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From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes

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Abstract

The family Theridiidae is one of the most diverse assemblages of spiders, from both a morphological and ecological point of view. The family includes some of the very few cases of sociality reported in spiders, in addition to bizarre foraging behaviors such as kleptoparasitism and arancophagy, and highly diverse web architecture. Theridiids are one of the seven largest families in the Araneae, with about 2200 species described. However, this species diversity is currently grouped in half the number of genera described for other spider families of similar species richness. Recent cladistic analyses of morphological data have provided an undeniable advance in identifying the closest relatives of the theridiids as well as establishing the family's monophyly. Nevertheless, the comb-footed spiders remain an assemblage of poorly defined genera, among which hypothesized relationships have yet to be examined thoroughly. Providing a robust cladistic structure for the Theridiidae is an essential step towards the clarification of the taxonomy of the group and the interpretation of the evolution of the diverse traits found in the family. Here we present results of a molecular phylogenetic analysis of a broad taxonomic sample of the family (40 taxa in 33 of the 79 currently recognized genera) and representatives of nine additional araneoid families, using approximately 2.5 kb corresponding to fragments of three nuclear genes (Histone 3, 18SrDNA, and 28SrDNA) and two mitochondrial genes (16SrDNA and Col). Several methods for incorporating indel information into the phylogenetic analysis are explored, and partition support for the different clades and sensitivity of the results to different assumptions of the analysis are examined as well. Our results marginally support theridiid monophyly, although the phylogenetic structure of the outgroup is unstable and largely contradicts current phylogenetic hypotheses based on morphological data. Several groups of theridiids receive strong support in most of the analyses: latrodectines, argyrodines, hadrotarsines, a revised version of spintharines and two clades including all theridiids without trace of a colulus and those without colular setae. However, the interrelationships of these clades are sensitive to data perturbations and changes in the analysis assumptions.
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1. Introduction

The spider family Theridiidae, popularly known as comb-footed or cobweb spiders, ranks as one of the most species-rich families of spiders, currently including 2209 species grouped in 79 genera (Platnick, 2002). One of the primary factors thought to contribute to species numbers in the Theridiidae is the diversity of foraging and lifestyle strategies (Avilés, 1997; Barmeyer, 1975; Buskirk, 1981; Carico, 1978; Cavalieri et al., 1987; Elgar, 1993; Gillespie and Oxford, 1998; Holldobler, 1970; Maretic, 1977a,b; Oxford, 1983; Oxford and Gillespie, 2001; Porter and Eastmond, 1982; Shear, 1986). Very few spiders species are social. Most spiders are solitary and highly intolerant of conspecifics, but several

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theridiid spiders are social, ranging from maternal care, to quasisociality, or even eusociality, with little gene flow between established colonies and a high degree of genetic structure (Avilés, 1986, 1997; Lubin and Crozier, 1985; Roeloffs and Riechert, 1988; Smith and Hagen, 1996). Argyrodine theridiids are famous for kleptoparasitism (Elgar, 1993; Shear, 1986), in which individuals steal food from the webs of other, unrelated and usually larger web-building spiders (Cangialosi, 1991; Smith Trail, 1980; Vollrath, 1984; Whitehouse, 1997; Whitehouse and Jackson, 1998). In both sociality and kleptoparasitism, conspecific adults share webs and display atypical tolerance that may be homologous at some level (Agnarsson, 2002). Likewise, web architectures in the Theridiidae range from complex to simple (Benjamin and Zschokke, 2003) and web reduction has occurred in many groups, presumably associated with specialization on specific prey (Eberhard, 1990). In particular, the genera *Spintharus* Hentz, 1850, *Episinus* Walckenaer, in Latreille, 1809, and some *Chrosiothes* Simon, 1894 have reduced webs and prey on arboreal pedestrian arthropods (Stowe, 1986). In *Euryopis* (Carico, 1978; Levi, 1954; Porter and Eastmond, 1982) and *Dipoena* Thorell, 1869 (Levi and Levi, 1962) the web is highly reduced or lost, and the spiders appear to feed exclusively on ants (Carico, 1978). *Latrodectus* Walckenaer, 1805 has evolved neurotoxins inimical to vertebrates, which has obvious health implications (Maretic, 1977a). Some theridiid genera contain spectacularly polymorphic species such as *Enoplognatha* Pavesi, 1880 (Oxford and Shaw, 1986) and *Theridion* Walckenaer, 1805 (Gillespie and Oxford, 1998; Gillespie and Tabashnik, 1989; Oxford and Gillespie, 1996a,b,c) and others contain asymmetric "one-palp" males, e.g. *Echinotheridion* Levi, 1963, and *Tidarren* Chamberlin & Ivie, 1934 (Branch, 1942; Knoflach, 2002; Knoflach and van-Harten, 2000, 2001).

In order to understand how these different traits evolved, a clear picture of phylogenetic relationships is required. A phylogenetic context is also essential to understand patterns of diversification of specific lineages, and the key attributes that may be involved in generating these patterns.

1.1. Family-level relationships

The advent of quantitative cladistic techniques has yielded major advances in our understanding of the phylogenetic structure of spider families to date, with most attention having been focused on the Araneoidea. The currently accepted morphologically based, family-level araneoid phylogeny places the former family Hadrotarsidae (Forster et al., 1990) within the Theridiidae and establishes the outgroup structure for the Theridiidae (Griswold et al., 1998) (Fig. 1). Nesticidae and Theridiidae form a clade that is sister to Synotaxidae plus Cyatholipidae are then together sister to the families Theridiidae and Nesticidae, a clade called the 'spineless femur clade' by Griswold et al. (1998). With the linyphioids these then form the 'araneoid sheet-web weavers,' suggesting a single loss (transformation) of the orb web.

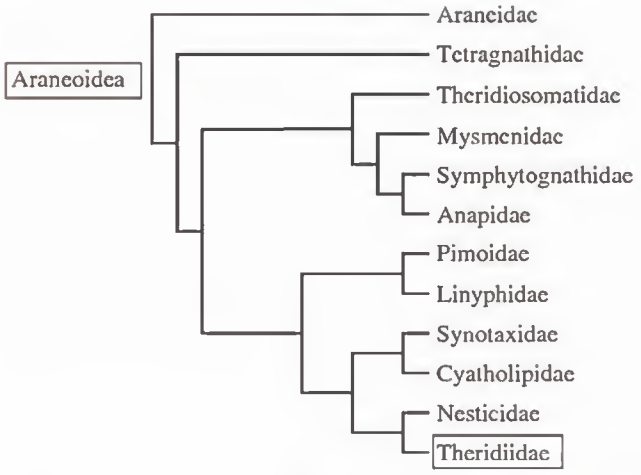

Fig. 1. Current hypothesis of the phylogenetic relationship of the Araneoid families based on morphology (modified from Griswold et al., 1998).

1.2. Internal phylogenetic structure

Only two other studies (Forster et al., 1990; Levi and Levi, 1962) have even marginally addressed theridiid interrelationships. Neither included an explicit cladistic analysis, but their arguments can be presented in tree-like form (Fig. 2). Both were based on a few character systems and differ mainly in the relative stress given to different characters. Levi and Levi (1962) emphasized the progressive reduction of the colulus, while Forster et al. (1990) called attention to the position of the paracymbial hook (tegular-cymbial locking mechanism). Phylogenies based entirely on one character system are unlikely to reflect global optima when many characters are considered.

In addition to the problem of generic relationships, some theridiid genera are poorly delimited and probably poly- or paraphyletic. The genera *Achaearanea* Strand, 1929 and *Theridion* seem to have been used as the dumping ground for species with no colulus that do not fit in other, better defined, genera. The genus *Argyrodes* Simon, 1864 includes several formerly valid genera that span an amazing diversity in morphology and foraging behaviors (Exline and Levi, 1962). Yoshida (2001a), elevated the *Argyrodes* complex to a subfamily and revalidated *Ariamnes* and *Rhomphaea*. Several formerly valid genera were also merged in the genus *Anelosimus*, which, as currently defined, includes species displaying all different levels of sociality (Levi and Levi, 1962).

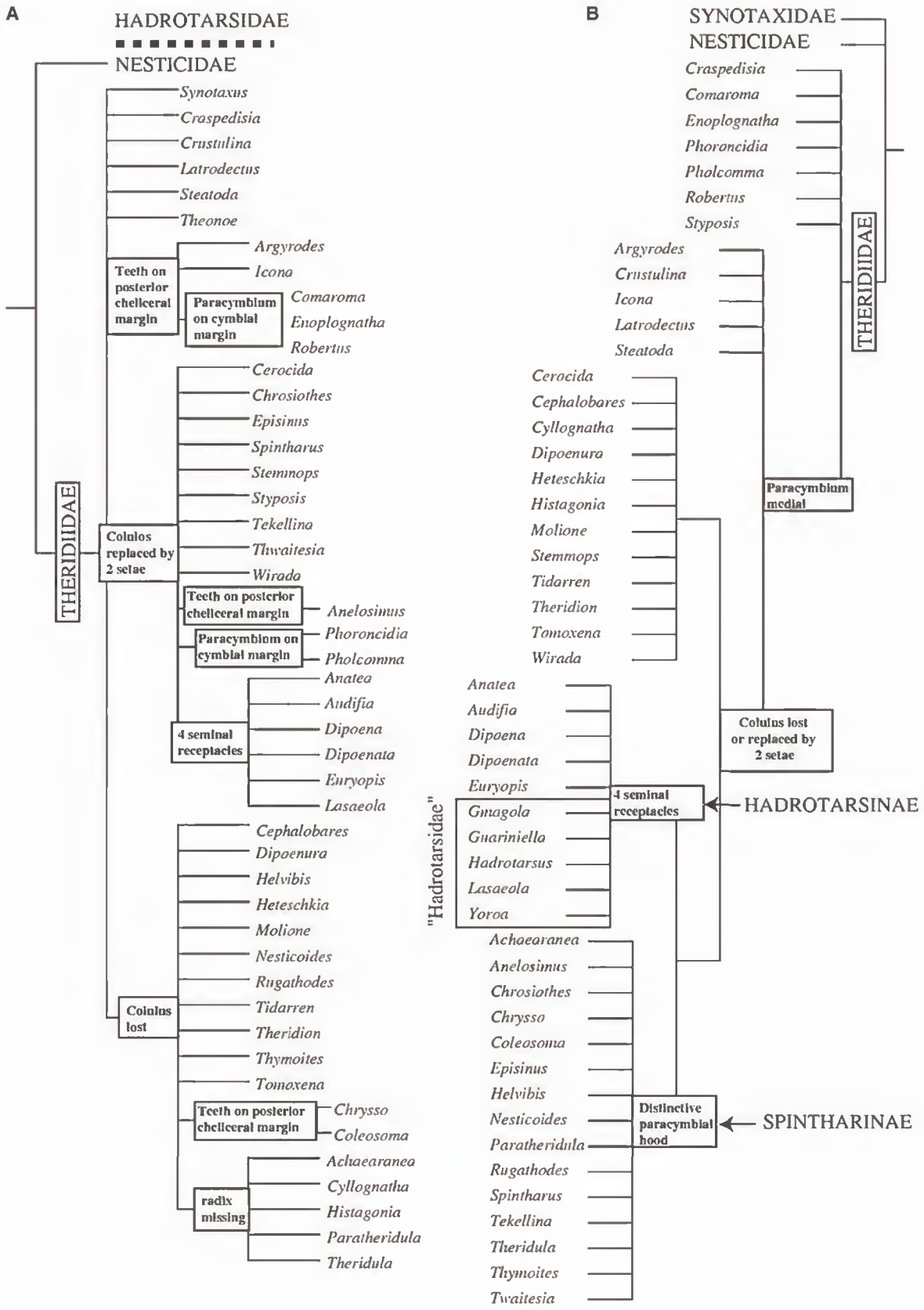

Fig. 2. Two morphology-based hypotheses of the phylogenetic structure of the family Theridiidae. Neither is based on an explicit cladistic analysis, but the proposed relationships have been redrawn in tree-like form for the sake of clarity and comparison. (A) Levi and Levi (1962); (B) Forster et al. (1990).

Table 1
Taxonomic and geographical information of the specimens included in the present study and GenBank accession number of the gene fragments sequenced for each specimen

Family	Genus	Species	Country	Locality	Code	CO1	16S	18S	28S	H3
Araneidae	*Argiope*	*argentata*	USA	HI: Kauai, Kokee S.P.	MS92	AY231021	AY230937	AY230889	AY231068	AY230981
Cyatholipidae	*Alaranea*	*merina**	Madagascar		X133	AY231022	AY230942	AY230890	AY231074	AY230982
Linyphiidae	*Linyphia*	*triangularis*	Denmark	Baelum Sonderskov	G48	AY078693	AY078664	AY078668	AY078682	AY078702
Mysmenidae	*Mysmena*	sp.	Guyana	S of Gunns Landing	MS91	AY231023		AY230891	AY231071	AY230983
Nesticidae	*Nesticus*	sp.	China		X132	AY231024	AY230941	AY230892	AY231073	AY230984
Pimoidae	*Pimoa*	sp.	China		X131	AY231025	AY230940	AY230893	AY231072	AY230985
Synoyaxidae	*Synotaxus*	sp.	Guyana	S of Gunns Landing	MS90	AY231026	AY230943	AY230894	AY231075	AY230986
Tetragnathidae	*Tetragnatha*	*mandibulata*	USA	HI:	A90	AY231027	AY230938	AY230895	AY231069	AY230987
Theridiosomatidae	*Theridiosoma*	*gemmosum**	USA	NC: Macon Co.,	MS107	AY231028	AY230939	AY230896	AY231070	AY230988
Theridiidae	*Achaearanea*	*tepidariorum*	USA	NC: Macon Co., Highlands B.S.	MS15	AY231029	AY230955	AY230897	AY231088	AY230989
Theridiidae	*Ameridion*	sp.	Costa Rica	Cartago, Cerro de la Muerte	MS83	AY231030	AY230944	AY230898	AY231076	AY230990
Theridiidae	*Anelosimus* [*Kochiura**]	*aulicus*	Spain	Almeria: Punta Entinas	MS105	AY231045	AY230949	AY230914	AY231082	
Theridiidae	*Anelosimus*	*eximius**	Guyana	S of Gunns Landing	MS95	AY231031	AY230956	AY230899	AY231089	AY230991
Theridiidae	*Anelosimus* [*Selkirkiella*]	sp.	Chile	Osorno, P. Nat. Puyehue	MS81	AY231055	AY230972	AY230924	AY231107	AY231012
Theridiidae	*Ariamnes*	*attenuata*	Guyana	S of Gunns Landing	MS101	AY231033	AY230946	AY230901	AY231078	AY230993
Theridiidae	*Argyrodes*	*argentatus*	USA	HI: Oahu	A80	AY231032	AY230957	AY230900	AY231090	AY230992
Theridiidae	*Argyrodes* [*Faiditus*]	*chickeringi*	Guyana	S of Gunns Landing	MS100	AY231043		AY230912	AY231081	AY231002
Theridiidae	*Argyrodes* [*Neospintharus*]	*trigonum*	USA	HI	MR2	AY231048	AY230945	AY230917	AY231077	AY231006
Theridiidae	*Cerocida*	*strigosa**	Guyana	S of Gunns Landing	MS97	AY231034	AY230958	AY230902	AY231091	AY230994
Theridiidae	*Chrosiathes*	cf. *jocosus*	Guyana	S of Gunns Landing	MS109	AY231035	AY230959	AY230903	AY231092	AY230995
Theridiidae	*Chrysso*	sp.	Colombia	Iguaque	MS2	AY231036		AY230904	AY231093	AY230996
Theridiidae	*Crustulina*	*sticta*	USA	NY: Yonkers	A91		AY230947	AY230906	AY231079	
Theridiidae	*Dipoena*	cf. *hortoni*	Guyana	S of Gunns Landing	MS94	AY231038	AY230961	AY230907	AY231095	AY230998
Theridiidae	*Enoplognatha*	*caricis*	Japan	Tokyo	X136	AY231040	AY230962	AY230909	AY231096	AY231001
Theridiidae	*Episinus*	*angulatus*	UK	England: Yorkshire, Strensall	A41	AY231041	AY230963	AY230910	AY231097	
Theridiidae	*Euryopis*	*funebris*	USA	SC: Pickens Co., L. Issaqueena	MS20	AY231042	AY230964	AY230911	AY231098	AY231000
Theridiidae	*Helvibis*	cf. *longicauda*	Guyana	S of Gunns Landing	MS98	AY231044	AY230965	AY230913	AY231099	AY231003
Theridiidae	*Keijia*	*mneon*	USA	HI: Kauai, Hanalei	X43	AY231037	AY230960	AY230905	AY231094	AY230997
Theridiidae	*Latrodectus*	*mactans*	USA	NC: Jackson Co., Cullowhee	MS25	AY231046	AY230966	AY230915	AY231100	AY231004
Theridiidae	*Neottiura*	*bimaculata**	Slovenia	500 m N of Cmice	X51	AY231047	AY230967	AY230916	AY231101	AY231005
Theridiidae	*Nesticodes*	*rufipes*	USA	HI: Oahu, Hawaii Kai	X6	AY231049	AY230968	AY230918	AY231102	AY231007
Theridiidae	*Pholcomma*	*hirsutum*	USA	NC: Swain Co.,	MS105	AY231050	AY230969	AY230919	AY231103	AY231008
Theridiidae	*Phoroncidia*	sp.	Tanzania	Iringa Distr., Kihanga strm.	MS39	AY231051	AY230970	AY230920	AY231104	
Theridiidae	*Robertus*	*neglectus**	Denmark	Hestehaven, 22 km NE Arhus	X50	AY231053	AY230971	AY230922	AY231105	AY231010
Theridiidae	*Rugathodes*	*sexpunctatus**	USA	NC: Haywood Co., Cataloochee	X98	AY231054		AY230923	AY231106	AY231011

Theridiidae	*Rhomphaea*	*metallissima*	Guyana	S of Gunns Landing	MS102	AY231052	AY230950	AY230921	AY231083	AY231009
Theridiidae	*Spintharus*	*flavidus**	USA	NC: Macon Co., Blue Valley	MS9	AY231056	AY230973	AY230925	AY231108	AY231013
Theridiidae	*Steatoda*	*bipunctata**	UK	England: Yorkshire	A28	AY231057	AY230951	AY230926	AY231084	AY231014
Theridiidae	*Stemmops*	cf. *sertus*	Guyana	S of Gunns Landing	MS99	AY231058	AY230974	AY230927	AY231109	
Theridiidae	*Stypostis*	*selis*	Argentina	Misiones: PN Iguazu	MS87	AY231059	AY230975	AY230928	AY231110	AY231015
Theridiidae	*Theridion*	cf. *frondeum*	USA	NC: Macon Co., Highlands B. S.	X31	AY231060	AY230953	AY230929	AY231086	
Theridiidae	*Theridion*	*grallator*	USA	HI: Hawaii, Puu Makaala	X67	AY231061	AY230952	AY230930	AY231085	AY231016
Theridiidae	*Theridion*	*longipedatum*	Colombia	Iguaque	X54	AY231062	AY230954	AY230931	AY231087	
Theridiidae	*Theridion*	*varians*	UK	England: Yorkshire	A34	AY231063	AY230976	AY230932	AY231111	AY231017
Theridiidae	*Theridula*	*opulenta**	USA	NC: Macon Co., Horse covc	MS4	AY231064	AY230977	AY230933	AY231112	
Theridiidae	*Thwaitesia*	sp.	Australia	Queensland, Gold Creek Reservoir	X80	AY231065	AY230980	AY230934	AY231113	AY231018
Theridiidae	*Thymoites*	*unimaculatus*	USA	MA: Middlesex Co., Pepperell	A58	AY231066	AY230978	AY230935	AY231114	AY231019
Theridiidae	*Tidarren*	*sisyphoide**	USA	SC: Pickens Co., L. Issaqueena	MS21	AY231067	AY230979	AY230936	AY231115	AY231020
Theridiidae	*Trigonobothrys*	*nustelinus*	Japan	Tokyo, Hino-shi	A68	AY231039	AY230948	AY230908	AY231080	AY230999

An asterisk after the species name indicates it is the type species of the genus.

1.3. Aim

This paper provides the first phylogenetic hypothesis for theridiid intrageneric relationships based on molecular data. The results allow us to test current hypotheses of relationships based on morphology, and provide a framework to analyze the great array of ecological and behavioral traits displayed by the family.

2. Materials and methods

2.1. Taxonomic sampling

Theridiid terminals included 40 species representing 33 of the most species-rich and ecologically diverse genera. Important genera, in terms of species diversity and morphological distinctiveness that could not be sampled in the present study include: *Carniella* Thaler & Steinberger, 1988 (8 species); *Coleosoma* O.P.-Cambridge, 1882 (10 species); *Coscinida* Simon, 1895 (13 species); *Echinotheridion* Levi, 1963 (9 species); *Hadrotarsus* Thorell, 1881 (5 species); *Moliore* Thorell, 1892 (3 spp.); *Moneta* O.P.-Cambridge, 1870 (18 species); *Paidiscura* Archer, 1950 (4 species); *Takayus* Yoshida, 2001 (13 species); *Tekellina* Levi, 1957 (6 species.); *Theonoe* Simon, 1881 (6 species). Only the genus *Trigonobothrys* Simon, 1889 have been included from the recently resurrected hadrotarsine genera (e.g., Yoshida, 2002) which, although diverse, are poorly defined and have dubious species composition.

Representatives of nine additional araneoid families were included to test theridiid monophyly. In all the analyses, exemplars from the family Arancidae were used as the primary outgroup under the assumption of their sister-group relationship to the remaining araneoids (Griswold et al., 1998). More than one species of the genera *Argyrodes, Theridion,* and *Anelosimus* were included in the analysis to test some contrasting views on their taxonomic limits. The list of the specimens sampled in the present study is shown in Table 1.

2.2. Characters

Live specimens were collected in the field and fixed in 95% ethanol, except when fresh material was not available, in which case specimens from museum collections (preserved in 75% ethanol) were used for extractions, with success mostly dependent on the time since preservation. Only one or two legs were used for extraction, except for specimens preserved in 75% EtOH, for which as many as four legs plus the carapace were used. The remainder of the specimen was kept as a voucher (deposited at the National Museum of Natural History, Smithsonian Institution, in Washington, DC and the Essig Museum of Entomology, University of California

at Berkeley). Total genomic DNA was extracted following the phenol/chloroform protocol of Palumbi et al. (1991) or using Qiagen DNeasy Tissue Kits. The approximate concentration and purity of the DNA obtained was evaluated through spectophotometry and the quality was verified using electrophoresis in agarose/TBE (1.8%) gel. Partial fragments of the mitochondrial genes cytochrome *c* oxidase subunit I (CO1) and 16S rRNA (16S) and the nuclear genes 18S rRNA (18S), 28S rRNA (28S) and Histone H3 (H3) were amplified using the following primer pairs: [CO1] C1-J-1751 and C1-N-2191 (designed by R. Harrison's lab, Simon et al., 1994), [16S] LR-N-13398 (Simon et al., 1994) and LR-J-12864 (CTCCGGTTTGAACTCAGATCA, Hsiao, pers. comm.), [18S] 5F or 18Sa2.0 and 9R (Giribet et al., 1999), [28S] 28SA and 28SB (Whiting et al., 1997), and [H3] H3aF and H3aR (Colgan et al., 1998). The thermal cyclers Perkin–Elmer 9700, Perkin–Elmer 9600, and Bio-Rad iCycle were used indiscriminately to perform either 25 (mitochondrial genes) or 40 (nuclear genes) iterations of the following cycle: 30 s at 95 °C, 45 s at 42–58 °C (depending on the primers, see below), and 45 s at 72 °C, beginning with an additional single cycle of 2 min at 95 °C and ending with another one of 10 min at 72 °C. Positive amplification for CO1 and 16S primers was achieved at annealing temperatures ranging from 42 to 45 °C. For the 28S and H3 a single annealing temperature of 48 °C yielded positive amplifications in most cases. For the 18S primer a "touchdown" strategy was applied, beginning at 58 °C and lowering proportionally the temperature in each cycle for 20 cycles down to 45 °C and keeping that annealing temperature for an additional 20 cycles. The PCR reaction mix contained primers (0.48 μM each), dNTPs (0.2 mM each), and 0.6 U Perkin–Elmer AmpliTaq DNA polymerase (for a 50 μl reaction) with the supplied buffer and, in some cases, adding an extra amount of $MgCl_2$ (0.5–1.0 mM). PCR results were visualized by means of an agarose/TBE (1.8%) gel. PCR products were cleaned using Geneclean II (Bio 101) or Qiagen QIAquick PCR Purification Kits following the manufacturer's specifications. DNA was sequenced directly in both directions through the *cycle sequencing* method using dye terminators (Sanger et al., 1977) and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction with AmpliTaq DNA Polymerase FS kit. Sequenced products were cleaned using Princeton Separations CentriSep columns and run out on an ABI 377 automated sequencer. Sequence errors and ambiguities were edited using the Sequencher 3.1.1 software package (Gene Codes). Sequences were subsequently exported to the program GDE 2.2 (*Genetic Data Environment*) (Smith et al., 1994) running on a Sun Enterprise 5000 Server, and manual alignments built, for management purposes, taking into account secondary structure information from secondary structure models available in the literature for 16S (Arnedo et al., 2001), 28S (Ajuh et al., 1991), and 18S (Hendriks et al., 1988). Alignment of the protein-coding genes was trivial since no length variation was observed in the sequences.

2.3. Analysis

2.3.1. Alignment

Insertions and deletions (hereafter called either indels or gaps) are common events in the evolution of non-protein-coding DNA sequences, as inferred from different length fragments resulting from amplification of homologous DNA regions across different taxa. Indel events present two main challenges in phylogenetic analysis of DNA sequence data: positional homology (i.e., alignment) and indel treatment (Giribet and Wheeler, 1999). Unlike nucleotide bases, indels are not observable characters but gaps inserted to accommodate homologous DNA sequences of unequal length to define the putative homologous characters amenable to phylogenetic analysis. Although homologous landmarks (e.g., secondary structure in structural genes) can facilitate manual sequence alignment, they almost never resolve all the ambiguity and subjectivity in the position assignment. As a result of these problems, a common approach is to avoid or discard regions that have experienced such events (Lee, 2001). However, gaps can contain important phylogenetic information that can have dramatic effects on tree topology and clade support (Simmons et al., 2001). Alternative methods for incorporating indels include using automatic algorithms to evaluate objective optimality function. In particular, programs using the dynamic programming algorithm of Needleman and Wunsch (1970) provide methods of aligning sequences that are repeatable. Automatic alignment algorithms require explicit parameter costs (e.g., gap opening, extension costs, and transition/transversion ratio) and thus provide a comparative framework to investigate the effects of changes in these parameters. Regardless of the actual method or parameter cost scheme employed, the final outcome is one or several alignments that are then subject to phylogenetic analysis.

The most common way to incorporate gaps into the analysis once an alignment is obtained is by considering them either missing data or as a state in addition to the four nucleotides (i.e., gaps as 5th state). The reasons cited for treating gaps as missing data include the lack of a proper treatment to deal with events that are the results of processes different from those acting on base substitution (Swofford et al., 1996) or their lack of reliable phylogenetic information (Simmons et al., 2001). Recently, Simmons and Ochoterena (2000) have criticized the gaps as 5th state approach because gaps do not constitute alternative forms of bases but are essentially a different form of change. In addition, scoring gaps as a

5th state can result in treating contiguous gap positions as multiple independent characters although they are most parsimoniously considered as a single indel event. These authors recommended that indels be scored as additional absence/presence characters, according to a set of rules based on gap overlaps and sharing of the 5′ and/or the 3′ termini (Simmons and Ochoterena, 2000).

This two-step procedure (alignment followed by analysis) for analyzing DNA sequences of different length considers alignment and tree search as logically independent steps (Simmons and Ochoterena, 2000). Alternatively, the alignment can be considered as an integral part of the phylogenetic analysis. This approach considers indels as transformations not observations and thus claims it is logically inconsistent to consider them as characters.

2.3.2. Optimization alignment

The optimization alignment method, also referred as the direct optimization method (Wheeler, 1996), circumvents this inconsistency, and the whole alignment issue, by incorporating indels as one of the possible transformations during the optimization process linking ancestral and descent nucleotide sequences. Unlike the standard two-step procedure where a "static" alignment (Wheeler, 2001) is constructed and submitted to phylogenetic analysis, optimization alignment produces an alignment that is tree-dependent and thus the homology statements are dynamic instead of fixed or static.

In the current study, optimization alignment analyses were performed with the computer program POY v. 2.0 (Gladstein and Wheeler, 1997). Due to computational demands most of the analyses were run in a twenty-eight 1 GHz processor Beowulf cluster running PVM (Parallel Virtual Machine) (ca. 28 Gflops) based at the Department of Organismic & Evolutionary Biology at Harvard University. The heuristic search strategy implemented consisted of 500 random iterations (1000 for the simultaneous analyses of all the combined gene fragments under equal parameters) of the following unit: 32 independent trees were built by random addition of taxa with a subsequent round of SPR branch swapping and the best tree submitted to successive rounds of SPR and TBR branch swapping, followed by successive rounds of tree fusion (10 fusing pairs, each pair including a minimum of 5 taxa, and saving a maximum of 100 fused trees) and tree drifting (10 SPR followed by 10 TBR branch-swapping rearrangements keeping trees equal or better than the originals under a criterion based on character fit and tree length with 30 topological changes accepted per drift round and subsequently subjected to full SPR and TBR branch swapping accepting only minimal trees) (Goloboff, 1999). In order to speed up the computation, POY uses certain shortcuts to calculate tree length that can occasionally result in the miscalculation of the exact length. To circumvent this problem, all cladograms found within 0.5% of the minimum tree length were examined, and an extra TBR branch-swapping round was applied to all cladograms found within 1% of the minimum tree length. Bremer support (BS) (Bremer, 1988, 1994) was used as a measure of clade support by implementing searches to disagree with constraints that corresponded to the clades obtained in the analysis under equal parameters. Forty-six (as many as clades) constrained searches were run locally on a PC Pentium 4 at 1.7 GHz. A less exhaustive search strategy (10 replicates of 5 iterations of random addition of taxa, otherwise the same as the general searches) was used to speed up computation, which could result in an overestimation of the actual Bremer values. The contribution to the combined tree of each data partition was measured by means of the partitioned Bremer support (PBS) (Baker and DeSalle, 1997). PBS values were obtained by inferring the length of the trees obtained in the constrained searches for each individual data set. Optimization alignment analyses were performed under different parameter cost combinations. Gap costs of 1, 2, 4, and 8 (the version of POY available at the time the analyses presented in this study were performed did not implement gap extension costs, but this option has been incorporated in more recent versions of the program) were combined with transition/transversion ratios of 1/1, 1/2, and 1/4. The robustness of clades to changes in parameter costs was assessed by the ratio of parameter combinations where an equal cost clade was observed (at least in one of the trees obtained under that particular parameter combination) to the total number of combinations assayed.

2.3.3. Static alignments

Static alignments, i.e., alignments with fixed homology statements, were also constructed to explore the sensitivity of the results to alternative phylogenetic treatments of gaps and alternative phylogenetic inference frameworks. Static alignments for the ribosomal genes were constructed following the method of Hedin and Maddison (2001). Multiple automatic alignments corresponding to different combinations of gap opening and gap extension costs (8/2, 8/4, 20/2, 24/4, and 24/6, transition weight fixed to 0.5) were built with ClustalX (Thompson et al., 1997). Alignments for each particular set of parameters were built using a single guide tree as currently implemented in Clustal and as it is the common practice in the literature. However, it is well known that automatic alignments are order-dependent and a different addition series of taxa may result in different alignments. Moreover, many equally optimal alignments may exist for the same set of sequences (Giribet et al., 2002). The use of a single guide tree precludes the exploration of such alternative alignments. We chose gap opening/extension cost ratios that favored both relatively gappy (e.g., 8/2) and compressed (e.g., 24/6)

alignments (Hedin and Maddison, 2001). A particular gap opening/extension cost alignment was chosen based on topological congruence to the elision matrix (Wheeler et al., 1995) obtained by appending all the alignments constructed for a given gene fragment. Topological congruence was measured by the number of nodes in common between the consensus tree of each individual matrix and the elision matrix and by calculating the average symmetric-difference distances (Swofford, 2001) between all the trees from the individual matrix and the elision matrix. All the former analyses were run considering gaps as a 5th state. A combined matrix was then constructed by adding the best alignment selected for each ribosomal gene plus the COI and H3 fragments. The combined static matrix was then analyzed by considering gaps as either missing data or as 5th character-state. The same combined data matrix was recoded using the gap coding method of Simmons and Ochoterena (2000) to explore the effect of coding gaps (indels) as presence/absence characters rather than as a 5th state. The computing program GapCoder (Young and Healy, 2002) facilitates the automatic recoding of an alignment using the simple indel coding version of Simmons and Ochotorena's method.

Parsimony analyses of static alignments were conducted using PAUP* (Swofford, 2001) and NONA (Goloboff, 1993) computer programs, and manipulations of the data matrices and trees were performed with MacClade (Maddison and Maddison, 2000) and WinClada (Nixon, 2002). Unless otherwise stated, all the static matrices were analyzed using a heuristic search with 100 random additions, keeping a maximum of 10 trees per iteration and an overall maximum of 1000 trees. Branch support was assessed by means of Bremer support and bootstrap proportions (Felsenstein, 1985). In PAUP*, Bremer support was implemented through the TreeRot program (Sorenson, 1999). Bootstrap proportions were obtained from 100 replicates of a heuristic search with 15 iterations of random addition of taxa holding 20 trees per iteration. Analyses in a parametric statistical framework were performed using Bayesian inference as implemented in the computer program MrBayes (Huelsenbeck and Ronquist, 2001). Bayesian inference was favored over standard Maximum Likelihood because it is less demanding computationally and because the posterior probability of the trees provide a natural and more intuitive measure of node support (hereafter referred to as posterior probability value) (Leache and Reeder, 2002; Lewis and Swofford, 2001). It should be pointed out that posterior probability values have recently been criticized as being excessively liberal when using concatenated gene sequences, and has been suggested that bootstrap probabilities are more suitable for assessing the reliability of phylogenetic trees than posterior probabilities (Suzuki et al., 2002). However, other studies have reached opposite conclusions, also based on simulations, that have led their authors to claim that Bayesian support values represent much better estimates of phylogenetic accuracy than do nonparametric bootstrap support values (Wilcox et al., 2002).

The computer program Modeltest (Posada and Crandall, 1998) was used to assess the model of evolution that best fit the combined data. The parameters corresponding to the model selected were treated as unknown variables with equal a priori probability and estimated as part of the analysis based on Bayesian inference. Four MCMC (Markov Chain Monte Carlo) chains (one cold and three heated) were run simultaneously on random starting trees for 1.5×10^6 generations with results sampled every 100 (printed every 1000) generations. The $-\ln$ of the trees was plotted against generations to determine the number of generations required to achieve stability of the results and the trees obtained in generations below the stability value were discarded as "burn-in."

To explore sensitivity of the results to changes in parameter costs, alignment construction, and gap treatment was explored by taking as a reference the topology obtained from optimization alignment analysis under equal parameter costs (gap = transversion = transition) as a reference because we prefer this method in theory (see Section 4). We assessed similarity between analyses in two ways. Topology similarity is the ratio of the number of shared clades between the trees found in each analysis to the total number of clades obtained in the reference analysis. Second, we calculated the increase in length of the topologies found by each analysis under the assumptions of the equal costs optimization alignment method (i.e., diagnosed length of a particular tree under equal cost optimization alignment minus the minimum length of the equal cost optimization alignment analysis). The different values obtained were rescaled by dividing them by the difference between the maximum and the minimum possible lengths of the equal cost optimization alignment analysis. This index, which we call Rescaled Length Increase (RLI), is reminiscent of the Retention Index (Farris, 1989) and measures the overall decrease in fit of characters to a non-optimal topology under a particular set of assumptions.

We also constructed an additional index to measure the decrease in fit of each character partition when analyzed simultaneously with other partitions. We call this measure the partition-based retention index (PRI), and calculate it by subtracting the minimum possible length of a data partition from the actual length of the partition in the topology obtained from the analyses of the combined data set. It is even closer to Farris' Retention Index. We scaled the raw value by dividing the differences between the maximum and the minimum possible lengths of that partition. In the static alignment, the

maximum and minimum values are the sum of the maximum and minimum steps for each character, while for the optimization alignment they are estimated from actual searches.

3. Results

The gene fragments sequenced yielded the following lengths (primers excluded): CO1 472 bp, H3 328 bp, 18S 779–829 bp, 28s 297–320 bp, and 16S 428–467 bp. Sequences have been deposited in GenBank and their accession numbers are listed in Table 1.

Table 2 summarizes some of the results of the optimization alignment analyses and Table 3 gives clade support measures. Analysis of the combined data matrix under equal parameter costs resulted in 1 tree of 7283 steps, consistency index (CI) (all values reported with uninformative characters removed) = 0.29, retention index (RI) = 0.42, depicted in Fig. 3. The implied alignment yielded 2825 positions, 916 of which were parsimony informative. This tree supports theridiid monophyly, including the Hadrotarsinae genera, as do all the analyses with gap costs of one or two, but the outgroup structure contradicts previous hypotheses based on morphology. For the most part, outgroup relationships are very sensitive to changes in parameter costs, with the only exception of the sister-group relationship of cyatholipids (*Alaranea merina*) and theridiosomatids (*Theridiosoma gemmosum*), which is supported in all optimization alignment analyses. A clade formed by synotaxids (*Synotaxus* sp.) plus nesticids (*Nesticus* sp.) is found in 75% of the analyses but only receives a Bremer support value under equal costs of 3. If the outgroup topology is constrained to the current morphological hypothesis 4 trees result that are 58 steps longer (0.0254 increase in informative variation). Outgroups and basal theridiid clades typically have well-developed coluli (black circles in Fig. 3); reduced coluli cluster in more distal lineages (grey circles in Fig. 3), although reduction is not monophyletic. Support for the nodes concerned (42,9) is low: only 33.3% of the cost schemes explored (gap costs below 4) and Bremer support values of 7 and 11, respectively. Conversely, genera lacking a colulus and colular setae (node 12, hereafter referred as the "lost colular setae (LCS) clade," white circles in Fig. 3) are monophyletic in all analyses regardless of parameter values, and received a Bremer support of 23. Spintharinae sensu Forster et al. (1990) is not supported under any circumstance. The dramatically redefined Spintharinae, including *Stemmops* but excluding formerly assigned species in at least eight genera, contains taxa with reduced coluli and is supported under most parameter combinations and by a Bremer value of 16. Genera without coluli or setae formerly assigned to Spintharinae

Table 2
Statistics of the trees obtained in the different analyses performed

Matrix	Gap cost	tv/ts	Length	Trees	Diagnosed length	RLI	% Shared clades
Optimization							
	1	1	7283	1	7283	0	100
	1	2	11,464	1	7371	0.0340	47.8
	1	4	19,492	1	7355	0.0278	54.3
	2	1	7912	1	7318	0.0135	67.4
	2	2	12,631	1	7357	0.0285	56.5
	2	4	21,892	1	7363	0.0309	56.5
	4	1	8925	5	7401.8	0.0458	47.8
	4	2	14,573	4	7450.25	0.0645	39.1
	4	4	25,715	3	7494	0.0814	36.9
	8	1	10,661	4	7552.5	0.1040	21.7
	8	2	18,081	1	7644	0.1393	26.1
	8	4	32,595	1	7695	0.1590	21.7
Static							
	?	1	7066	3	7326.6	0.0168	60.9
	1	1	7975	2	7396.5	0.0438	54.3
	A/P	1	7772	1	7357	0.0285	54.3
	—	GTR	—	—	7348	0.0251	60.9

Optimization: Analyses performed with optimization alignment. *Static*: analyses performed on the combined fragments aligned with Clustal with the following alignment parameters (in all cases transition cost set to 0.5): 18S = 8:2 (gap opening cost:gap extension cost), 28S = 8:2, 16S = 8:4 (see text for justification of the selection of these values). Gap cost: For the optimization alignment analyses refers to the gap cost used in the analyses, and for the static analyses refers to the gap treatment. A/P: Gaps recoded as absence/presence characters. tv/ts: Transversions/transition ratio. GTR: Values of the different base transformation derived from the General Time Reversible model. Trees: Numbers of trees obtained. Diagnosed length: Length of the topologies obtained from each analysis under optimization alignments with equal cost parameters. RLI: Rescaled Length Increase (see text for details). % Shared clades: Percentage of the clades supported in the optimization alignment under equal costs present in at least one of the trees obtained in each of the remaining analyses.

Table 3
Measures of support of the clades supported in the optimization alignment under equal costs

Optimization alignment									Static alignment						
Node	NH	SA	BS	PBS					Miss.		5th		A/P		BI
				CO1	H3	18S	28S	16S	BP	BS	BP	BS	BP	BS	PP
1	2	8.3	7	−6	10	2	−4	5	—	—	—	—	—	—	—
2	3	16.7	12	11	−3	−4	1	7	—	—	—	—	—	—	—
3	4	16.7	10	10	−6	3	3	0	0	2	—	—	—	—	—
4	5	100.0	23	−4	6	18	−5	8	55	3	—	—	0	3	23
5	3	25.0	10	−7	2	9	−1	7	—	—	—	—	—	—	—
6	4	33.3	6	−7	1	6	0	6	—	—	—	—	—	—	—
7	5	75.0	3	3	3	10	−6	−7	—	—	—	—	—	—	—
8	2	50.0	14	−2	10	8	−7	5	—	—	—	—	—	—	73
9	3	33.3	11	12	−14	3	−6	16	—	—	—	—	—	—	71
10	4	8.3	14	−3	0	13	5	−1	—	—	—	—	—	—	—
11	5	50.0	17	−5	2	15	3	2	95	12	96	16	99	15	100
12	6	75.0	23	5	−7	13	2	10	95	11	57	1	70	10	100
13	7	66.7	14	−6	5	−1	3	13	0	1	0	1	0	2	83
14	8	33.3	18	0	−11	0	−1	30	0	1	54	2	—	—	100
15	9	50.0	12	0	10	8	−9	3	0	1	52	1	0	2	72
16	10	41.7	14	6	−6	−2	6	10	85	13	89	11	87	13	100
17	10	41.7	11	−7	2	5	4	7	71	5	79	5	76	4	99
18	9	33.3	13	0	−2	1	0	14	0	1	74	2	71	5	98
19	8	16.7	7	6	−1	1	0	1	—	—	—	—	—	—	—
20	9	41.7	4	−1	0	0	2	3	—	—	—	—	—	—	69
21	9	66.7	19	−5.5	3	6.5	−0.5	15.5	—	—	—	—	—	—	92
22	10	83.3	7	−7	6	7	−7	8	0	1	0	2	—	—	55
23	10	100.0	13	5	2	9	0	−3	94	6	99	9	98	9	100
24	7	83.3	13	−4	9	0	−2	10	—	—	—	—	—	—	68
25	8	83.3	10	14	−6	−2	7	−3	93	7	100	12	95	14	99
26	6	50.0	20	5	−9	24	3	−3	80	9	51	1	69	8	100
27	5	8.3	12	5	8	10	−4	−7	—	—	—	—	—	—	—
28	6	41.7	13	4	−1	−7	1	16	68	13	53	0	77	9	99
29	4	16.7	8	4.5	−5.5	7	2.5	−0.5	0	0	0	2	0	4	—
30	5	83.3	18	7	7	8	0	−4	58	6	57	2	51	5	100
31	6	100.0	57	20	−4	27	3	11	100	39	100	41	100	42	100
32	7	8.3	9	−10	15	9	−2	−3	—	—	0	1	0	1	—
33	8	8.3	1	−3	3	3	−1	−1	—	—	0	1	0	1	—
34	7	58.3	27	−12	9	15	−1	16	87	7	90	8	81	9	100
35	5	25.0	18	−3	−3	19	−4.5	9.5	72	6	73	8	74	10	100
36	6	75.0	16	9	−4	−1	2	10	—	—	—	—	—	—	100
37	7	58.3	8	−3	−2	13	−6	6	—	—	—	—	—	—	—
38	8	33.3	10	−2	6	7	−1	0	66	4	66	7	77	11	—
39	8	8.3	7	−1	2	−2	5	3	53	5	57	5	66	2	—
40	6	25.0	15	−0.5	3	13.5	5	−6	55	1	55	0	63	0	100
41	7	75.0	17	2	10	−2	−3	10	—	—	—	—	—	—	100
42	3	33.3	7	−4	8	6	−7	4	0	2	—	—	—	—	98
43	4	16.7	8	0	−2	−1	1	10	0	0	—	—	—	—	—
44	5	100.0	40	−1	−7	24	1	23	100	34	100	49	100	50	100
45	6	66.7	7	0	−8	−3	−1	19	0	0	82	3	78	5	—
46	5	83.3	6	−8	6	3.5	5	−0.5	0	2	88	8	66	4	—
			629	16.5	46.5	301.5	−14.5	279							

Node: Node number in Fig. 3. NH: Node height. SA: Sensitivity analysis support expressed as percentage of the analyses under different parameter costs (total = 12) that supported the particular clade. PBS: Partial Bremer supports of the optimization alignment under equal costs analysis. Miss.: Gaps as missing data. 5th: Gaps as 5th state. A/P: Gaps recoded as absence/presence characters. BI: Bayesian inference analysis. BP: Bootstrap proportions. BS: Bremer support. PP: Posterior probability.

remain in the LCS clade. Hadrotarsinae is monophyletic with a Bremer support of 15, although contradicted by most analyses under differential parameter costs. The same is true for the Hadrotarsinae–Spintharinae clade (node 35, Fig. 3). Neither *Theridion* nor *Anelosimus* is monophyletic. Conversely, argyrodine monophyly (= *Argyrodes* sensu Exline and Levi, 1962, see Yoshida, 2001a) and its sister-group relationship to *Enoplognatha* is supported by all analyses. However, *Argyrodes* apart from *Rhomphaea* and *Ariannes* (see Yoshida, 2001a) is

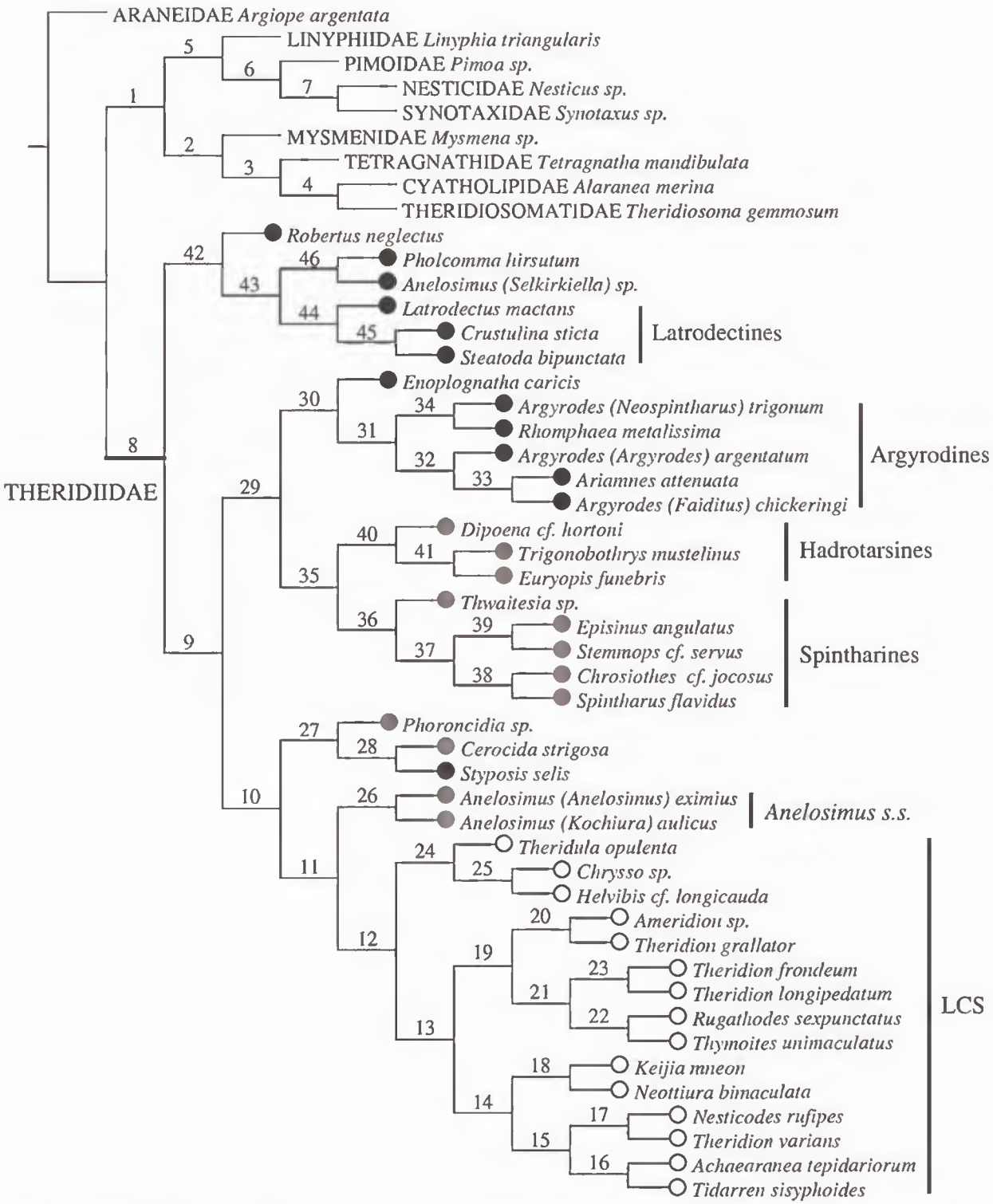

Fig. 3. Single cladogram obtained from the optimization alignment of all gene fragments combined, with uniform parameter costs (gap = transitions = transversions = 1). Figures above branches refer to clade numbers. Tree statistics are included in Table 3, and different measures of clade support are shown in Table 2. Circles at the tips of the branches refer to the degree of developments of the colulus [according to Levi and Levi (1962) with additional modifications based on scanning electron microscope images, Agnarsson in prep.] in the corresponding theridiid taxon: Black circle denotes well-developed colulus, grey circle denotes colulus reduced or substituted by two setae, white circle denotes no trace of colulus or colular setae. LCS, Lost colular setae clade.

not monophyletic. The Latrodectinae (*Latrodectus*, *Steatoda*, and *Crustulina*) is the only other major clade supported by all optimization alignment analyses. The sister relationship of LCS clade (node 12) to *Anelosinus* sensu *strictu* (i.e., all *Anelosinus* species apart from the ones formerly included in *Selkirkiella*) form the 'lost colulus (LC) clade' (node 11) and received Bremer support of 17 and occurred in half of the different parameter costs analyses. Trees resulting from the search with outgroups constrained to the topology based on current morphological knowledge, largely agree with equal cost results for the ingroup. The only difference is that *Phoroncidia* + *Cerocidia* + *Styposis* (clade 27 in Fig. 3) joins at node 9.

When the independent gene fragments are analyzed separately, the 18S and 16S genes performed best in terms of percentage of shared clades (18S = 23.9%, 16S = 22.2%, 28S = 21.7%, H3 = 14.6%, and CO1 = 11.1%) when compared to the simultaneous analyses, while the protein-coding genes performed the worst. However, according to the PRI the protein-coding fragments and 18S, are the ones that best fit the combined tree for most analyses (Table 4). The 18S and 16S are also the genes that contribute the most to the total Bremer support of the simultaneous analyses, but the ribosomal 28S contribution is negative (Table 3). Partitioned Bremer support of the different gene fragments do not show any clear relationship with time of divergence as measured by node depth, suggesting that they contribute information in all time windows.

As parameter cost increase results diverge increasingly from those under different costs (Table 2). However, this trend is neither proportional nor monotonic. For a constant gap cost, both RLI and percent shared clades suggest that doubling the transition/transversion ratio (ts/tv) from 1 to 2 has more effect than from 2 to 4 (where similarity to the reference analysis actually improved under gap cost 1). For constant ts/tv ratio, trends vary: at ts/tv = 1, doubling gap costs loses about a third of shared clades each time, but at 2 and 4 doubling gap cost increases both measures but quadrupling it decreases both. In any case, results are quite sensitive to parameter choice: roughly 50% of the reference clades are lost if any value is changed.

ClustalX alignments with gap opening cost 8 and extension gap penalty of 2 were selected as the ones that best represented the elision matrix for both the 18S and the 28S gene fragments under the optimality criteria adopted (see Section 2). For the 16S alignment optimal parameter costs depended on the criteria used.

Table 4
Statistics of the partial analysis of the different gene fragments

Matrix	*L*	*T*	*L*combined	Δ*L*	Max*L*	Min*L*	PRI
Optimization							
COI	1736	19	1797	61	2285	402	0.0324
H3	1005	12	1082	77	1509	225	0.0600
18S	1382	1	1428	46	2226	1382	0.0545
28S	747	49	818	71	1226	747	0.1482
16S	2103	1	2158	55	2708	2103	0.0909
Static, miss.							
COI	1736	227	1808	72	2285	402	0.0382
H3	1003	>1000	1077.6	74.6	1509	225	0.0581
18S	1335	>1000	1392.3	57.3	2213	609	0.0357
28S	667	18	730	63	1094	300	0.0793
16S	1949	>1000	2059	110	2779	599	0.0505
Static, 5th							
COI	1736	227	1822.5	86.5	2285	402	0.0459
H3	1003	>1000	1079	76	1509	225	0.0592
18S	1501	242	1616.5	115.5	2482	714	0.0653
28S	807	>1000	892	85	1247	381	0.0982
16S	2458	>1000	2565	107	3701	826	0.0372
Static, AIP							
COI	1736	19	1851	115	2285	402	0.0611
H3	1005	12	1119	114	1509	225	0.0888
18S	1501	728	1757	256	2484	704	0.1438
28S	770	54	908	138	1217	368	0.1625
16S	2304	74	2488	184	3372	838	0.0726

Matrix: gene fragment. *Optimization*: optimization alignments. *Static, miss.*: Clustal-based alignments, gaps as missing data. *Static, 5th*: Clustal-based alignments, gaps as 5th state. *Static, AIP*: Clustal-based alignments, gaps recoded as absent/present characters. *L*: Tree length. *T*: Number of trees. *L* combined: Number of steps of the tree obtained in the combined analysis of all the gene fragments, for a particular gene fragment. Δ*L*: Difference in length of the combined tree and the best tree for each particular gene fragment. Max*L*: Maximum possible length of a particular gene fragment. Min*L*: Minimum possible length of a particular gene fragment. PRI: Partition-based retention index (see text for details).

Using percent shared clades, gap opening 8 and gap extension 4 were optimal. Using average symmetric-difference distance criterion selected gap opening 8 and gap extension 2 were optimal. Because the 8/4 alignment shared more clades than the 8/2 (34–23) and was only marginally worse under average symmetric-difference distance (15–14), we chose this alignment to merge the static data in the combined data matrix. The number of characters for each of the preferred alignments was 871, 343, and 549 for the 18S, 28S, and 16S, respectively. The static alignment of the five gene fragments combined yielded 2562 positions. Under gaps as missing data (975

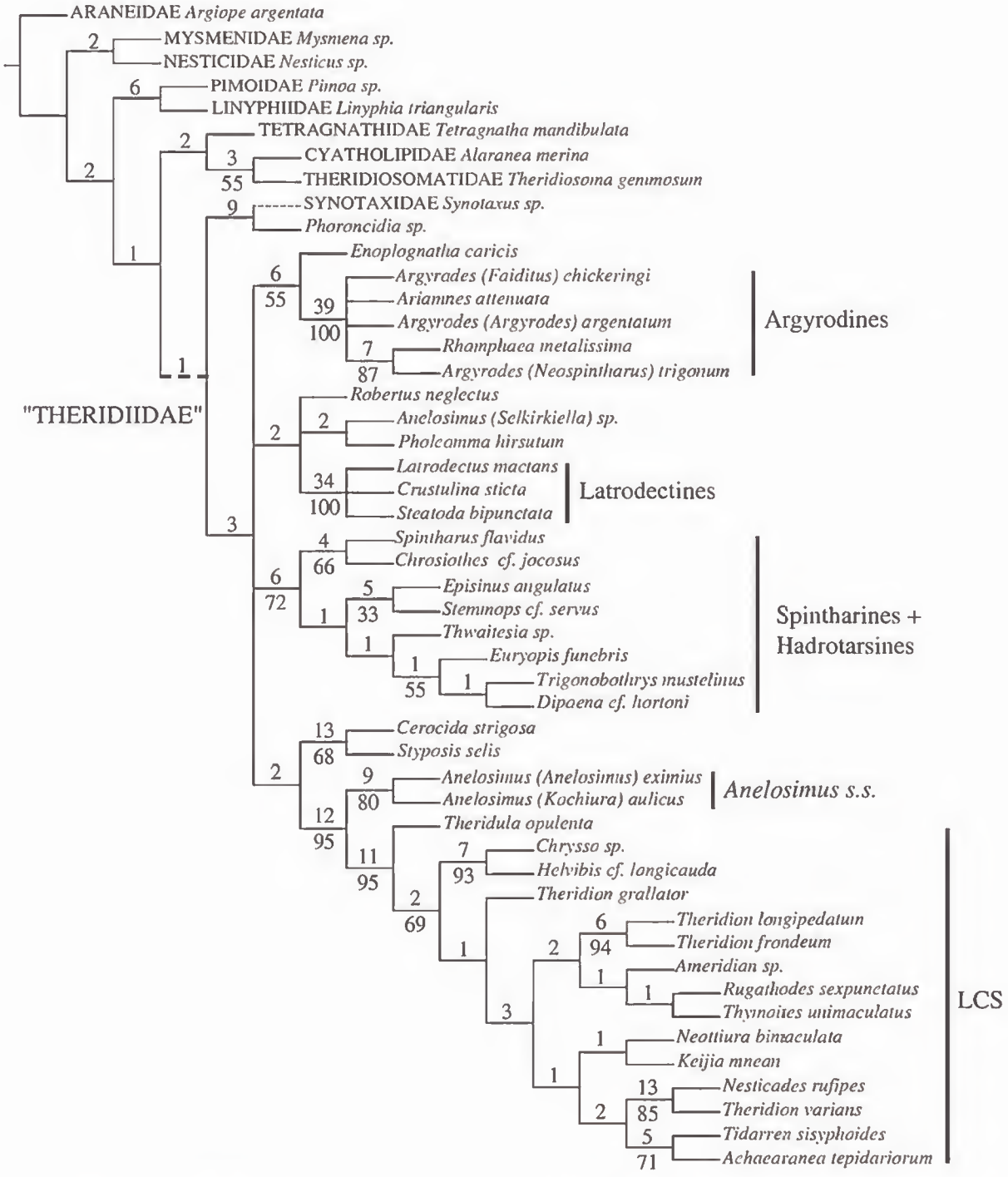

Fig. 4. Strict consensus of the 3 trees resulting of the parsimony analysis of the static combined alignment with gaps as missing data. Numbers above branches are Bremer support and below branches bootstrap proportions. Additional statistics and support showed in Tables 2 and 3. LCS, Lost colular setae clade.

informative positions) parsimony analysis resulted in three trees of length 7066, CI = 0.26, RI = 0.36 (Fig. 4). Under 5th state gap coding (1092 informative positions) the same matrix yielded 2 trees of length 7975, CI = 0.27, RI = 0.37 (Fig. 5). The Simmons and Ochotorena's simple indel coding method added 95, 71, and 238 indel absence/presence characters to 18S, 28S, and 16S gene fragments, respectively, for a total combined alignment of 2967 positions (1153 informative). Analysis of the recoded matrix resulted in 1 tree of 7772 steps, CI = 0.26, RI = 0.37 (Fig. 6). Surprisingly, "gaps as missing data" analysis is much more similar to the optimization alignment analysis under equal costs than to other static gap-coding methods (Table 2). The PRI-measured performance of the independent gene fragments if analyzed separately varies drastically depending on the gap treatment (Table 4). When gaps were coded as missing data, the 18S most resembles the combined

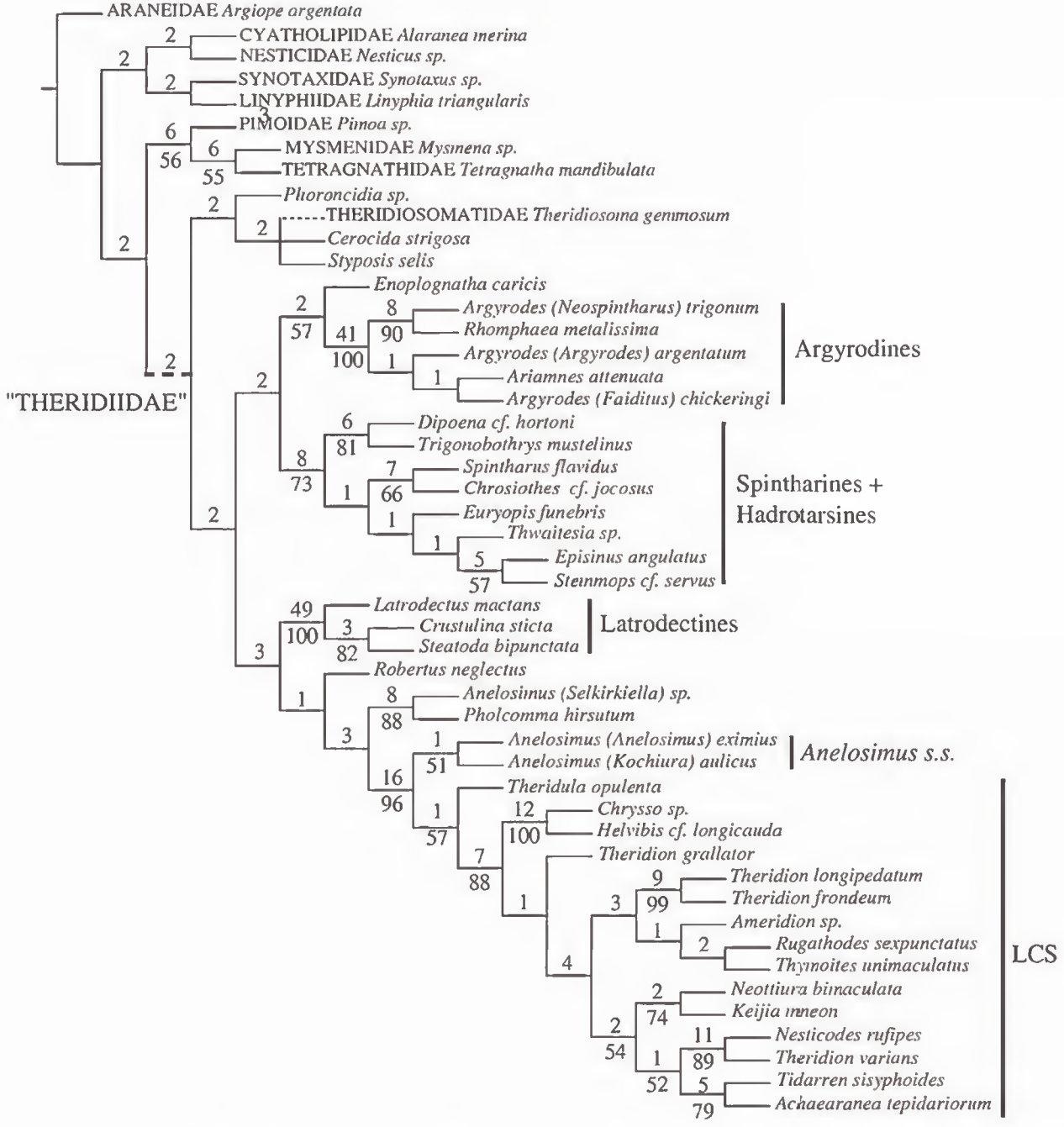

Fig. 5. Strict consensus of the 2 trees resulting of the parsimony analysis of the static combined alignment with gaps as 5th state. Numbers above branches are Bremer support and below branches bootstrap proportions. Additional statistics and support showed in Tables 2 and 3. LCS, Lost colular setae clade.

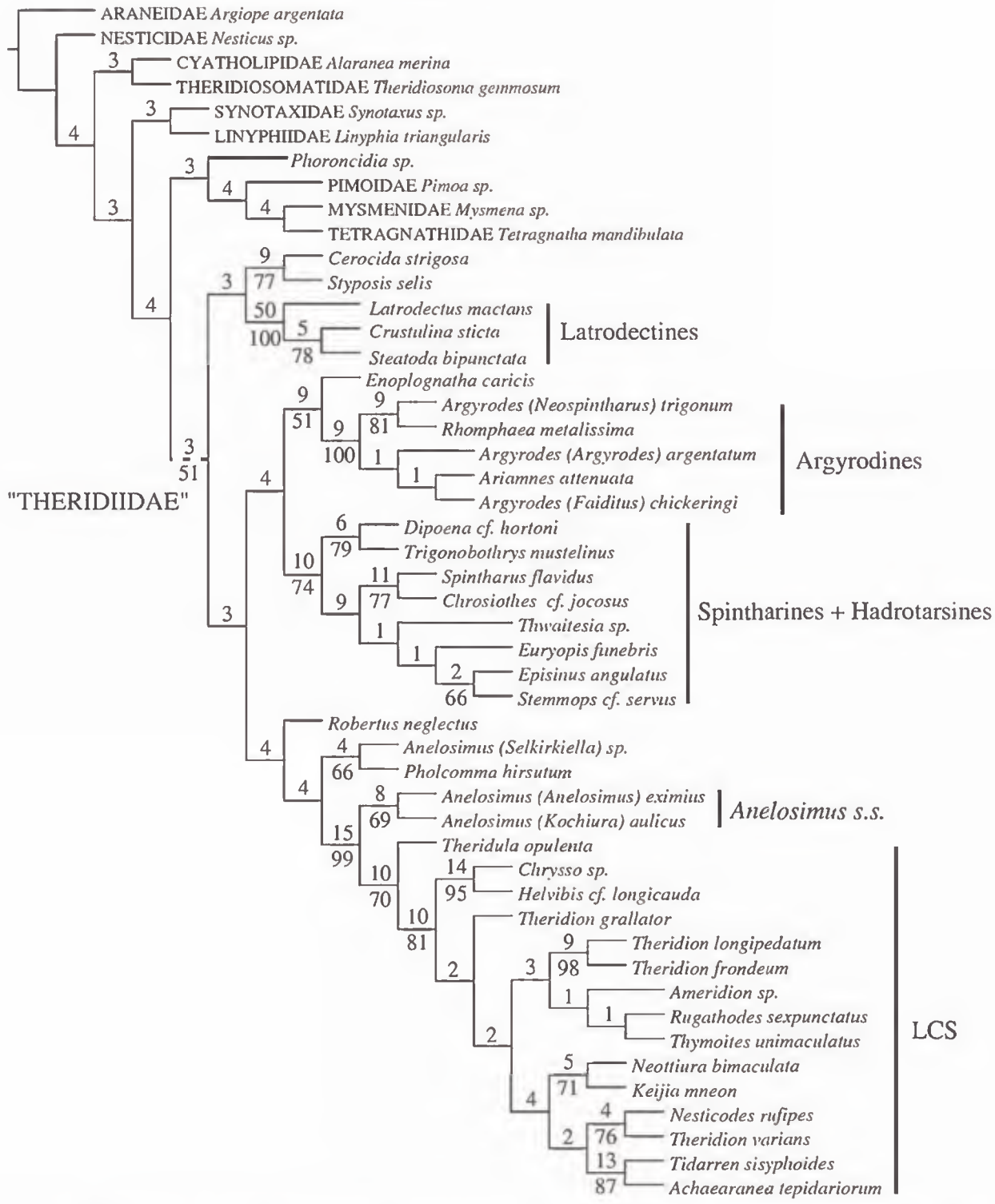

Fig. 6. Strict consensus of the single tree resulting of the parsimony analysis of the static combined alignment with gaps recoded as absence/presence characters (see text for details). Numbers above branches are Bremer support and below branches bootstrap proportions. Additional statistics and support showed in Tables 2 and 3. LCS, Lost colular setae clade.

results. However, 16S performs better under 5th state coding and under absence/presence CO1 has the lowest PRI. In all cases, 28S performs the worst.

All parsimony static analyses of the combined data set agree with the optimization alignment under equal costs in supporting the monohlyly of Latrodectinae, the Hadrotarsinae + Spintharinae + *Stemmops* clade (although Hadrotarsinae and Spintharinae are not monophyletic in some gap treatments explored), the Argyrodinae and *Enoplognatha* + Argyrodinae, *Anelosimus* sensu stricto,

and the sister relation between the latter and LCS clade. The Hadrotarsinae + Spintharinae + *Stemmops* clade is sister to the *Enoplognatha* + Argyrodinae clade only in some trees with gaps as missing data. Unlike optimization alignment analyses, parsimony analyses on the static alignments refuted theridiid monophyly, and for gaps as a 5th state or absent/present, Hadrotarsinae. Clades under static analyses that disagree with the equal cost optimization alignment tended to be poorly supported (Table 3).

Both the hierarchical likelihood ratio test and the Akaike information criterion preferred the GTR + I + Γ (Yang, 1994) as the model for the static combined data. The Bayesian inference results for the static combined matrix is shown in Fig. 7. The only well-supported outgroup clade is *Pimoa* sp.—*Linyphia triangularis*,

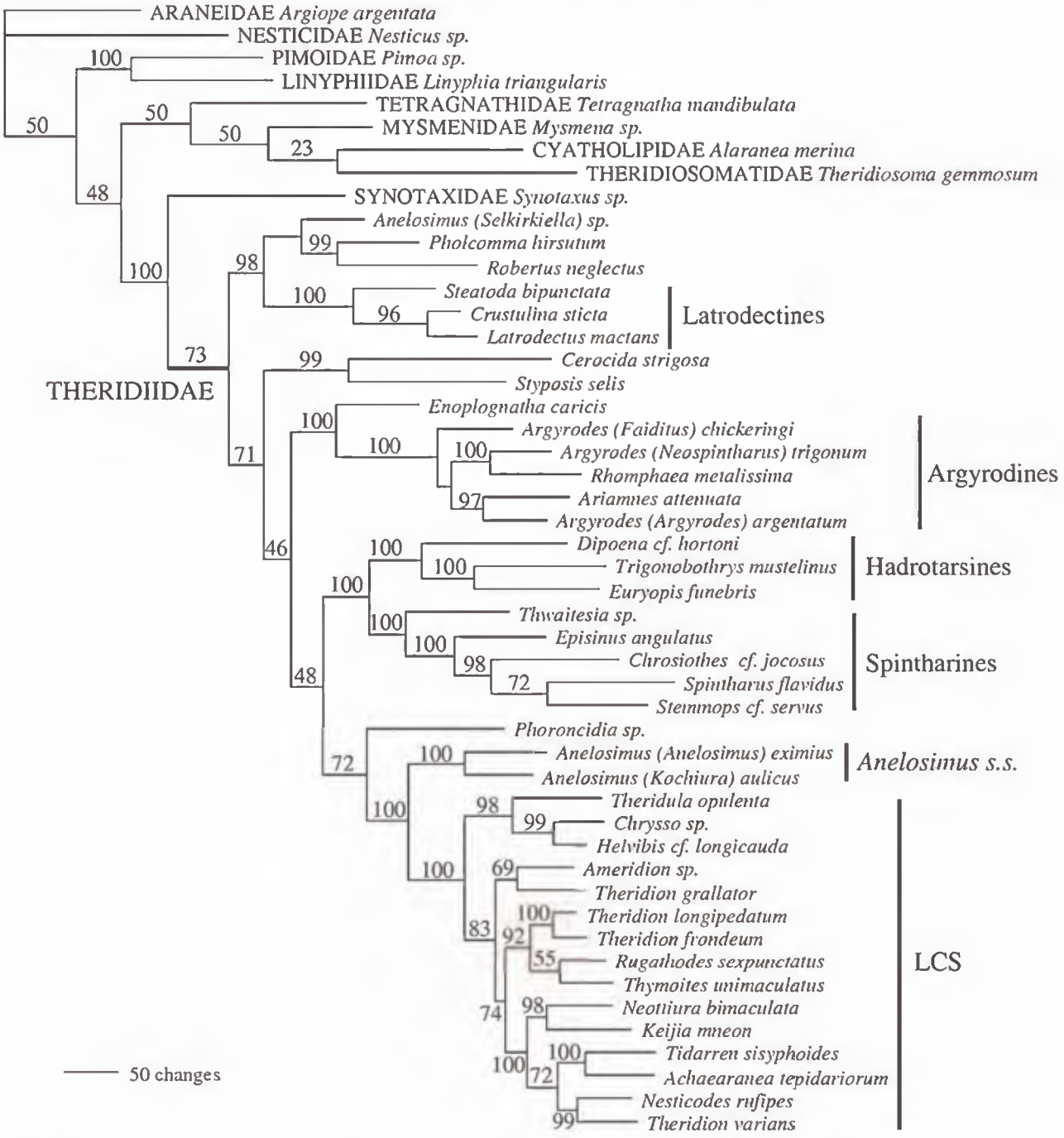

Fig. 7. The 50% majority rule consensus of the trees obtained from the Bayesian analysis (GTR + I + Γ model) of the static combined alignment (500 first generations of the MCMC burn in). Numbers above branches are posterior probability values. Additional statistics and support showed in Tables 2 and 3. LCS, Lost colular setae clade.

concordant with current morphological analyses. *Synotaxus* sp. is shown as the sister to all theridiids, contra morphology that places nesticids as sister to theridiids and synotaxids as sister to cyatholipids (here *A. merina*). The tree supports most of the ingroup clades obtained by the equal costs optimization alignment. Theridiid monophyly receives low posterior probability as do the interrelationship of most subfamilies, but subfamily posterior probabilities are high. Support for Argyrodinae + *Enoplognatha*, hadrotarsines + spintharines + *Stemmops*, *Anelosimus* sensu stricto, the LC clade and the LCS clade is high. At lower levels, the internal arrangements within these clades differ and *Cerocida* + *Styposis* jumps from *Phoroncidia* to basal theridiids. Most of the discrepancies receive low support, as measured by the posterior probability values.

4. Discussion

4.1. Alignments

Unsurprisingly, different assumptions of alignment construction, gap treatment, or phylogenetic inference method yielded conflicting phylogenetic hypotheses (Morrison and Ellis, 1997; Wheeler, 1995). In this study, we preferred a particular inference method a priori for two reasons. First, more detailed and explicit hypotheses are more easily falsified. Second, picking a reference tree simplifies the sensitivity analysis by reducing the number of comparisons to be performed. Preference for a particular method with specific assumptions should precede the actual analysis to avoid the pitfalls, and circularity, of preferring those results that better suit our preconceptions. We favor optimization alignment for epistemological reasons. It is superior to static alignment because it more accurately treats indel events as transformations rather than as character states. Although alignment construction and tree search are arguably independent by analogy to the primary homology concept in morphological data (Simmons and Ochoterena, 2000), in practice static alignments usually come from automatic alignment algorithms, with or without subsequent manual modifications, all of which are in turn based on a guide tree (Frost et al., 2001). We preferred equal costs during optimization alignment because, absent an objective criterion to choose across differential costs (Faith and Trueman, 2001), equal costs seem simpler, add less to background knowledge, and thus maximize explanatory power (Kluge, 1997a,b).

Although the results of a particular analysis may be favored a priori, it is still worth exploring the effect of assumptions on these results.

Molecular systematists commonly use taxonomic congruence across different methods of data analysis as a measure of clade robustness. Our varied analyses generally shared more than 50% of the clades, except optimization alignment with gap costs of 4–8 (Table 2). Static alignments that dismiss gap information (gap as missing and Bayesian inference, Table 2) are most similar to the equal cost optimization alignment results. This surprising result may relate to the way optimization alignment treats gaps. Optimization alignment minimizes the numbers of indel events necessary to explain the data for given gap and base transformation costs. Our analyses corroborate this point, although at first sight it may seem the opposite. Optimization alignment implied more characters than did static Clustal (2825 and 2562, respectively). However, shorter alignments do not necessarily result in most parsimonious explanations of the data. In this particular situation, optimization and static alignments are difficult to compare because parameter costs vary (gap cost 1, ts/tv 1 in the former, gap opening 8, gap extension 2 or 4, ts/tv 0.5 in Clustal). The number of informative characters is a less tricky comparison. Implied alignment yields fewer number of informative characters (916) than the static alignment, regardless of the gap treatment (975 as missing data, 1092 as 5th state). Fewer informative indels (gaps in a static alignment) probably affect the final output less, making the results more similar to the static alignment analyses when gaps are not considered. Of course, the generality of these comments must await further research on alternative alignment methods and in additional taxa and genes.

4.2. Morphological implications and evolutionary patterns

Different analyses chiefly disagree on the basal part of the tree, outgroup relationships and theridiid monophyly. The Theridiidae is an extremely diverse family, and instances of non-monophyly as a result of a few odd taxa incorrectly placed in the family might be expected. However, is it likely that *Phoroncidia*, a genus with many classic theridiid features, is not a theridiid? *Phoroncidia*, shares many theridiid synapomorphies, e.g., absence of a basal, ectal paracymbium, distal cymbial hook present and involved in cymbial-lock mechanism, and grossly flattened aggregated gland (AG) spigots. On the other hand, *Phoroncidia* lacks some typical theridiid features: male palpal tibial rim regularly and strongly hirsute and facing palpal bulb, abdominal stridulatory picks, and theridiid-type tarsal comb. It is definitely aberrant, and although morphology supports its placement within theridiids, its exact phylogenetic position remains inconclusive.

Outgroup topology in this study was highly sensitive to parameter change and mostly disputes morphological evidence. No analyses support theridiid–nesticid monophyly. This sister-group relationship is based on morphology and behavior (Coddington, 1986, 1989; Forster et al., 1990; Griswold et al., 1998; Heimer and

Nentwig, 1982). The list of synapomorphies includes: exactly two colular setae present, reduced posterior lateral spinnerets (PLS) piriform spigot field, lobed PLS aggregate (AG) glands, cobweb, and sticky silk placed on gumfoot lines. Nesticid somatic and genital morphology differs considerably from theridiids, but, linyphioids and cyatholipoids are equally divergent. The placement of nesticids in this study is unlikely to endure. Similarly, no analyses supported 'araneoid sheet-web weavers' or 'spineless femur clade' monophyly, both well supported morphologically (Griswold et al., 1998). This incongruence may be caused by several factors, including sparse outgroup taxa and use of genes that perform better at lower (species/genus) taxonomic levels.

These results confirms the monophyly of Hadrotarsinae and Argyrodinae, but dispute *Argyrodes* sensu Yoshida (2001a), Spintharinae sensu Forster et al. (1990) and Theridiinae sensu Yoshida (2001b). The latter two include essentially the same taxa, but our results suggest that *Spintharus* is not closely related to the lost colular clade. Spintharinae comprises mainly taxa with reduced, highly specialized, webs.

None of the genera represented by more than one species is monophyletic in all analyses. Both *Theridion*, and *Argyrodes* (sensu Yoshida, 2001a) are polyphyletic regardless of the gap treatment or method of inference used. The *Theridion* result confirms a problem that has long been suspected (e.g., Forster et al., 1990; Levi and Levi, 1962); *Theridion* is a 'waste basket' group that urgently needs revisionary work. Yoshida's (2001a) attempted to improve the classification of argyrodines by recognizing two very distinctive clades: *Rhomphaea* and *Ariamnes*. This, however, rendered the remaining *Argyrodes* paraphyletic, which could be remedied by recognizing *Faiditus* and *Neospintharus* as well. The five groups are highly distinct, and differ strikingly in morphology and behavior. The monophyly of *Anelosimus eximius* and *A. aulicus* is only contradicted by optimization alignment analyses with high gap costs (>4), while the Chilean '*Anelosimus*' (=*Selkirkiella*) does not cluster with the other *Anelosimus* under any condition.

Our study suggests novel relationships among the argyrodine genera that affect interpretations of the origin and evolution of kleptoparasitism and araneophagy (Fig. 8). The species *Argyrodes* (*Faiditus*) *chickeringi* nests deep within argyrodines here, suggesting that its rather generalized prey catching strategy is derived. The specialized araneophagic genera *Ariamnes* and *Rhomphaea* are not sister here; Whitehouse et al. (2002) also suggested that their unique hunting strategy was probably convergent. They also favored the hypothesis that kleptoparasitism evolved once at the base of Argyrodinae. Given the distribution of kleptoparasitism in Fig. 8, three optimizations are possible: gain at the argyrodine node with loss in *Rhomphaea* and *Ariamnes*, three convergent gains, and an intermediate optimization of two gains and a loss. The first reconstruction is consistent with the Whitehouse et al. (2002) hypothesis and preserves homology of kleptoparasitism across argyrodines. Similarly, araneophagy can be optimized either at the argyrodine node with subsequent loss in *Argyrodes*, or as two convergent gains. Not all *Faiditus* are araneophages, however, which could alter these optimizations. Regardless, our results do not support the idea that either behavior arose directly from the other (Smith Trail, 1980; Vollrath, 1984).

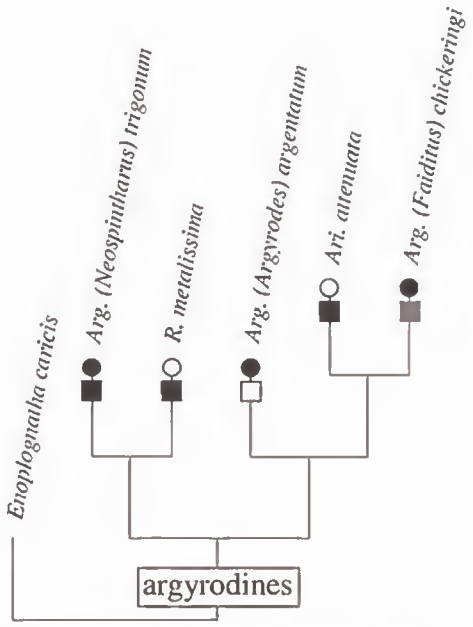

Fig. 8. Topology for the argyrodines supported by the single cladogram obtained from the optimization alignment of all gene fragments combined, with uniform parameter costs (gap = transitions = transversions = 1). Circles at the tips of the branches refer to the presence (black) or absence (white) of kleptoparasitic behavior in that particular lineage. Squares at the tips of the branches refer to the presence (black) or absence (white) of araneophagic behavior in that particular lineage. The grey square denotes the lack of knowledge about araneophagy in *Argyrodes* (*Faiditus*) *chickeringi* specifically although other species in this group are araneophagic.

The monophyly of the lost colulus and the lost colular setae clades are confirmed. Among other genera, it includes *Anelosimus*, *Theridion*, and *Achaearanea*, which contain all social theridiids. Agnarsson (2002) already noted that instances of sociality are unexpectedly clustered among theridiids.

We have here presented the first detailed phylogenetic hypothesis of Theridiidae. In many cases our results are congruent with morphological knowledge and reinforce the arguments based on previous studies. In other cases these findings starkly contest hypotheses based on morphology alone and challenge both morphological observations and interpretations, providing new and often unexpected insight into the evolution of the diverse traits of theridiid spiders.

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