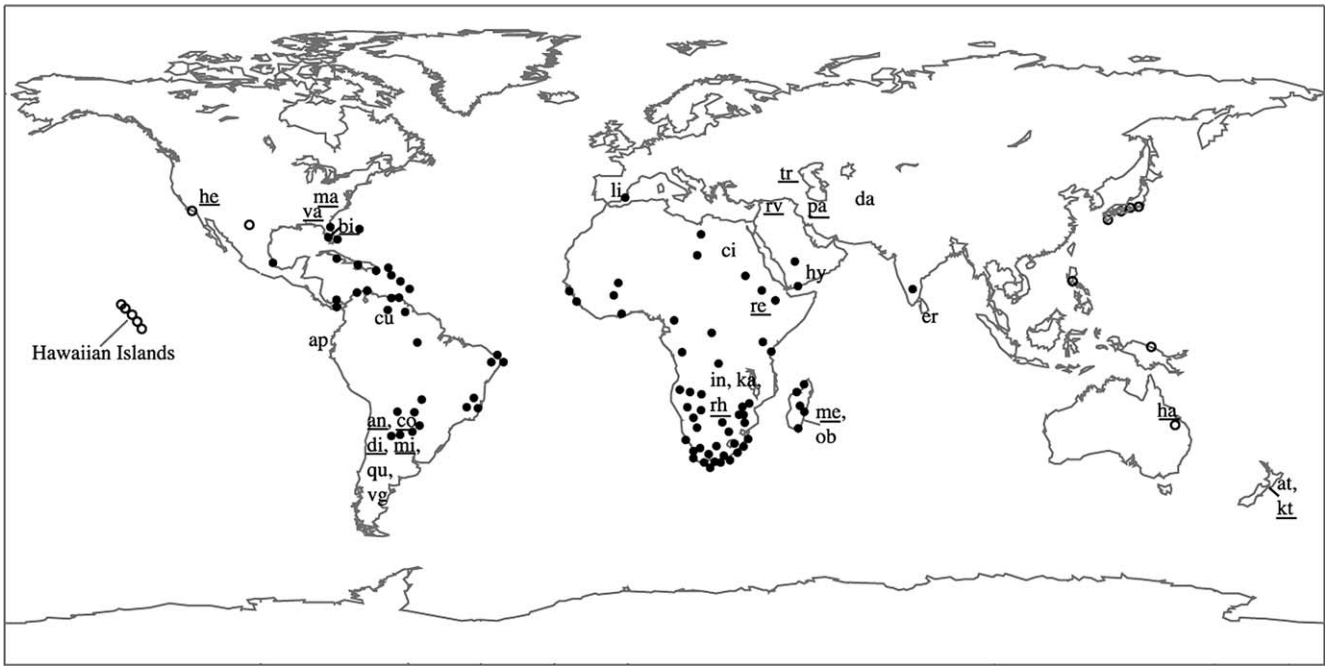


Fig. 1. Map showing distribution of *Latrodectus* species, marked at approximately the center of their known distribution. Underlined taxa are included in the current study; an, *L.antheratus*; ap, *L.apicalis*; at, *L.atritus*; bi, *L.bishopi*; ci, *L.cinctus*; co, *L.corallinus*; cu, *L.curacaviensis*; da, *L.dahli*; di, *L.diaguita*; er, *L.erythromelas*; ha, *L.hasselti*; he, *L.hesperus*; hy, *L.hystrix*; in, *L.indistinctus*; ka, *L.karooensis*; kt, *L.katipo*, li, *L.lilianae*; ma, *L.mactans*; me, *L.menavodi*; mi, *L.mirabilis*; ob, *L.obscurior*; pa, *L.pallidus*; qu, *L.quartus*; re, *L.renivulvatus*; rv, *L.revivensis*; rh, *L.rhodesiensis*; tr, *L.tredecimguttatus*; vg, *L.variegatus*; and va, *L.variolus*. Distribution of *L. geometricus* indicated by solid circles with open circles indicating sites considered human introductions based on Levi (1959), Lotz (1994), Pinter (1980), Ono (1995), Reed and Newland (2002), and J. Kempf (pers. comm.: Southern California locality).

starting point for investigating the phylogenetic relationships among species of *Latrodectus*. In this study we develop a phylogenetic hypothesis of relationships among species in the genus *Latrodectus* and representatives from closely related genera based on the mitochondrial (mt) gene cytochrome *c* oxidase subunit I (COI). In addition to evaluating relationships among species of the genus, we further sample *L. geometricus* from multiple and distantly separated localities, to assess levels of genetic divergence, because we hypothesize that limited genetic divergence exhibited within this species across continents provides corroborative evidence of its recent establishment and probable human mediated dispersal.

2. Materials and methods

2.1. Taxon sampling

The 30 recognized *Latrodectus* species and their geographic range, including the 18 sampled in this study, are listed in Appendix A. These taxa were selected to span the widest spectrum of morphological variation exhibited by members of the genus and to cover a diversity of geographic localities, including North and South America, Africa, Madagascar, the Iberian Peninsula, the Middle East, Hawaii, Australia, and New Zealand. Wherever possible we sampled two individuals per species. To examine relationships among populations of the widespread *L. geometricus*, we also included individuals from Africa, Argentina, North America, and Hawaii. Information from morphological characters suggests that the closest outgroups to *Latrodectus* are the theridiid genera *Steatoda* Sundevall, 1833 and *Crustulina* Menge, 1868 and perhaps also *Enoplognatha* Pavesi, 1880 and *Robertus* Cambridge, 1879 (Forster et al., 1990; Levi and Levi, 1962) as they all share a large colulus (a vestigial pair of spinnerets). Analyses of sequence data from the nuclear genes histone subunit H3 (H3), ribosomal 28S rRNA and 18S rRNA, as well as mitochondrial genes 16S rRNA and COI sampled across the family Theridiidae indicates that *Latrodectus* spiders share a close phylogenetic affinity with members of the genera *Steatoda* and *Crustulina*, with these three genera comprising the well-supported Latrodectinae clade (Arnedo et al., in press). COI sequences from *Crustulina* were not available for this study. Accordingly, we included representatives of *Steatoda* and *Robertus* in addition to the sampled *Latrodectus* as outgroups in our phylogenetic analysis. In summary, 43 individuals were examined in this study, representing 18 species of *Latrodectus*, 3 species of *Steatoda*, and 1 of *Robertus* (Appendix A).

2.2. DNA preparation and sequencing

Genomic DNA was extracted from 1–2 legs of each specimen using either the phenol–chloroform preparation of Palumbi et al. (1991) or the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA). The remainder of each specimen was retained as a voucher in 95% EtOH and deposited in UC Berkeley's Essig Museum (http://www.mip.berkeley.edu/essig/). Portions of COI were amplified by PCR in overlapping fragments using either universal primers LCOI 1498: 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and LCOI 2198: 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer et al., 1994), to produce a ~700 base-pair (bp) fragment, or universal primers C1-J-1718: 5′-GGA GGA TTT GGA AAT TGA TTA GTT CC-3′ and C1-N-2191: 5′-CCC GGT AAA ATT AAA ATA TAA ACT TC-3′ (Simon et al., 1994), generating a ~473 bp fragment. PCR amplifications were generated using two different thermocylcers: (1) the Bio-Rad iCycler and (2) Perkin–Elmer Applied Biosystems' GeneAmp 9700. Conditions to amplify either COI segments included an initial 95 °C denaturation of 90 s, followed by 35 cycles of 30 s at 94 °C, 40 s ranging from 45 to 55 °C, 45 s at 72 °C, followed by a final 10 min 72 °C extension. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and sequenced directly in both directions using either ABI 377 or ABI 310 automated sequencers (Applied Biosystems, Foster City, CA) with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Text and chromatogram files produced for each DNA sequence were compiled and edited in Sequencher 3.1 (Gene Codes, Ann Arbor, MI). Each text file was compared visually against chromatograms and rechecked against complementary strands. The protein-coding COI sequences were translated into their corresponding amino acids in order to identify codon positions. These sequences were easily aligned manually due to the conserved nature of their 1st and 2nd codon positions and because they contained no length variation. The final aligned data matrix consisted of 428 continuous bp of COI from the 43 sampled individuals. GenBank (www.ncbi.nlm.nih.gov) Accession numbers for each of the 43 sequences are listed in Appendix A.

2.3. Phylogenetic analyses

We tested for homogeneity among observed base frequencies (uncorrected for phylogeny) at 1st, 2nd, and 3rd codon positions as well as over the entire molecule and excluding invariant sites. Uncorrected as well as maximum likelihood (ML) estimates of sequence divergence (Yang and Kumar, 1996) were calculated for each pairwise taxon comparison. A best-fit model of sequence evolution and model parameters to calculate ML divergence was determined by evaluating nested hypotheses of evolutionary models using the likelihood ratio test as implemented in the program MODELTEST 3.06 (Posada and Crandall, 1998). Calculated divergence estimates were employed to assess levels of saturation among codon position sites of COI by plotting the number of transitions and transversions in the 1st, 2nd, and 3rd positions against the ML corrected genetic distance (Moritz et al., 1992).

The best-fit model of sequence evolution and model parameters suggested by MODELTEST, as described above, was utilized to find maximum-likelihood tree(s), employing the heuristic search algorithm in PAUP * β10 (Swofford, 2002). Searches were initiated by step-wise addition of taxa, followed by TBR branch swapping rearrangement. Because the order of taxon addition affects the ability of heuristic tree searches to find the globally optimal tree(s) (Maddison, 1991), 100 step-wise random taxon addition replicates were conducted to improve tree searches. Attempts to assess clade support using ML bootstrap pseudo-replicates were deemed too computationally time consuming. Instead, we performed Bayesian analyses to estimate clade posterior probabilities, as an alternative method to evaluate clade support using a likelihood approach (Huelsenbeck et al., 2001; Leaché and Reeder, 2002). With Bayesian analysis a greater amount of likelihood "tree space" may be quickly sampled and evaluated based on a particular nucleotide substitution model. The number of times a clade reappears in trees sampled subsequent to stabilization of likelihood values (stationarity), is the clade posterior probability (*PP*) and may be considered a measure of confidence in that clade (Huelsenbeck et al., 2001). Suzuki et al. (2002) have argued that posterior probability values overestimate statistical confidence in particular clades, while bootstrap values are more conservative measures of support. However, Wilcox et al. (2002) suggested that clade posterior probability values are also somewhat conservative measures of clade support (though far less conservative than bootstrap values) and are better estimates of phylogenetic accuracy, as the bootstrap values they computed from simulated DNA sequence data (for a given topology) more frequently failed to support correct branches as compared to posterior probability values. Using the program Mr. Bayes 3.0 (Huelsenbeck and Ronquist, 2001), likelihood tree space was explored, evaluating sampled trees based on the GTR + I + Γ model of sequence evolution with model parameters estimated during searches. Three independent searches were conducted to ensure that log likelihood values ($-\ln L$) were converging on similar levels of stationarity (Huelsenbeck and Bollback, 2001). Searches were initiated with the "random tree" option, running four Markov Chain Monte Carlo chains for 1,000,000 generations, saving a tree every 1000 generations. Following each of the three runs, likelihood values of sampled trees were plotted against generation time to determine stationarity

(Huelsenbeck and Ronquist, 2001). "Burn-in," or stabilization of likelihood values, was reached well before 100,000 generations for each independent run and converged on similar values following stabilization (range of $-\ln L$ following stationarity across three runs = 3371.51–3276.87). Thus, for each run the first 100 sampled trees were discarded and a 50% majority rule consensus of the remaining 901 trees was generated to compute clade posterior probabilities. Congruence of clade posterior probabilities across each independent run was assessed to determine whether each run, while converging on similar likelihood values, also supported similar nodes (Huelsenbeck and Imennov, 2002; Huelsenbeck et al., 2001; Leaché and Reeder, 2002).

PAUP* (Swofford, 2002) was also used to search for the most parsimonious tree(s), where all characters were treated as unweighted, reversible and unordered. Heuristic parsimony searches (MP) were conducted for 1000 step-wise random replicates followed by TBR branch swapping. Whenever multiple equally most parsimonious trees (MPTs) were recovered in a search, a strict consensus of the trees was computed. Branch support was assessed by 1000 replicates of non-parametric bootstrapping (Felsenstein, 1985) consisting of 10 random replicates each, and by calculating decay indices (Bremer support), or the number of extra steps required to collapse a branch (Bremer, 1988), using MacClade V. 4.0 (Maddison and Maddison, 2000) in concert with PAUP * (Swofford, 2002). *Robertus neglectus* (Cambridge, 1871) was used to root all resulting phylogenetic trees.

3. Results

3.1. Sequence variation

Of the 428 bp of mt COI collected for this study, 182 were variable across the sampled taxa and 134 of these 182 (73.6%) variable sites were located in 3rd codon positions, followed by 38 (20.8%) in the 1st codon positions and 10 (5.5%) in 2nd codon positions (summarized in Table 1). A χ^2 test of base homogeneity, uncorrected for phylogeny, indicated that overall base composition was not significantly different across all sites ($P = 0.99$). However, upon exclusion of invariable sites, base composition was deemed significantly heterogeneous ($P < 0.01$), and on average exhibited substantial A + T skew (A + T = 0.78). Moreover, nucleotide frequencies differed among codon sites. Base composition did not differ significantly among 1st and 2nd codon positions. However, among 3rd codon positions, base composition was heavily A + T skewed (A = 0.37, C = 0.04, G = 0.15, T = 0.45), and significantly heterogeneous ($P < 0.01$).

Scatter plots of transitions and transversions in 1st, 2nd, and 3rd codon positions against the corrected pairwise ML distance showed evidence of saturation for transitions and transversions at the 3rd codon positions beyond an ML corrected divergence of 0.5 (roughly corresponding to an uncorrected distance of ~14%; Fig. 2). Signal conflict, due to substitutional saturation, may thus account for difficulties associated with recovering levels of relationships between taxa exhibiting levels of uncorrected sequence divergence of 14% or greater. Maximal pairwise uncorrected genetic distance across all sampled taxa was 24.5%, and as much as 19.6% within the genus *Latrodectus* (Table 2).

3.2. Phylogenetic analyses

The best-fit model of sequence evolution determined by MODELTEST under the hierarchical likelihood ratio test criterion was GTR + I + Γ (Rodríguez et al., 1990; Yang et al., 1994). Estimates for the model parameters employed in heuristic maximum-likelihood searches included estimated base frequencies (A = 0.32, C = 0.07, G = 0.14, T = 0.48), rate parameter estimates ([A<>C] = 0.65; [A<>G] = 18.18; [A<>T] = 0.74; [C<>G] = 6.50; [C<>T] = 26.67; [G<>T] = 1.00), proportion of invariable sites ($I = 0.52$), and Γ-shape parameter ($\alpha = 0.668$). A heuristic maximum-likelihood search using 100 random taxon addition replicates yielded a single tree ($-\ln L = 3238.06$). In the resulting phylogram (Fig. 3), all sampled *Latrodectus* were united as

Table 1
Characteristics of cytochrome *c* oxidase subunit I in this study

Partition	# of sites	# Variable sites[a]	# PI sites[b]	Mean base frequencies				*P* (Homogeneity test)[c]
				A	C	G	T	
All sites	428	182	153	0.254	0.137	0.202	0.407	0.99
Variable sites only	182	182	153	0.354	0.049	0.175	0.422	< 0.01
1st Codon position	143	38	25	0.259	0.102	0.289	0.349	1.00
2nd Codon position	143	10	6	0.135	0.272	0.168	0.425	1.00
3rd Codon position	142	134	122	0.367	0.037	0.148	0.448	< 0.01

[a] Refers to number of variable sites in that partition.
[b] Refers to number of parsimony informative sites.
[c] Probability values resulting from χ^2 test of homogeneity, uncorrected for phylogeny, determined using PAUP* (Swofford, 2002).

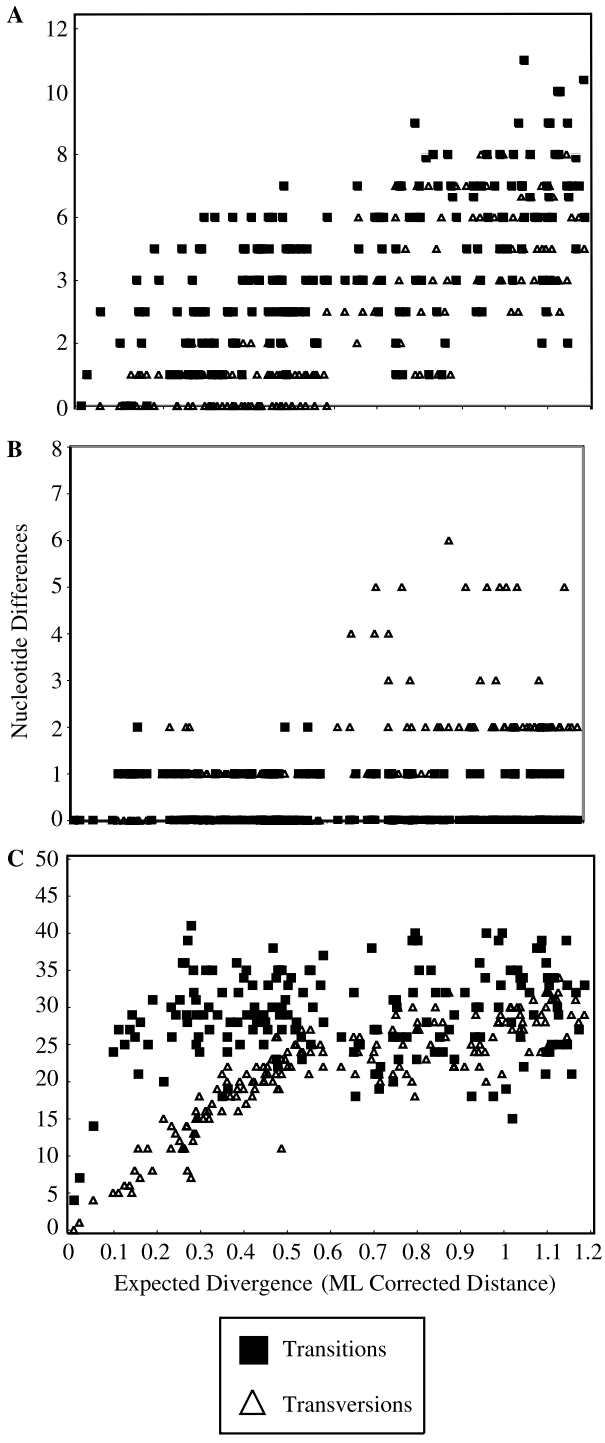


Fig. 2. Saturation plots of transitions and transversions for (A) first, (B) second, and (C) third codon positions of portion of cytochrome *c* oxidase subunit I sampled in this study plotted against expected divergence (maximum-likelihood corrected distance, GTR + I + Γ model).

monophyletic to the exclusion of *Steatoda* and *Robertus*. The likelihood topology was comprised of two reciprocally monophyletic clades of *Latrodectus*. The first was a clade uniting *Latrodectus geometricus* (from all its samples localities) as sister to *Latrodectus rhodesiensis*

Table 2
mtDNA COI pairwise sequence divergence within clades

Clade[a]	Uncorrected distance ave. (range)	Expected divergence[b] ave. (range)
L. geometricus	0.015 (0–0.02)	0.013 (0–0.02)
geometricus clade	0.054 (0–0.12)	0.105 (0–0.27)
mactans clade	0.112 (0–0.17)	0.349 (0–0.88)
Latrodectus	0.130 (0–0.20)	0.583 (0–1.40)
All sampled taxa	0.138 (0–0.25)	0.713 (0–3.98)

[a] Clades correspond to those noted in Fig. 3 and text.
[b] Expected divergence based on maximum-likelihood corrected distances, GTR + I + Γ model.

Mackay (1972) from South Africa; we will refer to this as the *geometricus* clade (*L. geometricus* having taxonomic priority over *L. rhodesiensis*). The second clade consisted of all other sampled *Latrodectus* species including those sampled from South Africa, Israel, Spain, Australia, New Zealand, and North and South America, hereafter refer to as the *mactans* clade. Additional clades corresponding to particular biogeographic regions were recovered within the *mactans* clade. For example, taxa sampled from (1) South America ($PP = 0.98$), (2) North America ($PP = 0.99$), and (3) Australia + New Zealand ($PP = 1.00$) formed monophyletic clades recovered with strong support within the *mactans* clade. However, relationships between these clades, and between those taxa sampled from Spain, Africa, Madagascar, and Israel, comprising the "deeper" relationships within the *mactans* clade, remained poorly supported. Each sampled species was monophyletic in the ML tree, with the exception of *L. tredecimguttatus* and two species from South America. The specimen of *L. tredecimguttatus* from Spain appeared more closely related to *L. renivulvatus* Dahl (1902) from South Africa than to the other sampled specimen of *L. tredecimguttatus* collected in Israel. *Latrodectus mirabilis* (Holmberg, 1876) and *L. corallinus* Abalos (1980) came out as paraphyletic lineages relative to *L. variegatus* Nicolet (1849), and *L. diaguita* Carcavallo (1960), respectively, these four species being restricted to South America.

Estimates of clade posterior probabilities (*PP*) for nodes appearing in the ML tree (for each of the three-independent Bayesian searches) are reported in Table 3. Across the three runs, *PP* values were similar. Inspection of each of the 50% majority rule consensus tree generated from the three runs (post burn-in), supported identical clades with one exception. In one of the three Bayesian runs, the consensus tree united *Steatoda grossa* (Koch, 1838) as sister to the *geometricus* clade (*PP* value = 0.52), rendering *Latrodectus* paraphyletic. However, the other two Bayesian consensus trees both left the relationship between *S. grossa*, the *geometricus* clade and the *mactans* clade, as an unresolved trichotomy. While the node uniting all *Latrodectus* as monophyletic in the ML tree was poorly supported ($PP < 0.50$), both the *geometricus*

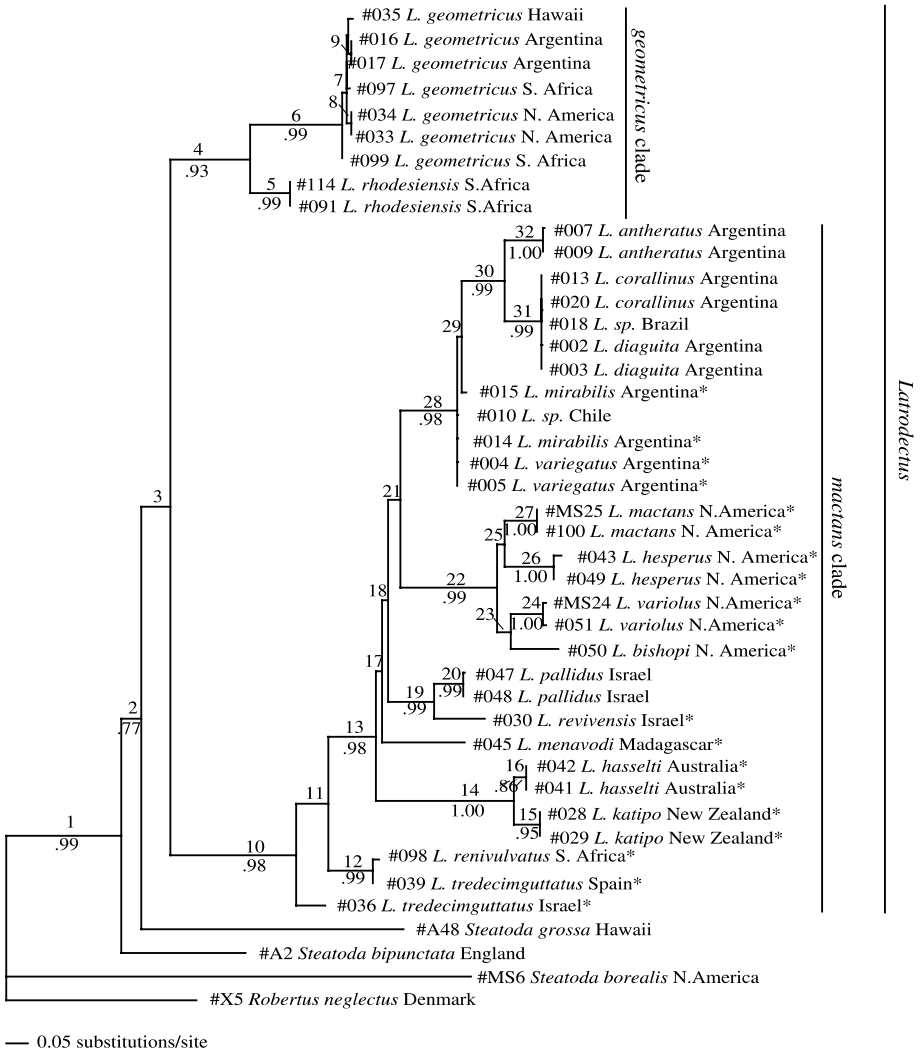


Fig. 3. Phylogenetic hypothesis for *Latrodectus:* phylogram ($-\ln L = 3238.06$) recovered from a maximum-likelihood heuristic search of COI data, using 100 random taxon addition replicates, with a GTR + I + Γ model. Numbers below nodes indicate clade posterior probabilities, numbers above branches correspond to nodes referred to in Table 3, where MP bootstrap support and decay indices are reported. Asterisks indicate taxa formerly synonymized with *L. mactans*. Black bars denote clades referred to in Table 2 and text.

clade and the *mactans* clade appear well supported, each having clade $PP \geqslant 0.93$ in all three analyses.

A heuristic parsimony search of the data resulted in 42 equally parsimonious trees (length = 632, CI = 0.446, RI = 0.772, RC = 0.344, 153 parsimony informative characters). A strict consensus tree computed from the 42 MPTs (not shown) was similar in topology to the presented ML tree. The parsimony consensus tree did not contain any nodes contradicting those presented in the ML tree; the only difference was that the parsimony consensus tree was less well resolved. Bootstrap (BS) support and decay indices (DI) for nodes recovered in the parsimony consensus tree are also reported in Table 3 (nodes in Table 3 having dashes in decay indices column were collapsed in the parsimony consensus tree). The parsimony consensus tree similarly recovered both the *geometricus* (BS = 71, DI = 3) and *mactans* clades (BS = 66, DI = 4). However, a node uniting these clades, making *Latrodectus* monophyletic was not recovered. Instead, relationships between the *geometricus* clade, *mactans* clade, *Steatoda grossa*, and *S. bipunctata* (L., 1758) were equivocal. Clades corresponding to particular geographic regions appearing in the *mactans* clade of the likelihood tree were also recovered in the parsimony consensus tree, including the clades uniting taxa from (1) South America (BS = 87, DI = 5), (2) North America (BS = 98, DI = 8), and (3) Australia + New Zealand (BS = 100, DI = 19). Relationships between these three clades were similarly poorly resolved.

In all analyses, populations of *L. geometricus* were consistently recovered as monophyletic. Given the extremely widespread distribution of the sampled individuals, average uncorrected genetic distance across *L. geometricus* was relatively small (average = 1.5%, maxi-

Table 3
Comparison of support values for nodes appearing in ML phylogram (Fig. 3) across phylogenetic analyses

Node[a]	Clade posterior probability[b]			MP bootstrap[c]	Decay index[d]	Clade name
	Run 1	Run 2	Run 3			
1	.99	1.00	1.00	64	3	
2	.77	.68	–	–	–	
3	–	–	–	–	–	*Latrodectus*
4	.93	.99	.99	71	3	*geometricus clade*
5	.99	1.00	1.00	100	7	
6	.99	1.00	1.00	100	15	
7	–	–	–	–	–	
8	.99	.99	.99	99	1	
9	.93	.94	.92	81	2	
10	.98	1.00	1.00	66	4	*mactans clade*
11	–	.73	.75	–	–	
12	.99	1.00	1.00	100	8	
13	.98	.98	.97	–	–	
14	1.00	1.00	1.00	100	19	
15	.95	.99	.99	100	7	
16	.86	.98	.96	100	10	
17	–	–	–	–	1	
18	–	–	–	–	–	
19	.99	1.00	1.00	84	3	
20	.99	1.00	1.00	100	13	
21	–	–	–	–	1	
22	.99	1.00	1.00	98	8	
23	–	.69	.69	–	1	
24	1.00	1.00	1.00	100	12	
25	.83	.73	.75	–	–	
26	1.00	1.00	1.00	100	14	
27	1.00	1.00	1.00	100	11	
28	.98	1.00	1.00	87	5	
29	–	–	.54	–	–	
30	.99	.99	.99	–	–	
31	.99	1.00	1.00	100	8	
32	1.00	1.00	1.00	100	12	

[a] Node number corresponds to those labeled in Fig. 3.
[b] Three values correspond to clade posterior probabilities recorded for three independent runs of Mr. Bayes, each starting from a random tree; dashes represent nodes with clade posterior probabilities lower than 0.50.
[c] Non-parametric bootstrap support for nodes recovered in consensus of maximum parsimony trees (MPTs), dashes represent nodes having lower than 50% bootstrap support.
[d] Decay index for nodes recovered in MP search, those with dashes represent nodes that are collapsed in the strict consensus of the MPTs.

mum = 2.3%; Table 2) and comparable to the genetic distances within other species of the genus that have more restricted geographical distributions. The uncorrected genetic distance between the two sampled African *L. geometricus* was 1.4%, roughly the same amount of difference as exhibited between the sampled Hawaiian (specimen #035) and either Argentine (#016 and #017) individual (each comparison being 1.5% different), and between an African (#099) and either sampled North American individual (1.7% different). In the ML tree one individual (#099) from Africa was basal to the rest of the other sampled *L. geometricus*, with the other African individual (#97), appearing more closely related to individuals from other localities (Fig. 3). However, this node was poorly supported and within the parsimony consensus tree the relationships between the African individuals and those sampled from other localities were equivocal.

4. Discussion

Despite past difficulties in identifying discrete morphological boundaries between widow spider species, our results based on molecular markers reveal considerable underlying phylogenetic structure across the genus *Latrodectus* and substantial amounts of genetic divergence between its members. Phylogenetic trees generated from the sampled data consistently recovered two well-supported reciprocally monophyletic clades within the genus *Latrodectus*: (1) the *geometricus* clade, placing the cosmopolitan brown widow, *L. geometricus*, as sister to the African *L. rhodesiensis*, and (2) the *mactans* clade containing other *Latrodectus* species sampled from Africa, Israel, Spain, Australia, New Zealand, and North and South America. The geographic area covered by *L. geometricus* is worldwide, similar to that covered by the entire *mactans* clade. However, the clade containing

all *L. geometricus* displays a smaller amount of uncorrected sequence divergence (2.3%) than the *mactans* clade (17.3%). This difference suggests that the *mactans* clade is comprised of taxa that have been geographically isolated over a considerably longer period of time, while *L. geometricus* has expanded its range relatively recently.

4.1. Latrodectus

Monophyly of the genus *Latrodectus*, while recovered in the ML tree, was weakly supported and the relationship between the *geometricus* clade, the *mactans* clade, *Steatoda grossa*, and *S. bipunctata* were unresolved in the parsimony consensus tree. However, monophyly of the *Latrodectus* genus has never been questioned in the literature; its members are recognized by the presence of a large colulus, lack of cheliceral teeth, widely separated lateral eyes and in the distinct structure of the male genitalia (Levi, 1959; Levi and Levi, 1962). That the genus was not recovered as monophyletic or poorly supported may be explained rather easily as a result of substitutional saturation, in that changes in the 3rd codon positions of COI occur too rapidly to recover relationships between distantly related taxa. Moreover, the limited number of sampled characters (428 bp) may provide an insufficient number of variable, yet slowly evolving characters suitable for resolving deeper relationships within the genus. We recognize that the presented phylogeny, though generally well resolved, should be interpreted with some caution as it is derived from a single gene region. Misleading or weak phylogenetic signal may arise as a consequence of sampling error when few characters are considered, or from excessive homoplasy related to the evolutionary dynamics of the selected molecule, including problems such as substitutional saturation mentioned above. For example, Hedin and Maddison (2001a) found that a COI based phylogeny for the jumping spider subfamily Dendryphantinae visibly conflicted with those derived from other mitochondrial and nuclear genes (16S rRNA, ND1, and 28S rRNA) as well as from morphological information, an outcome attributed to homoplasy in third codon positions of COI possibly due to variable selective constraints at the amino acid level. Combination of multiple molecular loci can improve resolution when phylogenetic signal from individual gene regions is weak (Baker and Desalle, 1997; Edgecombe et al., 2002; Hormiga et al., 2003), and provides a method to corroborate relationships proposed by any single locus. Ribosomal genes such as mitochondrial 12S rRNA and 16S rRNA and nuclear 18S rRNA and 28S rRNA, in addition to the nuclear protein coding gene elongation factor 1-α, are more conserved than the third codon positions of mitochondrial COI and these loci have been successfully employed for clarifying relationships among spiders at different hierarchical levels (e.g., Arnedo et al., 2001; Hedin, 2001; Hedin and Maddison, 2001b; Hormiga et al., 2003; Tan et al., 1999). These gene regions may be similarly applied to address phylogenetic relationships within the genus *Latrodectus* and its position relative to closely related theridiid genera.

Although the monophyly of *Latrodectus* was not strongly supported, there is support for the highly divergent, reciprocally monophyletic *geometricus* and *mactans* clades. Lotz's (1994) taxonomic revision of African *Latrodectus* recognized two species groups: the *geometricus* group, comprised of *L. geometricus* and *L. rhodesiensis*, with females having parallel seminal receptacles and the *tredecimguttatus* group, where he placed all other African *Latrodectus*, or those having receptacles that form a V-shape. In our results, recovery of a distinct *geometricus* clade is consistent with the species groups recognized by Lotz (1994). However, whether the shape of the seminal receptacles is a phylogenetically consistent (as opposed to homoplastic) character across the genus requires an investigation of this trait in all other *Latrodectus* species not included in Lotz's revision.

4.2. The mactans clade

The presented phylogeny indicates that the *mactans* clade consists of several well-supported monophyletic groups consistent with geographic boundaries that are separated by high levels of genetic divergence. However, nearly all of the species in the *mactans* clade represent taxa that were synonymized with *L. mactans* by Levi (1959) due to their similarity in genitalic structure and apparent lack of morphological boundaries (Fig. 3). Thus it appears that only slight changes in genitalic structure have occurred during a period when this clade experienced substantial lineage diversification.

Latrodectus species once synonymized with *L. mactans* by Levi (1959) were not monophyletic in the resulting trees (Fig. 3). This in part reflects the fact that two of the four species that were not synonymized with *L. mactans* (*L. corallinus* and *L. diaguita*) had not yet been described at the time of Levi's (1959) revision. However, Levi (1959) recognized *L. pallidus* Cambridge, 1872 as distinct from *L. mactans*, while our results strongly support *L. pallidus* as sister to *L. revivensis* Shulov (1948) nested within species formerly synonymized with *L. mactans*. Levi (1959) considered *L. pallidus* distinct from *L. mactans* (based solely on female specimens), principally because of differences in coloration, and based on previous literature that reported *L. pallidus* as restricted to desert plains, living in shrubs and primarily feeding on ants (Schulov, 1940). Szlep (1965) subsequently described the web of *L. pal-*

lidus as specialized and distinct from *L. revivensis* and *L. tredecimguttatus*, these three species having overlapping distributions. Thus it appears that the specific characteristics of *L. pallidus* may be derived from the pleisiomorphic morphological and ecological features retained by the majority of *Latrodectus* species in the *mactans* clade. Levi (1959) also synonymized *L. antheratus* (Badcock, 1932) with *L. curacaviensis* (Müller, 1776) rather than *L. mactans*, due to differences in embolus coil morphology (male intromittent organ). Levi's (1959) revision, based largely on differences in embolus coil number, synonymized *Latrodectus* species characterized by three embolus coils with *L. mactans*, those with two coils with *L. curacaviensis*, and diagnosed *L. geometricus* as having four coils. However, Levi (1983) subsequently recognized that he overemphasized the importance of genitalic differences for diagnosing *Latrodectus* species and concluded that he had incorrectly lumped several species with *Latrodectus mactans*.

Ábalos and Báez (1967) similarly used male genitalic morphology to revise *Latrodectus* species of South America. In addition to *L. geometricus*, they identified two other species groups of *Latrodectus* in Argentina differing in the number of embolus coils: group "*mactans*" comprised of *L. mirabilis*, *L. diaguita*, *L. corallinus*, and *L. quartus* Abalos (1980) having three coils; and group "*curacaviensis* " comprised of *L. antheratus* and *L. variegatus*, having two coils. In all of our trees, *L. mirabilis* and *L. corallinus* appear as paraphyletic lineages relative to *L. variegatus* and *L. diaguita* respectively, indicating that the species groups proposed by Ábalos and Báez (1967) are inconsistent with our results and that embolus coil number may be homoplasious. Moreover, the two paraphyletic assemblages: (1) *L. mirabilis* and *L. variegatus* and (2) *L. corallinus* and *L. diaguita* share nearly identical sequences within each group. This result suggests the possibility that the recognition of multiple species within each group is a taxonomic artifact, and that morphological features utilized to designate *L. mirabilis* as distinct from *L. variegatus* (such as embolus coil number) may instead represent polymorphic traits exhibited by different individuals within or between populations. Interestingly, Kaston (1970) cautioned against the use of embolus coil number to designate *Latrodectus* taxa, as male siblings he reared from the same egg sac appeared to differ in their coil number.

Ábalos and Báez (1967) also identified *L. corallinus* as further differing from the other Argentine species in egg case morphology. Specimens of *L. corallinus* included in this study were collected along with their characteristically "spiked" egg case (Ábalos and Báez, 1967), while *L. diaguita* were associated with smooth egg cases. Nevertheless, sequences between these taxa included in this study were identical. Because the mitochondrial genome is maternally inherited, a phylogeny generated solely from its gene sequences may not reflect the true relationships between populations or species, particularly for closely related taxa if there is incomplete sorting of haplotypes, interspecific hybridization, or if mating is non-random (Maddison, 1997; Moore, 1995). Thus paraphyly of species (such as exhibited by the South American taxa), as well as shared haplotypes across species may also be explained as a consequence of incomplete lineage sorting and/or hybridization. A phylogeny based on nuclear markers may allow assessment of the role, if any, of these effects. The collection of additional data is clearly needed to more comprehensively address the phylogenetic distribution of morphological characters and taxonomic boundaries within the South American *Latrodectus*.

The phylogeny generated from the current study similarly did not unite the two sampled specimens of *L. tredecimguttatus.* Instead, the specimen of *L. tredecimguttatus* from Spain (#039) appeared more closely related to *L. renivulvatus* from South Africa than to the other included *L. tredecimguttatus* sampled from Israel. *Latrodectus tredecimguttatus* from Spain and *L. renivulvatus* from South Africa were also surprisingly similar in sequence (differing at 4 sites), despite being collected from distant geographic regions. The *L. tredecimguttatus* specimen from Spain was initially identified as *L. schuchii* (L. Lotz, pers. comm.). Subsequently, Melic (2000) synonymized *L. schuchii* with *L. tredecimguttatus* (Schmidt et al., 2001). In Melic's (2000) revision of *Latrodectus* of the Iberian Peninsula, he further proposed a new species, *L. lilianae*, bringing the number of currently recognized species from the Iberian Peninsula to two (*L. tredecimguttatus* and *L. lilianae*). The type locality of *L. lilianae* is the same as the collection locality of the *L. tredecimguttatus* we have included from Spain, casting some doubt on the identity of this specimen. However, regardless of the exact identity of this specimen, the relationship uniting it with *L. renivulvatus* is particularly interesting as females of *L. renivulvatus*, *L. tredecimguttatus* and *L. lilianae* are characterized by spermathecal ducts having four loops (Lotz, 1994; Melic, 2000), whereas all other African *Latrodectus* species are characterized by three loops (Lotz, 1994). In both *L. tredecimguttatus* and *L. lilianae* the fourth loop is situated between loop two and three, while in *L. renivulvatus* the fourth loop follows loop three (Lotz, 1994; Melic, 2000). The difference between the former two species (*L. lilianae* and *L. tredecimguttatus*) with respect to the spermathecal ducts, is related to the shape of the third loop, as the third loop folds to form a nearly complete circle in *L. lilianae* while in *L. tredecimguttatus* the third loop barely forms a half-circle (Melic, 2000). The close relationship between *L. renivulvatus* with *L. tredecimguttatus* is corroborated by the occurrence of four loops in the spermathecal ducts.

4.3. The geometricus clade

The low levels of genetic divergence exhibited by the brown widow, *L. geometricus*, from extremely disparate localities corroborates the hypothesis that *L. geometricus* has been introduced only recently to many areas where it currently occurs. Although the exhibited divergence is low considering its widespread distribution, these values are not insubstantial as they maximally are as much as 2.3% (uncorrected difference). Sequences (particularly mt COI sequences) compared between individuals of an introduced species might be expected to be identical or only slightly different. However, our results, that the amount of sequence difference exhibited within one locality (South Africa) is as much as that found between distant localities (such as between Hawaii and Argentina), are consistent with a scenario in which members of a genetically heterogeneous population, or set of populations is subsequently introduced to a number of distant localities.

Latrodectus geometricus is considered to have been introduced to Hawaii (Pinter, 1980), Australia (Forster and Forster, 1999; Raven and Gallon, 1987), Southern California (J. Kempf, pers. comm.), and Japan (Ono, 1995) as it was detected relatively recently in each of these locations and in association with urban environments. Despite its broad distributional range, Levi (1959) hypothesized that the native range of *L. geometricus* lies within Africa, primarily because it is extremely widespread there, yet occurs in highly disjunct and narrow ranges elsewhere (see Fig. 1). At the time of its description, *L. geometricus* was known to occur in South America as well as in Africa. Consequently, it is not certain whether its native range includes South America as well as Africa. That our phylogenetic hypothesis places the African *L. rhodesiensis* as sister to *L. geometricus* suggests the possibility of a shared African ancestor. A shared African ancestor is further supported by the ML tree topology, as it places one African *L. geometricus* (#099, Fig. 3) as sister to all other sampled *L. geometricus* haplotypes. However, this node is weakly supported and the consensus of the MPTs was equivocal regarding the relationship between this individual with respect to the other sampled *L. geometricus*. Thus, the occurrence of *L. geometricus* in Africa may be equally well explained by our results as a secondary colonization from one of the other localities in which it is found. A confident assessment of the historical movements of *L. geometricus* requires a more comprehensive assessment of the genealogy of its constituent populations (especially employing multiple loci, e.g., Davies et al., 1999; Tsutui et al., 2001), involving a greater sampling of individuals from different localities in which it occurs, including those not sampled in this study (e.g., *L. geometricus* introduced to Australia and Japan).

4.4. Latrodectus range expansion

The distributions of a number of *Latrodectus* species, in addition to *L. geometricus*, have clearly expanded as a result of human transport. For example, *L. hesperus* Chamberlin and Ivie (1935) considered native to western North America, has been intercepted multiple times in New Zealand in association with grapes imported from California (Reed and Newland, 2002). Further, the red-back spider, *L. hasselti*, has been introduced to both Japan (Ono, 1995) and New Zealand (Forster, 1992; Raven and Gallon, 1987), from Australia (its presumed native range). *L. hasselti*, while already established in some areas of New Zealand, has subsequently been intercepted multiple times in New Zealand on grapes imported from Australia (Reed and Newland, 2002). The impact of *L. hasselti* on the endemic New Zealand widow spider *L. katipo* Powell, 1870 may be a further cause for concern. *Latrodectus katipo* is restricted to coastal beach habitats of New Zealand and is considered threatened due to beachfront development and displacement by *Steatoda capensis* Hann (1990), another introduced theridiid spider (Forster and Forster, 1999; Hann, 1990). *Latrodectus katipo* and *L. hasselti* differ markedly in aspects of their mating behavior, as a male of *L. hasselti*, unlike *L. katipo*, performs a stereotyped "somersault" behavior, presenting the female with its abdomen for consumption during mating (self-sacrifice) (Forster, 1992). Although laboratory studies examining interspecific interactions have shown that *L. hasselti* females are not receptive to courtship attempts by *L. katipo* males (Forster, 1992, 1995), we found that *L. hasselti* and *L. katipo* were closely related (4.9% uncorrected genetic divergence). Moreover, female *L. katipo* readily mate with *L. hasselti* males (males perform the "somersault," but females do not eat them) and produce fertile F1 and F2 offspring (Forster, 1992, 1995). This unidirectional potential for hybridization presents a further threat to the persistence of *L. katipo*, as introgression may homogenize the two species if in time *L. hasselti* invades the native range of *L. katipo*.

4.5. Latrodectus biogeography

While it is certain that multiple *Latrodectus* species have expanded their range through human mediated transport, it appears that *Latrodectus* spiders are also naturally present in all continents excepting Antarctica. However, it should be noted that Raven and Gallon (1987) have suggested that widow spiders (specifically, *L. hasselti*) may not be native to Australia, as they were first detected there at seaports. Nevertheless, the widespread biogeographic distribution of *Latrodectus* spiders is particularly interesting

given that the genus is a member of the relatively derived spider family Theridiidae. The presented phylogeny suggests the existence of clades restricted to particular geographic regions that had not previously been detected based on morphological evidence. For example, members of the *mactans* clade were monophyletic in both North and South America. A weakly supported node further united the South American and North American clades, suggesting the possibility that members of the North American *mactans* clade may be derived from a South American ancestor or vice versa. That this South American + North American clade is nested within the *mactans* clade, with species from Africa, the Middle East, Australia, New Zealand, and the Iberian Peninsula appearing in more basal positions suggests that the *mactans* clade may have arrived in North or South American from one of these areas. However, because of the limited resolution among the deeper level relationships within the *mactans* clade and exclusion of several species that may be of particular relevance for explaining the present distribution of the genus, it may be premature to infer biogeographic patterns. It is likely that much of the present day distribution of *Latrodectus* spiders is attributable to dispersal events. Many spiders (including theridiids) can disperse over long distances via ballooning as juveniles, explaining the prevalence of native spiders on remote oceanic islands (Gillespie et al., 1994). Thus it is plausible that *Latrodectus* spiders have occasionally colonized distant landmasses within which subsequent lineage diversification has occurred. If *L. geometricus* is native to Africa and African members of the *mactans* clade are ultimately found to be basal with respect to the remaining species of the *mactans* clade, it would strongly suggest the possibility that *Latrodectus* spiders originated in Africa.

A tremendous amount of data has been collected related to the morphology, ecology, behavior, and biochemistry of *Latrodectus* spiders, including studies of habitat selection (Lubin et al., 1993), development (Kaston, 1970), diet (Hódar and Sánchez-Piñero, 2002), and venom (Ashton et al., 2001; Orlova et al., 2000) to cite a few. Moreover, arguments for the evolutionary significance of a number of variable traits have been presented in the absence of a phylogenetic hypothesis for the group. For example, red pigmentation appearing on the abdomen of many *Latrodectus* spiders (often against a black background) has long been suspected an example of aposematic coloration, with variation in this feature indicative of venom toxicity (Bristowe, 1946; Kaston, 1970). The phylogenetic hypothesis presented for the genus *Latrodectus* provides the opportunity to examine the evolution of this and other fascinating features exhibited by members of the genus and further provides a basis for future population genetic work to track the source and spread of ongoing invasions of these spiders.

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Appendix A

Recognized species of *Latrodectus*, distribution, included specimens, their locality and GenBank Accession Number

Species[a]	Distribution[b]	Specimen #[c]	Collecting locality[d]	GenBank Accession #
Recognized Latrodectus species				
L. antheratus (Badcock, 1932)	Paraguay, Argentina	#007	Santiago del Estero, Argentina	AY383047
		#009	Santiago del Estero, Argentina	AY383048
L. apicalis (Butler, 1877)	Galapagos Is.	NA	–	–
L. atritus (Urquhart, 1890)	New Zealand	NA	–	–
L. bishopi (Kaston, 1938)	Florida, US	#050	Florida, US	AY383060
L. cinctus (Blackwall, 1865)	Cape Verde Is., Africa, Kuwait	NA	–	–
L. corallinus (Abalos, 1980)	Argentina	#013	Santiago del Estero, Argentina	AY383061
		#020	Cruz del Eje, Argentina	AY383062
L. curacaviensis (Müller, 1776)	Lesser Antilles, South America	NA	–	–

Appendix A (*continued*)

Species[a]	Distribution[b]	Specimen #[c]	Collecting locality[d]	GenBank Accession #
L. dahli (Levi, 1959)	Middle East to Central Asia	NA	–	–
L. diaguita (Carcavallo, 1960)	Argentina	#002	Cartamarca, Argentina	AY383063
		#003	Cartamarca, Argentina	AY383064
L. erythromelas (Schmidt and Klaas, 1991)	Sri Lanka	NA	–	–
L. geometricus (Koch, 1841)	Cosmopolitan	#035	Honolulu, Hawaii	AY383046
		#099	Roodepoort, South Africa	AY383049
		#097	Roodepoort, South Africa	AY383050
		#034	Florida, US	AY383067
		#033	Florida, US	AY383068
		#016	Santiago del Estero, Argentina	AY383065
		#017	Santiago del Estero, Argentina	AY383066
L. hasselti (Thorell, 1870)	SE Asia, Australia, N. Zealand	#042	Queensland, Australia	AY383051
		#041	Queensland, Australia	AY383069
L. hesperus (Chamberlin and Ivie, 1935)	Western US	#043	California, US	AY383070
		#049	New Mexico, US	AY383071
L. hystrix (Simon, 1890)	Yemen, Socotra	NA	–	–
L. indistinctus (Cambridge, 1904)	Namibia, South Africa	NA	–	–
L. karrooensis (Smithers, 1944)	South Africa	NA	–	–
L. katipo (Powell, 1870)	New Zealand	#029	Kaitorete spit, New Zealand	AY383053
		#028	Kaitorete spit, New Zealand	AY383052
L. lilianae (Melic, 2000)	Spain	NA	–	–
L. mactans (Fabricius, 1775)	North America	#MS25	North Carolina, US	AY383054
		#100	Mississippi, US	AY383072
L. menavodi (Vinson, 1863)	Madagascar, Comoro Is.	#045	Madagascar	AY383075
L. mirabilis (Holmberg, 1876)	Argentina	#014	Buenos Ares, Argentina	AY383076
		#015	Buenos Ares, Argentina	AY383077
L. obscurior (Dahl, 1902)	Cape Verde Is., Madagascar	NA	–	–
L. pallidus (Cambridge, 1872)	Libya to Russia, Iran	#047	Beer Sheeva, Israel	AY383055
		#048	Beer Sheeva, Israel	AY383056
L. quartus (Abalos, 1980)	Argentina	NA	–	–
L. renivulvatus (Dahl, 1902)	Africa, Saudi Arabia, Yemen	#098	Gauteng Province, S. Africa	AY383057
L. revivensis (Shulov, 1948)	Israel	#030	Sde Boqer, Israel	AY383078
L. rhodesiensis Mackay, 1972	Southern Africa	#114	Namibia/S. Africa border	AY383058
		#091	Namibia/ S. Africa border	AY383079
L. tredecimguttatus (Rossi, 1790)	Mediterranean to China	#036	Sayeret Shaaed, Israel	AY383081
		#039	Zaragoza, Spain	AY383080
L. variegatus (Nicolet, 1849)	Chile, Argentina	#004	Santa Cruz, Argentina	AY383083
		#005	Bariloche, Argentina	AY383084
L. variolus (Walckenaer, 1837)	USA, Canada	#MS24	South Carolina, US	AY383059
		#051	Maryland, US	AY383082
Included unidentified Latrodectus taxa				
L. sp.Brazil	–	#018	Salvador, Bahia, Brazil	AY383073

Appendix A (*continued*)

Species[a]	Distribution[b]	Specimen #[c]	Collecting locality[d]	GenBank Accession #
L. Chile	–	#010	Chile	AY383074
Included outgroups				
Robertus neglectus (Cambridge, 1871)	Palearctic	#X5	Arhus, Denmark	AY231053
Steatoda bipunctata (L., 1758)	Holarctic	#A2	Yorkshire, England	AY231057
Steatoda borealis (Hentz, 1850)	North America	#MS6	North Carolina, US	AY383085
Steatoda grossa (C.L. Koch, 1838)	Cosmopolitan	#A48	Molokai, Hawaii	AY383086

[a] All recognized species of *Latrodectus* as obtained from Platnick, N. I. 2003. The world spider catalog, version 3.5. American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog81-87/index.html, bold font indicates taxon sampled in this study.

[b] Species ranges also obtained from Platnick (2003) and include non-native ranges.

[c] Taxa number codes correspond to individual specimen code in authors' voucher database, letter codes refer to vouchers deposited at the National Museum of Natural History.

[d] More specific information regarding collection locality is available upon request.

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