Cladistics **14**, 173–192 (1998)
WWW http://www.apnet.com
Article i.d. cl980061

The Phylogeny of the Extant Chelicerate Orders

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Accepted 24 January 1998

The phylogeny of the extant chelicerate orders is examined in the light of morphological and molecular evidence. Representatives from each of the chelicerate "orders" and mandibulate and onychophoran outgroups are examined. Molecular (small and large ribosomal subunit DNA) and morphological information is combined in a total evidence regime to determine the most consistent picture of extant chelicerate relationships for these data. Multiple phylogenetic analyses are performed with variable analysis parameters yielding largely consistent results. A normalized incongruence length metric is used to assay the relative merit of the multiple analyses. The combined analysis with lowest character incongruence yields the scheme of relationships (Pycnogonida+(Xiphosura+((Opiliones+((Solifugae+Pseudoscorpiones)+Scorpiones))+((Ricinulei+Acari)+(Palpigradi+((Thelyphonida+Schizomida=Uropygi)+(Amblypygi+Araneae)))))). This result is fairly robust to variation in analysis parameters, with the placement of solifugids and the status of the pedipalps responsible for most disagreement.

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INTRODUCTION

The phylogeny of the chelicerate orders has been the subject of argument for over a century. The basal extant lineages—Xiphosura (horseshoe crabs), Pycnogonida (sea spiders), and Scorpiones—have been discussed mainly in the context of arthropod relationships (Snodgrass, 1938; Weygoldt, 1986; Wheeler et al., 1993) while the arachnid orders (Araneae: spiders; Amblypygi: tailless whipscorpions; Thelyphonida: vinegaroons; Schizomida: tartarids; Palpigradi: micro whip scorpions; Solifugae: sun or wind scorpions; Ricinulei; and Acari: mites and ticks; Pseudoscorpiones: false scorpions; and Opiliones: daddy-long-legs/harvestmen) have a distinct literature. This has resulted in shortcomings in both arenas. Chelicerate-level discussions frequently rely on the assumption that scorpions are the sister taxon to the remaining arachnids, hence can be used as the generalized, basal condition of the group. Although widely held (Pocock, 1893; Weygoldt and Paulus, 1979b; Weygoldt, 1986), this view has been questioned (Savory, 1971; Yoshikura, 1975; van der Hammen, 1977a, 1977b, 1979, 1982, 1985, 1986; Shultz, 1990). A parallel assumption in many arachnid studies is that *Limulus* or some hypothetical construct is adequate to determine character polarity within Arachnida. Pycnogonids, though

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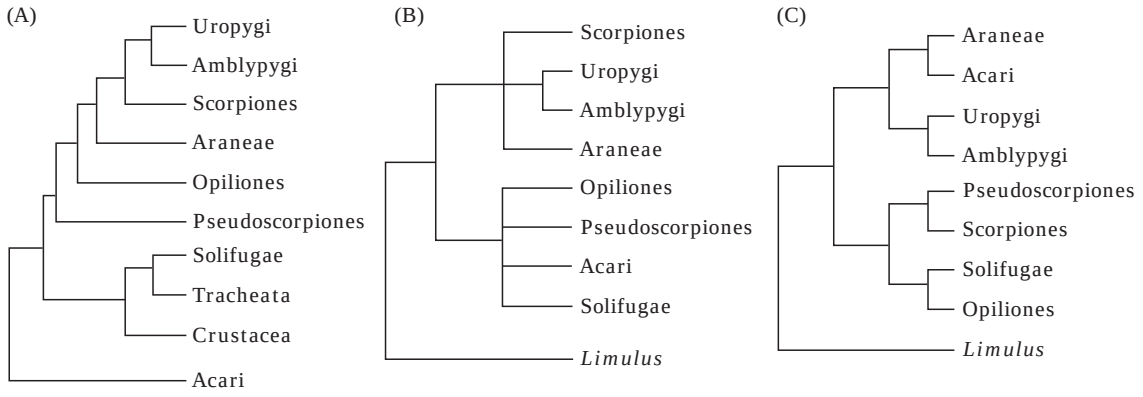

FIG. 1. Arachnid phylogeny of (A) Thorell (1877) and (B) Lankester (1881) classifications. (C) "Genealogical" tree.

certainly bizarre, may well affect arachnid groundplan notions. This study attempts to elaborate chelicerate ordinal relationships by examining basal and derived lineages simultaneously. This is accomplished by sampling pycnogonid, xiphosuran, and arachnid lineages as well as mandibulate and onychophoran outgroups with both morphological and molecular data. We hope that through improved taxon sampling and the integration of morphological and molecular data ("total evidence": Kluge, 1989), a more consistent and robust picture of chelicerate relationships will emerge.

BACKGROUND

In his initial study of arachnid classification, Thorell (1877) erected a ladder-like progression from basal mites and ticks (Acari) to the most derived scorpions (Fig. 1A). This was based on Thorell's notions of morphological complexity and specialization leading to "higher" and "lower" taxa. Unfortunately, Arachnida is (at best) paraphyletic with respect to the mandibulates. Lankester (1881) maintained the grouping of Scorpiones, Aranae, and Pedipalpi (Uropygi+Amblypygi) naming them the Aerobranchia (respiratory lamellae filled with air) while elevating the basal slurry to the Lipobranchia (tracheate arachnids) in his classification (Fig. 1B). Furthermore, the Lipobranchia contain the solifugids, which Thorell had consigned to lie with the hexapods and myriapods, but not the derived "lipobranchiate" spiders. His "genealogical" tree, however (Fig. 1C), scrambles these groupings by placing the Acari with the spiders and the scorpions with the pseudoscorpions. Even Pocock (1893) wondered as to the naturalness of non-genealogical groupings. Lankester also was the first to unite Xiphosura with arachnids via his discussion of book-gills in *Limulus* and book-lungs in *Scorpio.*

Pocock (1893) criticized Thorell's placement of the Solifugae as "quite unintelligible" and the general ordering (scorpions highly derived) as absurd. To Pocock, the presumed annelid ancestor of arthropods logically required that creatures which were more possessed of this serially homonymous arrangement of body segments be primitive (or basal). He also denied the restriction of the "lipobranchiate" condition, presumably believing it to be primitive or at least widespread. In Pocock's scheme, scorpions (Ctenophora) were for the first time placed in their cherished position outside the remainder of the group (Lipoctena). As character support, Pocock cited the number of embryonic "abdominal" appendages (six in Scorpiones and no more than four in the remaining arachnids). He also placed the spiders with amblypygids, schizomids, and thelyphonids together in the Caulogastra (Fig. 2). This leaves Lankester's Lipobranchia intact, but splits the Aerobranchia into the Ctenophora (scorpions) and Caulogastra (spiders and kin). This scheme is based on Pocock's notion that scorpions possess more structures arranged in serially segmented fashion, hence resemble the presumed Ur-arachnid to a greater extent than other taxa.

Before Pocock, Thorell had unified Thelyphonida (sometimes referred to as the Uropygi themselves) and Schizomida into the Uropygi. This grouping, based on a unique pattern of trichobothria and mating behavior, has been one of the few constants in arachnid phylogeny.

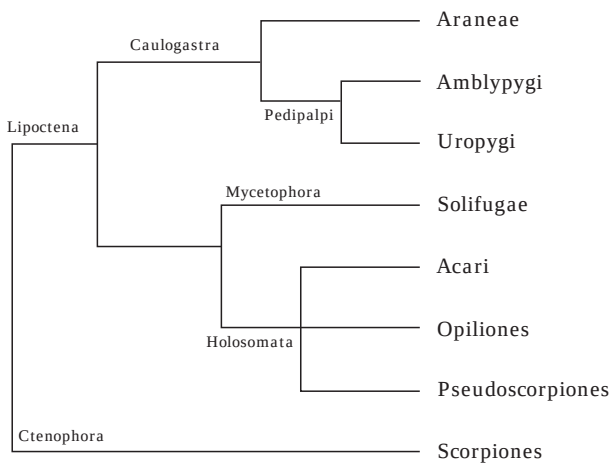

FIG. 2. Arachnid phylogeny of Pocock (1893).

On the chelicerate level, Börner (1904, 1912, 1932) placed the pycnogonids as sister to Xiphosura and Arachnida based mainly on the presence of chelicerae. It was Snodgrass (1938), however, who created the primary divisions in the Arthropoda that we recognize today. The basic distinction between chelicerates on one side and mandibulates on the other sets the stage for subsequent discussion of arthropod subgroups (Fig. 3A). Furthermore, his establishment of the scheme (Pycnogonida+Xiphosura+Arachnida) on firm character basis is still robust. Stormer (1944) felt the placement of pycnogonids within Chelicerata to be unwarranted and so removed the sea spiders to their own higher taxon. Pycnogonids were now not only outside Xiphosura+Arachnida, but also outside the non-chelicerate trilobites.

Petrunkevitch (1955), in his summary of arachnid classification, supported several novel groups. Among these are the Labellata (Araneae+Amblypygi) and the Caulogastra (those taxa with a constriction between the prosoma and opisthosoma—Pocock, 1893) enlarged to include the palpigrades and redefined to include solifugids and ricinuleids. The Labellata group was erected based on perceived similarities between the circum-oral structures ("mouth anteroventral between 2 lips") in amblypygids and spiders. He placed this large assemblage in opposition to the "Latigastra" composed of the scorpions, pseudoscorpions, Opiliones, and Acari. These taxa were united based on the broad juncture between the prosoma and opisthosoma. Disagreeing with Petrunkevich, Sharov (1966) asserted that scorpions had a separate origin from the remaining arachnids. This diphyly could be interpreted to maintain the basal position of scorpions proposed by Pocock but to resurrect the grade leading to spiders with monophyletic pedipalps (as opposed to Labellata) as their sister taxon, then connected to solifugids and Acarina (Acari) at the base.

Savory (1971) cited the Cyphophthalmi as intermediate to (and linking) the Acari and Opiliones and based many of his ideas on features of the presumed arachnid ancestor. Agreeing with the emerging Labellata–Caulogastra standard, he added ((Opiliones+Acari)+Ricinulei) as the sister group to this collection. At the base of the arachnids, the scorpions and pseudoscorpions are linked with the solifugids (Fig. 3B). Savory also held that Arachnida were not monophyletic. By this, he seems to mean that the arachnid condition (for want of a better phrase) arose several times independently. He suggests no genealogical kinship between any particular arachnid and any non-arachnid group. Hence, this Arachnida (the taxon) are monophyletic no matter how one looks at it.

In an exhaustive study of extant chelicerates, Firstman (1973) included pycnogonids, *Limulus*, and the previously under-examined palpigrades and ricinuleids (Fig. 3C). On the basis of variation in the arterial system and its relationship to the endosternite, he erected a scheme again maintaining the basal status of scorpions and dividing the pedipalps to make the Amblypygi sister to the Araneae based on the number and position of endosternite suspensors. This group, Labellata, was first put forward by Petrunkevitch (1955). Firstman suggested that the pycnogonid vascular septum is homologous with the endosternite of Xiphosura and arachnids, placing the sea spiders as the sister taxon to the remaining extant chelicerates.

Soon after Firstman, Yoshikura (1975; Fig. 3D) examined mainly embryological characters and embraced the Labellata–Caulogastra arrangement of Petrunkevitch (1955). In his discussion, however, Yoshikura states that the Uropygi and Amblypygi are most "similar" and hence a group, which differs from his dendrogram of relationships. He added the scorpions and pseudoscorpions as sister group to this clade, segregating the remaining taxa to a group very similar to Pocock's (1893).

After nearly a century of monophyly, van der Hammen (1977a, 1977b, 1979, 1982, 1985, 1986)—like

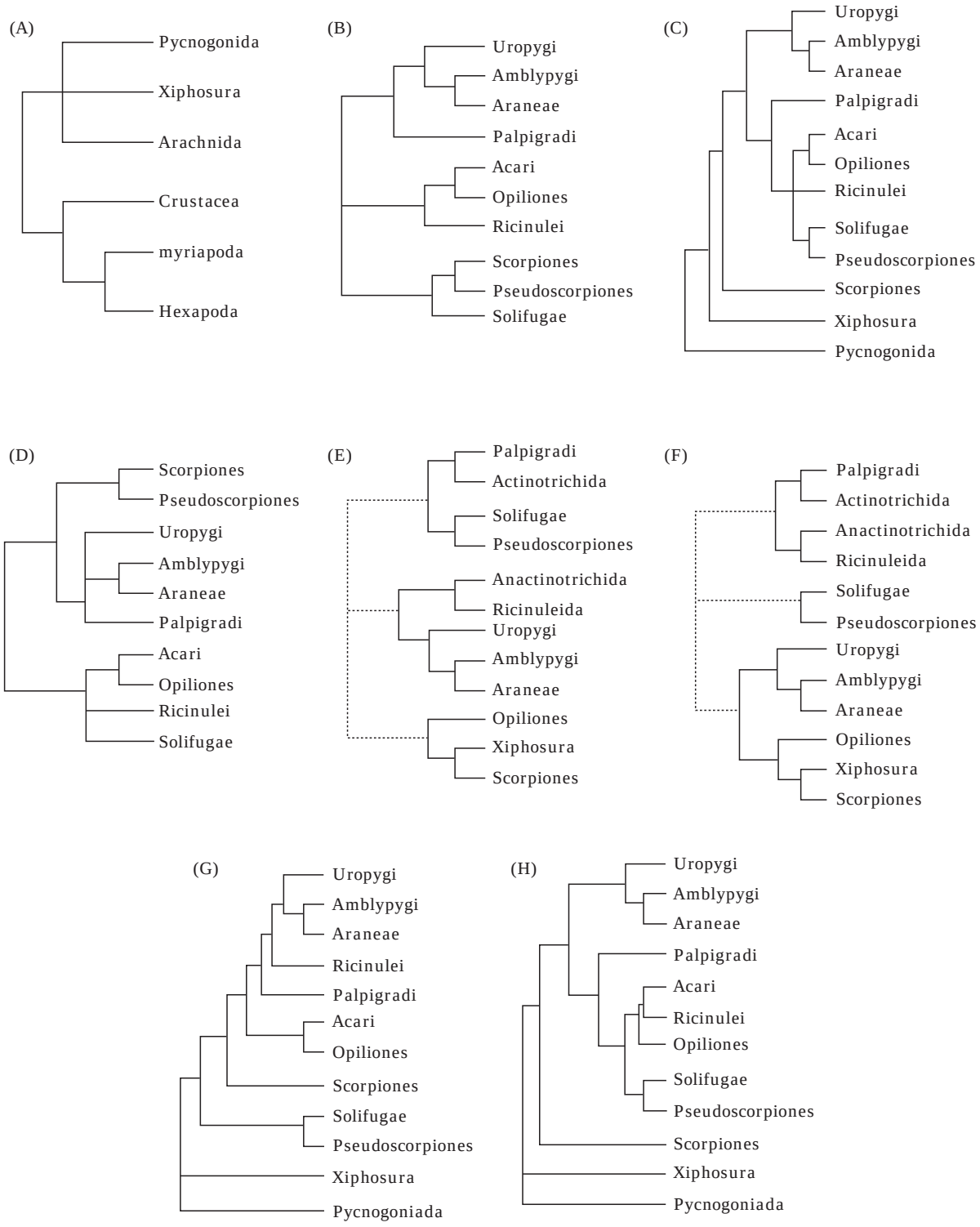

FIG. 3. (A) Arthropod phylogeny of Snodgrass (1938), and arachnid phylogenies of (B) Savory (1971), (C) Firstman (1973), (D) Yoshikura (1975), (E) van der Hammen (1985), (F) van der Hammen (1986), (G) Grasshoff (1978), and (H) Weygoldt and Paulus (1979b). The stippled lines of (E) and (F) denote the doubted nature of arachnid monophyly according van der Hammen. The taxon "myriapoda" is in lower case due to the uncertainty of its status.

Savory—proposed multiple origins of the arachnids. Over the next 10 years, he settled on a scheme of arachnid relationships, although he denied their monophyly (Fig. 3E, F). The most salient feature of this scheme is the diphyly of the Acari. Van der Hammen placed the Actinotrichida (non-parasitic mites) with the palpigrades and the remaining parasitic forms (Anactinotrichida) with the ricinuleids. Much of the support for these notions is based on "laws underlying" every aspect of chelicerate evolution. These "laws", coupled with the bald assertion of the independent transformation of lobopodia into arachnid legs, exclude much character evidence from consideration. This view has not found many adherents and has been questioned thoroughly by Lindquist (1984) who provided several acaran synapomorphies.

In explicitly rejecting Hennig's systematic notions, Grasshoff (1978) denied the ability to reconstruct phylogeny for the chelicerates. His character analysis, however, returned to the arachnid monophyly rejected by van der Hammen (Fig. 3G). After accepting the monophyly of the Labellata (derived from similar sucking specializations of the pharynx and the narrowed prosoma-opisthosoma juncture) as joined with the Uropygi, he added (Pseudoscorpiones+Ricinulei) as their sister group. He also moved the solifugids outside scorpions to the most basal lineage of arachnids, echoing Thorell (1877). Grasshoff also supported a notion of Chelicerata which included pycnogonids as the basalmost lineage. Although rejecting Hennigian character analysis, Grasshoff presented one.

The explicit Hennigian (1966) paradigm and character argumentation rationale came to chelicerate systematics through the efforts of Weygoldt and Paulus (1979a, 1979b). Weygoldt and Paulus gathered the character information generated over the previous century, added their own, then through superior analysis produced a scheme of chelicerate relationships based on synapomorphy (Fig. 3H). The Pycnogonida were placed incertae sedis at the base of Chelicerata. Xiphosurids were then the sister taxon to the Arachnida. Weygoldt and Paulus' scheme is very similar to that of Pocock (1893). The basic divisions between Scorpiones (Ctenophora) and the Lipoctena and the Caulogastra (Araneae, Amblypygi, and Uropygi) versus Apulmonata (Solifugae, Pseudoscorpiones, Acari, Ricinulei, and Palpigradi) are supported. They also support the Labellata of Petrunkevitch (1955—although for different reasons) and agreed largely with the apulmonate relations of Firstman (1973).

In his cladistic analysis of arthropods, Weygoldt (1986) directly placed the pycnogonids as the sister group of the Eucheliceratа (Xiphosura+Arachnida). Although the observations Weygoldt systematicized (two tagmata, lack of antennae, and presence of chelicerae) were discussed by Snodgrass (1938), the placement of the sea spiders had not been made securely until this study.

Through the inclusion of functional morphological data, Shultz (1990) resurrected the Pedipalpi (Amblypygi+Uropygi) and, like other studies presented an amalgam of previously elaborated groups (Fig. 4A). His basic scheme includes the Caulogastra of Pocock (1893), to which are added the palpigrades to equal the Arachnoidea of Savory (1971). Shultz also supports Savory's Scorpionomorpha, but with the pseudoscorpions allied with the solifugids. The main difference between Shultz and Savory (other than methodology) is the division of the Opilionoidea. Shultz places the Acari+Ricinulei with the Arachnoidea(=Megoperculata of Börner, 1902) and the Opiliones with the Scorpionomorpha.

There have been two molecular studies which related to chelicerate relationships. Both studies (Turbeville et al., 1991; Wheeler et al., 1993) concerned themselves mainly with arthropod relationships, hence the arachnid samples were desultory. Wheeler et al. (1993) supported Weygoldt's (1986) position of the Pycnogonida as sister to their Eucheliceratа, Xiphosura+Arachnida (Fig. 4B).

While there is some consensus on the placement of pycnogonids (at least as regards living taxa), the phylogeny of the arachnids is less well agreed upon. Although there has been considerable disagreement since Thorell (1877), there is at least one common thread in these schemes and that is the enlarged Caulogastra of Pocock (1893, Figure 2). Whether the "Pedipalpi" are monophyletic or not, their alliance with the spiders and the palpigrades is a theme which pervades most analyses. The placements which are most unstable are the basal position (or not) of the pycnogonids and scorpions and the interrelationships of the opiliones, solifugids, and pseudoscorpions. This study aims to achieve the robust placement of these taxa.

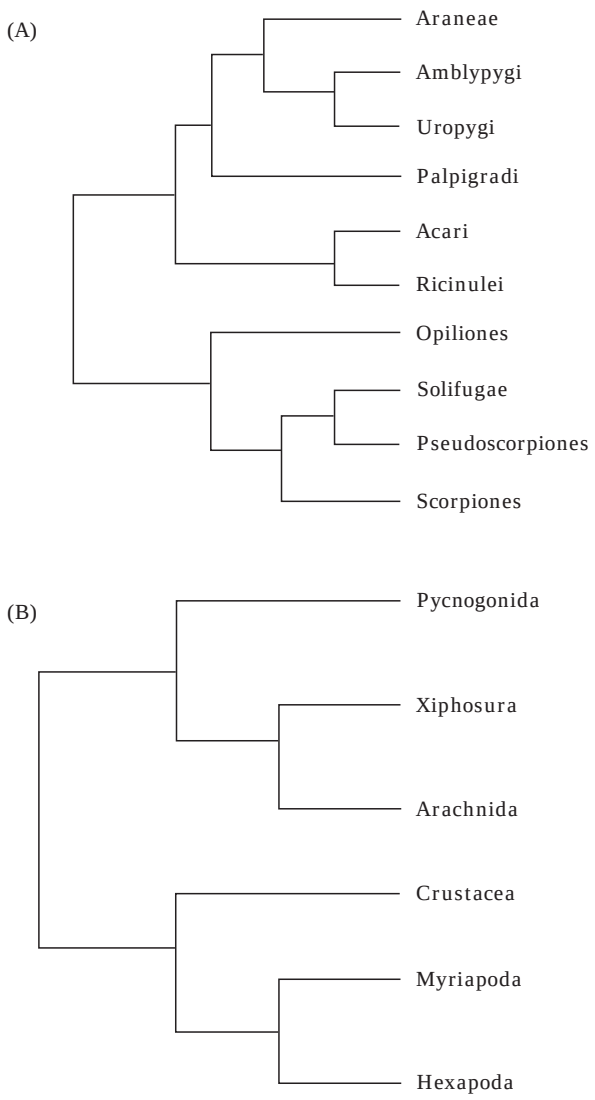

FIG. 4. (A) Arachnid phylogeny of Shultz (1990) and (B) chelicerate phylogeny of Wheeler et al. (1993).

THE DATA

Taxa

In order to form a more perfect estimate of the basal conditions and variation within groups, multiple representatives of chelicerate lineages were examined where possible. This gave a total of 25 samples to represent the chelicerate orders and nine to represent onychophoran, crustacean, myriapod, and hexapod outgroups (Table 1) . Most of these lineages have multiple representatives and only one, Palpigradi, was unavailable for molecular analysis. Each of these lineages is extant. No extinct taxa are included and no character coding based on extinct taxa is used (e.g. book-lungs in scorpions). Such reliance on current information must limit this discussion, but a complete analysis including extinct taxa would of necessity include trigonotarbids, architarbids, anthrocomartins, haptopods, kustarachnids, eurypterids, and other more basal arachnate lineages and is beyond the scope of this study.

The three sources of data used in this study are anatomy, and sections of both the small (18S rDNA) and the large subunit ribosomal DNAs (28S rDNA).

Morphology

The morphological data matrix was derived from literature sources and resulted in 93 characters, all of which were treated as unordered (non-additive—Tables 2 and 3). The primary sources for this information were Snodgrass (1938), Yoshikura (1975), Weygoldt and Paulus (1979a, 1979b), Weygoldt (1979, 1986) and Shultz (1990). These characters were scored as ground-plan or presumed basal conditions in the 13 extant chelicerate and four outgroup taxa as coded in the referenced literature. The codings were taken as presented by the cited authors with the exception that, where conflict occurred between authors, the coding of Shultz (1990) was used. The only exception to this was the book-lungs of scorpions mentioned above. Since the non-homology of book-lungs in scorpions and other arachnid taxa is based on Paleozoic taxa, it was not used here. This analysis is restricted to living taxa, hence the book-lungs are treated as at least potentially homologous. Several characters appear to be autapomorphic (e.g. those for Araneae) in the morphological matrix. When the several representatives of these lineages are analysed, however, these features are no longer unique and are informative.

Molecular

Approximately 1000 bases of the 18S rDNA and 350 bases of the 28S rDNA were determined as described by Whiting et al. (1997). The small subunit sequences of some taxa have been published previously and were included. All of the areas within the contiguous segments of DNA were used. Total genomic DNA was isolated from fresh, ETOH-preserved, and dried specimens by homogenization in an extraction buffer (10 mM Tris, 25 mM EDTA, 0.5% SDS, 100 mM NaCl, 0.1 mg / ml proteinase K).

TABLE 1
Taxa Used in the Study

Higher group	Taxon	18S rDNA	28S rDNA
Onychophora			
Peripatopsidae	*Peripatopsis caperisis*	Here	Here
Chelicerata			
Pycnogonida	*Anoplodactylus portus*	Wheeler	Here
	Anoplodactylus lentus	Here	Here
	Colossendeis sp.	Here	ND
Xiphosura	*Limulus polyphemus*	Wheeler	Here
Scorpiones	*Centruroides hentzii*	Wheeler	Here
	Androctonus australis	Chalwatzis	ND
	Hadrurus arizonensis	Here	Here
	Paruroctonus mesaensis	Here	Here
Araneae	*Hypochilus pococki*	Here	Here
	Gea heptagon	Here	Here
	Eurypelma californica	Friedrich	Friedrich
	Thelechoris striatipes	Here	Here
	Heptathela kimurai	Here	Here
	Liphistius bristowei	Here	Here
Palpigradi	Morphology only	ND	ND
Pseudoscorpiones	*Americhenernes* sp.	Here	Here
Solifugae	*Chanbria regalis*	Here	Here
Opiliones	*Vonones ornata*	Here	Here
	Leiobunum sp.	Here	Here
Acari	*Amblyomma americanum*	Turbeville	ND
	Rhiphicephalus sanguineus	Here	Here
	Tetranychus urticae	Here	Here
Ricinulei	Ricinoididae (juvenile)	Here	Here
Amblypygi	Amblypygid sp.	Here	Here
Thelyphonida	*Mastigoproctus giganteus*	Wheeler	Here
Schizomida	*Trithyreus pentapeltis*	Here	Here
Crustacea			
Reptantia	*Callinectes* sp.	Wheeler	Here
Anostraca	*Artemia salina*	Nelles	Friedrich
Thoracica	*Balanus* sp.	Wheeler	Here
Myriapoda			
Chilopoda	*Scutigera coleoptrata*	Wheeler	Here
Diplopoda	*Spirobolus* sp.	Wheeler	Here
Hexapoda			
Odonata	*Agrion maculatum*	Whiting	Whiting
Hymenoptera	*Monobia* sp.	Whiting	Whiting

Chalwatzis: Chalwatzis, N., Kinzelbach, R. and Zimmermann, F. K. (unpublished, Genbank Accession Number X74761; Friedrich: Friedrich and Tautz (1995); Nelles: Nelles et al. (1984); Sharp: Sharp and Li (1987); Turberville: Turberville et al. (1991); Wheeler: Wheeler et al. (1993); Whiting: Whiting et al. (1997); ND: no data; Here: this study, Genbank Accession Number AF062943–AF062995.

TABLE 2
Morphological Character Descriptions

1. Postoral antennae: absent (0), present (1); (Snodgrass, 1938; Weygoldt and Paulus, 1979a, b).
2. First appendage chelicerae (or cheliphores): undifferentiated (0), chelicerae (1); (Snodgrass, 1938; Weygoldt and Paulus, 1979a, b).
3. Tagmosis of body segments into prosoma and opisthosoma without distinct head: absent (0), present (1); (Weygoldt and Paulus, 1979a, b).
4. Enlarged proboscis with terminal triangular mouth: absent (0), present (1); (Snodgrass, 1938; King, 1973; Weygoldt and Paulus, 1979b). Snodgrass (1952) noted the similarity of the pycnogonid proboscis to the sucking pharynx of the Araneae and Amblypygi.
5. Inverse retina in four median eyes: not inverse (0), inverse (1); (Weygoldt and Paulus, 1979b).
6. Opisthosoma greatly reduced forming a slender tube emerging from between the posteriormost legs with a terminal anus: not reduced (0), reduced (1); (Snodgrass, 1952).
7. Number of median eyes four (0), two (1), absent (2); (Weygoldt and Paulus, 1979b).
8. Extraintestinal digestion; absent (0), present (1); (Weygoldt and Paulus, 1979b).
9. Endodermal Malpighian tubules: absent (0), present (1); (Weygoldt and Paulus, 1979b).
10. Lateral eyes: compound (0), aggregate with six facets (1), aggregate with four facets (2), vestigial (3), absent (4); (Weygoldt and Paulus, 1979b; coded as in Shultz, 1990).
11. Slit sensillae: absent (0), present (1); (Weygoldt and Paulus, 1979b).
12. Eyes with a network of rhabdomeres; absent (0), present (1); (Weygoldt and Paulus, 1979b).
13. Spermatazoa with coiled axoneme: absent (0), present (1); (Weygoldt and Paulus, 1979b).
14. First leg morphologically differentiated for use as a tactile organ: undifferentiated (0), differentiated (1); (Weygoldt and Paulus, 1979b; coded as in Shultz, 1990).
15. Subchelate chelicerae with two segments: three segments (0), subchelate (1), segmented chelate (2); (Weygoldt and Paulus, 1979b).
16. 9+3 microtubule arrangement in spermatazoan axoneme: absent (0; usually 9+2), present (1); (Weygoldt and Paulus, 1979b).
17. Fused pedipalpal coxae: free (0), fused (1); (Weygoldt and Paulus, 1979b).
18. Prenymph and four postnymphial instars: absent (0), present (1); (Weygoldt and Paulus, 1979b).
19. Female grasps male during mating: absent (0), present (1); (Weygoldt and Paulus, 1979b).
20. First opisthosomal segment: broad (0), narrow (1), petiolus (2; segment extremely narrowed); (Pocock, 1893, 1902).
21. Large post-cerebral "sucking" pharynx: absent (0), present (1); (Weygoldt and Paulus, 1979b).
22. Book lungs: absent (0), present (1); (Weygoldt and Paulus, 1979b). Scorpions are coded as present since all extant taxa have book lungs. Although it is often said that paleozoic scorpions had external book-gills, the analyses performed here are based on extant taxa. Extinct lineages could well be included, but as separate taxa.
23. Tracheae: absent (0), present (1); (Weygoldt and Paulus, 1979b). Embryonic origin and microstructure make those of Onychophora, Chilopoda, Diplopoda, and Hexapoda not homologous to those found in some arachnids. When taxa are diverse (as in Araneae) the presumed basal condition of the groups is used. This state is assigned to all exemplars of that taxon, since they are intended as sample exemplars, not terminal lineages. The coding here is as in Shultz (1990).
24. Opisthosomal flagellum: absent (0), present (1); (Weygoldt and Paulus, 1979b).
25. Aflagellate spermatazoan with specialized acrosome: absent (0), present (1); (Weygoldt and Paulus, 1979b).
26. Anterior genital opening: absent (0), present (1); (Weygoldt and Paulus, 1979b).
27. Six-legged larvae and three nymphal stages: absent (0), present (1); (Weygoldt and Paulus, 1979b).
28. Perineural membrane enveloping arterial sinus: present (0), no adult connection between arterial system and endosternite (1); (Firstman, 1973).
29. Midgut ceca of prosoma: simple (0), branched (1); (Yoshikura, 1975).
30. Position of the ganglia of the subesophageal nerve mass: present in the opisthosoma (0), restricted to the prosoma (1); (Yoshikura, 1975).
31. Lateral organ: present (0), absent (1); (Yoshikura, 1975).
32. Egg teeth on the dorsal side of the pedipalp coxae: present (0), absent (1); (Yoshikura, 1975).
33. Embryonic number of opisthosomal segments: ≤11 (0), 12 (1), 13 (2); (Yoshikura, 1975).
34. Egg structure: isolethical or telolecithal (0), centrolecithal (1); (Yoshikura, 1975). Since scorpions exhibit both iso- and telolecithal eggs, the states are combined.
35. Two pairs of ostia: absent (0), present (1); (Weygoldt, 1986).
36. Pharynx with x-shaped lumen: absent (0), present (1); (Clark, 1979).
37. Gonads: reticulum of fine tubules as in *Limulus* (0), ladder type (1), saccular type (2); (Clark, 1979).
38. Pectines: absent (0), present (1); (summarized by Shultz, 1990).
39. Copulatory organ on the male pedipalp: absent (0), present (1); (summarized by Shultz, 1990).
40. Cheliceral venom glands: absent (0), present (1); (summarized by Shultz, 1990).
41. Opisthosomal silk glands: absent (0), present (1); (summarized by Shultz, 1990).
42. Absence of the trochanterofemoral depressor muscle in walking legs: absent (0), present (1); (summarized by Shultz, 1990).
43. Elongation of leg 2 to form tactile organs: absent (0), present (1); (summarized by Shultz, 1990).
44. Trochanterofemora joint with vertical bicondylar articulation: absent (0), present (1); (summarized by Shultz, 1990).
45. Paired tracheal stigmata on genital segment: absent (0), present (1); (summarized by Shultz, 1990).
46. Prosomal defense glands: absent (0), present (1); (summarized by Shultz, 1990).
47. Hexapodal prelarva: absent (0), present (1); (summarized by Shultz, 1990).
48. Carapace: undivided (0), transverse segmental furrows (1), divided (2); (Shultz, 1990).
49. Carapacal pleural margin: well developed (0), poorly developed (1); (Shultz, 1990).

Continued.

Continues.

50. Intercoxal sternal region: broad throughout (0), narrow posteriorly (1), narrow throughout (2); (Shultz, 1990).
51. Prosomal sternite: uniform (0), with distinct sclerites (1); (Shultz, 1990).
52. Prosomal endosternite segmental components: five (0), four (1), three (2), two (3), one (4), absent (5); (Shultz, 1990).
53. Dorsal endosternal suspensor of fourth postoral segment with anterolateral carapacal insertion: absent (0), present (1); (Shultz, 1990).
54. Fenestrate endosternite: absent (0), present (1); (Shultz, 1990).
55. Direction of mouth: posterior (0), antroventral (1); (Shultz, 1990).
56. Tritosternum: absent (0), present (1); (Shultz, 1990).
57. Chelicerocarapacal articulation: absent (0), present (1); (Shultz, 1990).
58. Stomotheca: absent (0), present (1); (Shultz, 1990).
59. Rostrum: absent (0), present (1); (Shultz, 1990).
60. Scorpionid pedipalpal chelae: absent (0), present (1); (Shultz, 1990).
61. Raptorial pedipalps: absent (0), present (1); (Shultz, 1990).
62. Pedipalpal coxae: free (0), fused medially (1); (Shultz, 1990).
63. Movable subcapitulum: absent (0), present (1); (Shultz, 1990).
64. Movable coxae: absent (0), present (1); (Shultz, 1990).
65. Musculi laterales: absent (0), present (1); (Shultz, 1990).
66. Coxal endites: absent (0), present (1); (Shultz, 1990).
67. Coxotrochanteral joint: simple (0), complex(1); (Shultz, 1990).
68. Femur of third and fourth legs: divided (0), undivided (1); (Shultz, 1990).
69. Femorpatellar joint: hinge (0), bicondylar (1), monocondylar (2); (Shultz, 1990).
70. Femorpatellar flexor muscle insertion: symmetrical (0), asymmetrical (1); (Shultz, 1990).
71. Posterior transpatellar muscle origin: dorsoposterior surface of femur and / or posterior surface of patella (0), distal process of femur (1), absent (2); (Shultz, 1990).
72. Patellotibial extensor muscle: absent (0), present (1); (Shultz, 1990).
73. Anterior transpatellar muscle insertion on tibia: anterior (0), ventral (1), absent (2); (Shultz, 1990).
74. Patellotibial joint: monocondylar (0), hinge (1), bicondylar (2); (Shultz, 1990).
75. Anterior patellotibilar muscle insertion on tibia: anterior (0), ventral (1), absent (2); (Shultz, 1990).
76. Posterior patellotibial muscle: absent (0), present (1); (Shultz, 1990).
77. Telotarsus with three tarsomeres: absent (0), present (1); (Shultz, 1990).
78. Claw depressor muscle tibial head: absent (0), present (1); (Shultz, 1990).
79. Claw depressor muscle patellar head: absent (0), present (1); (Shultz, 1990).
80. Claw depressor muscle origin on posterior wall of patella: absent (0), present (1); (Shultz, 1990).
81. Empodium in adult: absent (0), present (1); (Shultz, 1990).
82. Appendages on first opisthosomal segment: absent (0), present (1); (Shultz, 1990).
83. Genital sternite overlapping third opisthosomal sternite: absent (0), present (1); (Shultz, 1990).
84. Postgenital appendages: opercular / lemellar (0), poorly sclerotized / eversible (1), absent (2);(Shultz, 1990).
85. Pygidium: absent (0), present (1); (Shultz, 1990).
86. Pygidial defence glands: absent (0), present (1); (Shultz, 1990).
87. Tibial trichobothria with 2–2–1 distribution: absent (0), present (1);(Shultz, 1990).
88. Sternal stigmata on third and fourth opisthosomal segments: absent (0), present (1); (Shultz, 1990).
89. Spermatozoan nucleus with microtubule array: absent (0), present (1); (Shultz, 1990).
90. Gonoporal brood sac: absent (0), present (1); (Shultz, 1990).
91. Ovipositor: absent (0), present (1); (Shultz, 1990).
92. Leg three coxal gland orifice: present (0), absent (1); (Shultz, 1990).
93. Leg one coxal gland orifice: absent (0), present (1); (Shultz, 1990).

All characters unordered.

After 12+ hours of incubation with agitation at 55°C, the DNAs were cleaned with a standard series of phenol / chloroform extraction followed by ethanol precipitation and resuspension in water. If tissues were rare, the precipitation was replaced by supernatant in separation columns (Centricon 100) to increase the total DNA yield and quality. Double-stranded template suitable for sequencing was prepared for 18S and 28S rDNA via the polymerase chain reaction (PCR) amplification with conserved primers (Whiting et al., 1997). For most 18S sequences, the entire region was amplified and sequenced with internal primers. 18S rDNA sequencing was carried out by using ^{35}S-ATP; the primers used for PCR amplification and internal primers; the modified T7 DNA polymerase Sequenase^{TM} (version 2.0, U.S. Biochemical Corp.); and the accompanying reagents following standard protocols; and with the PRISM cycle sequencing kit (ABI) and run on the ABI 373A automated sequencer. In all cases, complementary strands of all fragments were independently amplified and sequenced to ensure accurate results. If complementary strands disagreed, the product was reamplified and sequenced to resolve any discrepancies.

The combination of lineage sampling and data collection resulted in approximately 1500 observations for each of 34 terminal taxa. However, since some of the taxa were unknown for 28S or unknown from molecular data entirely (palpigrades) there were some missing data. The effect of missing data can be insidious (Nixon and Davis, 1991; Platnick, 1991) but the levels here were rather low (<5%).

TABLE 3

17 taxa and characters

Onychophora	10000-210-0000-0-000000-000-?????????0000000000----------
Crustacea	10000-00000000-00000000-000-?????????000000000000-1---00-0000000?000--------0???0-----0-??000
Hexapoda	10000-00000000-00000000-000-?????????000000000000-1---00-0000000?000--------0???0-----0-??000
Myriapoda	10000-00000000-00000000-000-?????????000000000000-1---00-0000000?000--------0???0-----0-??000
Pycnogonida	011111000-0000000000000-0000?1???????0000000001120-00000?000000?1000????1??10000-----0-00000
Xiphosura	01100010000?000000000000000???1??0??000000000000000000000000000000010000000000000000-0000000
Scorpiones	01100011101000000000010000011100200011000000000112020010010100010001101112111110010000-0000000
Araneae	01100011111111011000211000001100l?10020111100000010011011000000001111100000000111001110-0010001
Amblypygi	01100011111111111000211000001101111101?000000000001011101100001000011101000000210001111100011001
Thelyphonida	01100011111111111111101010001111011011000000000001111111100001100111101000000211101121110110l1
Schizomida	01100021131111111111101010001011011001000000000021111011100001100011101202001211101121110110l1
Palpigradi	01100021040-010-000100010000001??0?10?000000000021010101100000000010100000000110001111000?011
Ricinulei	011000211-1-10201001001011100???00?000000000001003001100000111010000202110110001021000100l1
Acari	011000111111000-1000001011100000011120000000001?100?00110000011001000000100110001010-0000111
Opiliones	01100010041110000000001011001000011O?000001111011202001001000000000110100200111001020-0000100
Psuedoscorpiones	01100021021110200000001000000000100120000000001120200101011000101011011122111l011020-0101000
Solifugae	0110001112110l2-000100100000111001002000000000021204--10101000010100202011111111011020-0100010

All characters unordered/non-additive.

PHYLOGENETIC ANALYSIS AND RESULTS

The character data were analysed using parsimony to elucidate efficiently Hennigian synapomorphy schemes (Hennig, 1966), that is, the simplest or most parsimonious result was taken to be the best summary representation of variation in the studied taxa. This was accomplished in two ways. The morphological data on their own were examined using Goloboff's parsimony based NONA (1995). TBR branch swapping was performed to generate nine equally parsimonious trees of length 201 (CI=0.56, RI=0.66; Fig. 5).

The molecular data were analysed using OY (Gladstein and Wheeler, 1996) to construct phylogenetic hypotheses directly. This is performed by optimizing the nucleic acid sequences without the intervening step of multiple sequence alignment (Wheeler, 1996). This methodology assigns cladogram lengths directly to competing hypotheses. In essence, entire sequences (or fragments) are treated as characters with many character states. In this way, a generalized character optimization can be performed to determine parsimony tree lengths. When "total evidence" analysis was performed the morphological characters received various weights corresponding to various notions of "equal" weighting (see below). For all analyses, as with morphological data, TBR branch swapping was performed.

The data were combined directly, i.e. all characters were weighted equally without regard to source. The character transformations, however, were weighted differentially in a number of different schemes to see how they affected phylogenetic conclusions. The morphological transformations were weighted as equal to indels, base changes, and assigned a constant fraction of tree length based on the number of

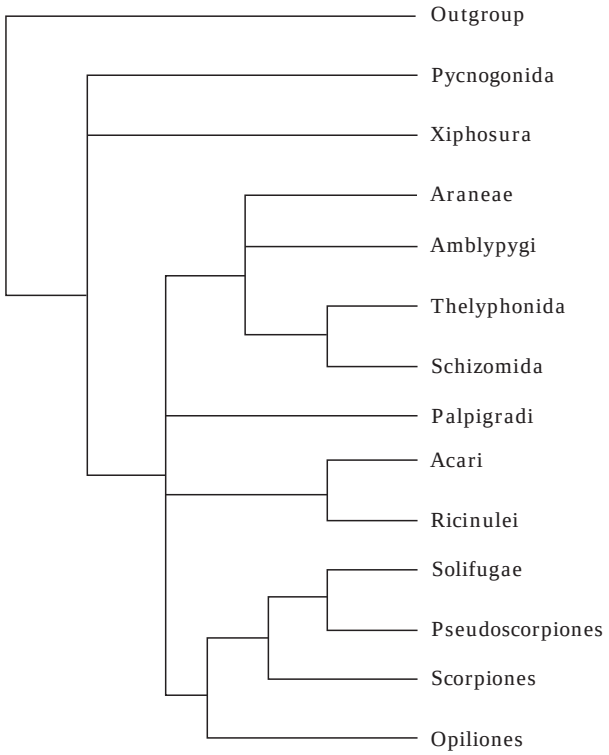

FIG. 5. Consensus cladogram of chelicerate relationships derived from the morphological data collected here. The strict consensus of nine equally parsimonious cladograms of length 201 (CI=56, RI=66) were derived from the 93 characters of Tables 2 and 3.

characters to examine the sensitivity of results to the relative weights assigned to different character sources. Since phylogenetic results can depend critically on the assumptions made to perform the analysis (Wheeler and Gladstein, 1992, 1994; Wheeler, 1995), multiple analyses were performed to examine the effect of variation in three parameters on phylogenetic outcome. These parameters, insertion–deletion cost (indel), transversion–transition ratio, and relative weight of morphology, were varied and analysed simultaneously and separately. The insertion–deletion cost was applied as the relative cost of the insertion or deletion of a base versus a base change. In other words, if an indel ratio of 2:1 was specified, two base changes would be taken as having an equal cost to a single insertion event. When the overall cost of a phylogenetic topology is determined, the weighted sum of the events is minimized. The analyses performed here varied the relative indel cost from equal to base substitutions to twice, four, eight, and sixteen times as costly. Analogously, the transversion–transition weights are specified and employed the same way, except that instead of a final 16:1 ratio a transversion-only scheme (transition cost=0) was used. With a transition–transversion of one, all base substitutions are treated equally whereas a ratio of 4:1 would count four transitions as equal to a single transversion. These values were chosen not to represent some notion of absolute values, but to span the range of possible values (Wheeler, 1995).

When the morphological characters were analysed by OY, the results were identical (albeit at a slower pace) to those derived from NONA and Hennig86 (Farris, 1988). These characters were still treated as unordered characters in the standard fashion. Their combination with the molecular data in a simultaneous analysis, however, allows them to participate in the determination of the homology schemes for the molecular data in a way that other methods will not. In essence, the morphological information helps to determine dynamically the best putative homology and synapomorphy scheme for each topology. The molecular homologies are not fixed a priori as they would be with a multiple alignment procedure.

The notion of "equal" weighting is both central and ill-defined in the rationale of total evidence. When all character transformation events are treated homogeneously—transitions equal to transversions equal to indels—the choice seems clear that morphological changes should also be treated in the same way. Identical weighting is but one of the possible weighting schemes explored here. How is "equal weighting" defined when things are not so obvious? Three immediate possibilities present themselves. First the morphological characters could be weighted identically to the indel cost, second to the base change cost, and third to some value determined by the relative number of evolutionary events presented by the data. Each of these options can be defended, but there seems to be no a priori way to distinguish among the possibilities. Hence, each was explored. The morphological characters were assigned the same weight as indels (L), as base changes (C), and weighted such that the morphological characters contributed approximated 10% of the total tree length (M).

The five values which were used for both the insertion–deletion cost and transversion–transition ratio and the three for the morphological character weight resulted in 75 sets of assumptions and 75 results (Table 4). In each case, the character incongruity was calculated (ILD of Mickevich and Farris, 1981) for the combination of molecular, morphological, and total analyses (Table 4). A rescaled ILD (RILD for want of a better acronym) was also calculated for each analysis. This value is calculated along the lines of the retention index by normalizing homoplasy levels with respect to maximum and minimum possible levels of incongruity. Where the ILD is calculated by dividing the difference between the overall tree length and the sum of its data components:

$$\mathrm{ILD}=(\mathrm{Length}_{\mathrm{Combined}}-\mathrm{Sum\ length}_{\mathrm{Individual\ sets}})/\mathrm{Length}_{\mathrm{Combined}},$$

the rescaled value uses the same numerator but the denominator is the difference between the maximum tree length from the combined data (bush) and the minimum (sum of the individual lengths):

$$\mathrm{RILD}=(\mathrm{Length}_{\mathrm{Combined}}-\mathrm{Sum\ length}_{\mathrm{Individual\ data}})/(\mathrm{Max\ length}_{\mathrm{Combined}}-\mathrm{Sum\ length}_{\mathrm{Individual\ sets}}).$$

The benefit of this rescaled index is that it does exhibit the trivial minima (0) as data set weights become increasingly disproportionate.

Both the total (morphological+18S+28S) and molecular (18S+28S) analyses achieved minimum incongruence with indel cost of twice that of base transversions and transitions equal to one-half

TABLE 4
Morphological Character Descriptions

IndelC	Tv/Ti	MoW	Total	Mol	Morph	18	28	MolW	18W	28W	ILDTot	ILDMM	ILDMol	TotR	MMR	MolR
1	1	LFC	2322	2077	201	1082	966	684	1570	1302	0.0314	0.0140	0.0189	0.0559	0.0352	0.0344
	2	LFC	3626	3175	402	1608	1518	1368	2364	2056	0.0270	0.0154	0.0135	0.0434	0.0379	0.0222
	4	LC	6145	5269	804	2616	2533	2736	3916	3433	0.0312	0.0228	0.0117	0.0465	0.0545	0.0179
		F	5951	5269	603	2616	2533	2736	3916	3433	0.0334	0.0228	0.0133	0.0459	0.0545	0.0188
	8	LC	11223	9413	1608	4622	4543	5472	7002	6292	0.0401	0.0263	0.0180	0.0563	0.0601	0.0261
		F	10579	9413	1005	4622	4543	3420	7002	6292	0.0387	0.0263	0.0152	0.0625	0.0601	0.0256
	∞	LC	10048	8336	1608	3984	4040	5472	6152	5616	0.0414	0.0374	0.0104	0.0547	0.0833	0.0143
		F	9292	8336	804	3984	4040	2736	6152	5616	0.0499	0.0374	0.0164	0.0817	0.0833	0.0283
2	1	L	2860	2432	402	1199	1175	1368	1772	1572	0.0294	0.0238	0.0091	0.0434	0.0598	0.0138
		F	2659	2432	201	1199	1175	684	1772	1572	0.0316	0.0238	0.0098	0.0578	0.0598	0.0186
		C	2659	2432	201	1199	1175	684	1772	1572	0.0316	0.0238	0.0098	0.0578	0.0598	0.0186
	2	L	4691	3830	804	1838	1906	2736	2728	2558	0.0305	0.0225	0.0122	0.0412	0.0558	0.0168
		F	4292	3830	402	1838	1906	1368	2728	2558	0.0340	0.0225	0.0140	0.0582	0.0558	0.0248
		C	4292	3830	402	1838	1906	1368	2728	2558	0.0340	0.0225	0.0140	0.0582	0.0558	0.0248
	4	L	8304	6598	1608	3079	3272	5472	4604	4479	0.0415	0.0374	0.0118	0.0523	0.0904	0.0154
		F	7282	6598	603	3079	3272	2052	4604	4479	0.0450	0.0374	0.0111	0.0785	0.0904	0.0206
		C	7487	6598	804	3079	3272	2736	4604	4479	0.0443	0.0374	0.0114	0.0712	0.0904	0.0192
	8	L	15450	12046	3216	5559	6007	10944	8332	8368	0.0432	0.0398	0.0122	0.0519	0.0935	0.0152
		F	13440	12046	1206	5559	6007	4104	8332	8368	0.0497	0.0398	0.0140	0.0832	0.0935	0.0249
		C	13851	12046	1608	5559	6007	5472	8332	8368	0.0489	0.0398	0.0142	0.0752	0.0935	0.0231
	∞	L	14328	10904	3216	5000	5488	10944	7632	7760	0.0436	0.0382	0.0145	0.0494	0.0848	0.0170
		F	12132	10904	1005	5000	5488	3420	7632	7760	0.0527	0.0382	0.0184	0.0873	0.0848	0.0323
		C	12744	10904	1608	5000	5488	5472	7632	7760	0.0508	0.0382	0.0182	0.0739	0.0848	0.0278
4	1	L	3862	2999	804	1400	1503	2736	2090	2063	0.0401	0.0320	0.0153	0.0487	0.0768	0.0191
		F	3258	2999	201	1400	1503	684	2090	2063	0.0473	0.0320	0.0178	0.0889	0.0768	0.0354
		C	3258	2999	201	1400	1503	684	2090	2063	0.0473	0.0320	0.0178	0.0889	0.0768	0.0354
	2	L	6642	4933	1608	2236	2513	5472	3351	3523	0.0429	0.0373	0.0152	0.0476	0.0866	0.0174
		F	5435	4933	402	2236	2513	1368	3351	3523	0.0523	0.0373	0.0184	0.919	0.0866	0.0344
		C	5435	4933	402	2236	2513	1368	3351	3523	0.0523	0.0373	0.0184	0.919	0.0866	0.0344
	4	L	12176	8733	3216	3880	4482	10944	5785	6504	0.0491	0.0425	0.0186	0.0513	0.0945	0.0201
		F	9712	8733	804	3880	4482	2736	5785	6504	0.0562	0.0425	0.0180	0.0932	0.0945	0.0319
		C	9712	8733	804	3880	4482	2736	5785	6504	0.0562	0.0425	0.0180	0.0932	0.0945	0.0319
	8	L	23177	16199	6432	7133	8442	21888	10915	12201	0.0505	0.0385	0.0236	0.0509	0.0827	0.0244
		F	18268	16199	1608	7133	8442	5472	10915	12201	0.0594	0.0385	0.0252	0.0951	0.0827	0.0428
		C	18268	16199	1608	7133	8442	5472	10915	12201	0.0594	0.0385	0.0252	0.0951	0.0827	0.0428
	∞	L	22024	15008	6432	6496	7840	21888	10032	11568	0.0570	0.0448	0.0265	0.0553	0.0925	0.0265
		F	16854	15008	1407	6496	7840	4788	10032	11568	0.0659	0.0448	0.0260	0.1044	0.0925	0.0440
		C	17072	15008	1608	6496	7840	5472	10032	11568	0.0661	0.0448	0.0267	0.1014	0.0925	0.0436
8	1	L	5750	4013	1608	1742	2080	5472	2667	3003	0.0557	0.0476	0.0224	0.0560	0.1034	0.0234
		F	4554	4013	402	1742	2080	1368	2667	3003	0.0725	0.0476	0.0305	0.1173	0.1034	0.0530
		C	4304	4013	201	1742	2080	684	2667	3003	0.0651	0.0476	0.0207	0.1201	0.1034	0.0416
	2	L	10419	6904	3216	2905	3635	10944	4547	5397	0.0636	0.0527	0.0287	0.0596	0.1069	0.0278
		F	7748	6904	603	2905	3635	2052	4547	5397	0.0781	0.0527	0.0311	0.1247	0.1069	0.0537
		C	7493	6904	402	2905	3635	1368	4547	5397	0.0735	0.0527	0.0250	0.1261	0.1069	0.0467
	4	L	19733	12514	6432	5160	6680	21888	8471	10063	0.0740	0.0539	0.0399	0.0660	0.1007	0.0366
		F	14341	12514	1206	5160	6680	4104	8471	10063	0.0903	0.0539	0.0433	0.1350	0.1007	0.0696
		C	13836	12514	804	5160	6680	2736	8471	10063	0.0862	0.0539	0.0374	0.1382	0.1007	0.0651
	8	I	38317	23817	12864	9670	12778	43776	15840	20104	0.0784	0.0575	0.0427	0.0677	0.1014	0.0380
		F	27260	23817	2211	9670	12778	7524	15840	20104	0.0954	0.0575	0.0452	0.1383	0.1014	0.0706
		C	26429	23817	1608	9670	12778	5472	15840	20104	0.0898	0.0575	0.0380	0.1367	0.1014	0.0628
	∞	L	37096	22784	12864	9008	12176	43776	15144	19632	0.0822	0.0702	0.0390	0.0685	0.1177	0.0337

Continued

TABLE 4
Morphological Character Descriptions (continued)

IndelC	Tv/Ti	MoW	Total	Mol	Morph	18	28	MolW	18W	28W	ILDTot	ILDMM	ILDMol	TotR	MMR	MolR
		F	25668	22784	2010	9008	12176	6840	15144	19632	0.0964	0.0702	0.0341	0.1343	0.1177	0.0520
		C	24992	22784	1608	9008	12176	5472	15144	19632	0.0880	0.0702	0.0240	0.1260	0.1177	0.0378
16	1	L	9513	5891	3216	2355	3165	10944	3900	4958	0.0817	0.0630	0.0427	0.0702	0.1111	0.0380
		F	6781	5891	603	2355	3165	2052	3900	4958	0.0970	0.0630	0.0423	0.1375	0.1111	0.0650
		C	6263	5891	201	2355	3165	684	3900	4058	0.0865	0.0630	0.0273	0.1418	0.1111	0.0496
	2	L	17962	10598	6432	4124	5765	21888	7002	9557	0.0914	0.0669	0.0519	0.0742	0.1063	0.0435
		F	12151	10598	1005	4124	5765	3420	7002	9557	0.1034	0.0669	0.0451	0.1384	0.1063	0.0654
		C	11375	10598	402	4124	5765	1368	7002	9557	0.0953	0.0669	0.0330	0.1420	0.1063	0.0541
	4	L	34627	19983	12864	7581	10930	43776	13546	17991	0.0939	0.0737	0.0514	0.0740	0.1130	0.0419
		F	22834	19983	1809	7581	10930	6156	13546	17991	0.1101	0.0737	0.0456	0.1447	0.1130	0.0655
		C	21584	19983	804	7581	10930	2736	13546	17991	0.1051	0.0737	0.0369	0.1517	0.1130	0.0591
	8	L	68055	38641	25728	14556	21280	87552	25842	35889	0.0954	0.0726	0.0542	0.0740	0.1083	0.0434
		F	44437	38641	3618	14556	21280	12312	25842	35889	0.1121	0.0726	0.0490	0.1441	0.1083	0.0685
		C	41929	38641	1608	14556	21280	5472	25842	35889	0.1070	0.0726	0.0401	0.1507	0.1083	0.0623
	∞	L	66784	37376	25728	13896	20688	87552	24248	34816	0.0969	0.0747	0.0551	0.0750	0.1141	0.0441
		F	42656	37376	3216	13896	20688	10944	24248	34816	0.1138	0.0747	0.0484	0.1508	0.1141	0.0702
		C	40464	37376	1608	13896	20688	5472	24248	34816	0.1056	0.0747	0.0366	0.1507	0.1141	0.0579

IndelC=insertion–deletion cost ratio; Tv/Ti=transversion–transition cost ratio; MoW=morphological weight L—morph identical to Indel, C—morph identical to base change (or transversion), F—morph contributes approximately 10% of tree length; Total=weighted tree length (morph+18S+28S); 18=weighted tree length 18S; 28=weighted tree length 28S; MorW=worst morph tree; MolW=worst molecular tree; 18W=worst 18S tree; 28W=worst 28S tree; ILDTot=Mickevich and Farris (1981) incongruence metric for all (morph+18S+28S); ILDMM=incongruence metric for morph vs. molecular; ILDMol=incongruence metric for 18S vs. 28S; TotR=rescaled incongruence metric for all (morph+18S+28S); MMR=rescaled incongruence metric for morph vs. molecular; MolR=rescaled incongruence metric for 18S vs. 28S. "L" signifies that morphological characters were weighted as equal to indels; "M" that morphological characters were weighted such that they contributed approximately 10% of overall length; and 'F" that morphological characters were weighted as equal to base changes (transversions).

transversions (Figs 6–8). This cladogram is one of the nine equally parsimonious results of the morphological analysis alone. The morphological characters in this total evidence analysis were weighted equal to indels. When comparing morphological to molecular data (18S and 28S together), the minimum incongruence also occurred with an indel cost twice that of base changes, but with transversions weighted equal to transitions (Fig. 7).

Overall, the total analyses (morphological+18S+28S), where morphological character changes were weighted as equal to indels, exhibited the lowest levels of character incongruence. These comparisons were made to other weighting schemes with morphological changes weighted as equal to base changes or contributing a constant fraction (1/10 of molecular length contribution) of the overall tree length. These analyses were fairly stable (Fig. 8). Most of the variation in the cladograms is due to differences between those analyses with indels treated as equally costly to base changes and those where indels are more expensive.

For comparative purposes, the data were subjected to multiple alignment using MALIGN (Wheeler and Gladstein, 1992) using the parameters which gave the most congruent results in the direct analysis (indels=4, transversions=2, and transitions=1). When these aligned data were analysed by themselves or in combination with morphological data (weighted equally to indels—4), they resulted in grossly less parsimonious cladograms (5471 steps for multiple alignment versus 4691 for direct optimization) and demonstrated less congruence between morphological and molecular characters (5.3% for multiple alignment versus 1.2% for direct optimization) as measured by ILD (Mickevich and Farris, 1981; alignment available at ftp.amnh.org).

GROUP SUPPORT

Close on the heels of robustness is the notion of support. Support measures attempt to summarize the

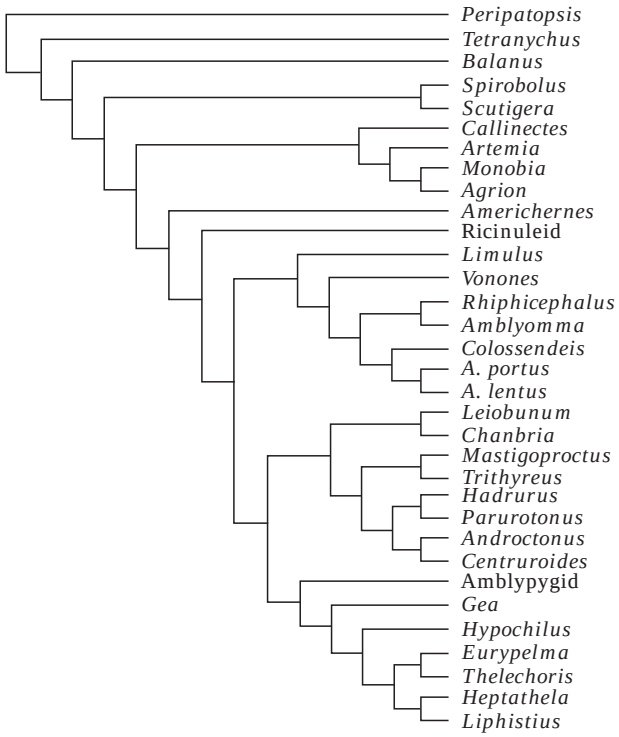

FIG. 6. Best supported (maximum character congruence) chelicerate cladogram based on molecular evidence—18S rDNA and 28S rDNA. (A) 18S+28S; (B) 18S; and (C) 28S. These cladograms are based on an insertion–deletion cost equal to that of base substitutions with transversions and transitions treated equally

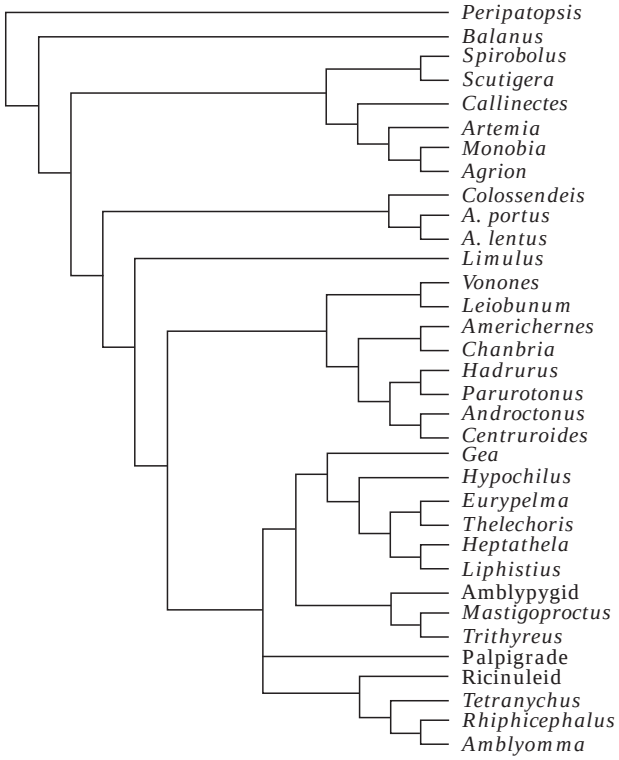

FIG. 7. Chelicerate cladogram which minimized character incongruence between morphological and molecular evidence. This cladogram is based on an insertion–deletion cost of twice that of base substitutions with transversions equal to transitions. In this scheme, the morphological transformations were weighted as equal to insertion–deletion events.

levels of character support for clades. Within the cacophony of metrics, Bremer support (or decay index—Bremer, 1994) and the number optimization independent apomorphies are the most closely linked to character distribution and are most intelligible in this context. On the "best" cladogram favored here (Figs 9 and 10) based on character incongruence, these two support values show great variation support levels (Fig. 9 and Table 5).

Those groups which are least well supported are also those least stable to variation in analysis parameters (i.e. Labellata–Amblypygi+Araneae: Bremer=8). The morphological data are ambiguous with respect to this hypothesis as they are at the chelicerate levels as well. The Labellata derive their support almost entirely from the molecular data with no unambiguously optimized morphological synapomorphies, but several from both the 18S and 28S rDNA. The placement of the scorpions with the pseudoscorpions and solifugids (Bremer=5) is almost completely derived from morphological data.

Those groups which are well supported in some cases derived their strength from different sources of information. The distinctions between pycnogonids, xiphorsurans, and arachnids are unresolved by these morphological data with no unambiguous apomorphies to link *Limulus* with Arachnida (Bremer=21). The combined information however, strongly supports (Bremer=21) this union at levels comparable with the hexapod taxa (Bremer=25) and solifugids+pseudoscorpions (Bremer=21).

CONCLUSIONS

The basal divisions of the Chelicerata, namely (Pycnogonida+(Xiphosura+Arachnida)), are strongly and robustly supported. Although the morphological data are agnostic by themselves (Fig. 5), taken with the molecular data the sum is strong support for both

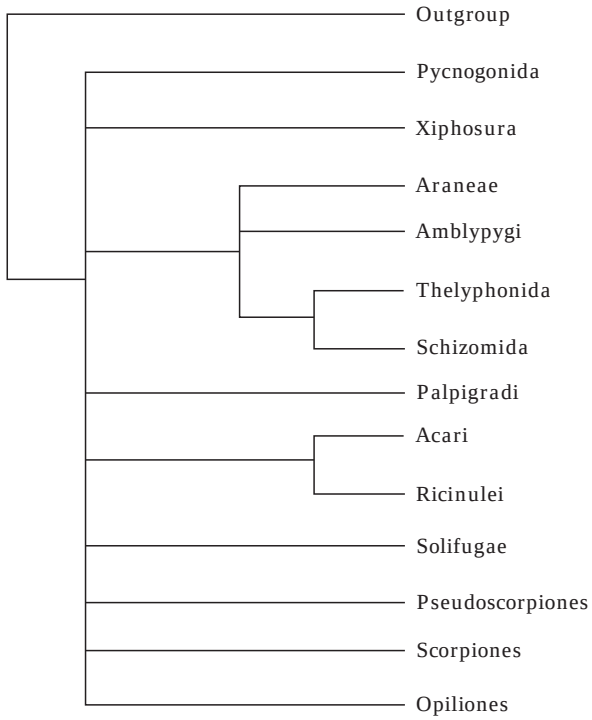

FIG. 8. Strict consensus cladogram of chelicerate relationships for all the results of all the 25 parameter sets (Table 4) where morphological characters were weighted as insertion–deletion events.

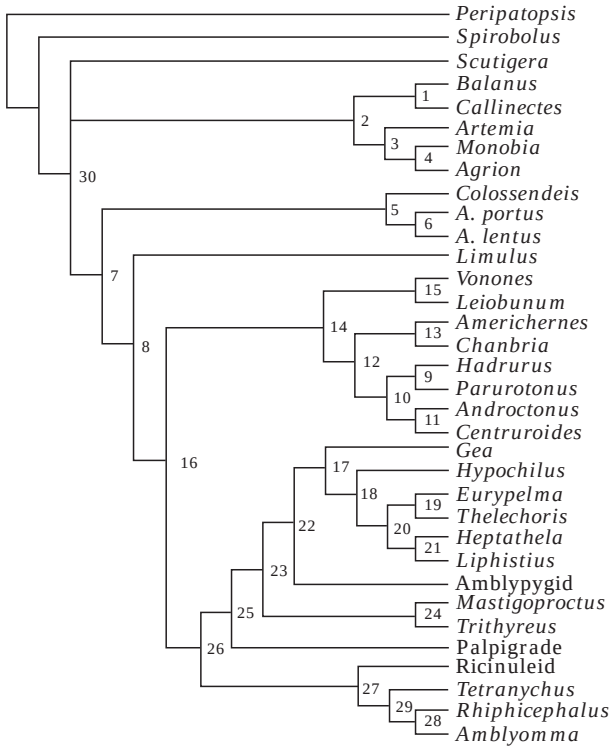

FIG. 9. Best-supported (maximum character congruence) chelicerate cladogram based on total evidence—morphology, 18S rDNA, and 28S rDNA. This cladogram is based on an insertion–deletion cost of twice that of base substitutions with transversions twice as costly as transitions. In this scheme, the morphological transformations were weighted as equal to insertion–deletion events. The numbers to the right of nodes corresponds to the HTU designations of Table 5. Branch lengths and support values can also be found in Table 5.

pycnogonids as chelicerates, and a sister-group relationship between Xiphosura (*Limulus*) and arachnids (Figs. 10, 11). This is coincident with most previous notions of chelicerate relationships, including the morphological analyses of Börner (1912) and Snodgrass (1938) and the previous molecular work of Wheeler et al. (1993).

Most of the disagreement among studies has concerned the interrelationships among arachnid groups. One of the more salient results of this analysis is the placement of the scorpions, not at the base of the arachnids but nested within a group containing solifugids, pseudoscorpions, scorpions and opiliones (Opiliones + (Scorpiones+(Solfugi+Pseudoscorpiones))). This placement agrees with Shultz (1990) and is at variance with Weygoldt and Paulus (1979b). This view, however, harkens back to the views of Petrunkevitch's (1955) Latigastra (minus the Acari). There is no support here for the Aracari+Opiliones or for a division of the Acari into the diphyly advocated by van der Hammen (Palpigrada+Actinotrichida=[*Tetranychus* here] versus Ricinulei+Anactinotrichida=[*Amblyomma* and *Rhiphicephalus* here]; 1977). However, the Apatellata (Solifugids+Pseudoscorpiones) that van der Hammen (1985) proposed did find support here. Shultz's attempt to resurrect the "Pedipalpi" is not supported by this analysis. Although this grouping was present in a majority of the combined analyses (65%), it was not present in those analyses with the greatest congruence among data.

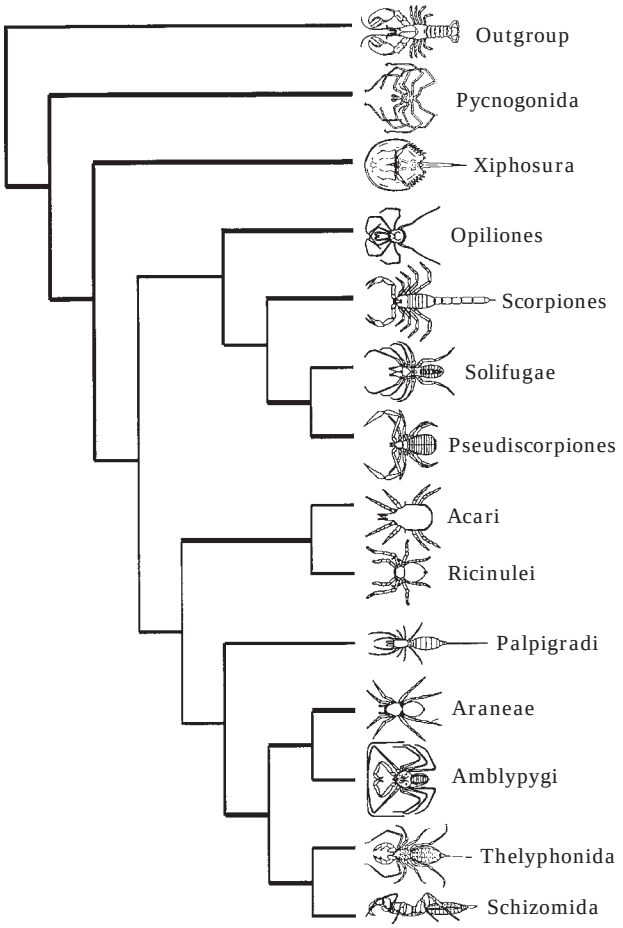

FIG. 10. Summary cladograms of chelicerate orders based on combined data set in Fig. 6.

DISCUSSION

As shown here and in previous studies (Fitch and Smith, 1983; Waterman et al., 1992; Wheeler, 1995), the phylogenetic analysis of DNA sequences is based on many untestable assumptions. Among these, indel costs and transversion–transition ratios are the most commonly discussed. All analyses are bound by these necessary assumptions. Furthermore, when morphological character data are included for simultaneous analysis, they must be accorded some weight. Even if we agree that all characters should be weighted equally, what does "equal" mean? Here we have examined three scenarios of such weighting. This raises the question of defining and quantifying an optimality criterion for comparing results which are themselves most parsimonious for their set of assumptions.

Character-based incongruence has been suggested as a criterion for phylogenetic analysis in general (Kluge, 1989; Wheeler, 1995). Since this concept is a generalization of the parsimony criterion for individual data sets, the logic is obvious and consistent. The character-incongruence metric of Mickevich and Farris (1981) provides a measure of this and, when appropriately rescaled, presents a simple, objective criterion for the phylogenetic analysis of multiple data sets. One of the benefits of choosing this metric is the ability to decide among unmeasurable assumptions. In the analysis here, several methods of weighting morphological data are possible and plausible. When morphological characters are weighted as indels, overall character incongruence was minimized. This provides evidence that this is the appropriate scheme for combining these data.

One of the advertised benefits of total evidence analysis is the potential complementarity of the contributions of the data. That is, individual data sets may weigh in on different areas of the cladogram. Where one set is agnostic or weak, another may be strong. The chelicerate groups here provide examples of this. The division of the Chelicerata into Pycnogonida+Eucheliceratais strongly supported by molecular data. There are transitions, transversions, and indels from both the 18S and 28S sequences which support this grouping (Fig. 9; Table 5)The distinction between the spider–acaran orders on one hand and the opilionid–scorpionid on the other is weakly supported by the molecular sequence data (only two are required) whereas six morphological synapomorphies are involved in this distinction (Fig. 9; Table 5).

The combination of the character incongruence metric and total evidence allows us to examine our assumptions and create joint hypotheses of phylogenetic relationships from multiple sources. Only this method of analysis offers the optimality of character congruence and the complementarity of total evidence.

TABLE 5
Character Support for "Best" Topology

	Bremer	Min Len	Max Len	Min Mor	Min 18 Ti	Min 18 Tv	Min 18 ID	Min 28 Ti	Min 28 Tv	Min 28 ID
HTU										
1	3	30	75	0	7	6	2	1	1	0
2	5	43	80	0	1	5	0	2	7	4
3	7	67	106	0	3	6	2	8	12	3
4	25	78	125	0	13	10	2	3	3	7
5	32	39	114	4	9	7	0	0	0	0
6	2	6	72	0	2	0	1	0	0	0
7	40	82	131	4	8	4	2	4	7	6
8	21	63	93	2	3	7	1	4	3	6
9	13	36	43	0	5	7	1	3	3	1
10	37	48	91	6	3	3	0	3	4	1
11	10	15	48	0	9	3	0	0	0	0
12	5	24	60	4	2	1	1	0	0	0
13	21	55	87	7	6	3	0	5	3	1
14	16	30	72	6	1	0	0	1	0	1
15	45	67	107	10	4	1	1	3	5	1
16	15	29	71	4	3	0	0	0	3	1
17	38	61	94	8	2	4	0	3	4	2
18	6	23	33	0	2	2	0	5	6	0
19	4	17	24	0	2	2	0	3	2	1
20	2	21	31	0	1	0	0	2	3	3
21	24	33	39	0	2	0	0	5	9	2
22	8	35	63	5	0	3	0	3	1	1
23	24	32	115	8	0	0	0	0	0	0
24	48	59	99	10	0	1	1	1	2	2
25	8	16	96	4	0	0	0	0	0	0
26	15	65	98	5	1	0	1	4	12	3
27	28	60	136	8	2	1	3	0	2	2
28	19	24	144	0	8	4	2	0	0	0
29	35	67	204	3	7	6	5	2	3	2
30	26	49	99	0	3	7	1	2	3	5
Terminal taxon										
Peripatopsis	NA	572	705	2	26	24	35	8	5	83
Balanus	NA	189	248	0	40	45	9	9	7	0
Callinectes	NA	68	86	0	24	9	2	6	4	1
Artemia	NA	134	178	0	28	15	2	11	20	4
Monobia	NA	70	103	0	13	9	4	9	1	3
Agrion	NA	133	192	0	16	15	6	7	2	13
Spirobolus	NA	80	115	0	11	8	1	11	15	2
Scutigera	NA	54	77	0	7	15	0	5	4	1
Colossendeis	NA	16	20	0	4	2	2	0	0	0
A. portus	NA	19	31	0	2	1	0	7	2	1
A. lentus	NA	67	79	0	1	3	1	8	12	6
Limulus	NA	39	88	0	11	3	0	6	4	2
Hadrurus	NA	34	40	0	5	5	3	3	2	0
Androctonus	NA	28	29	0	6	9	1	0	0	0
Centruroides	NA	4	40	0	2	1	0	0	0	0
Parurotonus	NA	23	28	0	0	1	0	1	2	4
Gea	NA	105	115	0	23	18	2	18	10	0
Hypochilus	NA	14	17	0	1	0	0	3	1	2
Eurypelma	NA	91	112	0	1	4	2	10	10	11
Thelechoris	NA	4	8	0	1	0	0	3	0	0

Continued.

TABLE 5
Character Support for "Best" Topology (continued)

	Bremer	Min Len	Max Len	Min Mor	Min 18 Ti	Min 18 Tv	Min 18 ID	Min 28 Ti	Min 28 Tv	Min 28 ID
Heptathela	NA	1	4	0	0	0	0	1	0	0
Liphistius	NA	11	14	0	2	0	0	5	2	0
Amblypigid	NA	70	106	1	9	10	1	3	5	5
Mastigoproctus	NA	42	52	2	5	3	0	11	4	1
Trithyreus	NA	67	78	7	3	2	2	8	6	1
Palpigrade	NA	16	36	4	0	0	0	0	0	0
Ricinulcid	NA	93	139	8	7	7	4	7	2	3
Amblyomma	NA	25	43	0	3	3	4	0	0	0
Rhiphicephalus	NA	8	51	0	4	2	0	0	0	0
Tetranychus	NA	125	217	0	15	15	5	24	12	3
Vonones	NA	56	74	0	6	7	0	16	10	0
Leiobunum	NA	73	88	0	6	16	1	9	5	3
Americhernes	NA	193	222	8	31	36	2	18	14	1
Chambria	NA	123	156	10	8	7	2	15	13	3

HTU=Node number of Fig. 6; Bremer=Bremer support; Min Len=minimum branch length; Max Len=Maximum branch length; Min Mor=minimum number of morphological character apomorphies; Min 18 Ti=minimum number of 18S rDNA transitions on branch; Min 18 Tv=minimum number of 18S rDNA transversions on branch; Min 18 ID=minimum number of 18S rDNA indels on branch; Min 28 Ti=minimum number of 28S rDNA transitions on branch; Min 28 Tv=minimum number of 28S rDNA transversions on branch; Min 28 ID=minimum number of 28S rDNA indels on branch. NA=Not applicable; terminal taxa cannot have Bremer support values.

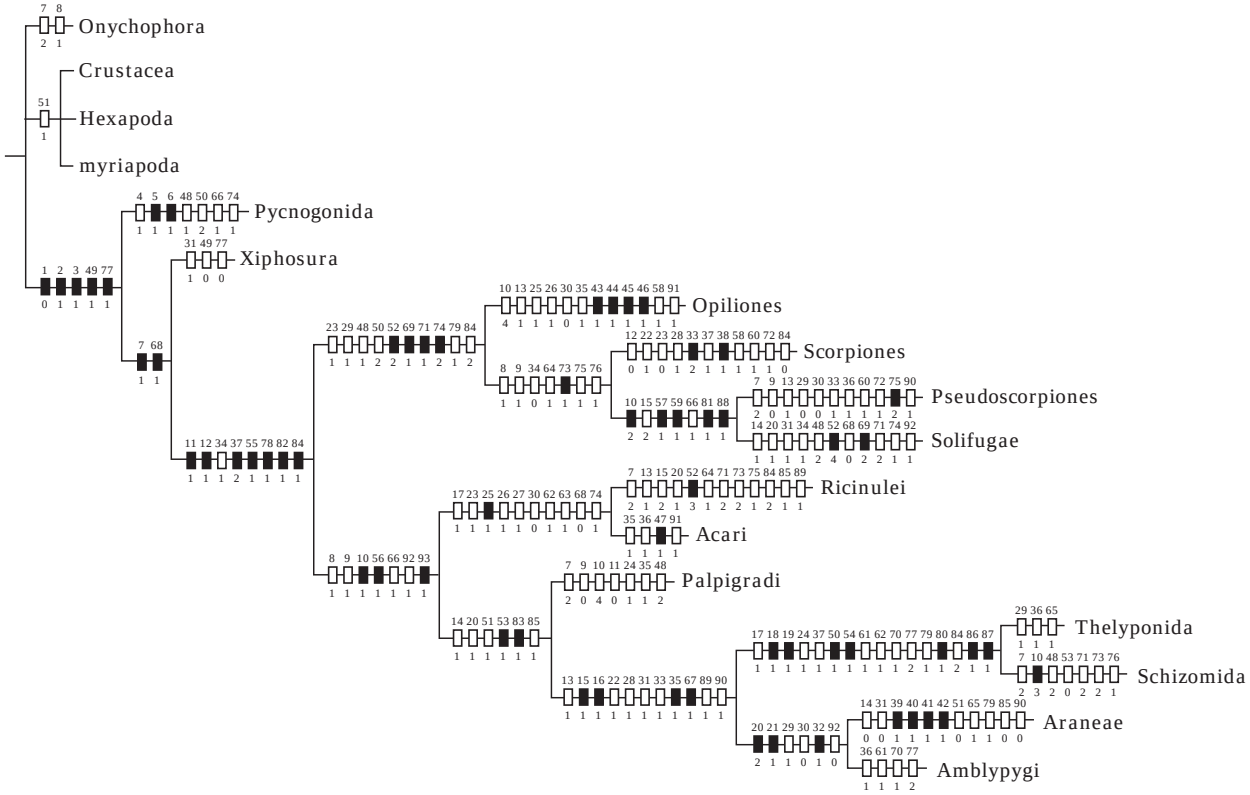

FIG. 11. Morphological character optimization for chelicerate orders. The cladogram is abstracted from Fig. 6. Characters were optimized using the default optimization of CLADOS (Nixon, 1992) which is basically delayed transformation optimization. Solid bars represent non-homoplastic characters and open bars homoplastic characters. The numbers above the boxes note the character number changing along that branch and the numbers below the state of the character in the HTU or terminal taxon.

ACKNOWLEDGEMENTS

We would like to thank Gonzalo Giribet for discussion and the use of some of his sequences; Mark Stowe, Graham Lowe, Wendell Icenogle, Gustavo Hormiga, and Norman Platnick for specimens; Michael Whiting, Amy Litt, Aloyisus Phillips, David Gladstein, Simon Braddy, and two anonymous reviewers for manuscript commentary; and Portia Rollings for artwork.

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