Molecular phylogeny of the leiobunine harvestmen of eastern North America (Opiliones: Sclerosomatidae: Leiobuninae)

Mercedes Burns [a,*], Marshal Hedin [b], Jeffrey W. Shultz [a]

[a] Department of Entomology, 4112 Plant Sciences Building, University of Maryland, College Park, MD 20740, USA
[b] Department of Biology, Life Sciences North, San Diego State University, San Diego, CA 92182, USA

ABSTRACT

Phylogenetic relationships among the leiobunine harvestmen or "daddy-longlegs" of eastern North America (*Leiobunum*, *Hadrobunus*, *Eumesosoma*) are poorly known, and systematic knowledge of the group has been limited largely to species descriptions and proposed species groups. Here we obtained mitochondrial (NADH1, 16S and 12S rDNA) and nuclear (28S rDNA, EF-1α introns and exons) DNA sequences from representatives of each genus, virtually all *Leiobunum* species from the USA and Canada, four western North American outgroup species and the distantly related *Phalangium opilio*. We applied Bayesian, maximum-likelihood and parsimony methods under various data-partition treatments to reconstruct phylogeny and to test taxonomy-based phylogenetic hypotheses. Results were largely congruent among methods and treatments and well supported by bootstrap and posterior probability values. We recovered *Leiobunum* as paraphyletic with respect to *Eumesosoma* and *Hadrobunus*. Most species were encompassed by five well-supported clades that broadly correspond to groups based on male reproductive morphology (*Hadrobunus* group, an early-season *Leiobunum* group, *L. vittatum* group, *L. politum* group and *L. calcar* group). Relationships within species groups were often ambiguous or inconsistent with morphology, suggesting the presence of gene introgression or deep coalescence and/or the need for taxonomic revision.

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1. Introduction

The harvestman or "daddy-longlegs" fauna of eastern North America (i.e., eastern and central USA and adjacent Canada) is dominated by the subfamily Leiobuninae (Sclerosomatidae), with four genera (*Leiobunum*, *Nelima*, *Hadrobunus*, *Eumesosoma*) encompassing about 35 described species. *Leiobunum* and *Nelima* are species-rich genera that also occur in Mesoamerica, East Asia and the Euro-Mediterranean Region. *Hadrobunus* and *Eumesosoma* contain few species and are endemic to North America. Few explicit phylogenetic hypotheses have been proposed for these harvestmen, with most taxonomic treatments describing species and occasionally noting similarities among them (e.g., Roewer, 1923; Crosby and Bishop, 1924; Bishop, 1949a; Davis, 1934; Edgar, 1966). The recognition of four genera is one of the few implicit phylogenetic hypotheses.

In a largely unpublished dissertation on the *Leiobunum* of the region, McGhee (1970, 1975) circumscribed several species groups based on male genitalia. He recognized three penis types (sacculate, bulbate, lanceolate) using the morphology of a bilateral pair of subterminal cuticular structures (Fig. 1). He did not explore the sacculate group beyond noting its heterogenous membership. Eight sacculate species were widely recognized at the time (*L. aldrichi*, *L. aurugineum*, *L. bimaculatum*, *L. cretatum*, *L. flavum*, *L. nigripes*, *L. ventricosum*, *L. verrucosum*), although *L. nigripes* is now considered a junior synonym of *L. verrucosum* (Shultz, 2008). The bulbate group encompassed three species (*L. politum*, *L. bracchiolum*, *L. holtae*), with *L. holtae* being a distinctive outlier (McGhee, 1975). However, the "bulbs" of *L. politum* and *L. bracchiolum* are simply sacs with reduced lateral walls and no "bulb" is apparent in *L. holtae* (orig. obs.). The lanceolate group contained species that lack penial sacs or "bulbs", and McGhee (1970) recognized two subgroups, the *calcar* and *vittatum* species groups. The *calcar* group was characterized by a gradually tapered, dorsoventrally compressed penis (Fig. 1) and male palpal tibiae specialized for clasping the female during mating (Table 1). It included five species (*L. calcar*, *L. nigropalpi*, *L. serratipalpe*, *L. hoffmani*, *L. cumberlandense*), although *L. serratipalpe* was later synonymized with *L. calcar* (Cokendolpher, 1981) and descriptions of *L. hoffmani* and *L. cumberlandense* were never formally published. A recent revision of the group (Ingianni et al., 2011) resurrected the *L. serratipalpe* concept under the name *L. euserratipalpe*, formally described *L. hoffmani*, and synonymized *L. cumberlandense* with *L. calcar*. In the *vittatum* group, males tend to have long palpal femora with a proventral row of spines, and the penis (Fig. 1) has

* Corresponding author. Address: Department of Entomology, BEES Program, 4112 Plant Sciences Building, University of Maryland, College Park, MD 20742, USA. Fax: +1 301 314 9290.
E-mail address: mmburns@umd.edu (M. Burns).
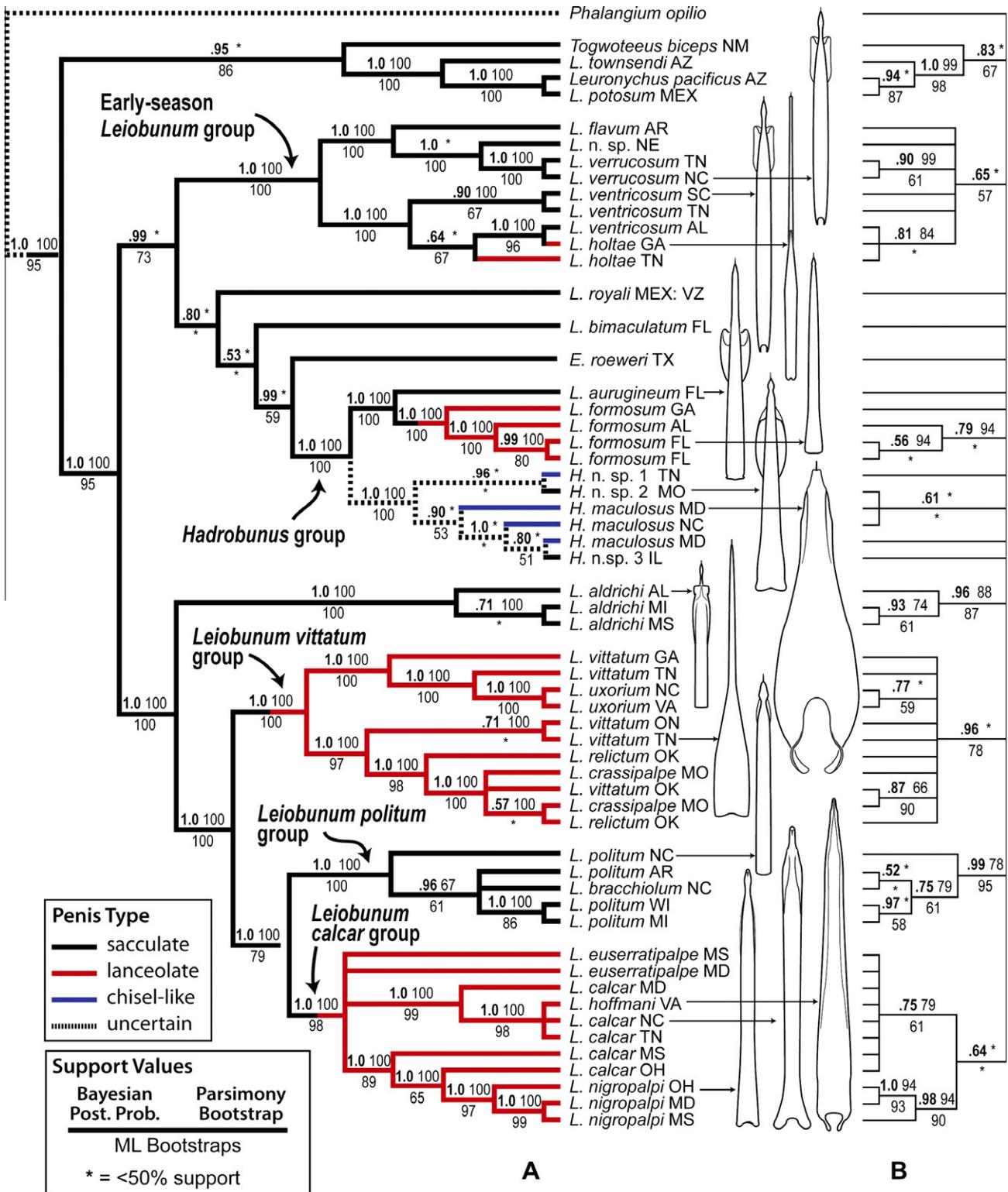
Fig. 1. Bayesian likelihood phylogeny obtained with MrBayes 3.1.2 for (A) the combined data set of nuclear and mitochondrial characters with seven model-tested partitions (jModelTest; Posada, 2008) and (B) the Bayesian likelihood phylogeny constructed with only nuclear genes 28S and EF1alpha. Values above nodes correspond to the Bayesian posterior probabilities and parsimony bootstrap percentages (left to right, respectively). Values below nodes indicate maximum likelihood bootstrap values. Support numbers for topologies derived from analyses of alternatively-partitioned data are given in supplementary information (see Fig. S1, Table S2). Figure center depicts dorsal perspective of penes for a selection of species to highlight genitalic diagnostic features (see also Table 1). Not to scale.

an expanded basal portion and thinner, usually cylindrical distal shaft (Table 1). McGhee placed four species in this group (*L. vittatum*, *L. speciosum*, *L. crassipalpe*, *L. formosum*). However, *L. uxorium* and *L. relictum* should also have been included. Indeed,

L. speciosum sensu McGhee seems to correspond to *L. uxorium* and the traditional view of *L. speciosum* (see Davis, 1934) appears to correspond to *L. vittatum*. The inclusion of *L. formosum* in the *vittatum* group was uncertain. The other widespread genus, *Nelima*,

Table 1
Diagnostic features and general distribution of the Leiobuninae included in analysis.

Group	Diagnostic features	Distribution[a]
Leiobuninae of eastern North America (ingroup)		
Early-season Leiobunum group	Overwinters as juvenile, not egg	
L. verrucosum (Wood, 1868)	Penis sacculate, dark trochanters	c-e USA, se Canada
L. flavum Banks, 1894	Penis sacculate, light trochanters, large	c-e USA
L. new species	Penis sacculate, light trochanters, small	USA: e NE
L. ventricosum (Wood, 1868)	Penis sacculate, elongate	c-e USA, se Canada
L. holtae McGhee, 1977	Penis lanceolate, elongate, very thin	USA: se TN, n AL, ne GA
Leiobunum vittatum group	Penis lanceolate; shaft thin, base broad	
L. vittatum (Say, 1821)	Male palpal femur elongate	c-e USA, sc-se Canada
L. uxorium Crosby and Bishop, 1924	Male palpal femur slightly elongate	se USA
L. relictum Davis, 1934	Male palps not elongate	USA: w OK: Wichita Mtns
L. crassipalpe Banks, 1900	Male palpal femur elongate, robust	USA: e OK, n AR, s MO
Leiobunum politum group	Penis sacculate, sac reduced laterally	
L. politum (Wood, 1868)	Larger body	c-e USA, se Canada
L. bracchiolum (McGhee, 1977)	Smaller body	USA: e PA to n GA
Leiobunum calcar group	Penis lanceolate, tapered; male palpal tibia modified proximally for clasping	
L. nigropalpi (Wood, 1868)	Male palpal femur thin, no distal spine cluster	e USA
L. euserratipalpe Ingianni et al., 2011	Male palpal femur thin, distal spine cluster	e USA
L. calcar (Wood, 1868)	Male palpal femur robust, distal spine cluster	c-e USA, sc-se Canada
L. hoffmani Ingianni et al., 2011	Male palpal femur massive, distal spine cluster	USA: nw NC, sw VA
Hadrobunus group	Retrorse scutal armature	
H. maculosus (Wood, 1868)	Penis chisel-like, massive; short legs	USA: ME to c NC
H. new species 1	Penis chisel-like, massive; short legs	USA: e TN, KY, WV, sw PA
H. new species 2	Penis sacculate, small, sacs large	USA: e KS, MO, s WI, w IL
H. new species 3	Penis sacculate, small, sacs small	USA: s IL, w TN
L. aurugineum Crosby and Bishop, 1924	Penis sacculate, sacs posterior, short legs	extreme se USA
L. formosum (Wood, 1868)	Penis lanceolate, weak armature, long legs	c-e USA
Other taxa		
Eumesosoma roeweri (Goodnight & Goodnight, 1943)	Penis sacculate, scutum tuberculate, short legs	c USA
Leiobunum aldrichi (Weed, 1893)	Penis sacculate, white band on tibia II	c-e USA, se Canada
L. bimaculatum Banks, 1893	Penis sacculate, large, light spots on carapace	extreme se USA, se VA
L. royali Goodnight & Goodnight, 1946	Penis sacculate	Mexico: HG, SL, VZ
Leiobuninae of Western North America (outgroup)		
Leiobunum townsendi Weed, 1893	Penis sacculate	sw USA – nc Mexico
L. potosum Goodnight & Goodnight, 1942	Penis sacculate	Mexico: DF, SL, PU, TL
Leuronychus pacificus (Banks, 1894)	Penis sacculate	West Coast: s AK to BJ
Togwoteeus biceps (Thorell, 1877)	Penis sacculate	nw Mexico – sw Canada

[a] *Abbreviations*: c, central; e, eastern; n, northern; s, southern; w, western. States: AK, Alaska; AL, Alabama; AR, Arkansas; BJ, Baja California; DF, Distrito Federal; GA, Georgia; HG, Hidalgo; IL, Illinois; KS, Kansas; KY, Kentucky; ME, Maine; MO, Missouri; NC, North Carolina; NE, Nebraska; OK, Oklahoma; PA, Pennsylvania; PU, Puebla; SL, San Luis Potosi; TL, Tlaxcala; TN, Tennessee; WI, Wisconsin; WV, West Virginia; VA, Virginia; VZ, Veracruz.

is characterized by the absence of pro- and retrolateral rows of coxal denticles but is otherwise similar to *Leiobunum*. The genus is represented in eastern North America by a single species, *N. elegans*, with clear affinities to *N. paessleri* and *L. exilipes* of western North America (Hedin et al., 2012).

The two endemic North American genera, *Eumesosoma* and *Hadrobunus*, differ from most *Leiobunum* and *Nelima* species in having relatively short legs and substantial dorsal armature. *Eumesosoma* contains six known species distributed in the southern and central United States (Cokendolpher, 1980). They are primarily ground-dwelling forms with a hard, tuberculate scutum and sacculate penes (Table 1). *Hadrobunus* includes three described species in eastern North America (*H. grandis*, *H. maculosus*, *H. fusiformis*), although the distinction between *H. grandis* and *H. maculosus* has never been clear. Two *Hadrobunus* species have been described from Mexico, the northeastern *H. knighti* and southwestern *H. davisi*, but the latter does not appear to belong to the genus (Shultz, 2010). *Hadrobunus* is currently united by having posteriorly curved (retrorse) spinules on the scutum. Many undescribed species of *Hadrobunus* are known to us, and the diversity of penial structure within the genus (Fig. 1) exceeds that of *Leiobunum*; there are sacculate and lanceolate types, and more commonly, massive chisel-like penes (Fig. 1: *H. maculosus*).

Until recently, there has been little understanding of the broader phylogenetic and biogeographic affinities of the Leiobuninae of eastern North America (but see Table 1; Cokendolpher and Lee, 1993). However, because non-leiobunine harvestmen from this region have close relatives in Europe, Mesoamerica and East Asia, it has long seemed possible (even likely) that leiobunines would also represent a phylogenetic mosaic with diverse biogeographic affinities. This expectation would seem to require any meaningful phylogenetic treatment of the group to include a significant representation of the entire holarctic leiobunine fauna, which would be an extremely daunting task. However, in a recent molecule-based phylogenetic analysis, Hedin et al. (2012) began to address this problem and showed that geographic proximity is often superior to current morphology-based taxonomy in predicting phylogenetic relationships among leiobunines. These authors found that *Leiobunum* and *Nelima* are polyphyletic syndromes that appear to have arisen independently in different geographic areas and that the leiobunines of eastern North America form a clade along with species from eastern Mexico. There is no evidence of any close relationship with Asian, European or even western North American clades. Indeed, among the eastern North American taxa, only *N. elegans* lies outside the clade. With this significant advance in our understanding of leiobunine phylogeny, a taxonomically meaningful phylogenetic analysis of eastern North American leiobunines can now be undertaken without intensive sampling of European, Asian or even western North American groups.

Table 2
Taxon sampling for phylogeny.

Species	Locality	Voucher no.	Collector	Date
Leiobunum calcar	USA: NC: Clay Co.	OP 1394	M. Hedin	16-August-2007
L. calcar	USA: MD: Frederick Co.	OP 1234	J.W. Shultz	06-July-2007
L. calcar	USA: MS: Tishomingo Co.	OP 830	M. Hedin	12-August-2005
L. calcar	USA: TN: Cocke Co.	OP 814	M. Hedin	27-August-2005
L. calcar	USA: OH: Summit Co.	OP 1091	J.W. Shultz	01-June-2005
L. n. sp. hoffmani	USA: VA: Grayson Co.	OP 1383	M. Hedin	11-August-2007
L. n. sp. euserratipalpe	USA: MS: Lafayette Co.	OP 1235	P. Miller, G. Stratton	10-June-2007
L. n. sp. euserratipalpe	USA: MD: Montgomery Co.	OP 1080	J.W. Shultz	01-July-2004
L. nigropalpi	USA: OH: Summit Co.	OP 1087	J.W. Shultz	01-June-2005
L. nigropalpi	USA: MD: Frederick Co.	OP 1075	J.W. Shultz	01-August-2004
L. nigropalpi	USA: AL: Cleburne Co.	OP 846	M. Hedin	13-August-2005
L. politum	USA: WI: Dodge Co.	OP 1414	M. McCormack	24-August-2007
L. politum	USA: MI: Calhoun Co.	OP 1076	J.W. Shultz	17-August-2002
L. politum	USA: NC: Haywood Co.	OP 819	M. Hedin	25-August-2005
L. politum	USA: AR: Lawrence Co.	Lpo-AR-A1	M. Hedin et al.	21-June-2009
L. bracchiolum	USA: NC: Guilford Co.	OP 1932	P. Nunez	19-September-2007
L. crassipalpe	USA: MO: Butler Co.	Lcr-MO-A1	J.W. Shultz	25-June-2009
L. crassipalpe	USA: MO: Ozark Co.	Lcr-MO-A2	M. Hedin et al.	22-June-2009
L. vittatum	CANADA: ON: Osawa Island	OP 1242	P. Miller et al.	02-July-2007
L. vittatum	USA: TN: Cumberland Co.	OP 835	M. Hedin	27-August-2005
L. vittatum	USA: OK: Cleveland Co.	Lvi-OK-A1	J.W. Shultz	25-June-2004
L. vittatum	USA: GA: White Co.	OP 1411	M. Hedin	16-August-2007
L. vittatum	USA: TN: Davidson Co.	OP 1405	M. Hedin	13-August-2007
L. uxorium	USA: VA: Smythe Co.	OP 1423	M. Hedin	13-August-2007
L. uxorium	USA: NC: Guilford Co.	OP 1934	P. Nunez	19-September-2007
L. relictum	USA: OK: Comanche Co.	OP 1078	J.W. Shultz	27-June-2004
L. relictum	USA: OK: Comanche Co.	Lre-OK-A1	J.W. Shultz	27-June-2004
L. aldrichi	USA: MI: Calhoun Co.	OP 1069	J.W. Shultz	17-August-2002
L. aldrichi	USA: MS: Tishomingo Co.	OP 829	M. Hedin	12-August-2002
L. aldrichi	USA: AL: Marshall Co.	OP 821	M. Hedin	17-August-2005
L. holtae	USA: GA: Dade Co.	OP 1382	M. Hedin	15-August-2007
L. holtae	USA: TN: Cumberland Co.	OP 1379	M. Hedin	10-August-2007
L. ventricosum	USA: SC: Oconee Co.	OP 1440	M. Hedin	20-August-2007
L. ventricosum	USA: AL: Winston Co.	OP 840	M. Hedin	13-August-2005
L. ventricosum	USA: TN: Blount Co.	OP 815	M. Hedin	27-August-2005
L. verrucosum	USA: NC: Buncombe Co.	OP 817	M. Hedin	25-August-2007
L. verrucosum	USA: TN: Cumberland Co.	OP 1412	M. Hedin	10-August-2007
L. n. sp.	USA: NE: Lancaster Co.	Lne-NE-A1	S. Schwartz et al.	30-October-2006
L. flavum	USA: AR: Garland Co.	OP 833	M. Hedin	10-August-2005
L. formosum	USA:GA: Dade Co.	OP 1476	M. Hedin	14-August-2007
L. formosum	USA: FL: Jackson Co.	OP 841	M. Hedin	15-August-2005
L. formosum	USA: FL: Jackson Co.	OP 842	M. Hedin	15-August-2005
L. formosum	USA: AL: Jefferson Co.	OP 827	M. Hedin	13-August-2005
L. aurugineum	USA: FL: Alachua Co.	Lau-FL-A1	K. Prestwich	Summer 2009
L. royali	MEXICO: Veracruz, Xalapa	OP 1162	R. Macías Ordóñez	?
L. bimaculatum	USA: FL: Jackson Co.	Lbi-FL-A1	P. Miller	27-November-2009
L. townsendi	USA: AZ: Cochise Co.	OP 1081	B. Tomberlin	August-2004
L. potosum	MEXICO: Tlaxcala, Ixtacuixtla	OP 1161	R. Macías Ordóñez	?
H. maculosus	USA: NC: Pitt Co.	Hgr-NC-A1	A. Bailey et al.	07-August-2007
H. maculosus	USA: MD: Howard Co.	Hgr-MD-A1	J.W. Shultz	12-June-2004
H. maculosus	USA: MD: Howard Co.	Hgr-MD-A2	J.W. Shultz	12-June-2004
H. n. sp. hoffmani	USA: TN: Sevier Co.	OP 1060	M. Hedin, J. Cokendolpher	31-July-2000
H. n. sp. milleri	USA: MO: Ozark Co.	Hmi-MO-A1	M. Hedin et al.	22-June-2009
H. n. sp. hedini	USA: IL: Johnson Co.	Hhe-IL-A1	M. Hedin et al.	20-June-2009
Eumesosoma roeweri	USA: TX: Wichita Co.	OP 1058	J. Cokendolpher	2000
Togwoteeus biceps	USA: NM: Taos Co.	OP 1068	M. Hedin	03-July-2007
Leuronychus pacificus	USA: AZ: Cochise Co.	Leuropac-AZ-A1	J. Cowles	February-2009
Phalangium opilio	USA: MD: Montgomery Co.		J.W. Shultz	10-September-2003

2. Materials and methods

2.1. Taxon sample

The ingroup sample included all non-problematic species of *Leiobunum* from the eastern and central United States, except *L. cretatum* (see Tables 1 and 2). Taxonomically ambiguous species not represented in the analysis are *Leiobunum peninsulare* Davis, 1934 (probably a junior synonym of *L. bimaculatum*), *L. leiopenis* Davis, 1934 (probably a junior synonym of *L. formosum*), and *L. speciosum* (historically problematic and likely synonymous with *L. bimaculatum*). All specimens corresponding to *Leiobunum speciosum sensu* Davis (1934) were included as *L. vittatum*. *Leiobunum davisi* and *L. zimmermani* are probably synonymous with species included here; the original descriptions were poor, the types appear to have been lost, and type localities are historically well collected. Four species of *Hadrobunus* were sampled, including *H. maculosus* and three previously undescribed species. *Eumesosoma* was represented by *E. roeweri*. The Mexican *Leiobunum royali* was also included because Hedin et al. (2012) showed it to be closely related to USA and Canadian leiobunines. Outgroup sampling included four leiobunines from western North America (*Togwoteeus*

biceps, *Leiobunum potosum*, *Leiobunum townsendi* and *Leuronychus pacificus*) as well as the distantly related phalangiid *Phalangium opilio*.

2.2. DNA extraction, amplification and sequencing

Total DNA was extracted using the DNeasy Blood and Tissue kit (QIAGEN, www.qiagen.com). Eight gene fragments were targeted, corresponding to mitochondrial genes (namely, NADH dehydrogenase 1, 16S ribosomal DNA, the 5′ end of the 12S ribosomal subunit and their intervening tRNAs, leucine and valine), and the two nuclear genes 28S ribosomal DNA and elongation factor-1α (EF1α) exons and introns. Gene fragments were PCR amplified with 35 cycles (94° for 30 s, 55° for 30 s, 72° for 1 min). Primer sequences and combinations are presented in Table 3. PCR products were run out on a 1% low-melt electrophoresis gel with ethidium bromide added to visualize amplicons, and product bands were excised and purified using the Wizard Genomic DNA Purification kit (Promega, www.promega.com). Amplicons were fluorescently labeled and sequenced with a 3730× DNA Analyzer (Applied Biosystems) under the Long50_Z protocol with KBbasecaller setting using the same primers.

2.3. Phylogenetic analyses

Sequences were manually edited using BioEdit v.7.0.1 (Hall, 1999) and aligned using the multiple alignment programs Clustal X (Larkin et al., 2007) and ProAlign (Löytynoja and Milinkovitch, 2003) to assess the effect of alignment method on results. Data were uploaded and exported into Nexus format using Mesquite (Maddison and Maddison, 2008). jModelTest (Posada, 2008) and MrModelTest (Nylander, 2004) were used to evaluate each gene for the appropriate likelihood models using the MrModelTest hierarchies and the Akaike Information Criteria (AIC) (Akaike, 1974) to select the best model. See Table S1 in the Supplementary Information for the best models per fragment. Separate analyses treating each partition scheme with a general time reversible model with an estimated proportion of invariable sites and a gamma distribution (GTR + I + Γ_4) were also applied to demonstrate a lack of difference between reconstructions used with this and other selected models (see Table S2). We applied maximum likelihood (GARLI-part-0.97; Zwickl, 2006) with 1000 bootstrap replicates to assess the relative robustness of taxon bipartitions and Bayesian likelihood inference methods (MrBayes v.3.1.2; Ronquist and Huel-

Table 3
Gene and primer information for amplified target DNA. Standard ambiguity codes apply.

Gene	Fragment	Primer (5′-3′)
ND1	mtDNA 1 (F)	CCTWATAAACTAATCATTTAGC
ND1	mtDNA 1 (R)	GAGTCTGARCTTGTYTCYGG
ND1	mtDNA 2 (F)	CCRGARACAAGYTCAGACTC
ND1	mtDNA 2 (R)	GGGTATATTCAAATTCGAAAAGG
tRNA Leu + 16S	mtDNA 3 (F)	TAGATAGAAACCAACCTGGC
tRNA Leu + 16S	mtDNA 3 (R)	GTGCWAAGGTAGCATAATCA
16S	mtDNA 4 (F)	CCTTTTCGAATTTGAATATACCC
16S	mtDNA 4 (R)	TGACCTCGATGTTGAATTAA
16S + tRNA Val	mtDNA 5 (F)	TGATTATGCTACCTTWGCAC
16S + tRNA Val	mtDNA 5 (R)	ACAAATCGCCCGTCACTCTG
12S	mtDNA 6 (F)	TGTAAATAAATGGCTTAAAGCTTCA
12S	mtDNA 6 (R)	GGCGGTATCTTATCCTYATAGAGG
28S	28S 1 (F)	ACCCGCTGAATTTAAGCATAT
28S	28S 1 (R)	GCTATCCTGAGGGAAACTTCGG
EF-1α intron II	EF-1α 1 (F)	GATTTCATCAARAACATGATYAC
EF-1α intron II	EF-1α 1 (R)	CTTTGTTCCAACATGTTATCTCC
EF-1α intron III	EF-1α 2 (F)	TACATYAAGAAGATTGGTTA
EF-1α intron III	EF-1α 2 (R)	GAACTTGCAAGCAATGTGAGC

senbeck, 2003; Huelsenbeck and Ronquist, 2001) to concatenated sequences modeled using one, three (mitochondrial DNA, 28S and EF-1α), or seven partitions (all genes and tRNAs). Bayesian analysis was performed using Markov-chain Monte Carlo with two independent runs of four Metropolis-coupled chains of ten million generations each, to estimate the posterior probability distribution. Topologies were sampled every 1000 generations, and a majority-rule consensus tree was estimated after discarding the first 250,000 generations. Maximum parsimony was applied to un-partitioned sequences using PAUP* v4.0b10 (Swofford, 2002) or TNT (Goloboff et al., 2008) with 1000 bootstrap replicates. Table 4 gives GenBank accession numbers by sample.

3. Results and discussion

3.1. General findings

Analyses of alternative partitioning schemes of the complete concatenated data produced very similar topologies, and the tree derived from analysis of seven partitions (each having a separate program-tested model) was selected to illustrate this result (Fig. 1A). Findings from other analytical conditions are summarized in Table S2. Most nodes were strongly supported by bootstrap values and posterior probabilities, with lower values and poor resolution being limited primarily to several terminal nodes. Analyses differed in their relative placement of *Leiobunum bimaculatum* and *L. royali*, although their placement was weakly supported under all analytical conditions. The tree derived from nuclear genes (Fig. 1B) was largely congruent with that generated from the complete data but recovered fewer well-supported nodes, and these tended to correspond to major species groups. Taken together, these findings indicate that most of the phylogenetic signal within species groups was derived from the mitochondrial data.

Among the 15 species in our analysis that included multiple geographic examplars, only five were consistently reconstructed as monophyletic, namely *Leiobunum verrucosum*, *L. formosum*, *L. aldrichi*, *L. uxorium* and *L. nigropalpi*. Likely explanations for species non-monophyly vary. For example, the apparent paraphyly of the widely distributed and/or morphologically diverse *Leiobunum vittatum* and *L. calcar* with respect to distinctive species *L. uxorium* and *L. hoffmani*, respectively, may reflect the need for more intensive taxonomic work. However, the recovery of morphologically apomorphic and geographically restricted species as polyphyletic (e.g., *Leiobunum holtae*, *L. relictum*, *L. crassipalpe*) probably reflects either problems in data quality, gene introgression, or deep coalescence impacting mitochondrial data. The latter factors likely explain non-monophyly of species in the *vittatum* group, where mixed species clades tend to cluster by geographic proximity. Deep coalescence may also account for results that are counter-intuitive from a morphological perspective, such as the apparent derivation of a sacculate species, *Hadrobunus* n. sp. 3, from a highly derived and paraphyletic *H. maculosus*. Similarly, results from the full data set recover the more generalized *L. nigropalpi* from the more derived *L. calcar*, although the nuclear-only data resolves these taxa in a manner consistent with expectations derived from morphology. Thus, while the general phylogenetic structure of the Leiobuninae of eastern North America is well-supported, resolving relationships within species groups will require additional data, probably from rapidly evolving nuclear genes.

3.2. Taxonomic implications

3.2.1. Eastern North America Leiobunum is not monophyletic

Results from a recent molecular analysis of sclerosomatid harvestmen (Hedin et al., 2012) indicate that *Leiobunum*, the largest

Table 4
GenBank accession numbers for taxa listed in Table 2. Accession numbers GQ870643–GQ870668; GQ872152–GQ872185 are derived from Hedin et al. (2010).

Sample	Voucher no.	ND1, tRNA-Leu, 16S rDNA, tRNA-Val, partial 12S rDNA; mitochondrial DNA	28S cytoplasmic ribosomal DNA	Elongation factor 1-α introns I and II
Leiobunum calcar	OP 1394	GQ870653, JQ432316	JQ432258	GQ872157
L. calcar	OP 1234	JQ432317	JQ432259	JQ432223
L. calcar	OP 830	JQ432319	JQ432261	
L. calcar	OP 814	GQ870655, JQ432320	JQ432262	GQ872158
L. calcar	OP 1091	JQ432318	JQ432260	
L. n. sp. hoffmani	OP 1383	GQ870654, JQ432315	JQ432257	GQ872159
L. n. sp. euserratipalpe	OP 1235	JQ432321	JQ432263	
L. n. sp. euserratipalpe	OP 1080	GQ870656, JQ432322	JQ432264	GQ872160
L. nigropalpi	OP 1087	JQ432323	JQ432265	JQ432224
L. nigropalpi	OP 1075	JQ432324	JQ432266	JQ432225
L. nigropalpi	OP 846	JQ432325	JQ432267	JQ432226
L. politum	OP 1414	JQ432326	JQ432268	JQ432227
L. politum	OP 1076	JQ432327	JQ432269	JQ432228
L. politum	OP 819	JQ432328	JQ432270	JQ432229
L. politum	Lpo-AR-A1	JQ432329	JQ432271	
L. bracchiolum	OP 1932	JQ432330	JQ432272	JQ432230
L. crassipalpe	Lcr-MO-A1	JQ432331	JQ432273	
L. crassipalpe	Lcr-MO-A2	JQ432332	JQ432274	JQ432231
L. vittatum	OP 1242	JQ432333	JQ432275	JQ432232
L. vittatum	OP 835	GQ870651, JQ432334	JQ432276	GQ872155
L. vittatum	Lvi-OK-A1	JQ432335	JQ432277	JQ432233
L. vittatum	OP 1411	JQ432336	JQ432278	JQ432234
L. vittatum	OP 1405	GQ870652, JQ432337	JQ432279	GQ872156
L. uxorium	OP 1423	JQ432339	JQ432281	JQ432235
L. uxorium	OP 1934	JQ432338	JQ432280	JQ432236
L. relictum	OP 1078	JQ432340	JQ432282	JQ432237
L. relictum	Lre-OK-A1	JQ432341	JQ432283	
L. aldrichi	OP 1069	GQ870650, JQ432342	JQ432284	GQ872154
L. aldrichi	OP 829	GQ870649, JQ432343	JQ432285	GQ872153
L. aldrichi	OP 821	JQ432344	JQ432286	JQ432238
L. holtae	OP 1382	JQ432345	JQ432287	JQ432239
L. holtae	OP 1379	JQ432346	JQ432288	JQ432240
L. ventricosum	OP 1440	JQ432348	JQ432290	
L. ventricosum	OP 840	JQ432349	JQ432291	JQ432242
L. ventricosum	OP 815	JQ432350	JQ432292	JQ432243
L. verrucosum	OP 817	JQ432351	JQ432293	JQ432244
L. verrucosum	OP 1412	JQ432347	JQ432289	JQ432241
L. n. sp.	Lne-NE-A1	JQ432352	JQ432294	
L. flavum	OP 833	JQ432353	JQ432295	JQ432245
L. formosum	OP 1476	JQ432354	JQ432296	
L. formosum	OP 841	JQ432356	JQ432298	JQ432247
L. formosum	OP 842	JQ432355	JQ432297	JQ432246
L. formosum	OP 827	JQ432357	JQ432399	JQ432248
L. aurugineum	Lau-FL-A1	JQ432358	JQ432300	JQ432249
L. royali	OP 1162	JQ432367	JQ432309	JQ432254
L. bimaculatum	Lbi-FL-A1	JQ432366	JQ432308	
L. townsendi	OP 1081	JQ432369	JQ432311	
L. potosum	OP 1161	JQ432370	JQ432312	
Hadrobunus sp.	Hgr-NC-A1	JQ432360	JQ432302	
H. maculosus	Hgr-MD-A1	JQ432361	JQ432303	JQ432251
H. maculosus	Hgr-MD-A2	JQ432362	JQ432304	
H. n. sp. hoffmani	OP 1060	JQ432359	JQ432301	JQ432250
H. n. sp. milleri	Hmi-MO-A1	JQ432364	JQ432306	JQ432252
H. n. sp. hedini	Hhe-IL-A1	JQ432363	JQ432305	
Eumesosoma roeweri	OP 1058	JQ432365	JQ432307	JQ432253
Togwoteeus biceps	OP 1068	JQ432371	JQ432313	
Leuronychus pacificus	Leuropac-AZ-A1	JQ432368	JQ432310	JQ432255
Phalangium opilio		NC010766	JQ432314	JQ432256

genus in Leiobuninae, is artificial. This conclusion will surprise few harvestman systematists. The genus is traditionally defined by the presence of long, thin legs without femoral nodules (pseudoarticulations), rows of coxal denticles at least on the prolateral surface of coxa I and retrolateral surface of coxa IV, and poorly armed dorsal cuticle. This combination of traits has evolved independently in different regions of the Northern Hemisphere, with the type species of the genus, *L. rotundum* (Latreille 1789), occurring in Europe. The "*Leiobunum*" of East Asia and North America are more closely related to other sympatric genera than to the European *Leiobunum*, and they will inevitably be transferred to new genera once the phylogenetic understanding of the group has stabilized. This situation is complicated further by the fact that regional "*Leiobunum*" appear to have given rise to new genera. Thus, aside from *L. rotundum* and its close relatives, *Leiobunum* is polyphyletic at deeper, intercontinental levels of leiobunine phylogeny and often paraphyletic at shallower, regionally restricted levels. These problems are well illustrated by the results of our analysis, where *Leiobunum* is clearly paraphyletic with respect to *Eumesosoma* and *Hadrobunus* in the east and *Leuronychus* in the west (Fig. 1).

3.2.2. The early-season Leiobunum group

This group encompasses five species (Fig. 1; Table 1) and was recovered under all analytical conditions with high support in

analyses that include all mitochondrial and nuclear sequences. The early-season group has no obvious morphological synapomorphies, although its members appear to be unique in overwintering as juveniles rather than eggs and in reaching sexual maturity in the late spring and early summer rather than mid- to late summer. This aspect of the life cycle has been determined for *L. verrucosum*, *L. flavum*, *L.* n. sp. and *L. ventricosum* by original observation (JWS), but the life cycle of *L. holtae* has not been specifically investigated. McGhee (1975) noted that adult specimens of *L. holtae* are present from spring to fall, which suggests the life history of this species is similar to that of *L. ventricosum* in maturing in late spring and persisting as adults for several months.

With the exception of *L. holtae*, species in the early-season group have a pair of subterminal, distally opened sacs on the penis (Fig. 1: *L. verrucosum*, *L. ventricosum*) used to deliver a nuptial secretion to the female during mating (Shultz, 2005; Macías-Ordóñez et al., 2010; MMB and JWS, orig. obs.). Sacs are useful in identification but are symplesiomorphic for Leiobuninae. The penis in *L. holtae* (Fig. 1) lacks sacs and is otherwise highly specialized, with a long, thin, laterally compressed distal shaft and broad, somewhat dorsoventrally compressed proximal portion. Despite its unique male genitalia, *L. holtae* is similar to *L. ventricosum* in body size, relative leg length, and coloration. It is likely that *L. ventricosum* will eventually emerge as being paraphyletic with respect to *L. holtae*.

3.2.3. Leiobunum vittatum group

The *vittatum* group was recovered with high support under all analytical conditions (Fig. 1). Our analysis included three well-delimited morphospecies, namely *L. uxorium*, *L. crassipalpe* and *L. relictum*. The taxonomic status of *Leiobunum vittatum* requires more thorough morphological and molecular study. This species occurs throughout the eastern and central United States and adjacent southern Canada (Cokendolpher and Lee, 1993) and shows considerable variation in color, relative leg length and male palpal armature. It is unclear whether the geographic variants reflect taxonomically objective boundaries. The problematic species *L. speciosum*, as described by Davis (1934), appears to correspond to one such *L. vittatum* variant; specimens in the type series are clearly *L. bimaculatum* (JWS, orig. obs.). Another distinctive species, *L. denticulatum*, is a heretofore unrecognized member of the group (JWS, orig. obs.) known from a few museum specimens from south-central Mexico. We were unable to obtain specimens for molecular work. The *vittatum* group is united by synapomorphic features associated with reproduction. The penis lacks all evidence of sacs or alae. The distal half to two-thirds of the penis shaft is a thin cylinder (broader in *L. crassipalpe*) and the proximal region is thicker (Fig. 1: *L. vittatum*). In all species except *L. relictum*, the femur and patella of the male pedipalp are long and a proventral row of spines extends along the femur, patella and tibia.

As noted above, the internal phylogenetic structure of the *vittatum* group recovered here does not match implicit taxonomic predictions: *L. vittatum* and *L. crassipalpe* are recovered as paraphyletic and *L. relictum* as diphyletic. A literal interpretation of our result suggests that the *L. vittatum* concept corresponds to a widespread ancestral form that has persisted and has also given rise to several distinctive, regional forms. However, the interpretation of non-monophyly in the morphologically distinct and geographically restricted *L. crassipalpe* and *L. relictum* may stem from other factors, such as quality of the DNA templates, low phylogenetic signal (note low support values in Fig. 1 and Table S2), standing genetic variation, or deep coalescence of mitochondrial haplotypes. The latter factors are consistent with the geographic proximity of related terminals. Clearly, the internal structure of the *L. vittatum* species group, especially *L. vittatum*, demands a more thorough taxonomic and phylogenetic treatment.

3.2.4. Leiobunum politum group

This group contains two named species, *L. politum* and *L. bracchiolum*. They are united by a pair of "bulbs" on the penis that represent modifications of the primitive sacs, apparently through reduction of the lateral walls. In addition, the male is unique among North American *Leiobunum* in having a labrum greatly inflated at the tip. McGhee (1975) interpreted the transitional region between the laterally compressed and dorsoventrally compressed parts of the penile shaft of *L. holtae* as a "bulb" and placed it with *L. politum* and *L. bracchiolum* in a "bulbate" species group. We find no morphological or phylogenetic evidence to support this hypothesis. Our results indicate that the widespread *L. politum* is paraphyletic with respect to *L. bracchiolum*, which is limited to the eastern Appalachian Region, Piedmont, and portions of the Atlantic Coastal Plain (McGhee, 1975; JWS, orig. obs.). The two species are morphologically distinct where their ranges overlap (McGhee, 1975). In Maryland, for example, *L. politum* is found in woodlands with or without understory and *L. bracchiolum* occurs in herbaceous vegetation usually associated with trees. They can occur together but are readily distinguished by body size (McGhee, 1975) and coloration.

3.2.5. Leiobunum calcar group

The group is united by several morphological features and is recovered here as monophyletic with strong support (Fig. 1). Two species have been recognized historically, the morphologically uniform *L. nigropalpi* and the morphologically diverse *L. calcar*. Ingianni et al. (2011) described two additional species, *L. euserratipalpe* and *L. hoffmani*. The *calcar* group is united by a suite of reproductive features: the penis lacks subterminal sacs, although a pair of variably developed alae are often present (Fig. 1), and the proximal region of the male palpal tibia is inflated and denticulate for use in clasping trochanter I of the female during mating (Bishop, 1949b). The internal phylogenetic structure of the *calcar* group is not well resolved and is inconsistent with morphology, with the latter supporting *Leiobunum nigropalpi* as the plesiomorphic sister group to the remaining species (Table 1). In male *L. nigropalpi*, the palpal femur retains a retrolateral row of denticles rather than a distal cluster, the palps are gracile rather than robust, and the penis is relatively unspecialized (e.g., it retains a demarcation between glans and shaft) (Fig. 1). The inference derived from morphology is supported by analyses of the nuclear sequences, and it is possible that the conflicting mitochondrial signal results from deep coalescence and/or genetic introgression.

3.2.6. Hadrobunus group

Our analysis strongly recovers two *Leiobunum* species, *L. aurugineum* and *L. formosum*, as the monophyletic sister group to *Hadrobunus*. Like *Hadrobunus*, *L. aurugineum* has relatively short, robust legs and the opisthosomal dorsum is armed with retrorse spinulate tubercles. Our placement of *L. aurugineum* is therefore not surprising and, indeed, many museum specimens of *L. aurugineum* are already labeled "*Hadrobunus grandis*" (JWS, orig. obs.). In contrast, *L. formosum* is typical of *Leiobunum* in having long, thin legs and a weakly armed or unarmed scutum, although some populations also have retrorse armature (JWS, orig. obs.). An on-going revision of *Hadrobunus* has revealed additional morphological similarities between *Hadrobunus*, *L. aurugineum* and *L. formosum* that support the eventual transfer of the two *Leiobunum* species to *Hadrobunus*.

3.3. Future directions

Our results indicate that the leiobunine fauna of the eastern North America contains two major clades. The early-season *Leiobunum* clade encompasses mostly species that retain the plesiomorphic sacculate penis and the second clade contains mainly

species that lack sacs (Fig. 1). A strong connection between eastern US and eastern Mesoamerican leiobunines has already been established (Hedin et al., 2012) and the current evidence suggests that the major lineage diversified in Mesoamerica before entering the US region and then diversified further. Future work will continue to explore cryptic diversity, especially in the *Hadrobunus* and *L. vittatum* groups. We will also focus on the evolution of penile traits in North American leiobunines, utilizing comparative methods to describe the evolution of reproductive morphology in male and female harvestmen and the corresponding mating systems across the phylogeny.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.12.025.

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