

## Lapsiines and hisponines as phylogenetically basal salticid spiders (Araneae: Salticidae)

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### Abstract

Increased phylogenetic resolution of the basal lineages of salticid spiders will help us understand their early evolution and provide better outgroups for phylogenetic studies within the major clades. We gathered sequences of nuclear and mitochondrial gene regions (28S, 18S, Histone 3, 16S-ND1, CO1) and used them to reconstruct salticid phylogeny by parsimony, likelihood and Bayesian methods. Our results confirm that lapsiines and hisponines are among the basal salticids, i.e. outside the major clade Salticoida. The lapsiines are resolved as sister group to the spartaeines. The precise placement of hisponines is unclear, but they may represent a deep-branching lineage independent from the spartaeines.

**Key words:** Araneae, Salticidae, *Thrandina*, *Galianora*, *Hispo*, *Massagris*, *Tomocyriba*, *Goleba*, lapsiines, Hisponinae, Spartaestinae, Lyssomaninae, jumping spider, basal groups, phylogeny

### Introduction

Morphological and molecular data have begun to resolve the basal phylogenetic structure of salticid spiders (Wanless, 1980, 1982, 1984, Rodrigo & Jackson, 1992, Maddison, 1988, 1996, Wijesinghe, 1992, 1997, Maddison & Hedin, 2003). One of the best corroborated clades is the Salticoida (Maddison & Hedin, 2003), within which falls the vast majority of salticids, about 95% of the approximately 5000 described species (Platnick, 2005). Excluded from the Salticoida are three much smaller groups: the lyssomanines, the spartaeines, and the *Cocalodes* group. Six extant Old World and 2 New World genera are placed in the Lyssomaninae (Wanless, 1980, Logunov, 2004); 15 genera, entirely from the Old World, are placed in the Spartaestinae (Wanless, 1984, Wijesinghe, 1992, Žabka &

Kovac, 1996); 4 Old World genera are in the *Cocalodes* group (Wanless, 1982, 1985). We will refer to these non-salticoid groups as "basal salticids", not to imply that they have predominantly primitive characteristics, merely to indicate that they fall outside of the speciose clade Salticoida.

Basal salticids offer special insight into early salticid evolution for two reasons. First, if the basal salticids do not form a single clade, but diverged successively from the line leading to the Salticoida, then they will have a strong influence over inference of the family's ancestral states, whether parsimony or likelihood methods are used. Second, even if basal salticids do form a single clade, which therefore would stand equal to the Salticoida as an indicator of ancestral states, each sampled basal species would have more influence on ancestral state inference than each sampled salticoid species. For these reasons the unusual predatory behaviour (Jackson & Pollard, 1996, Li, 2000) and eye anatomy (Blest *et al.*, 1990) of basal salticids are of particular interest in understanding the origins of salticid diversity. By recognizing what species are among the basal groups and how they are related, we will be able to characterize better the early evolution of salticids. In addition, we will have more complete outgroup information for reconstructing phylogeny within the Salticoida.

In this paper we present molecular data to examine whether two little-studied groups of salticids might belong with the lyssomanines and spartaeines as basal salticids: the lapsiines (Maddison, 2006) and hisponines (Wanless, 1981, Prószyński & Żabka, 1983, Wesołowska, 1993). Salticid systematists have made few comments on where these groups belong. Simon (1901) apparently considered lapsiines and hisponines relatively primitive, but he also considered various salticoids as equally primitive. Simon conferred suggestive names on one lapsiine ("*Lapsias cyrboides*") and one hisponine ("*Tomocyriba*") that hint to similarities with a spartaeine, *Cyrba* Simon. Prószyński & Żabka (1983) placed *Tomocyriba* Simon within the Euophryinae on the basis of the spiraled embolus.

The neotropical lapsiines include *Lapsias* Simon and two recently discovered genera (Maddison, 2006). Their basal phylogenetic placement is suggested by the presence of a tarsal claw on the female palpus, loss of which is considered a synapomorphy of the salticoids (Maddison & Hedin, 2003). In addition, the male palp has an extra sclerite associated with the tegulum, presumably homologous to the median apophysis of *Cocalodes* Pocock and *Holcolaetis* Simon (Wanless, 1982, 1985). The salticoids and spartaeines are characterized by the loss of this sclerite. If the lapsiines are confirmed as basal salticids, it would show that the New World has a previously unrecognized radiation of basal salticids.

Hisponines are Old World salticids primarily from Africa. The three extant genera—*Hispo* Simon (Wanless, 1981), *Massagris* Simon (Wesołowska, 1993) and *Tomocyriba* Simon (Prószyński & Żabka, 1983)—are distinctive for a constriction on the carapace just behind the posterior median eyes. Our attention was drawn to hisponines by two observations. First, as in the lapsiines, the tegulum in many species has a small sclerite that

may be homologous to the median apophysis of other basal salticids. Second, Baltic amber salticids are dominated by two body forms, the first with the small eyes unusually large (placing them among the basal salticids), the second with a distinctive constriction behind the small eyes. As implied by Prószyński & Żabka (1983), this constriction suggests that the Baltic amber genera *Gorgopsina* Petrunkevitch and *Prolinus* Petrunkevitch are hisponines. If Baltic amber has hisponines but not salticoids, hisponines may have a relatively ancient divergence from other salticids.

We therefore obtained molecular data from hisponines and lapsiines to determine whether, as hinted by their morphology, they lie outside the Salticoida. We also include data from two nuclear genes not previously used in salticids (18S, Histone 3) and for several other genera not previously studied.

## Material and methods

### Taxon sampling

In addition to sequences obtained previously (Maddison & Hedin, 2003), we analyzed sequences obtained from 26 species (Appendix 1). The primary targets of the study were lapsiines of the genera *Thrandina* Maddison and *Galianora* Maddison (Maddison, 2006) and hisponines of the genera *Hispo*, *Massagris*, and *Tomocyriba*. In addition, we sequenced two genera of lyssomanines (*Lyssomanes* Hentz, *Goleba* Wanless), two genera of spartaeines (*Portia* Karsch, *Phaeacius* Simon), *Holcolaetis*, ten salticoids, and four outgroups. The sampled species of *Thrandina*, *Galianora* and *Lyssomanes* include the type species of their genera; those of the other hisponine, lyssomanine and spartaeine genera are closely similar to the respective type species except *Massagris schisma* Maddison & Zhang and *Tomocyriba andasibe* Maddison & Zhang. The salticoids were chosen to include representatives of the major groups recognized by Maddison & Hedin (2003): marpissoids (*Dendryphantes* C.L.Koch, *Ghelna* Maddison), euophryines (*Euophrys* C.L.Koch, *Zenodorus* Peckham & Peckham), amycoids (*Sitticus* Simon), plexippoids (*Evarcha* Simon, *Pellenes* Simon), and two unplaced genera (*Salticus* Latreille, *Orthrus* Simon). Whether or not the salticoid species sampled are type species of their higher taxa or close thereto is not vital as long as they are well scattered among the salticoids. This is demonstrated in our results (e.g., Figure 10). Additional sequences from Maddison & Hedin (2003) were added to most analyses. Authors of species names are listed in Appendix 1. Voucher specimens are accompanied by labels with their voucher numbers (as shown in the second column of Appendix 1); the label is marked "WPM voucher DNA".

We were able to identify specimens to species with a few exceptions. Several are described by Maddison (2006) and Maddison & Zhang (2006). Our *Hispo* (called here *Hispo* cf. *frenata*) and one of the *Massagris* (called here *Massagris* cf. *honesta*) may

represent undescribed species, but without a thorough taxonomic review we are reluctant to describe them. Instead we offer figures of the genitalia (Figs. 11–12). The specimen of *Holcolaetis* is an immature female. Its markings are similar to adults of *H. zuluensis*, found in the same region. The specimen of *Orthrus* is one of the two used by Maddison & Hedin (2003). We resequenced its 28S because of concern that there may have been errors in the previous *Orthrus* sequence, suggested by its instability in their phylogenetic analyses. The "unidentified spartaeine" studied by Maddison & Hedin (2003) has since been identified as *Spartaeus uplandicus* Barrion & Litsinger.

### Sequencing

Specimens were preserved in 95% or 100% ethanol. From most specimens DNA was extracted from legs, although for some, other body parts were used. Otherwise-intact spiders are preserved as voucher specimens in the Spencer Entomological Museum, UBC, except for specimens borrowed from and returned to the California Academy of Sciences (see Appendix 1). Genomic DNAs were extracted from tissues using the QIAGEN DNeasy extraction kit.

Five gene regions were amplified by PCR and sequenced: the nuclear 28S (primers 28SO and 28SC from Hedin & Maddison, 2001), 18S (amplified and sequenced in three pieces by the primer pairs 1F-5R, 3F-7R, 4F-9R, from Giribet *et al.*, 1996), Histone 3 (primers H3AF and H3AR from Colgan *et al.*, 1998), and the mitochondrial 16S-ND1 (primers 12261 and 13398 from Hedin & Maddison, 2001) and CO1 (primers 1718 and 2776 from Hedin & Maddison, 2001). Histone 3 will hereafter be referred to as "H3". The 16S-ND1 fragment includes a transfer RNA in addition to parts of 16S and ND1 (Hedin & Maddison, 2001). PCR involved an initial 95°C denaturation followed by 35 cycles of 45 s at 95°C, 45 s at either 48–50°C (28S, CO1, H3), 44–48°C (16S-ND1), or 48°C (18S), 60 s at 72°C per cycle, with a final 10-min extension at 72°C.

Sequencing was done either by the University of British Columbia NAPS facility (ABI 377 sequencer) or by Macrogen, Inc. (ABI 3730 sequencer). The primers used in PCR were also used for sequencing. Three additional primers were used for *Galianora sacha* 28S to bypass a region that caused the sequencing reaction to stutter ("28S-G1F Forward" 5'-CGA AGG CAG TGC CTC ACG CCT G-3'; "28S-G2F Forward" 5'-CGC ACA CGT TGG GAC CCG AAA G-3'; "28S-G1R Reverse" 5'-CAG GCG TGA GGC ACT GCC TTC G-3'). Most base calls in all sequences were confirmed by reads in both directions except 16S-ND1 in *Thrandina parocula*, *Galianora bryicola*, and *Massagris* cf. *honestata*. For these three sequences only a single, although high quality, read was obtained and used.

Sequences were obtained from the chromatogram files using phred (Ewing & Green, 1998, Ewing *et al.*, 1998, Green & Ewing, 2002) and phrap (Green, 1999) as operated via the chromaseq package (D. Maddison & W. Maddison, in prep.) for Mesquite (W.

Maddison & D. Maddison, 2005). Phred was run with default options to read bases and assign quality scores; phrap was used with options "-qual\_show 20 -vector\_bound 0" to assemble the reads into contigs. Sequence ends were trimmed by chromaseq using a moving window analysis: the first window of 10 bases within which at least 6 were above quality score 20 was used as the start or end of the sequence. If a site had a secondary peak at least 0.3 the height of the primary peak, it was treated as ambiguous. Following this automatic processing and contig assembly, the resulting sequences were compared against the chromatograms by eye using chromaseq, to reconsider ambiguities and trimming. This resulted in few changes to the sequences, primarily adjustments of the extent of trimmed ends.

Proofread sequences were imported into ClustalX version 1.83 (Thompson *et al.*, 1997) for alignment. Multiple alignments were carried out with gap opening/gap extension costs set to 24/6 following Maddison & Hedin (2003). Following automatic alignment, minor editing of the alignments for the ribosomal sequences was done manually using MacClade (D. Maddison & W. Maddison, 2005). Manual editing was restricted to adjusting a few obviously misaligned regions near the sequence ends. Final sequences were submitted to GenBank (accession numbers in Appendix 1). The aligned all-genes matrix used in the Bayesian analysis for Figure 2 is deposited in TreeBASE (treebase.org).

Our sequences were supplemented with those of Maddison & Hedin (2003) for 9 of our taxa. Maddison & Hedin's sequences of 28S, 16S-ND1, and CO1 were used for *Lyssomanes* and each of the outgroups; their sequence of 16S-ND1 was used for *Salticus*; and their sequences of CO1 were used for *Evarcha* and *Orthrurus*. Their sequences of 16S-ND1 and CO1 were used for *Pellenes*, although this created a chimeric taxon (our sequences are from *P. peninsularis*, theirs from the closely related *P. shoshonensis* Gertsch). None of the other taxa are species chimeras. (The *Evarcha* used by Maddison & Hedin, reported as *E. hoyi*, was almost certainly *E. prószyńskii* as is ours, although their voucher specimen has not been relocated to confirm this.)

### Phylogenetic analysis

Phylogenetic analyses were performed on the gene regions separately and combined, and using various criteria (parsimony, likelihood, Bayesian posterior probability).

Phylogenetic analyses were done on both a large and a small taxon sample. The small taxon sample included 6 additional taxa from the data of Maddison & Hedin (2003): *Portia labiata* Thorell, *Spartaeus spinimanus* Thorell, *S. uplandicus*, *Helvetia* cf. *zonata* Simon, *Naphrys pulex* Hentz, and *Terralonus mylothrus* Chamberlin. The latter three salticoids were included because they belong in subfamilies for which some genes would be otherwise unrepresented. For instance, our *Heliophanus* sequences are 28S, 18S, and H3; the heliophanine *Helvetia* from Maddison & Hedin provides in addition 16S-ND1 and CO1, and also has 28S to provide the "glue" that would join the two heliophanine taxa

together in the course of analyses combining genes. We performed small taxon sample analyses for 28S, 18S, H3, 16S-ND1, and CO1 gene regions separately and together in various combinations. 16S-ND1 was used as a single partition not because of biological homogeneity (it is a mix of protein coding and non-coding regions) but because it is amplified and sequenced as a piece, and we wished to explore its value as a region to sequence in the future. We also performed analyses on a fused ND1-CO1 matrix translated to protein assuming the *Drosophila* mitochondrial genetic code. In analyses combining more than one gene region a taxon was deleted if sequences were lacking in some genes so as to give it 75% or more missing data.

The advantage of including few salticoids in the small taxon sample was that we could focus our efforts on sequencing more gene regions; the disadvantage was that even if we showed that the lapsiines and hisponines were outside the clade of sampled salticoids, they might still lie inside the clade of all salticoids. We mitigated this by choosing a broad sample of salticoids, but we also performed analyses on a much larger sample of taxa, though for fewer genes. The large sample included all remaining sequences of Maddison & Hedin (2003) for 28S, 16S-ND1, and CO1, with the exception of 28S in *Evarcha*, *Orthrus*, and *Salticus* and 16S-ND1 in *Evarcha* for which we used sequences presented here.

Bayesian analyses were done using MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003). The GTR invariant-gamma model was used throughout (nst=6 rates=invgamma). Models were permitted to vary among data partitions, with the partitions defined as follows: 28S; 18S; H3; 16S; ND1 first, second, and third codon positions; CO1 first, second, and third codon positions. When ND1 and CO1 were included together in an analysis, they were united to yield 3 partitions (ND1+CO1 first, second, and third codon positions) instead of 6. Each analysis was run for 10 million generations via the command "mcmc ngen= 10000000 printfreq=1000 samplefreq=1000 nchains=4 savebrlens=yes;", except for the individual analyses for CO1 and H3 which ran for only 5 million generations. Results are summarized by a majority rules consensus tree of sampled trees, having discarded generations up to 100,000 (by which point the posterior probabilities had stabilized), or up to 50,000 for the shorter CO1 and H3 runs.

Likelihood analyses were done using both RAxML-VI (Stamatakis *et al.*, 2005, Stamatakis 2005) and PAUP\* (Swofford, 2002). RAxML was used in general, with PAUP used in addition for the two All Genes analyses. With RAxML, most analyses were done by 20 separate runs with the default standard hill climbing, choosing the run with the best likelihood. For the All Genes analyses, however, 100 separate standard runs were done, and also simulated annealing was used with a time limit of 40 hours; the run with the best likelihood was chosen. The model GTRCAT was used for nucleotide matrices, JTT CAT for amino acid matrices. With PAUP\*, we used the same procedure as Maddison & Hedin (2003), with model parameters estimated on preliminary trees followed by 5 random addition sequence searches.

Parsimony analyses were done using PAUP\* (Swofford, 2002), treating character states as unordered (one step for any state change). Gaps were treated as missing data. For the small taxon sample analyses, initial searches consisted of 5,000 random addition sequence replicates, each saving at most 5 trees with each replicate in order to narrow the search (D. Maddison, 1991), using TBR branch swapping (Swofford, 2002). Only most parsimonious trees found were saved. These were used as input trees into a second round of TBR branch swapping that was not constrained except by MAXTREES of 100,000. For the large taxon sample analysis combining 28S + 16S-ND1 + CO1, the search was the same except that the initial phase consisted of 20,000 random addition sequence replicates. For the combined All Genes data matrix with the small taxon sample, replicability of clades was assessed by a PAUP\* non-parametric bootstrap analysis (Felsenstein, 1985) with 1000 replicates, on each of which 20 random addition sequence replicates obtained starting trees for TBR branch swapping holding no more than 1000 trees.

## Results

Seventy nine sequences were obtained from 26 taxa and five gene regions; they are listed in Appendix 1. Our new *Orthrhus* sequence for 28S is different at many sites from that obtained by Maddison & Hedin (2003) from a different specimen from the same locality (both vouchers reexamined, males, and confirmed as same species). We doubt that the differences represent polymorphism within the population; we have no explanation for this. Because of technological improvements we have more confidence in our current sequence and will use that.

The results of the phylogenetic analyses on the small taxon sample are summarized in Figure 1, which indicates support from the varied analytical methods and data partitions. We consider twelve clades resolved with reasonable confidence, and mark them by circled numbers. These twelve clades are recovered in the Bayesian analyses of All Genes combined (Fig. 2), and most are recovered in the parsimony analysis (Fig. 3).

For the All Genes likelihood analyses, PAUP\* and RAxML gave trees that were identical in the basal regions of the tree, differing only in placements within the Salticoida. Differences are not surprising because the two programs calculated likelihood slightly differently (e.g., gamma rate variation versus CAT) and also did not use identical search strategies. The models of substitution and rate variation used in PAUP\* likelihood searches, inferred from the data, were GTR plus gamma rate variation with a proportion invariant (small taxon sample,  $\text{rmatrix}=(0.89729\ 4.04401\ 5.58411\ 2.27794\ 5.73636)$   $\text{shape}=0.768635$   $\text{pinvar} = 0.508922$ ; large taxon sample,  $\text{rmatrix}=(1.43352\ 5.93829\ 6.45299\ 3.25682\ 8.16426)$   $\text{shape}=0.777875$   $\text{pinvar} = 0.313501$ ).

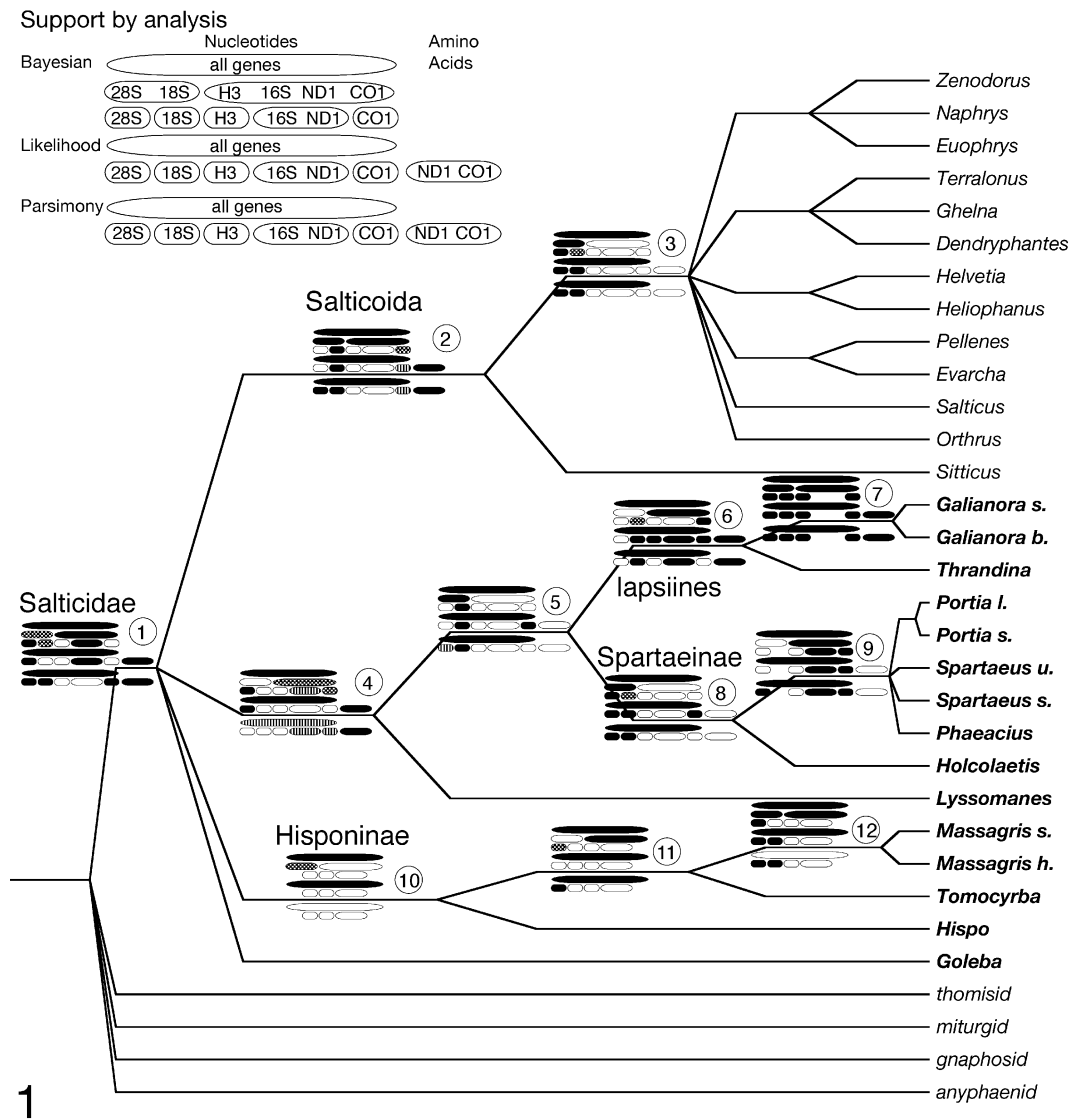
The individual gene regions analyzed separately provide independent support for many of these clades (Figs. 4–8). For instance, the exclusion of the lapsiines from the Salticoida is supported by 18S, 16S-ND1, and CO1. It is also supported by 28S except for

the placement of *Sitticus*. If 28S and 18S are combined into a single matrix, or if H3 + 16S-ND1 + CO1 are combined, this placement of lapsiines is also supported (there is no separate figure for these data combinations, although their results are indicated via the oval decorations in Fig. 1). The lapsiine placement is also supported in a matrix of CO1 and ND1 translated to amino acids (Fig. 9). The exclusion of the hisponines from the Salticoida is supported in the analysis of 18S alone, 28S alone (with the exception of *Sitticus*), the combined matrix 28S + 18S, the combined matrix H3 + 16S-ND1 + CO1, and with All Genes combined. In general there is strong support from varied analyses and data partitions for the primary conclusion of this paper, that the lapsiines and hisponines fall outside the Salticoida.

Results for the large taxon sample are shown in Figure 10. They confirm that the small taxon sample had been chosen well, as it includes representatives spanning the Salticoida. The Salticoida is resolved as monophyletic with the lapsiines and hisponines excluded. For legibility of the basal taxa, interrelationships within the Salticoida are de-emphasized in this figure; they will be treated in a subsequent paper using additional data. The relationships among the basal lineages matches in general those recovered from the small taxon sample analyses except for *Hispo*, *Tomocyriba*, and *Goleba*. *Hispo* fell with the miturgid in the likelihood and Bayesian analyses and with the salticoid heliophanines with parsimony. *Hispo* is lacking data from 28S, which was the single most informative gene region in the analyses of Maddison & Hedin (2003) as judged by its concordance with the All Genes analysis. *Tomocyriba* fell with the corinnid by parsimony. *Goleba* fell with the corinnid in all large taxon sample analyses. Given the strong support from various partitions for salticid monophyly in the small taxon sample analyses, and their unique and unreversed morphological synapomorphies, these placements would appear to be in error. The dominance of salticoids within the larger matrix may have degraded either the alignment in the basal regions of the tree, or the appropriateness of the estimated models of evolution for the basal regions of the tree (for likelihood and Bayesian methods). Otherwise, the basal relationships are as with the small taxon analyses, with lapsiines sister to spartaeines (including *Holcolaetis*), and *Lyssomanes* sister to the two.

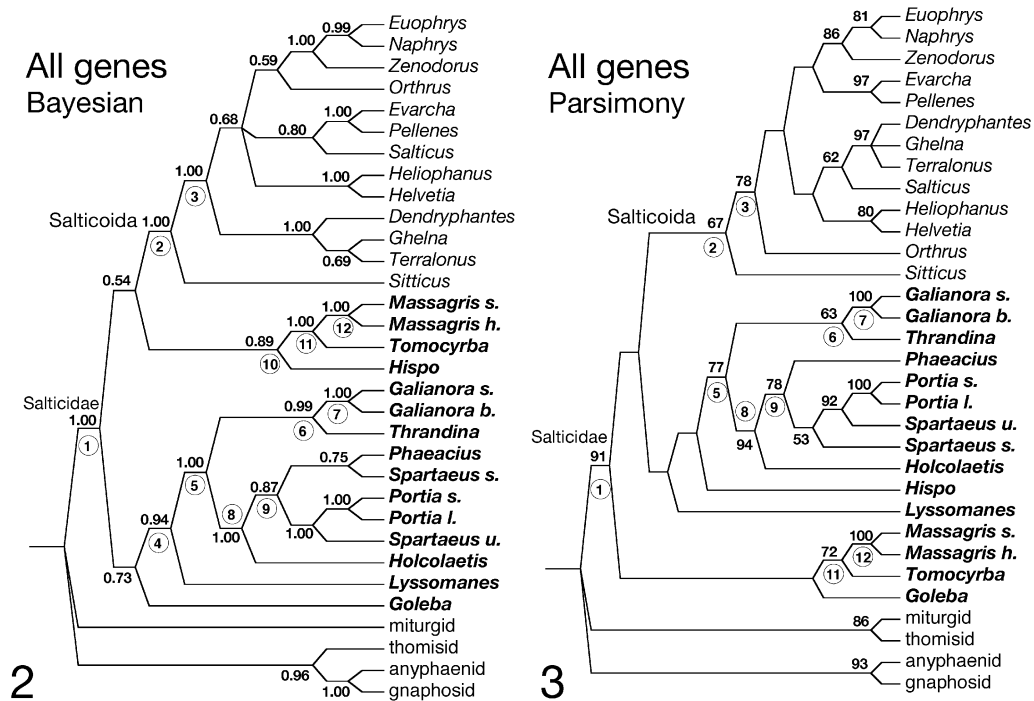
## Discussion

Our molecular data clearly support a placement of the lapsiines (*Galianora* and *Thrandina*) outside of the Salticoida. The All Genes analyses (Figs. 2–3) and various partitions support this (28S, 18S, 16S-ND1, CO1; Figs. 4–8), as does the large taxon sample analysis (Fig. 10). Some morphological support for the exclusion of lapsiines from the Salticoida has already been mentioned—the presence of the tarsal claw in females and median apophysis in males are both plesiomorphic and indicate lapsiines fall outside of the Salticoida. Also supporting the basal position of lapsiines is the more or less equal numbers of teeth on the anterior and posterior tarsal claws on the second pair of legs



**FIGURE 1.** Summary of phylogenetic analyses. Filled ovals indicate support from different data partitions and analytical methods. Bayesian analyses: black indicates posterior probability (p.p.) = 0.75; checkered indicates p.p. 0.51–0.75. Other analyses: black indicates the clade appears in the most parsimonious or highest likelihood trees; striped indicates the clade was supported but with one taxon excluded, as explained in the following notes. Notes concerning numbered clades: (1) 28S+18S p.p. = 0.74; 18S p.p. = 0.58. (2) CO1 p.p. = 0.56; clade by CO1 likelihood and parsimony excludes *Orthrus*. (3) 18S p.p. = 0.71. (4) H3+16S-ND1+CO1 p.p. = 0.69; CO1 p.p. = 0.67; clade by 16S-ND1 Bayesian and parsimony excludes *Tomocyrra*; clade by CO1 parsimony excludes *Thrandina*; clade by All Genes parsimony includes *Hispo*. (5) Clade by 28S parsimony excludes *Thrandina*. (6) 18S p.p. = 0.57. (7) 16S-ND1 sequence not obtained for *Galianora sachae*. (8) 18S p.p. = 0.56. (9) 18S sequences obtained only for *Portia s.* in this clade. (10) 28S+18S p.p. = 0.63; CO1 sequences not obtained for any hisponine. (11) 28S p.p. = 0.53.

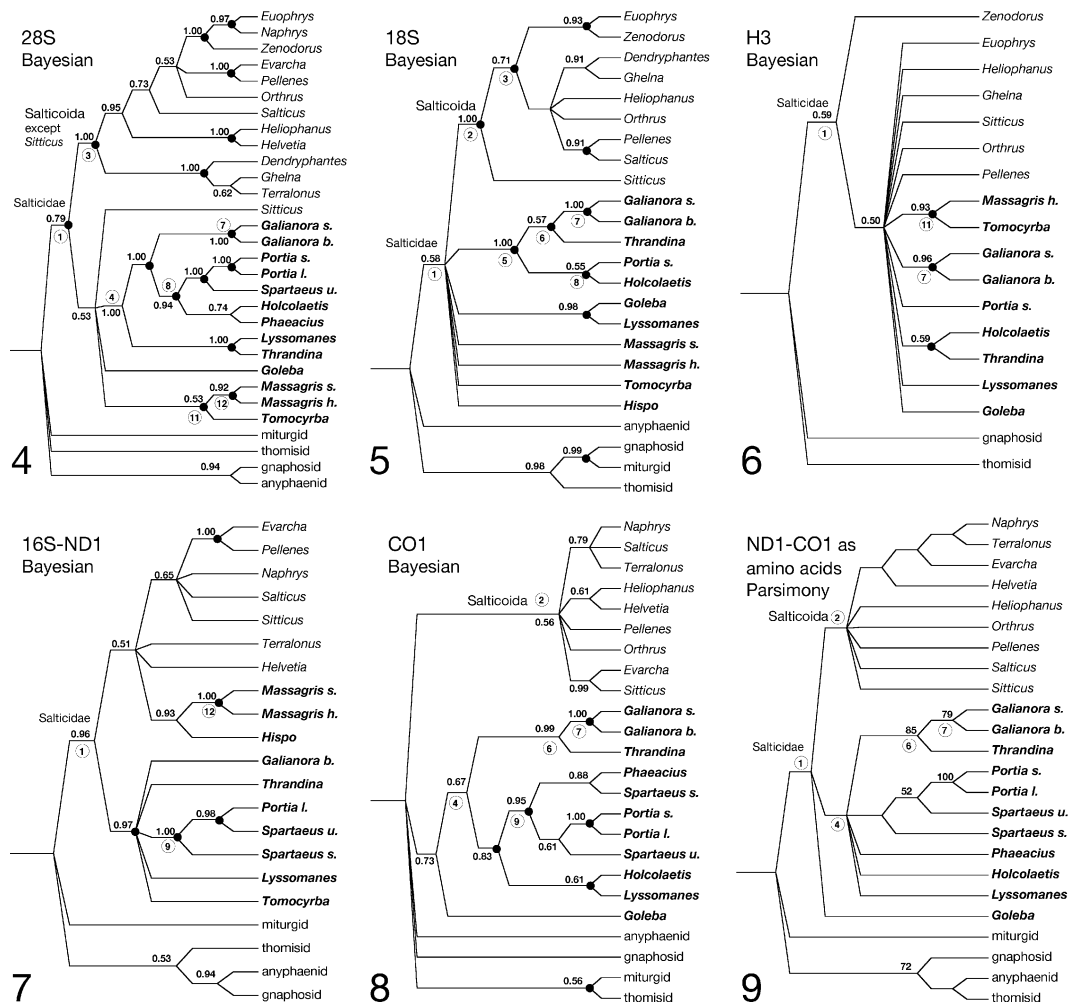
(Maddison, 2006; unequal in salticoids, Maddison, 1988, 1996). Two other plesiomorphies also indicating exclusion from the Salticoida are the relatively long fovea of the carapace and, in *Thrandina* at least, the large posterior median eyes (Maddison, 2006). Live specimens of *Thrandina* were observed to have a peculiar walk similar to that of spartaeines (Maddison, 2006); it might be either a synapomorphy uniting lapsiines with spartaeines or a plesiomorphy excluding both from the salticoids. Because so few lapsiine specimens are available, we did not examine several relevant characters requiring disarticulation (chelicerar mound, gnathocoxal glands, intercheliceral sclerite; Maddison & Hedin, 2003).



**FIGURES 2-3.** Summary of analyses using All Genes (28S + 18S + H3 + 16S-ND1 + CO1). Circled numbers mark clades from Figure 1. 2, Majority rule consensus tree of 9900 trees sampled from Bayesian analysis; shown are estimated posterior probabilities (from last 9900000 of 10 million generations). 3, Strict consensus of three most parsimonious trees (treelength 6871 steps); shown are bootstrap values, 1000 replicates.

To date no morphological synapomorphies of lapsiines have been found, although they are easily recognized among neotropical salticids by their plesiomorphic features (Maddison, 2006). The molecular data (node 6, Fig. 1), however, do provide support for the monophyly of the three species sampled. This demonstration of monophyly does not yet justify recognition of a formal taxon including these three species and *Lapsias*, because the latter has not yet been studied phylogenetically. Nonetheless we provisionally refer to our three sampled species as lapsiines (see Maddison, 2006). Our analyses (All Genes, 28S, 18S, 16S-ND1, CO1) give strong support for the lapsiines being in a clade including the spartaeines and possibly *Lyssomanes* (nodes 4 and 5, Fig. 1). Within the lapsiines, all

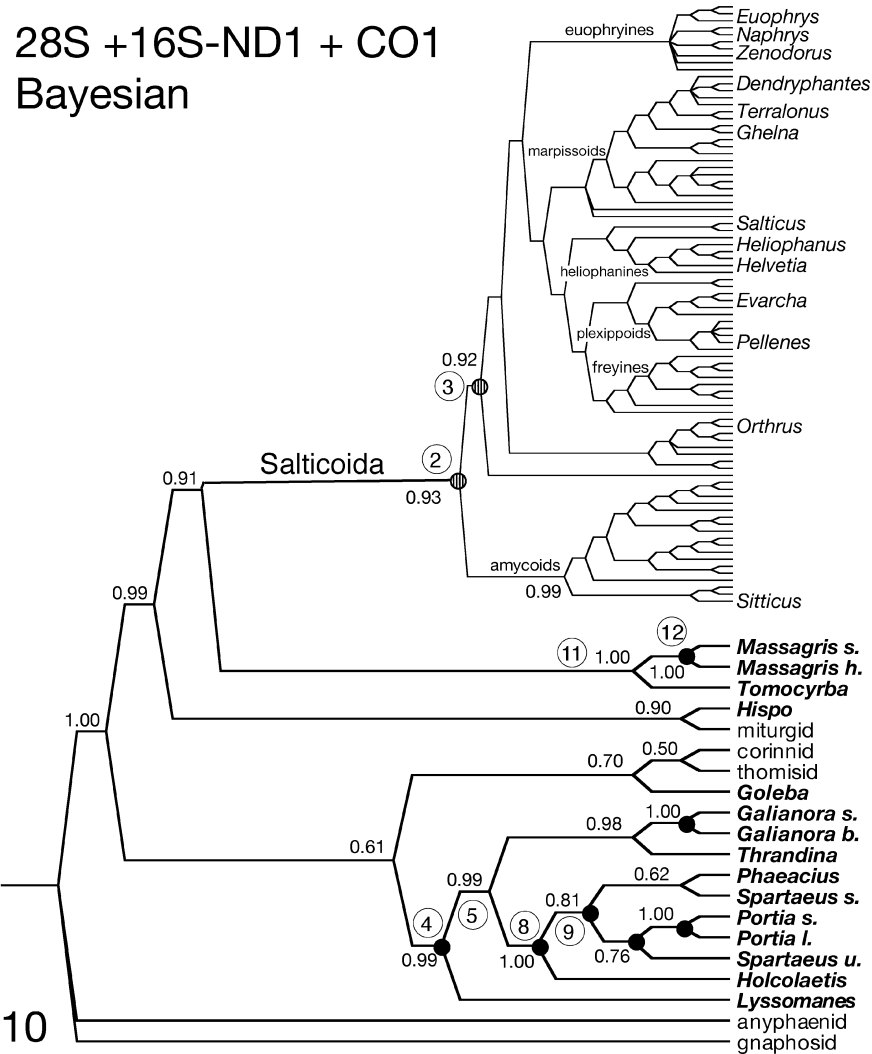
analyses agree on the two species of *Galianora* being sister taxa (node 7, Fig. 1). This is consistent with their sharing a round tegulum with peripheral embolus and reduced posterior median eyes (Maddison, 2006), both features derived with respect to the conditions in *Thrandina*.



**FIGURES 4-9.** Results from analyses on separate data partitions. Circled numbers mark clades from Figure 1. Figures 4-8 show majority rule consensus trees from sampled trees from Bayesian analysis, with posterior probabilities at nodes. Spots on nodes indicate clade was recovered also in parsimony analysis. Figure 9 shows strict consensus of nine most parsimonious trees (treelength 602) for ND1-CO1 amino acid matrix, with bootstrap values (1000 replicates).

Hisponines (*Massagris*, *Tomocyrrba*, *Hispo*) lie outside the Salticoida according to the All Genes analysis (all methods), 18S (all methods), and 28S (parsimony). They also lie outside the salticoids when the remaining gene regions (H3 + 16S-ND1 + CO1) are combined into a single analysis (Fig. 1). The precise relationships of the hisponines are unclear, however. Their monophyly is not supported universally in our analyses, but we

suspect this is a result of the sparse data we have for them, especially *Hispo*. There is little evidence that hisponines are near the spartaeines and lapsiines, but rather most analyses suggest they branch independently from the lineage leading to the Salticoida, possibly as the sister group to the salticoids (Figs. 2, 10). As noted above, their exclusion from the salticoids is supported by their retention of a median apophysis. In addition, Wanless (1981) notes that *Hispo* has a simple tracheal system as in lyssomanines and *Portia*, in contrast to the more complex salticoid tracheae.



**FIGURE 10.** Results from Bayesian analysis of large taxon sample, all genes combined. Shown is the majority rules consensus tree of 9900 sampled trees. Among the salticoids, only taxa also used in the small taxon sample are named; the unnamed taxa are those in Maddison & Hedin (2003). Circled numbers mark clades from Figure 1. Estimated posterior probabilities (last 9900000 of 10 million generations) marked for clades outside of the Salticoida. Maximum likelihood tree identical outside the Salticoida except it placed the corinnid/thomisid/*Goleba* group more basally. Black spots on nodes indicate clade was recovered also in parsimony analysis; hatched spots for nodes 2 and 3 indicate *Hispo* was placed within the salticoids by parsimony (as a heliophanine).



FIGURES 11–12. Ventral view of epigyna of hisponine females used. 11, *Hispo* cf. *frenata*; 12, *Massagris* cf. *honesta*.

Opinion on the relationships of the salticoids, the *Cocalodes* group, and the spartaeines has varied. A relationship of *Holcolaetis* to the spartaeines has been proposed based on sharing of apparent secretory organs on the abdomen (Wanless, 1985, Wijesinghe, 1992). Rodrigo & Jackson's (1992) formal morphological analysis placed the *Cocalodes* group (including *Holcolaetis*) with the spartaeines. However, Wijesinghe's (1997) phylogenetic reconstruction considered the Salticoida and Spartaeinae as sister groups, in part on the basis of their shared loss of the median apophysis. Salticids with median apophyses (e.g., lyssomanines, *Holcolaetis*) are a series of lineages branching at the base of Wijesinghe's tree. Our present results suggest instead that *Holcolaetis* is indeed related to the spartaeines, and thus that the median apophysis has been lost at least four times, once as a synapomorphy of the Salticoida, once within the spartaeines, once within the hisponines, and once within the lyssomanines (*Chinoscopus* Simon, Galiano 1998).

There was support for the placement of *Lyssomanes* with the spartaeines and lapsiines from the All Genes analysis and from the partitions 28S, 16S-ND1, and CO1 (node 4, Fig. 1). With this placement, *Lyssomanes* would be separate from the Old World "lyssomanine" *Goleba*, and thus the lyssomanines would be polyphyletic (or paraphyletic, depending on one's interpretation of ancestors). Wanless (1980) was correct therefore in being skeptical about the monophyly of the lyssomanines. Wanless suggested that one of the most distinctive features uniting lyssomanines, the transparent green or yellow colouration and long thin legs, may simply be a derived and convergent adaptation. This body form has arisen several times independently in other salticid groups (e.g., *Epeus* Peckham & Peckham and *Orthrus*). *Goleba*'s placement is unclear, but it may branch deeply, perhaps even as the sister group to all remaining salticids. Our results therefore indicate the lyssomanines should be divided into two groups. However, we will postpone revising their

classification until other Old World lyssomanine genera are better studied. The results also argue against placing the lyssomanines in a separate family, unless the spartaeines and lapsiines were to go with them.

Although our analyses were not designed to examine relationships within the salticoids, their deep relationships may be more easily resolvable now that we have a more complete representation of their outgroups, i.e. the basal salticids. The amycoids are resolved as sister group to the rest of the salticoids by 28S and 18S as well as by the All Genes analyses, although this result did not hold for the other genes individually.

Future work should seek to resolve the position of the hisponines and the decomposition of the lyssomanines. In addition, the finding that lapsiines are sister group to the spartaeines motivates work on their natural history. Do lapsiines share with their spartaeine relatives the behaviours, rare among salticids, of making webs and eating other spiders (Jackson & Pollard, 1996)? With these phylogenetic results it is apparent that there are new opportunities in the lapsiines and hisponines to study basal salticids and the family's early evolution.

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**APPENDIX 1.** Specimens sequenced for molecular phylogenetic analysis. All specimens deposited in Spencer Entomological Museum (UBC-SEM) except those marked CASENT, which are deposited in the California Academy of Sciences. Specimens (m) male, (f) female, and (j) juvenile as marked by species name. Columns marked 28S, 18S, H3, 16S-ND1, and CO1 show sequence length in parentheses and GenBank accession numbers. \* Specimen used by Maddison & Hedin, 2003.

Species	#	Locality	28S	18S	H3	16S-ND1	CO1
<b>Lapsiines</b>							
<i>Galianora sacha</i> Maddison (j)	d116	ECUADOR: Napó: S 1.067 W 77.617	(772) DQ665766	(1313) DQ665734	(331) DQ665716		(975) DQ665754
<i>Galianora bryicola</i> Maddison (m)	d124	ECUADOR: Napó: S 1.067 W 77.617	(756) DQ665771	(1313) DQ665741	(325) DQ665717	(692) DQ665727	(972) DQ665758
<i>Thrandina parocula</i> Maddison (m)	d123	ECUADOR: Morona Santiago: S 2.9227 W 78.4079	(773) DQ665779	(1320) DQ665751	(311) DQ665718	(793) DQ665726	(970) DQ665761
<b>Hisponinae</b>							
<i>Massagris schisma</i> Maddison & Zhang (m)	d081	SOUTH AFRICA: Northern Cape, Oorlogskloof Nature Reserve	(778) DQ665762	(1661) DQ665731		(898) DQ665728	
<i>Massagris cf. honesta</i> Wesolowska (f)	d082	SOUTH AFRICA: KwaZulu-Natal: Lake St. Lucia S 28.1021 E 32.4279	(597) DQ665772	(1677) DQ665743	(331) DQ665705	(661) DQ665722	
<i>Hispo cf. frenata</i> Simon (f)	d126	MADAGASCAR: Prov. Toamasina S 18.944 E 48.418 CASE NT 9005643		(1319) DQ665739		(736) DQ665724	
<i>Tomocyrra andasibe</i> Maddison & Zhang (m)	d127	MADAGASCAR: Prov. Toamasina S 18.944 E 48.418 CASE NT 9005649	(513) DQ665780	(1317) DQ665752	(229) DQ665706	(630) DQ665725	
<b>Lyssomaninae</b>							
<i>Goleba lyra</i> Maddison & Zhang (m)	d051	MADAGASCAR: Fianarantsoa: S 22.592 E 45.128 CASENT 9005863	(771) DQ665768	(1304) DQ665737	(323) DQ665707		(964) DQ665755

.....to be continued

## APPENDIX 1 (continued)

Species	#	Locality	28S	18S	H3	16S-ND1	CO1
<i>Lyssomanes viridis</i> Walckenaer (f)	d129	U.S.A.: Mississippi, Wall Doxey State Park		(1694) DQ665742	(327) DQ665715		
<b>Spartaeinae</b>							
<i>Holcolaetis</i> sp. ( <i>H. zuluensis</i> Lawrence?) (j)	d036	SOUTH AFRICA: Kwa-zulu-Natal: Lake St. Lucia S 28.2369 E 32.4100	(659) DQ665770	(1305) DQ665740	(213) DQ665721		(971) DQ665757
<i>Phaeacius</i> cf. <i>fimbriatus</i> Simon (m)	d111	MYANMAR: Yangon Division: N 17.045 E 96.095 CASENT 9019139	(789) DQ665775				(973) DQ665759
<i>Portia</i> cf. <i>schultzi</i> Karsch (f)	d131	MADAGASCAR: Fianarantsoa: S 22.592 E 45.128 CASENT 9005865	(642) DQ665776	(859) DQ665747	(326) DQ665708		
<b>Salticoida</b>							
<i>Dendryphantès</i> <i>hastatus</i> Clerck (f)	d043	POLAND: Siedlce N 52.127 E 22.271	(661) DQ665763	(1323) DQ665732			
<i>Euophrys</i> <i>monadnock</i> Emerton (m)	d029	CANADA: Nova Scotia: N 44.5279 W 64.6405	(596) DQ665764	(1471) DQ665733	(326) DQ665714		
<i>Evarcha</i> <i>proszynskii</i> Marusik & Logunov (m)	d096	CANADA: British Columbia: Richmond	(781) DQ665765			(939) DQ665723	
<i>Ghelna</i> <i>castanea</i> Hentz (f)	d005	U.S.A.: North Carolina	(744) DQ665767	(1686) DQ665735	(325) DQ665709		
<i>Heliophanus</i> <i>cupreus</i> Walckenaer (m)	d044	POLAND: Miełk, N 52.331 E 23.042	(534) DQ665769	(1573) DQ665738	(327) DQ665710		(975) DQ665756
<i>Orthrus</i> <i>bicolor</i> Simon *	S192	PHILIPPINES: Luzon	(773) DQ665773	(1441) DQ665745	(305) DQ665719		
<i>Pellenes</i> <i>peninsularis</i> Emerton (m)	d057	CANADA: Nova Scotia: N 45.5862 W 62.2271	(702) DQ665774	(1579) DQ665746	(325) DQ665712		
<i>Salticus</i> <i>scenicus</i> Clerck (j)	d003	CANADA: British Columbia: Mission	(699) DQ665777	(1488) DQ665748	(323) DQ665713		

.....to be continued

## APPENDIX 1 (continued)

Species	#	Locality	28S	18S	H3	16S-ND1	CO1
<i>Sitticus palustris</i>	d030	CANADA: Nova Scotia: N 44.4318 W 64.6075	(721) DQ665778	(1336) DQ665749		(770) DQ665729	(971) DQ665760
<i>Zenodorus cf. orbiculatus</i> Key-serling (m)	d008	AUSTRALIA: Queensland: S 16.2 E 145.4	(743) DQ665781	(1286) DQ665753	(294) DQ665711		
<b>Outgroups</b>							
Thomisidae: <i>Xysticus</i> sp. *	S316	U.S.A.: Colorado		(1680) DQ665750	(325) DQ665704		
Anyphaenidae: <i>Hibana</i> sp. *	S318	MEXICO: Sonora		(1534) DQ665730			
Gnaphosidae: <i>Cesonia</i> sp. *	S319	MEXICO: Sonora		(579) DQ665736	(320) DQ665720		
Miturgidae: <i>Cheiracanthium</i> sp. *	S321	MEXICO: Sonora		(822) DQ665744			

ZOOTAXA

1255