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Higher-level phylogenetics of linyphiid spiders (Araneae, Linyphiidae) based on morphological and molecular evidence

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Abstract

This study infers the higher-level cladistic relationships of linyphiid spiders from five genes (mitochondrial CO1, 16S; nuclear 28S, 18S, histone H3) and morphological data. In total, the character matrix includes 47 taxa: 35 linyphiids representing the currently used subfamilies of Linyphiidae (Stemonyphantinae, Mynogleninae, Erigoninae, and Linyphiinae (Micronetini plus Linyphiini)) and 12 outgroup species representing nine araneoid families (Pimoidae, Theridiidae, Nesticidae, Synotaxidae, Cyatholipidae, Mysmenidae, Theridiosomatidae, Tetragnathidae, and Araneidae). The morphological characters include those used in recent studies of linyphiid phylogenetics, covering both genitalic and somatic morphology. Different sequence alignments and analytical methods produce different cladistic hypotheses. Lack of congruence among different analyses is, in part, due to the shifting placement of *Labulla*, *Pityohyphantes*, *Notholepthyphantes*, and *Pocobletus*. Almost all combined analyses agree on the monophyly of linyphioids, Pimoidae, Linyphiidae, Erigoninae, Mynogleninae, as well as *Stemonyphantes* as a basal lineage within Linyphiidae. Our results suggest independent origins of the desmitracheate tracheal system in micronetines and erigonines, and that erigonines were primitively haplotracheate. Cephalothoracic glandular specializations of erigonines and mynoglenines apparently evolved independently. Subocular sulci of mynoglenines and lateral sulci (e.g. *Bathyphantes*) evolved independently but glandular pores in the prosoma proliferated once. The contribution of different character partitions and their sensitivity to changes in traditional analytical parameters is explored and quantified.

Introduction

Linyphiids are the most speciose family-level lineage of araneoid spiders, a large clade that includes, among others, the ecribellate orbweavers (Griswold et al., 1998) and close to 30% of the total described species diversity of spiders (Platnick, 2008) (Fig. 1). Although linyphiids have a worldwide distribution, they are most diverse in north temperate and colder regions. At higher latitudes they account for a large fraction of the spider species richness (e.g. 216 of the 523 spider species in Denmark are linyphiids; Scharff and Gudik-Sørensen, 2006). In the tropics, linyphiids can also be diverse (Scharff, 1990a, 1992; Miller, 2007) but they represent a much smaller fraction of the total spider diversity (Scharff, 1990b, 1992, 1993; Silva and Coddington, 1996; Sørensen et al., 2002; Floren and Deeleman-Reinhold, 2005). Most linyphiid species are generalist predators and build aerial or substrate webs to capture their prey. Linyphiid webs are often built as a sheet, with various elaborations such as an upper and lower scaffolding (or none at all) (Fig. 2), although only a very few detailed descriptions of their webs exist in the literature (Benjamin and Zschokke, 2004; Hormiga, 2007). Compared with other araneoid lineages, such as the members of the

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Fig. 1. Linyphiidae—variation in somatic morphology. (a) *Eperigone tridentata* (from USA), (b) *Pityohyphantes costatus* (from USA), (c) *Labulla thoracica* (from Denmark), (d) *Stemonyphantes lineatus* (from Denmark), (e) *Sphecozone* sp. (from Chile), (f) *Microneta viaria* (from Denmark), (g) *Orsonwelles falstaffius* (from Hawaii, USA), (h) *Dubiaranea* sp. (from Ecuador). Photos: Gustavo Hormiga (a–e), (g–h); Lars Bruun (f).

families Araneidae or Theridiidae, linyphiids have a relatively uniform somatic morphology (Fig. 1). However, the genitalia are among the most complex known for spiders, especially in males, and many male Erigoninae species have bizarre cephalic modifications (see Hormiga, 2000, figs 32–25).

Arachnologists currently recognize 109 extant families of spiders and about 40 000 described species (Platnick, 2008). Only jumping spiders (Salticidae) include more described species than Linyphiidae (5188 species in 560 genera versus 4345 species in 576 genera; Platnick, 2008). Given their cold and north temperate regional species richness, it is not surprising that most taxonomic work on Linyphiidae has been biased towards those latitudes, leaving the fauna of other regions relatively understudied. While the family has a long history of taxonomic descriptive work, with some of the species names going back to Clerck's *Svenska Spindlar* (Clerck, 1757), relatively few studies have addressed the phylogenetic structure of Linyphiidae, and even fewer have used cladistic methods to understand this problem (see Hormiga (2000) for a summary of the history of classification in Linyphiidae). Miller and Hormiga (2004) recently studied linyphiid phylogeny, emphasizing the subfamily Erigoninae, and building on the prior study by Hormiga (2000). A few other cladistic studies exist (e.g. Hormiga and Scharff, 2005), but all these attempts have suffered from relatively modest and rather biased taxonomic samples (admittedly by design). For example, Miller and Hormiga's (2004) data matrix included 70 linyphiid species (plus 12 outgroup taxa) scored for 176 almost exclusively morphological

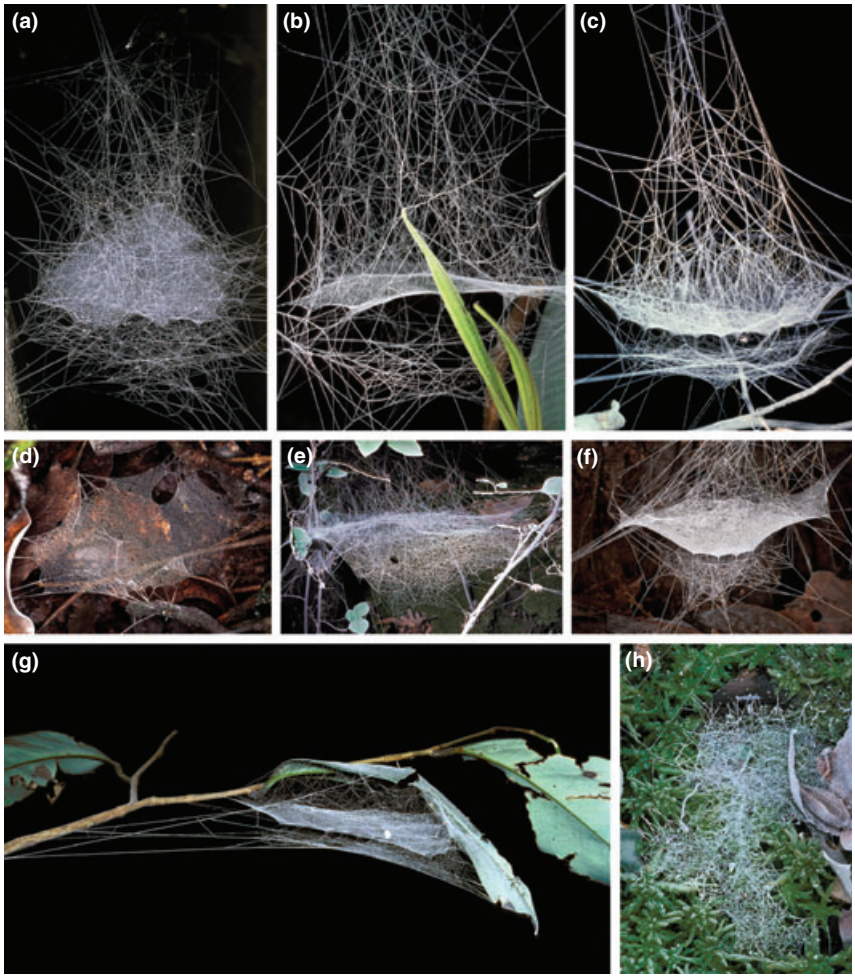

Fig. 2. Linyphiidae—variation in web architecture. (a) Unknown genus (from Thailand), (b) *Diplothyron* sp. (from Costa Rica), (c) *Frontinella communis* (from USA), (d) *Walckenaeria* sp. (from Costa Rica), (e) *Pityohyphantes costatus* (from USA), (f) *Exocora* sp. (from Costa Rica), (g) *Mecynidis* sp. (from Cameroon), (h) *Eperigone tridentata* (from USA). Photos: Gustavo Hormiga.

characters, but only seven terminals represented ''non-erigonine'' linyphiids. As a result, our present knowledge on the cladistic structure of linyphiids is fairly limited.

The monophyly of the family Linyphiidae is relatively well established on morphological synapomorphies (Hormiga, 1994b; Miller and Hormiga, 2004; Hormiga et al., 2005) as well as its sister group relationship to the relictual family Pimoidae (Wunderlich, 1986; Hormiga, 1993, 1994b, 2003, 2008; Hormiga et al., 2005; Hormiga and Tu, 2008). Within Linyphiidae, the monophyly of the subfamilies Mynogleninae and Erigoninae is also robustly supported, based on the results of several cladistic analyses of morphological data (Hormiga, 1993, 1994b, 2000; Miller and Hormiga, 2004). Some cladistic analyses (e.g. Hormiga, 1993, 1994a,b; Miller and Hormiga, 2004) suggest that the genus *Stemonyphantes* is sister to all other linyphiids, but this conjecture is not supported by other analyses (e.g. Hormiga, 2000, 2003; Hormiga et al., 2005; Hormiga and Scharff, 2005). So far, all phylogenetic analyses of linyphiids (e.g. Miller and Hormiga, 2004; Fig. 4) have supported the monophyly of a group that includes micronetines (''Micronetini'') plus *Linyphia* and its close relatives (the latter group is sometimes referred as ''Linyphiini''), usually in the subfamily Linyphiinae, although the composition and exact limits of this clade are far from clear (Hormiga and Scharff, 2005). As mentioned above, the cladistic studies cited were designed to address specific phylogenetic questions, such as erigonine relationships (Hormiga, 2000; Miller and Hormiga, 2004) or the placement of *Labulla* (Hormiga and Scharff, 2005), rather than explicitly inferring the main lineages of Linyphiidae. Cladistic work on linyphiids has helped to understand and test numerous hypotheses of homology, and has provided empirical support for some higher groups, but for the most part its core phylogenetic structure remains poorly known. What is the sister

group of the large subfamily Erigoninae? Is *Stemonyphantes* the most basal linyphiid lineage? Are the ''Linyphiini'' monophyletic? Have the specialized cephalic structures of mynoglenines and of male erigonines evolved independently? After more than a decade of quantitative phylogenetic work on linyphiids, the answers to these and many other related questions remain tantalisingly out of reach.

In discussing their progress in understanding the phylogeny of erigonines, Miller and Hormiga (2004) concluded that the addition of future data, especially the addition of new characters, would change their phylogenetic hypothesis, just as their own work changed the conclusions reached by previous studies. With the exception of the study of Hormiga et al. (2003), all linyphiid phylogenetic work to date has been based on morphology and a few behavioural homologies. The former gathered nuclear and mitochondrial sequences and morphology to resolve the internal and outgroup relationships of the Hawaiian endemic genus *Orsonwelles*, using relatively few linyphiines as terminals. In this paper we address the phylogenetic structure of Linyphiidae using molecular and morphological evidence to identify the main lineages of the family and their relationships. Currently, the monophyly of Erigoninae is not in question, for example, it has been empirically corroborated by the analyses of Hormiga (2000) and Miller and Hormiga (2004). This study includes the largest sample of ''non-erigonine'' linyphiid lineages thus far, as well as genetic data for them, although it is still a modest sample given the large size of the family.

Materials and methods

Taxon sampling

The taxonomic sample used in this study is based largely on our own morphological phylogenetic work in linyphioids to date, and has been expanded to better represent the diversity of linyphiids, including non-Holarctic representatives. It includes more erigonine outgroups but fewer erigonines than Hormiga (2000), which focused on that subfamily. We have attempted to include genera and/or species for which specimens were also available for DNA extraction, with the goal of maximizing the overlap between the morphological and molecular data partitions. In the absence of a detailed classification of the family Linyphiidae, we have used the schemes proposed by Millidge (1977, 1984, 1993), although Millidge himself reversed his views on several of these groups (for example, compare his 1977 classification based on male palp morphology with his 1984 ''epigynal groups''). Regarding the circumscription and composition of Micronetinae, we have followed Saaristo and Tanasevitch (1996). A similar approach to taxonomic sampling within Linyphiidae was followed in the studies of Hormiga (2000) and Miller and Hormiga (2004). Shortly after the completion of this study, Saaristo (2007) erected a new linyphiid subfamily, Ipainae, to include seven genera and 26 species. Although we do not have any representatives of the Ipainae in our matrix, we offer some comments on the phylogenetic relationships of ipaines in the ''Discussion'' section of this paper.

In total, the character matrix includes 47 taxa: 35 linyphiids and 12 outgroup species representing nine araneoid families (see below and Table 1).

Micronetinae were represented by *Agyneta ramosa*, *Bolyphantes luteolus*, *Drapetisca socialis*, *Floronia bucculenta*, *Lepthyphantes minutus*, *Meioneta rurestris*, *Microneta viaria*, and *Tenuiphantes tenuis*. Linyphiinae were represented by *Bathyphantes gracilis*, *Diplostyla concolor*, *Linyphia triangularis*, *Microlinyphia dana*, *Neriene variabilis*, *N. radiata*, *Orsonwelles polites*, *O. malus*, and *Pityohyphantes costatus*. The subfamily Erigoninae included the following taxa: *Erigone psychrophila*, *Gonatium rubens*, *Gongylidiellum vivum*, *Hilaira excisa*, *Oedothorax gibbosus*, and *Ostearius melanopygius*. The Mynogleninae were represented by *Haplinis diloris*, *Novafroneta vulgaris*, and *Pseudafroneta perplexa*. Dubiaraneinae are represented by *Dubiaranea distincta*. In addition, our sample included the following eight species (all of uncertain subfamilial affinities; e.g. see Millidge (1993) ''Miscellaneous genera''): *Frontinella communis*, *Stemonyphantes lineatus*, *Australolinyphia remota*, *Helophora insignis*, *Labulla thoracica*, *Laetesia* sp. (from Lamington National Park, Australia), *Notholepthyphantes australis*, and *Pocobletus* sp. (from Misiones, Argentina).

We studied 12 outgroup species representing nine araneoid families: *Argiope trifasciata* (Araneidae), *Theridiosoma gemmosum* (Theridiosomatidae), *Maymena* sp. (Mysmenidae), *Tetragnatha versicolor* (Tetragnathidae), *Nesticus cellulanus* (Nesticidae), *Theridion varians* (Theridiidae), *Steatoda grossa* (Theridiidae), *Synotaxus waiwai* (Synotaxidae), *Alaranea merina* (Cyatholipidae), *Weintrauboa contortipes* (Pimoidae), *Pimoa altioculata*, and *P. rupicola* (Pimoidae).

We succeeded in collecting sequence and morphological data from many of the same species, but we had no suitable material for sequencing *Hilaira* and *Weintrauboa* (Pimoidae). Furthermore, the species sequenced and scored morphologically differed in 11 genera: *Nesticus* (Nesticidae), *Pimoa* (Pimoidae), *Steatoda* (Theridiidae), *Tetragnatha* (Tetragnathidae), *Bolyphantes*, *Erigone*, *Gonatium*, *Oedothorax*, *Pseudafroneta*, and *Stemonyphantes* (Linyphiidae). The latter genus requires futher clarification. Morphology was scored for *Stemonyphantes blauveltae*, but molecular analyses were conducted on *S. lineatus* and *S. sibiricus*. For the combined analyses,

Table 1
Taxonomic and sequence information of the specimens included in the phylogenetic analyses

Family	Genus	Species	Country	Locality	CO1	16S	18S	28S	H3	Voucher
Araneidae	*Argiope*	*trifasciata*	USA	Hawaiian I, Kauai, Kokee SP	AY231021	AY230937	AY230889	AY231068	AY230981	Arnedo et al., 2004;
	Argiope	*argentata*								Alvarez-Padilla et al. (in press)
Cyatholipidae	*Alaranea*	*merina*	Madagascar		AY231022	AY230942	AY230890	AY231074	AY230982	Arnedo et al., 2004;
	Alaranea	*merina*	Madagascar	Toamasina Prov., PN Perinet						CAS
Mysmenidae	*Mysmena*	sp. (gu044)	Guyana	S of Gunns Landing	AY231023		AY230891	AY231071	AY230983	Arnedo et al., 2004;
Nesticidae	*Nesticus*	sp.	China		AY231024	AY230941	AY230892	AY231073	AY230984	Arnedo et al., 2004;
	Nesticus	*cellulanus*								Alvarez-Padilla et al., in press
Theridiidae	*Steatoda*	*bipunctata*	UK	Yorkshire	AY231057	AY230951	AY230926	AY231084	AY231014	Arnedo et al., 2004;
	Steatoda	*grossa*								Miller and Hormiga, 2004
	Theridion	*varians*	UK	Yorkshire	AY231063	AY230976	AY230932	AY231111	AY231017	Arnedo et al., 2004;
Synotaxidae	*Synotaxus*	*waiwai*	Guyana	S of Gunns Landing	AY231026	AY230943	AY230894	AY231075	AY230986	Arnedo et al., 2004;
Theridiosomatidae	*Theridiosoma*	*gemmosum*	USA	NC, Macon Co.	AY231028	AY230939	AY230896	AY231070	AY230988	Arnedo et al., 2004;
Tetragnathidae	*Tetragnatha*	*mandibulata*	USA	Hawaiian Is	AY231027	AY230938	AY230895	AY231069	AY230987	Arnedo et al., 2004;
	Tetragnatha	*versicolor*	USA	CA, Sikiyou Co., Yreka-Sahasta River						USNM
Pimoidae	*Pimoa*	sp.	China	Yunnan Prov., Gaoligong Shan	AY231025	AY230940	AY230893	AY231072	AY230985	Arnedo et al., 2004;
	Pimoa	*altioculata*	USA	OR, Lane Co., Cape Perpetua, Cook Ridge Trail, Siuslaw Ntl. Forest						USNM
	Pimoa	*rupicola*	Italy	Alpi Apuane						IZUI
	Pimoa	*rupicola*	Italy	Pemonte, Sampere						MCZ
	Weintrauboa	*contortipes*	Japan	No other locality data						MNHN
Linyphiidae	*Agyneta*	*ramosa*	Denmark	Bøgebakken in Tofte Skov	FJ838648	FJ838670	FJ838694	FJ838717	FJ838740	ZMUC
	Agyneta	*ramosa*	Denmark	Tørvekjær (ZMUC00008299)						ZMUC
	Agyneta	*ramosa*	Denmark	Tofte Skov (ZMUC00009661)						ZMUC

Table 1
(*Continued*)

Family	Genus	Species	Country	Locality	CO1	16S	18S	28S	H3	Voucher
	Australolinyphia	*remota*	Australia	QLD., O'Reilly's, Lamington, NP	FJ838649	FJ838671	FJ838695	FJ838718	FJ838741	MCZ, ZMUC
	Bathyphantes	*gracilis*	Denmark	Zealand, Dragerup, forest nr. Tjebberup	FJ838650	FJ838672	FJ838696	FJ838719	FJ838742	ZMUC
	Bathyphantes	*gracilis*	Denmark	Zealand, Vesterlyng, SW of Havnsø						MCZ, ZMUC
	Bathyphantes	*gracilis*	Denmark	Møn, Ulfshale						ZMUC
	Bolyphantes	*alticeps*	Denmark	Bælum Sønderskov	AY078691	AY078660	AY078667	AY078678	AY078700	Hormiga et al., 2003;
	Bolyphantes	*luteolus*	Spain	Huesca, Jaca, San Juan de la Peña						USNM
	Diplostyla	*concolor*	Denmark	Zealand, Kirkemose, Dyrehave, S of Ejby (ZMUC00004753)	FJ838651	FJ838673	FJ838697	FJ838700	FJ838743	ZMUC
	Diplostyla	*concolor*	Denmark	Falster, Idestrup (ZMUC00009899)						ZMUC
	Diplostyla	*concolor*	Denmark	Vejle, Munkebjerg (ZMUC00009700)						ZMUC
	Drapetisca	*socialis*	Denmark	Zealand, Dragerup, forest nr. Tjebberup	FJ838652	FJ838674	FJ838698	FJ838721	FJ838744	ZMUC, MCZ
	Dubiaranea	*distincta*	Chile	PN Puyehue, Aguas Calientes	FJ838653	FJ838675	FJ838694	FJ838722	FJ838745	USNM
	Erigone	*dentipalpis*	United Kingdom	Warwick, Wellesbourne	AY383538	–	–	–	–	Agustí et al., 2003;
	Erigone	*psychrophila*	USA	Alaska, Pt. Barrow						USNM
	Floronia	*bucculenta*	Denmark	Zealand, Kirkemose, Dyrehave, S of Ejby	FJ838654	FJ838676	FJ838700	FJ838723	FJ838746	ZMUC, MCZ
	Frontinella	*communis*	USA	MD, University Park	FJ838655	FJ838677	FJ838701	FJ838724	FJ838747	MCZ
	Gonatium	*rubellum*	Denmark	Zealand, Kirkemose, Dyrehave, S of Ejby	FJ838656	FJ838679	FJ838703	FJ838726	FJ838749	ZMUC
	Gonatium	*rubens*	USA	Massachusetts, Barnstable Co., Hatchville						USNM
	Gonatium	*rubens*	UK	Yorkshire, Allerston						AMNH
	Gongylidiellum	*vivium*	Denmark	Stubbegaard Sø	x	FJ838678	FJ838702	FJ838725	FJ838748	ZMUC
	Gongylidiellum	*vivium*	UK	Surrey						AMNH
	Haplinis	*diloris*	New Zealand	Cullen Creek	FJ838657	FJ838680	FJ838704	FJ838727	FJ838750	MCZ
	Haplinis	*diloris*	New Zealand	Fiordland Cascade						OM
	Helophora	*insignis*	Denmark	Bælum Sønderskov	FJ838658	FJ838681	FJ838705	FJ838728	FJ838751	ZMUC
	Helophora	*insignis*	Denmark	Lolland (ZMUC00008059)						ZMUC
	Helophora	*insignis*	Denmark	Jutland, Rønde, Hestehaven						ZMUC
	Helophora	*insignis*	Denmark	Jutland, Høstemark Skov (ZMUC00006473)						ZMUC
	Hilaira	*excisa*	UK	Brecon Beacons						AMNH
	Labulla	*thoracica*	Denmark	Bælum Sønderskov	AY078694	AY078662	AY078674	AY078680	AY078707	ZMUC
	Laetesia?	sp.	Australia	QLD., O'Reilly's, Lamington NP	FJ838659	FJ838682	FJ838706	FJ838729	FJ838752	ZMUC
	Laetesia?	sp.	Australia	QLD., Mt Tambourine NP, Witches Falls						MCZ
	Lepthyphantes	*minutus*	Denmark	Bælum Sønderskov	AY078689	AY078663	AY078673	AY078681	AY078705	ZMUC
	Lepthyphantes	*minutus*	Denmark	Hestehaven						ZMUC
	Lepthyphantes	*minutus*	Denmark	Zealand, Dragerup, forest nr. Tjebberup						ZMUC
	Linyphia	*triangularis*	Denmark	Bælum Sønderskov	AY078693	AY078664	AY078668	AY078682	AY078702	ZMUC
	Linyphia	*triangularis*	Denmark	Zealand, Kirkemose, Dyrehave						ZMUC
	Meioneta	*rurestris*	Denmark	Vesterlyng, SW of Havnsø	FJ838660	FJ838683	FJ838707	FJ838730	FJ838753	ZMUC
	Meioneta	*rurestris*	Spain	Gipuzkoa, Donostia						MCZ
	Meioneta	*rurestris*	Denmark	Gentofte						ZMUC

Table 1
(*Continued*)

Family	Genus	Species	Country	Locality	CO1	16S	18S	28S	H3	Voucher
	Meioneta	*rurestris*	Denmark	Zealand, Amager (ZMUC00004763)						ZMUC
	Microlinyphia	*dana*	USA	CA, Salt Point, SP	AY078690	AY078665	AY078677	AY078683		Hormiga et al., 2003;
	Microlinyphia	*dana*	USA	WA, Clallam Co. Elwha river nr Altaire Cmpgd. Olympic N. P						USNM
	Microneta	*viaria*	Denmark	Zealand, Kirkemose, Dyrehave, S of Ejby	FJ838661	FJ838684	FJ838708	FJ838731	FJ838754	ZMUC
	Microneta	*viaria*	Denmark	Jutland, Rønde, Hestehaven						ZMUC, MCZ
	Neriene	*variabilis*	USA	MD, Patuxent Wildlife Res. Ctr.	AY078699	AY078711	AY078669	AY078685	AY078706	Hormiga et al., 2003;
	Neriene	*variabilis*	USA	MD, Patuxent Wildlife Res. Ctr.						USNM
	Neriene	*radiata*	USA	MD, Patuxent Wildlife Res. Ctr.	AY078696	AY078710	AY078670	AY078684	AY078709	Hormiga et al., 2003;
	Neriene	*radiata*	USA	VA, Page Co., S of Luray						MCZ
	Neriene	*radiata*	USA	MD, Calvert Co., Matoka Beach						MCZ
	Notholepthyphantes	*australis*	Chile	PN Puyehue, Aguas Calientes	FJ838662	FJ838685	FJ838709	FJ838732	FJ838755	USNM
	Notholepthyphantes	*australis*	Chile	PN Nahuelbuta						USNM
	Novafroneta	*vulgaris*	New Zealand	Riccarton Bush	FJ838663	FJ838686	FJ838710	FJ838733	FJ838756	MCZ
	Novafroneta	*vulgaris*	New Zealand	S Brighton						OM
	Oedothorax	*apicatus*	Denmark	Zealand. Nr. Tisvildeleje	FJ838664	FJ838687	FJ838711	FJ838734	FJ838757	ZMUC
	Oedothorax	*gibbosus*	UK	Tentesmuir Dune						AMNH
	Orsonwelles	*malus*	USA	Hawaiian I., Kauai, Kokee SP., Nualolo trail	AY078697	AY078730	AY078676	AY078687	AY078708	Hormiga et al., 2003;
	Orsonwelles	*polites*	USA	Hawaiian I., Oahu, Palikea	AY078698	AY078726	AY078671	AY078686	AY078701	Hormiga et al., 2003;
	Ostearius	*melanopygius*	Denmark	Zealand, Ørslev Mose	x	FJ838688	FJ838712	FJ838735	FJ838758	ZMUC
	Ostearius	*melanopygius*	USA	Massachussetts, Peperell						USNM
	Ostearius	*melanopygius*	Bolivia	Oruro						AMNH
	Pityohyphantes	*costatus*	USA	MD, Patuxent Wildlife Res. Ctr.	AY078695	AY078666	AY078675			
	Pityohyphantes	*costatus*	USA	GA, Rabun Co., Ellicott Rock Wild. Area						USNM
	Pocobletus	sp.	Argentina	Misiones, PN Iguazu	FJ838665	FJ838689	FJ838713	FJ838736	FJ838759	MCZ
	Pocobletus	sp.	Brazil	SP, Salesopolis						MACN
	Pseudafroneta	*incerta*	New Zealand	Peel Forest	FJ838666	FJ838690	FJ838714	FJ838737	FJ838760	MCZ
	Pseudafroneta	*perplexa*	New Zealand	Flagstaff, Dunedin						MCZ
	Stemonyphantes	*blauveltae*	USA	MO, Callaway Co., Tucker Praire						USNM
	Stemonyphantes	*lineatus*	Denmark	Vesterlyng, SW of Havnsø	FJ838667	FJ838691	FJ838715	FJ838738	FJ838761	ZMUC
	Stemonyphantes	*sibiricus*	Russia	NE Siberia, nr. Magadan	FJ838668	FJ838692			FJ838762	MCZ
	Tenuiphantes	*tenuis*	Denmark	Zealand, nr. Tisvildeleje	FJ838669	FJ838693	FJ838716	FJ838739	FJ838763	ZMUC
	Tenuiphantes	*tenuis*	Spain	Huesca, Jaca, San Juan de la Peña						USNM

Sequences with codes FJ838648–FJ838763 have been generated for the present study. Specimens without sequence information have been studied only for morphology. Voucher specimen data for the taxa studied included from other studies are referred to Arnedo et al. (2004), given in that publication. In the combined analyses 11 genera were "chimaeras" in which the species sequenced and scored morphologically differed (see text for details).

AMNH, American Museum of Natural History, USA; CAS, California Academy of Sciences, USA; IZUI, Institute of Zoology, University of Innsbruck, Austria; MACN, Museo Argentino de Ciencias Naturales, Argentina; MCZ, Museum of Comparative Zoology, Harvard University, USA; MNHN, Muséum National d'Histoire Naturelle, France; OM, Otago Museum, New Zealand; USNM, National Museum of Natural History, Smithsonian, Institution, USA; ZMUC, Zoologisk Museum, University of Copenhagen, Denmark.

morphological information of *S. blauveltae* was merged with nucleotide sequence information of morphologically similar *S. lineatus*, while *S. sibiricus* morphology was scored as missing data.

Morphological data

Morphological methods are described in more detail by Hormiga (2000, 2002). Specimens were examined and illustrated using a Leica MZ16A or MZAPO stereoscopic microscope, with a camera lucida. Further details were studied using a Leica DMRXE or DMRM compound microscope with a drawing tube. Digital microscope images were recorded using a Nikon DXM1200F camera attached to a Leica MZ16A stereoscope and edited using the software package Auto-Montage®. A LEO 1340VP (at The George Washington University) or a JEOL JSM840 (at the Zoological Museum of the University of Copenhagen) scanning electron microscope was also used to study and photograph morphological features. Left structures (e.g. palps, legs, etc.) were usually studied and illustrated. The position of the metatarsal trichobothrium is expressed as in Denis (1949). Female genitalia were excised using surgical blades or sharpened needles. The specimen was then transferred to methyl salicylate (Holm, 1979) for examination under the microscope, and mounted temporarily as described by Grandjean (1949) and Coddington (1983). Male palps examined with the SEM were first excised and transferred to a vial with 70% ethanol and then cleaned ultrasonically for 1–3 min. The specimen was then transferred to absolute ethanol and left overnight. After critical point drying, the specimens were glued to rounded aluminium rivets using an acetone solution of polyvinyl resin (Paraloid B72) and then Au/Pd-coated for examination at the SEM (see also Alvarez-Padilla and Hormiga, 2008). Webs were photographed as described by Hormiga (2002). Most of the linyphiid morphological characters are from Hormiga (1994a,b, 2000, 2003), Hormiga et al. (2003), Hormiga and Scharff (2005) and Miller and Hormiga (2004), and outgroup characters from Griswold et al. (1998). A total of 149 characters were scored for the study taxa (Appendix 1). The data matrix was compiled and managed using the programs Mesquite ver. 2.0 (Maddison and Maddison, 2007) and WinClada ver. 1.00.08 (Nixon, 2002).

Phylogenetic analyses

The parsimony analyses of the morphological matrix were performed using the computer programs TNT ver. 1.1 (Goloboff et al., 2003) and PAUP* ver. 4.0 (Swofford, 2001). WinClada ver. 1.00.08 (Nixon, 2002) and Mesquite ver. 2.0 (Maddison and Maddison, 2007) were used to study character optimizations on the cladograms. Ambiguous character optimizations were usually resolved so as to favour reversal or secondary loss over convergence (Farris optimization or ACCTRAN); if not, the optimization scheme is discussed in the text. The 18 multistate characters were treated as non-additive (Fitch, 1971). Given the size of the data matrix, all parsimony analyses of the data set were done with heuristic methods and under both equal and implied weights. Implied weighting in TNT weights the characters according to a concave function of homoplasy (Goloboff, 1993). The concavity constant (k) is set by the user and negatively correlates with how strongly homoplasious characters are down-weighted. The current version of TNT sets the upper limit of k at 1000. For this sort of morphological data set, k values above 10–15 would be effectively equivalent to equal weights. We used k values of 3, 6, 10, and 15.

We did heuristic searches (''traditional search'') in TNT under equal weights, collapsing ''rule 3'' (tree collapsing = max. length 0; collapsing branches with no possible support), 1000 replications and holding 500 trees during each replication (using a tree bisection and reconnection (TBR) swapping algorithm). If the most parsimonious resolutions (MPR) were found in only a few replications we broadened the search to include more replications and holding more trees per replication. We did the same in PAUP* using heuristic methods, equal weights, and random addition sequence (1000 replications) holding 500 trees per replication. If more than one tree resulted, the solution set was filtered for compatible but polytomous topologies (using ''Do not retain a non-binary tree if a more highly resolved but compatible tree exists'' in the ''Filter Trees/New Filter'' menu) to eliminate compatible polytomies (Coddington and Scharff, 1996). TNT was also used to assess clade support. We used jackknifing (Farris et al., 1996), with 1000 jackknife pseudoreplicates and Bremer support indices (Bremer, 1988, 1994). Bremer support was calculated with TNT using the commands: hold 10 000 (to set aside room for 10 000 trees); sub 30 (to set the upper bound for suboptimal trees—up to 30 steps longer than optimal trees); bb = fillonly tbr (to find suboptimal trees and swap with tree bisection reconnection).

Molecular procedures

Total DNA was extracted from one or two legs of relatively large, freshly collected specimens fixed in 95% ethanol, or whole animals if small. In such cases, a puncture was made in the specimen with a needle and the exoskeleton recovered after lysis and stored in 75% ethanol as vouchers. Extraction, amplification, and sequencing followed the protocols described by Arnedo et al. (2004). 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-1490 and C1-N-2198 (Folmer et al., 1994) (676 bp), alternatively C1-J-1718 and C1-N-2191 (Simon et al., 1994) (472 bp); [16S] LR-N-13398 (Simon et al., 1994) and LR-J-12864 (Arnedo et al., 2004) (*c*. 450 bp), [18S] 5F or 18Sa2.0 and 9R (Giribet et al., 1999) (*c*. 800 and *c*. 600 bp, respectively), [28S] 28SO, or 28SA (Whiting et al., 1997), and 28SB (Whiting et al., 1997) (*c*. 800 and *c*. 300 bp, respectively), and [H3] H3aF and H3aR (Colgan et al., 1998) (328 bp). PCR annealing temperatures varied widely according to primers: 42–45°C for mitochondrial primers, 48°C for 28S and H3, and up to 58°C (implementing a ''touchdown'' strategy) for 18S. Laboratory and sequencing facilities were provided by the Laboratory of Molecular Systematics at The Natural History Museum of Denmark. Sequence errors and ambiguities were edited using the Staden Package ver. 1.4.0 (http://staden.sourceforge.net/). Sequences were managed in Bioedit ver. 7.0.5.2 (Hall, 1999). Taxonomic and sequence information of the study specimens are listed in Table 1.

Phylogenetic analyses

The inference of positional homology in homologous sequences of different length is not trivial and involves the insertion of gaps in one or more sequences to accommodate such differences (i.e. sequence alignment). In ribosomal genes, conservation of rRNA secondary structures may guide decisions about the assignment of homologous positions (e.g. Kjer, 1995), although the existence of non-canonical base pairs challenges the use of structural information. Even in the best case scenario, however, identifying stem and loop regions merely ameliorates the problem by constraining nucleotide homology within smaller fragments. In general, the use of manual methods, either by eye or by using structural information, is neither repeatable nor objective, two fundamental qualities of scientific endeavours (Giribet and Wheeler, 1999). Automatic multiple alignment programs use the Needleman–Wunsch dynamic algorithm (Needleman and Wunsch, 1970), which is guaranteed to align optimally any two sequences. Automatic alignment methods require the explicit definition of costs of the insertion and extension of gaps and nucleotide transformations. It is common practice to build fixed sequence alignments by incorporating gaps, which are subsequently subject to phylogenetic analyses. This two-step procedure approach has been defended by claiming that they are logically independent (Simmons and Ochoterena, 2000; Simmons et al., 2001; Simmons, 2004). Alternatively, homology assignment and phylogenetic inference have been considered to be intrinsically linked (Mindell, 1991). In accordance with this view, insertion/deletion events are better treated not as additional nucleotide bases, but as what they actually are: transformations and not observations (Wheeler, 1996, 2001). Direct optimization incorporates insertion/deletion events as one of the possible transformations along nucleotide substitutions during tree optimization and allows simultaneous estimation of alignment and phylogeny, avoiding biases toward the guide trees reported in progressive alignments. Ogden and Rosenberg (2007) have claimed that a two-step procedure (multiple sequence alignment with ClustalW followed by phylogenetic analysis) outperforms direct optimization, but such claims have recently been refuted. When affinity gap values are incorporated into the analyses, as we do in the present study, direct optimization yields more parsimonious solutions and better topological accuracy than fixed alignments (Lehtonen, 2008; Liu et al., 2008).

It has been claimed that phylogenetic results are more sensitive to differences in alignment strategies than to different tree-building methods (Morrison and Ellis, 1997). Therefore in this study we use both static (fixed two-step analysis) and dynamic alignment (direct optimization) approaches to assess their influence on results.

Fixed alignments

Automatic multiple alignments were built using pairwise progressive sequence alignment with ClustalX ver. 1.81 (Thompson et al., 1997). Several gap opening (GOP) and gap extension (GEP) costs were investigated to assess their influence on the results. The following parameter costs were employed (GOP/GEP): 8/2, 8/4, 20/2, 24/4, and 24/6. These values resulted in a set of alignments ranging from gappy (numerous short gaps) to compact (fewer and longer gaps) sequences. In all cases, transition/transversion ratio was kept to 0.5 (default). Following Hedin and Maddison (2001), selection of a particular parameter combination alignment for subsequent combined analyses was based on the similarity of the trees obtained from each alignment to the trees yielded by the elision matrix (Wheeler et al., 1995), as measured by the consensus fork index (Colless, 1980), and the symmetric distance, implemented in PAUP* ver. 4.0 (Swofford, 2001).

Gaps contribute a considerable portion of the potential phylogenetic information and are less prone to homoplasy (Simmons et al., 2001). However, considering gaps as a fifth character state is problematic if gaps are longer than a single position, because these positions are treated as independent of one another, although a single indel event, however, may be a most parsimonious explanation (Eernisse and Kluge, 1993). To avoid overweighting contiguous gap positions, all static parsimony and Bayesian analyses were conducted with gaps recoded as presence/absence characters using Simmons

and Ochoterena's simple code method (Simmons and Ochoterena, 2000), as implemented in the program Gapcoder (Young and Healy, 2002).

Analyses of the static alignment matrices under parsimony were run with TNT ver. 1.1 software (Goloboff et al., 2003) and the matrices and tree were manipulated with the program WinClada ver. 1.00.08 (Nixon, 2002). Parsimony analyses were performed under both equal weights and implied weights (see above). DNA sequence data are usually more homoplasious than morphological data. Large k values (> 10) for DNA sequence data are preferable to avoid extreme down-weighting (Goloboff, pers. comm.). We chose k values of 6, 50, and 100. Heuristic searches involved 100–500 rounds of random addition of taxa, holding five trees per round and a total maximum of 1000. Best overall trees were subjected to a new round of TBR branch swapping. Clade support was assessed using jackknifing (Farris et al., 1996), with 1000 jackknife pseudoreplicates. Individual searches consisted of 15 rounds of random addition of taxa, holding five trees per replicate and an overall maximum of 1000.

The program Modeltest ver. 3.7 (Posada and Crandall, 1998) was used to select the model of evolution that explained the data significantly better with fewer parameters using the Akaike's information criterion (Buckley et al., 2002). Model-based phylogenetic reconstruction was implemented through Bayesian inference analyses performed with MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). We prefer Bayesian inference to maximum likelihood analyses because the first can incorporate discrete qualitative data such as gap presence/absence or morphology. Analyses were run remotely either at Cornell's CBSU computer cluster (http://cbsuapps.tc.cornell.edu/mdiv.aspx) or the Bioportal computer resources (http://www.bioportal.uio.no). Unlinked nucleotide substitution models selected by Modeltest were specified for each gene fragment, and a standard discrete model was implemented for the gaps scored as absence/presence data. The substitution estimates were allowed to vary independently between each partition. Two independent runs with four simultaneous Markov chain Monte Carlo (MCMC) chains (one cold and three heated), each with random starting trees, were carried out simultaneously, sampling 1000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.05 (10 million generations). The program Tracer ver. 1.3 (Rambaut and Drummond, 2003) was used to ensure that the Markov chains had reached stationarity by examining the effective sample size values and also to determine the correct number of generations to discard as a burn-in for the analysis. Posterior probability (PP: frequency of a particular clade among the trees sampled during chain runs after burn-in removal) was used as a measure of support (Huelsenbeck et al., 2001; Lewis and Swofford, 2001). The relationship of PP to other measures of support (e.g. maximum likelihood or parsimony bootstrap) is controversial. Various authors have found that posterior probabilities in Bayesian analysis can overestimate support, especially when concatenated gene sequences are used (Suzuki et al., 2002; Wilcox et al., 2002; Simmons et al., 2004); but Alfaro et al. (2003) found that PP supports more correct monophyletic groups and is a less biased predictor of phylogenetic accuracy than bootstrapping methods.

Direct optimization

Phylogenetic analyses based on dynamic alignments were performed using the direct optimization method as implemented in POY ver. 3.0.11 (Wheeler and Gladstein, 2000). Analyses using different combinations of gap opening, gap extension, and transversion/transition costs (listed in Table 2) explored sensitivity to these assumptions (Wheeler, 1995). Thereafter, we used the incongruence length difference (ILD) (Mickevich and Farris, 1981) to select the parameter set that maximized congruence of data partitions as reference (Wheeler and Hayashi, 1998).

Analyses were run at the Zoological Museum of the University of Copenhagen (http://internal.binf.ku.dk/

Table 2
Summary of the results of the parsimony analyses of the alignments obtained with Clustal X under different parameter combinations and similarity values with the results of the elision matrix. The preferred combinations are given in bold

16S						18S						28S					
GOP/GEP	T	L	gaps	CF	SD	GOP/GEP	T	L	gaps	CF	SD	GOP/GEP	T	L	gaps	CF	SD
8/2	**1**	**1802**	**148**	**32**	**16**	**8/2**	**75**	**1138**	**62**	**22**	**17**	**8/2**	**53**	**596**	**42**	**19**	**15**
8/4	19	1816	138	7	57	8/4	88	1116	57	8	40	8/4	80	592	39	18	27
20/2	1	1835	107	9	62	20/2	12	1157	51	10	55	20/2	96	618	30	17	18
24/4	6	1869	88	8	58	24/4	12	1163	50	9	55	24/4	136	621	29	16	21
24/6	2	1868	88	8	58	24/6	4	1169	48	10	55	24/6	16	618	27	16	33
Elision	1	9221				Elision	2	5726				Elision	16	3050			

GOP/GEP, Gap opening cost/gap extension cost; T, number of most parsimonious trees; L, tree length; gaps, number of gap absence/presence characters added; CF, Consensus fork; SD, Symmetric difference.

IT:Cluster), using a cluster of 236 P4 processors at 2.4 GHz connected in parallel with PVM software and the parallel version of POY (commands `-parallel -controllers 3 in effect`). The heuristic search strategy involved quickly building 10 trees by random taxon addition (`-buildsperreplicate 10 -buildspr -buildtbr -approxbuild -buildmaxtrees 2`), followed by spr and tbr branch swapping holding one cladogram per round (`-sprmaxtrees 1 -tbrmaxtrees 1`). Two rounds of tree fusing (`-treefuse -fuselimit 10 -fusemingroup 5`) and tree drifting (`-numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10`) were added to increase efficiency, holding up to five trees per round (`-maxtrees 5`), and saving the most diverse cladograms from each island (`-fichtrees`). All cladograms found within 0.5% of the minimum tree length (`-slop 5 -checkslop 10`) were examined to exclude suboptimal trees due to tree-length miscalculations (POY uses some shortcuts to speed up tree evaluation). This strategy was repeated 100 times (`-random 100`) and a maximum of 50 trees was retained (`-holdmaxtrees 50`). Clade support was assessed by means of jackknife proportions using 100 randomly resampled matrices, with a probability of character deletion of $1/e$ (default option). Individual search strategies involved taking the best tree from five rounds of random additions of taxa. Cladograms obtained in each search under a particular parameter cost scheme were pulled together and subjected to a new run of tree fusion for each parameter set. We repeated this procedure until the same minimum length trees were obtained in two successive rounds. Clade support was assessed by means of jackknife proportions using 500 randomly resampled matrices, again with a probability of character deletion of $1/e$.

Simultaneous analyses of morphology and DNA sequence data

Morphological and molecular characters were concatenated in a single data matrix using WinClada. Taxa that could not be DNA sequenced or scored for morphology were completed by adding missing data. The combined morphological and molecular data matrix was analysed with parsimony and Bayesian inference, using the programs and searching strategies described above. The Mk (Markov k) model of Lewis (2001), as implemented in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003), was defined for the morphological and absence/presence gap partitions. This new model allows definition of ordered multistate characters and deals with the coding bias introduced by scoring only parsimony-informative characters (Nylander et al., 2004). All non-informative characters were thus removed from the morphology and gap data sets before running Bayesian searches.

Results

Morphological analyses

Equal weights. Heuristic searches in TNT with 1000 replications, holding 500 trees during each replication, generated 12 trees of length 658. Since optimal trees were found in only 442 out of 1000 replications, we broadened the search to include more replications and to hold more trees per replication. Various combinations, including extremes such as 5000 replications, holding 50 trees per replication, and 50 replications, holding 5000 trees per replication, resulted in the same 12 trees of length 658. Additional analyses carried out with TNT, using the Ratchet (Nixon, 1999) under "New Technology Search", with the default settings (up-weighting probability 4; down-weighting probability 4) and running until consensus stabilized 35 times and minimum length trees were found at least 100 times, found the same 12 trees. Heuristic searches in PAUP* using the same search parameters as in TNT also resulted in the same 12 trees, which comprised two tree islands (with four and eight trees, respectively). All 12 trees are fully resolved and disagree in only three areas of the cladogram, involving nine, four, and four genera, respectively. Figure 3 shows the strict consensus, and we discuss supported groups at length below. This analysis supported the monophyly of linyphioids, Pimoidae, Linyphiidae, and Erigoninae. *Stemonyphantes* was sister to all other linyphiids, within which Mynogleninae was sister to the rest. Micronetinae are paraphyletic with respect to erigonines and linyphiines are polyphyletic.

Implied weighting. Heuristic searches in TNT under implied weighting using the above search parameters found one tree for each k value studied (3, 6, 10, and 15). Optimal topologies under $k = 3$ and 6 differ considerably from those under equal weights (see below). The $k = 15$ tree is the same as tree 10 under equal weights, thus 658 steps long. The $k = 10$ tree is nearly the same (length 659), but *Synotaxus* jumps from being sister to *Mysmena–Alaranea* to become sister to *Nesticus*, *Theridion*, and *Steatoda*. Linyphiid phylogeny is exactly the same under equal and implied weighting with $k = 10$ or 15. Using k values of 50 and 100, we find the same linyphiid topology as the trees resulting from $k = 10$ and 15. Strongly down-weighted k values (3 and 6) find many of the same relationships as under equal weights: monophyly of linyphioids, Pimoidae, Linyphiidae, Erigoninae, Mynogleninae, basal placement of *Stemonyphantes* as sister to all other linyphiids, and mynoglenines basal lineage within the latter. But mynoglenines are sister to Erigoninae

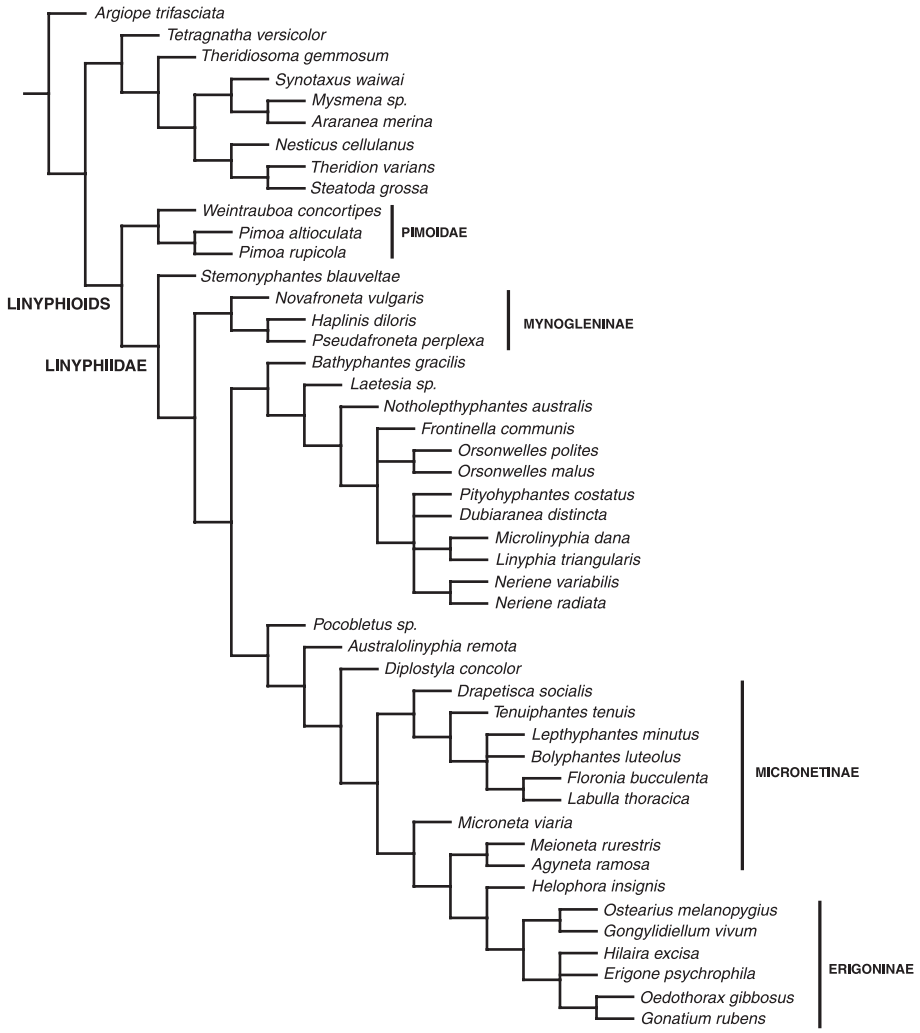

Fig. 3. Strict consensus of 12 most parsimonious trees of morphological data under equal weights (most parsimonious trees, L = 658; CI = 0.28; RI = 0.58).

under $k = 3$ and sister to *Australolinyphia* and a clade with *Orsonwelles* and *Frontinella* under $k = 6$. The $k = 3$ and $k = 6$ trees do not support linyphiine or micronetine monophyly.

Molecular analyses

Fixed analyses. Partial analyses of each gene fragment (mitochondrial genes were considered a single fragment as they are linked in the genome) yielded the following results: two trees of 3875 steps for the mitochondrial genes, eight trees of 749 steps for H3, eight trees of 1112 steps for 18S, and 56 trees of 593 steps for 28S (supporting information summarized in Fig. 4). For all ribosomal genes, gap costs of GOP = 8 and GEP = 2 best represented the elision matrix as measured by the consensus fork index and symmetric differences (Table 2). CO1 and H3 sequences were concatenated to the preferred alignment for each ribosomal gene. Analyses of the combined data matrix with gaps coded as present/absence resulted in eight trees of 6597 steps. Analyses under implied weights yielded one tree of fit 357.75850 (6615 steps), one tree of fit 75.81046 (6598 steps), and one tree of fit 1011.68 (6597 steps) for $k = 6$, 50, and 100, respectively. As expected, less down-weighting of homoplasy (higher k values) resulted in topologies more similar to equally weighted trees. Some equally weighted trees were topologically closer (as measured by the symmetric distance metric) to the implied weight trees than to other equally weighted trees. The $k = 100$ tree was identical to one of the eight equal weight trees (Fig. 4). The inclusion of the cyatholipid *Alaranea* renders Linyphiidae as paraphyletic, although none of the nodes linking *Alaranea* to more inclusive Linyphiidae clades is supported (jackknife < 50%). In general, the outgroup topologies do not correspond to current knowledge of araneoid systematics (Griswold et al., 1998; Lopardo and Hor-

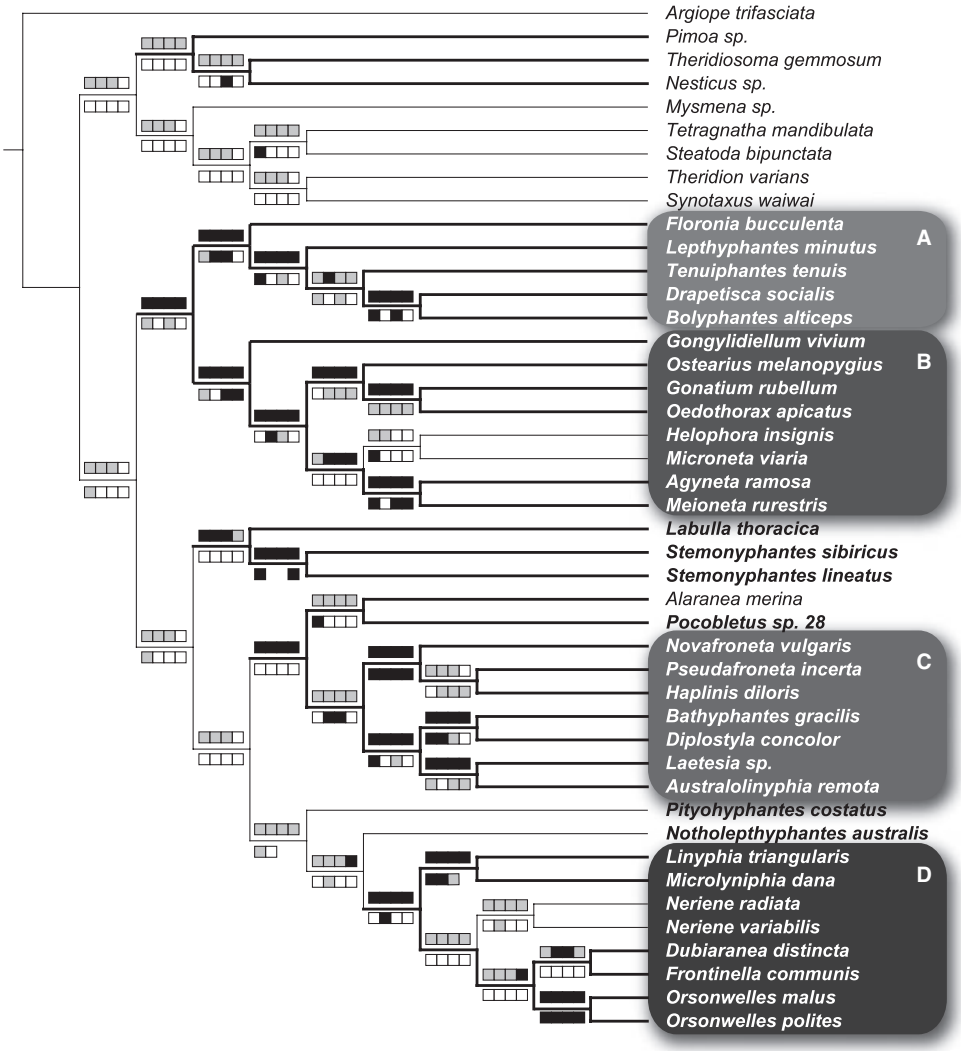

Fig. 4. One of eight most parsimonious trees (MPTs) obtained from the parsimony analyses under equal weights of the fixed, combined molecular data set with gaps coded as presence/absence and the single one obtained under implied weights ($k = 100$). Thick lines indicate branches that appear in the strict consensus of the eight MPTs. Squares above branches indicate support level recovered from analyses under equally weighted parsimony and implied weights with $k = 100$, 50 and 6, respectively. Squares below branches indicate support level recovered from partial analyses of each gene fragment: mitochondrial, 18S, 28S, and H3, respectively. Black squares indicate clades with parsimony jackknife support values above 70%; grey squares indicate clades recovered in the analyses but either not supported or with supports below 70%; white squares are clades contradicted by particular partial analyses. Clades discussed in the text are highlighted in shaded boxes.

miga, 2008). None of the clades, however, is supported by jackknife measures. Several highly supported clades occurred among Linyphiidae representatives. Clade A includes *Floronia*, *Lepthyphantes*, *Tenuiphantes*, *Drapetisca*, and *Bolyphantes*; clade B includes *Microneta*, *Helophora*, *Agyneta*, *Meioneta*, and the erigonines (the latter subfamily is never recovered as monophyletic). Clade C includes *Australolinyphia*, *Laetesia*, *Diplostyla*, *Bathyphantes*, and Mynogleninae; and, finally, clade D includes *Linyphia*, *Microlinyphia*, *Dubiaranea*, *Frontinella*, *Neriene*, and *Orsonwelles*. All analyses strongly support clade A as sister to clade B. The topology of the former shows varying levels of support. The positions of *Stemonyphantes*, *Labulla*, *Pityohyphantes*, *Notholepthyphantes*, and *Pocobletus* are poorly supported and sometimes contradicted under different weighting schemes.

The Bayesian analysis of the combined fixed molecular alignments closely resembled the parsimony result (Fig. 5): low support for the outgroup topology, paraphyly of Linyphiidae and Erigoninae, high support for clades A, B, C and D, and clade A sister to clade B. The placements of *Stemonyphantes*, *Labulla*, and *Pocobletus* were poorly supported. Unlike parsimony, the Bayesian analysis strongly supported *Pityohyphantes* and *Notholepthyphantes* in a group with clade D. Pimoidae

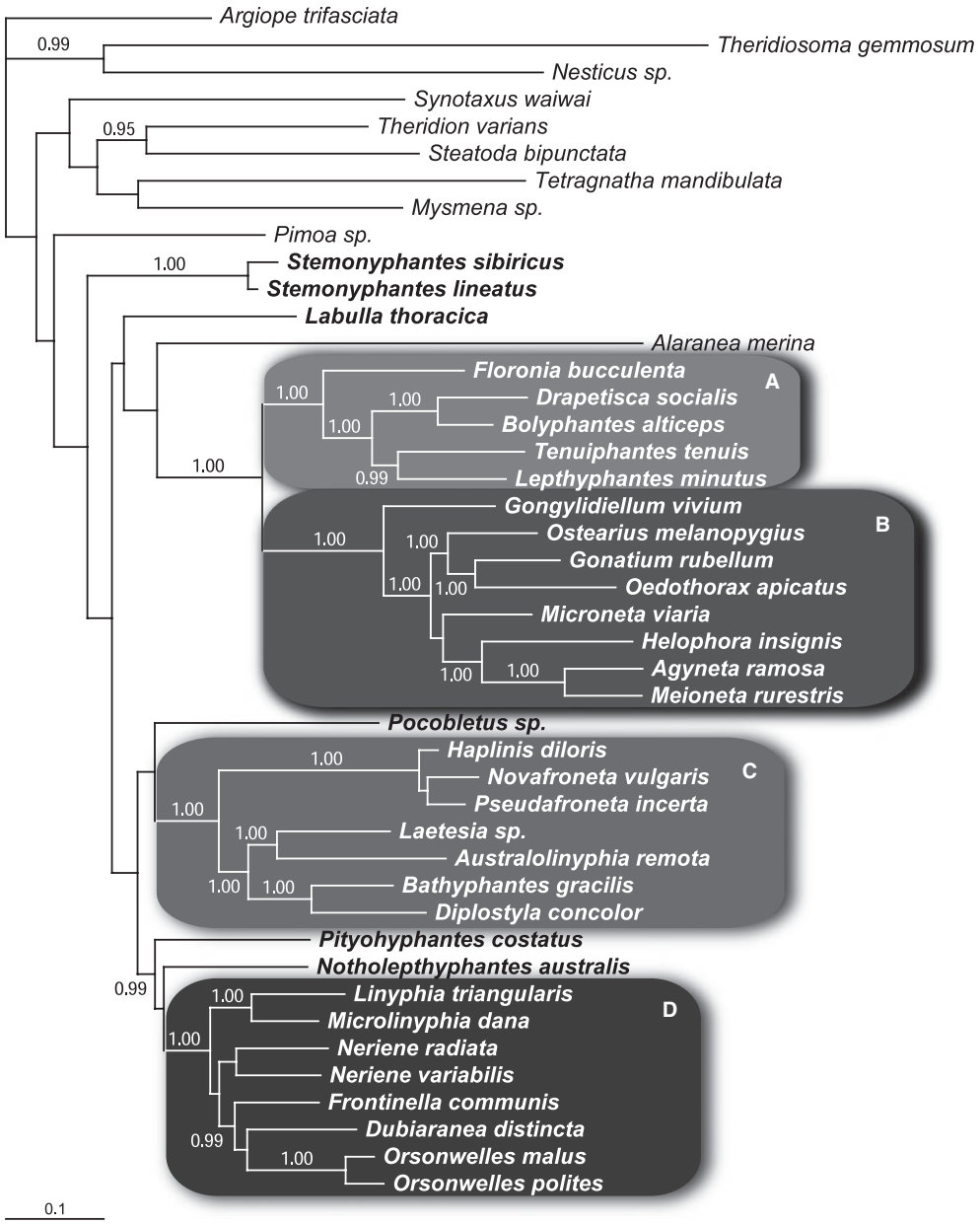

Fig. 5. Majority rule consensus of the trees sampled by the Bayesian MCMCMC (Metropolis-coupled Markov chain Monte Carlo) analyses of the combined fixed molecular alignments with unlinked models for each gene fragment and gaps scored as absence/presence. The first 10% of generations was removed as burn-in. Values at branches correspond to posterior probabilities above 95%.

and linyphiids + *Alaranea* were monophyletic, and *Stemonyphantes* was sister to the remaining linyphiids + *Alaranea*, albeit with low support.

Dynamic optimization analyses. Results for the five parameter cost schemes under direct optimization are summarized in Table 3. A gap opening cost twice the costs of gap extension, and nucleotide transformations maximized congruence among data sets as measured by the ILD (Fig. 6). The outgroup topology and linyphiid basal relationships were very sensitive to parameter values and poorly supported. Linyphioids, Linyphiidae and Erigoninae are not monophyletic. *Stemonyphantes* falls among the outgroups. On the other hand, clades A, B, A + B, C, and D are recovered and receive high jackknife support values in most parameter combinations.

Simultaneous analyses of morphology and DNA sequence data

Fixed analyses. All simultaneous analyses of morphology and fixed sequence alignments recovered

Table 3
Summary of the results of the analyses performed under direct optimization (dynamic homology)

	comb		mol		CO1		H3		16S		18S		28S		morph				
Param	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	ILD_m	ILD_{mm}	ILD_{ch612m}
1(1)11	8	8201	1	7448	15	1994	16	749	3	1868	40	1217	9	1288	12	658	0.0446	0.012	0.052
2(1)11	12	9333	3	7859	15	1994	16	749	3	2045	4	1299	47	1435	12	1316	0.0429	0.017	0.053
2(1)21	3	12520	1	11016		2958		1009	2	2978	34	1701	42	1858	12	1316	0.0465	0.015	0.056
4(1)21	3	14679	2	11731		2958		1009	3	3300	12	1850	24	2104	12	2632	0.0435	0.022	0.056
8(1)42	2	28673	1	22847		5916		2018	1	6254	1	3587	24	4062	12	5264	0.0442	0.020	0.055

Param, analysis parameters, gap opening (gap extension):transversion:transitions; Comb, Simultaneous analyses of morphology + molecules; mol, Simultaneous analyses of all gene fragments; T, number of most parsimonious trees; L, length of the most parsimonious trees; ILD_m, ILD among gene fragments; ILD_{mm}, ILD between the morphology and the molecular partitions; ILD_{ch612m}, ILD among all data sets separately; morph, partial analyses of morphology.

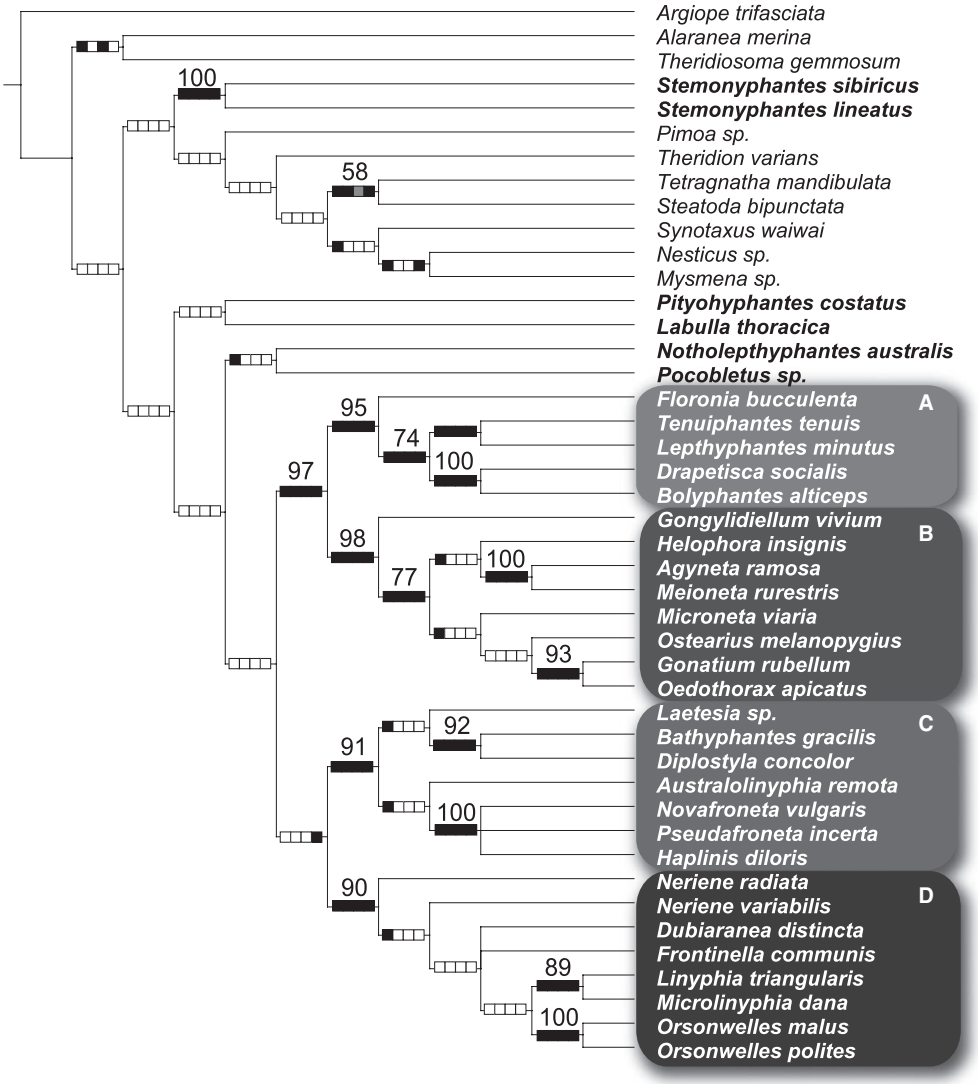

Fig. 6. Strict consensus of the three trees of 7859 steps resulting from direct optimization of all genes with gap opening (GOP) cost twice those of gap extension (GEP) and base transformations. Boxes indicated presence in all trees (black), some trees (grey), or no trees (white) of the particular clade under four cost combinations (GOP:GEP:TV:TS): 1111, 2121, 4121, and 8142. Values above branches indicate support based on 500 jackknife replicates under preferred cost scheme.

Linyphiidae as monophyletic and sister to Pimoidae, and *Stemonyphantes* as sister to remaining linyphiids, but only Bayesian inference (Fig. 7) strongly supported these groupings. Clades A, B, A + B, C, and D are strongly supported in all analyses. Conversely, the placement of *Labulla* is never strongly supported. *Notholepthyphantes* and *Pityohyphantes* form a monophyletic group with clade D with high PP but poor jackknife support, and *Pocobletus* is sister to clade C, also with poor jackknife support. The erigonines are paraphyletic because *Gongylidiellum* falls among micronetines (although remaining erigonines are monophyletic). Overall, Bayesian inference provides much higher support (posterior probabilities) than parsimony jackknife.

Dynamic optimization analyses. The direct optimization analysis of the molecular and morphological partition under equal costs for gap opening, extension,

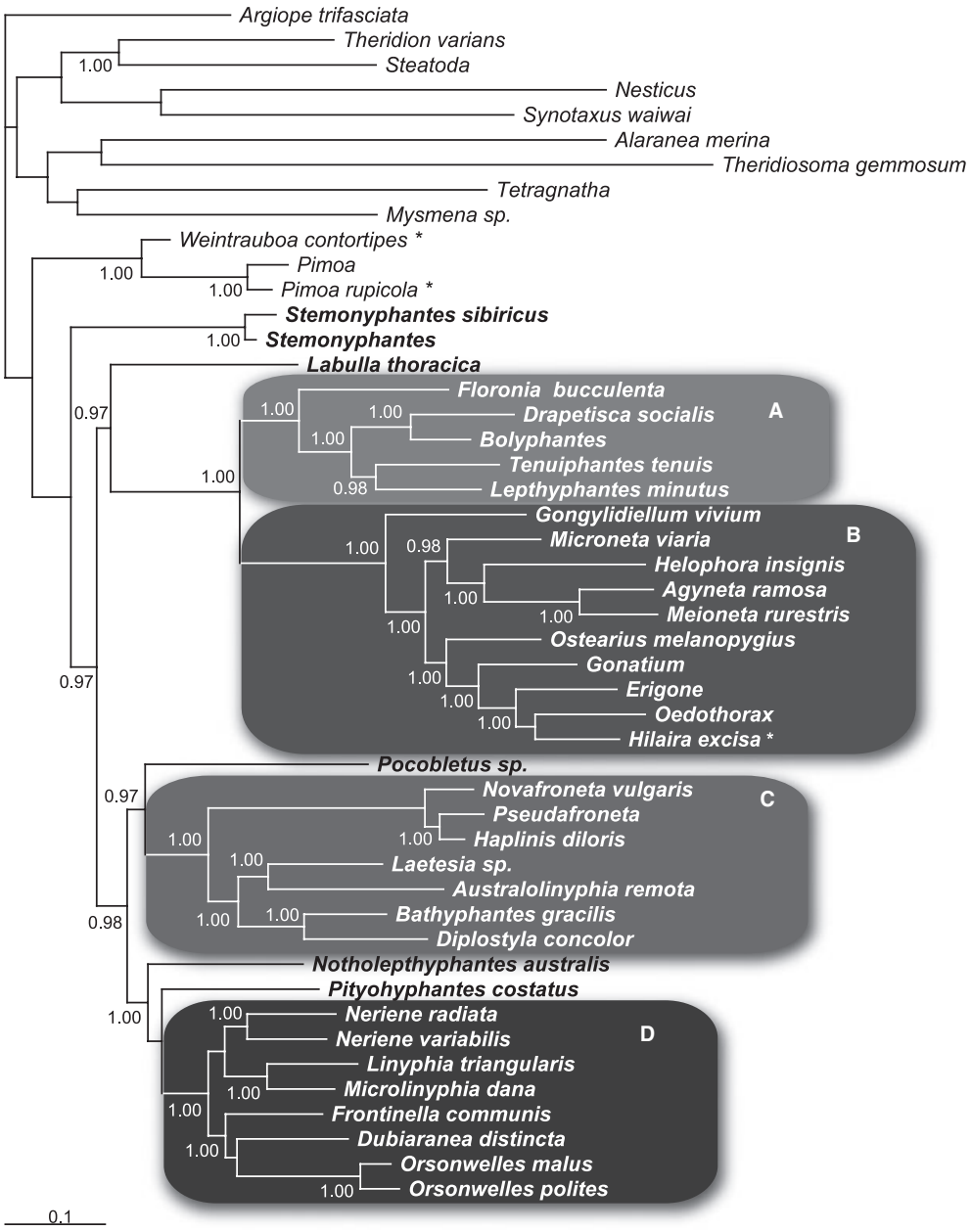

Fig. 7. Majority rule consensus of the trees sampled by the Bayesian MCMCMC analyses of the combined fixed molecular alignments and morphology, with unlinked models for each gene fragment, gaps scored as absence/presence, and the first 10% of generations removed as burn-in. Values at branches correspond to posterior probabilities above 95%. Values above branches are parsimony jackknife supports based on 1000 replicates with any taxa lacking any gene sequence removed (marked with an asterisk). Chimera taxa are referred to by the genus name.

and nucleotide transformations maximized congruence among data sets as measured by the ILD (Fig. 8). Results were quite sensitive to parameter values. Most basal relationships appear in only one or few parameter combinations. Jackknife support is low and mostly concentrated at more distal clades. Analyses are incongruent mainly due to *Labulla*, *Pityohyphantes*, *Notholepthyphantes*, and *Pocobletus* shifting among the different analyses. Most analyses do recover Linyphiidae, Erigoninae, and *Stemonyphantes* as sister to all other linyphiids, although the best parameter combination shows low jackknife support for all these groups. Some trees even reject Linyphiidae monophyly by finding *Stemonyphantes* as sister to, or inside, Pimoidae. Most parameter combinations recover clades A, B, A + B, C, and D.

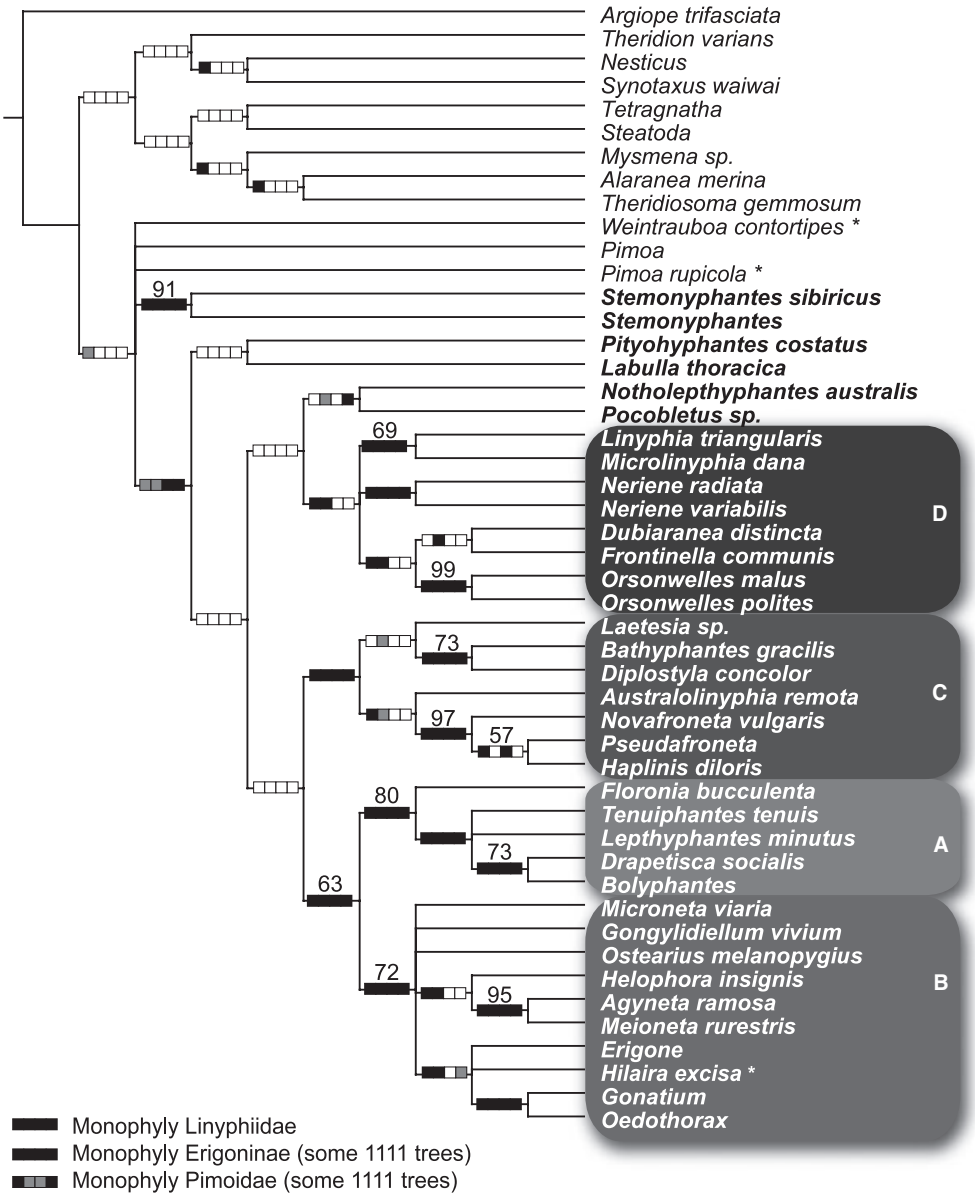

Fig. 8. Strict consensus of the eight trees of 820 steps resulting from direct optimization with equal cost for all parameters. Boxes indicated presence in all trees (black), some trees (grey) or no trees (white) of the particular clade under four cost combinations (GOP:GEP:TV:TS): 2111, 2121, 4121, and 8142. Support for clades not shown in the figure but recovered in alternative parameter cost analyses are shown at the bottom left. Values above branches indicate support based on 500 jackknife replicates under preferred cost scheme. Taxa lacking gene sequences are indicated with an asterisk. Chimera taxa are referred to by the genus name.

Discussion

This is the first time that DNA sequence data for all linyphiid subfamilies have been analysed. The present study also provides the broadest taxonomic sample, at the subfamilial level, analysed thus far using morphological data within a cladistic framework. Spider systematists in general, and araneoid workers in particular, have been relatively slow in using nucleotide sequences for phylogenetic inference compared with systematists working on other terrestrial arthropod groups (but see Gillespie et al., 1994, 1997; Hedin, 1997a,b; Hormiga et al., 2003; Garb et al., 2004; Arnedo et al., 2001, 2004, 2007; Rix et al., 2008; Alvarez-Padilla et al., in press; Blackledge et al., 2009). Multigene sequence data are particularly useful for linyphiid systematics because of the relatively homogeneous somatic morphology of linyphioids, at least compared with other araneoid lineages such as theridiids or araneids, which vary dramatically in both genitalic and somatic morphology (Scharff and Coddington, 1997; Agnarsson, 2004). In linyphiids, genitalic characters clearly have an "upper limit" when reconstructing the generic relationships in the family. Spider genitalic morphology can be extremely informative phylogenetically (Huber, 2004), and linyphiids have some of the most complex genitalia in spiders. For example, linyphiid male palps are made of numerous sclerites that seem to be evolving at different rates and, to a large extent, independently of other palpal sclerites (Hormiga, 2000, 2003; Hormiga and Scharff, 2005). Some male genitalic variation correlates with female genitalic morphology, most notably embolus length (the male intromittent sclerite) and female copulatory duct length. Unfortunately, at higher levels relatively few homologies can be hypothesized, and some of these entail long transformation series with numerous autapomorphic states. (This is, of course, why genitalic morphology works so well for species identification and circumscribing groups of species in genera.) An additional hurdle is the difficulty of establishing palpal sclerite homologies across species, especially at higher phylogenetic levels (Hormiga and Scharff, 2005; Ramírez, 2007; Agnarsson and Coddington, 2008). We do, however, stress that systematists have by no means exhausted the study of anatomical variation in linyphiids (e.g. electron microscopy data are available for a relatively small fraction of species). Clearly, new character systems, such as nuclear and mitochondrial gene sequences, will help to elucidate linyphioid phylogeny. Nevertheless, our taxonomic sample still seems fairly small when compared with the extraordinary taxonomic diversity of Linyphiidae (4345 species described so far, classified in 576 genera; Platnick, 2008) and consequently it can provide only a modest starting point towards the classification of the family.

We have analysed our data using a variety of analytical approaches and data partitions in order to better understand the phylogenetic patterns that underlie our observations. Our results demonstrate that these different approaches often produce different cladistic hypotheses, but this should hardly be surprising. We have preferred to use as a working hypothesis the results of the total evidence analysis under direct optimization. We have already discussed our preference for direct optimization (see our comments under "Materials and methods"). In addition, we have investigated the sensitivity of our results to analytical methods because we are aware that our approach is not universally shared, and that some methods, even if theoretically sound, may not have been implemented efficiently (e.g. Lehtonen, 2008). Our preference for simultaneous analysis of the total available evidence is based on the argument of maximization of explanatory power, and has been discussed profusely in the literature (e.g. Nixon and Carpenter, 1996; Kluge, 1998). Wheeler and Hayashi (1998) have argued that the parameter set-maximizing congruence should be chosen preferentially over others. This rationale is based on the premise that the cladogram that minimizes character incongruence will also be the most parsimonious, regardless of character partitions (Giribet and Wheeler, 2007), although there has been some philosophical criticism of that approach (e.g. Grant and Kluge, 2005). We prefer the combined analysis using direct optimization under 1111 costs as the working hypothesis for linyphiid relationships (Fig. 8; this is a strict consensus of the eight optimal trees), and the following discussion is based on that result. The morphological support for linyphioid higher groups (Fig. 9) is based on the optimization of those characters on one of the eight optimal trees. This cladogram was selected because it does not refute the monophyly of either Linyphiidae nor Pimoidae, although the base of linyphioids is a trichotomy (due to the position of *Stemonyphantes*). This cladogram (Fig. 9) is 89 steps longer than the 12 optimal cladograms resulting from the analysis of the morphological partition (strict consensus of which is depicted in Fig. 3).

Outgroups

Some of the phylogenetic relationships among the taxa outside the linyphioids are different from the cladistic hypothesis of Griswold et al. (1998) and Lopardo and Hormiga (2008). Our study has been designed to emphasize taxonomic sampling within linyphioids, but nonetheless we have included nine representatives of eight araneoid families outside linyphiids and pimoids. While we think that such a small taxonomic sample is insufficient to study araneoid interfamilial relationships, we note that previous and ongoing analyses that have included sequence data

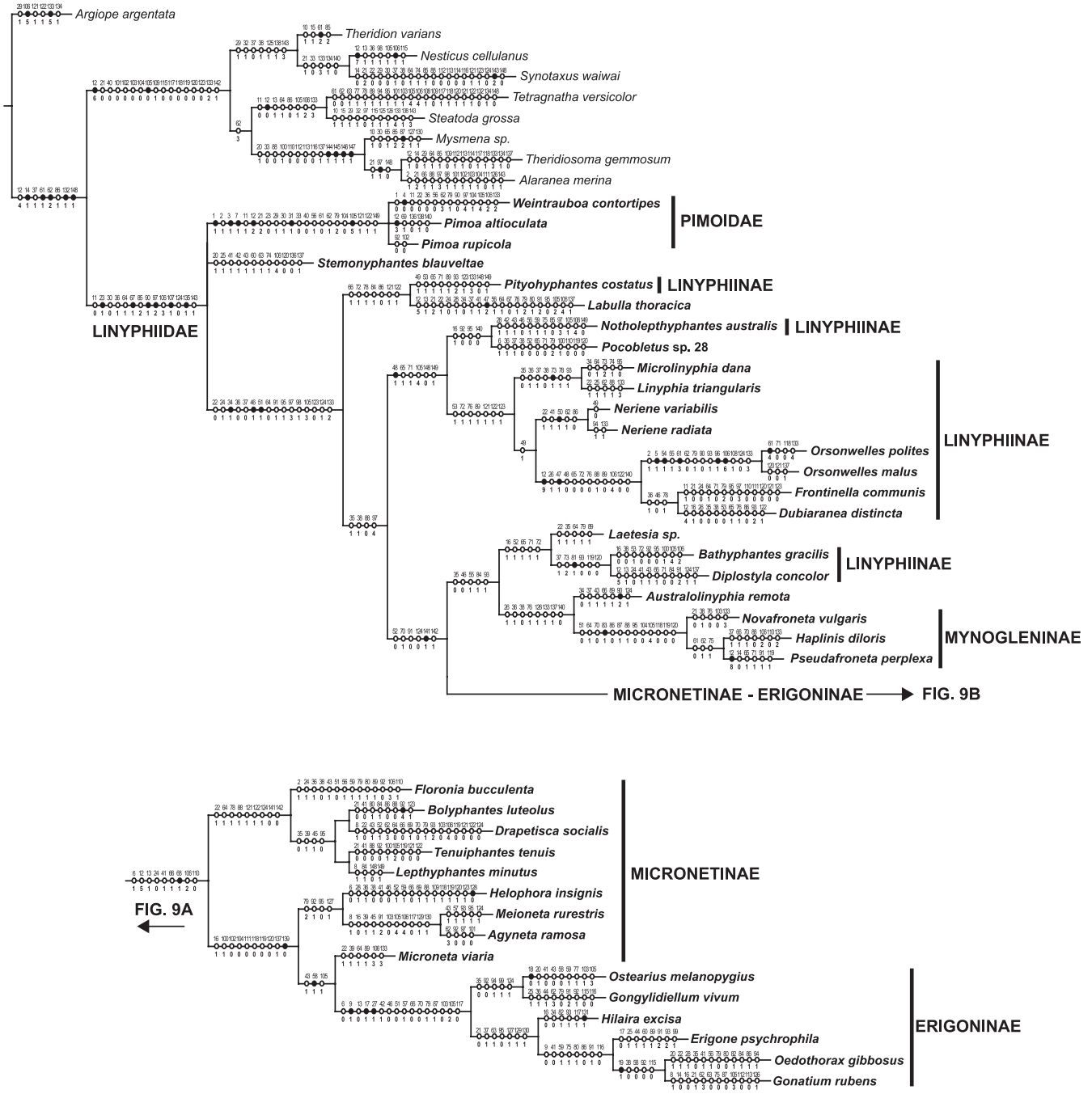

Fig. 9. One of the eight trees of 8201steps from Fig. 8 with morphological character changes mapped using Farris optimization (length 747 steps, CI = 0.25 and RI = 0.51). Morphological characters were not scored for *Stemonyphantes sibiricus*, and thus the species was removed from the tree. Chimera taxa are referred to by the genus name.

across araneoid families (Arnedo et al., 2004; Rix et al., 2008; Alvarez-Padilla et al., in press; Blackledge et al., 2009) have also failed to corroborate some of the clades proposed by Griswold et al. (1998). It seems that the current selection of molecular markers is appropriate for recovering relationships within araneoid families, but deeper cladogenetic events are proving more difficult to reconstruct with those loci. In addition, as discussed by Lopardo and Hormiga (2008) in reference to their own analysis (which was an extension of the work of Griswold et al.), "it would seem hardly surprising to find that addition of representatives of missing putative

araneoid families (such as mimetids or malkarids) and of new characters will change the phylogenetic relationships that result from our analyses''. Progress in araneoid interfamilial relationships will require a substantially higher sampling density, multiple data sets, and a thoughtful selection of taxa outside Orbiculariae. This is well beyond the goals of our study.

''Linyphioids''

Linyphioid monophyly (Pimoidae plus Linyphiidae) is generally supported by the combined analysis, although not all parameter sets agree. The morphological analysis also corroborates linyphioid monophyly, as do the quite similar matrices analysed by Hormiga and colleagues (Hormiga, 2003, 2008; Hormiga et al., 2003; Hormiga and Tu, 2008). When molecular data alone are analysed, neither linyphioid nor linyphiid monophyly is recovered (Fig. 6). Under direct optimization (DO; any cost explored), *Stemonyphantes* and *Pimoa* always fall among the outgroups, but no placement is robustly supported; remaining linyphiids form a clade (Fig. 6). The Bayesian analysis of the molecular partition places the cyatholipid *Alaranea* relatively basally within the linyphioids, but again none of the nodes involved has robust support (Fig. 5). Linyphioid morphological synapomorphies include the intersegmental paracymbium (reversed in some pimoids), sparse setae on the prosoma, cheliceral stridulatory striae, patella–tibia autospasy, enlargement of the mesal cylindrical gland spigot base on the female posterior lateral spinnerets, and the sheet web.

Pimoidae

Six out of the eight preferred cladograms (Fig. 8) support the monophyly of Pimoidae, as do the morphological data (Fig. 3). We lacked sequence data for *Weintrauboa* and therefore could not test pimoid monophyly with the molecular partition alone. Pimoid morphological synapomorphies include the ectal marginal cymbial process, cymbial cuspules (or modified macrosetae), pimoid cymbial sclerite, tegulum ventrally oriented relative to the subtegulum in the unexpanded palp, pimoid embolic process, absence of a column or a membraneous joint at the base of embolus, and two prolateral trichobothria in the male palpal tibia.

Linyphiidae

Linyphiid monophyly is supported by all DO combined analyses except 1111, but one of the 1111 trees shows a Pimoidae–*Stemonyphantes*–Linyphiidae trichotomy and thereby does not contradict linyphiid monophyly (Fig. 9). Neither the DO (Fig. 6) nor the Bayesian analysis (Fig. 5) of the molecular partition alone supports linyphiid monophyly (see comments under ''Linyphioids''). Linyphiids' morphological synapomorphies include the suprategulum, absence of the araneoid median apophysis and conductor, and presence of a radix in the embolic division (alternatively, the radix may have evolved at the base of Araneoidea and was independently lost in other lineages).

Linyphiid clades

Stemonyphantes has long been suggested as the most basal linyphiid lineage based on morphology (Wunderlich, 1986; Hormiga, 1994a; Miller and Hormiga, 2004). As noted above, the combined analysis under DO (using 1111) places *Stemonyphantes* as sister to pimoids or in an unresolved trichotomy (Figs 8 and 9). All other parameter sets (Fig. 8), and the Bayesian combined analysis (Fig. 7), recover *Stemonyphantes* as sister group to all other linyphiids, as does morphological evidence alone (Miller and Hormiga, 2004; see also Fig. 3). Synapomorphies for linyphiids except *Stemonyphantes* include the presence of an embolic membrane, a terminal apophysis and a lamella characteristica in the male palp, and the fused sternum–labium attachment. Instability in the placement of *Labulla* and *Pityohyphantes* affects the topological structure of the base of the linyphiid clade, distal to *Stemonyphantes* (Fig. 8). The phylogenetic placement of *Labulla* has long been an intriguing question (e.g. Millidge, 1993). In a recent morphological analysis based on many fewer taxa (micronetines were represented by *Tenuiphantes* and *Bolyphantes* in their study), Hormiga and Scharff (2005) found *Labulla* to be highly ''autapomorphic'' and sister to a micronetine clade. This morphological analysis (Fig. 3) also places *Labulla* with *Tenuiphantes* and *Bolyphantes*, but the combined analysis places it more basally as sister to *Pityohyphantes* (Fig. 8). Unfortunately, more characters and DNA sequences do not settle the question of *Labulla* because all placements are weakly supported (Fig. 8).

Few detailed cladistic hypotheses of relationships above the genus level exist for Linyphiidae, but the monophyly of Mynogleninae is not controversial. Mynoglenines can be easily diagnosed morphologically by the presence in both sexes of clypeal sulci (or pits) with cuticular pores served by glands; the group is restricted to Africa, New Zealand and Australia (Blest and Taylor, 1977; Blest, 1979; Hormiga, 1994b, 2000). In our study, mynoglenine monophyly is supported by virtually all analyses and data partitions. Our results suggest that the sister group of mynoglenines is the Australian monotypic genus *Australolinyphia*.

Erigoninae is the largest linyphiid subfamily by far in terms of species and genera. Most matrix-based phylogenetic work in linyphiids has focused on erigonine relationships (Hormiga, 2000; Miller and Hormiga,

2004). In our analyses, erigonines are monophyletic under all costs except three out of eight of the 1111 trees. Erigoninae are paraphyletic with respect to a micronetine clade in the Bayesian combined analysis (Fig. 7), the Bayesian molecules-only analysis, and the DO analysis (Figs 5 and 6, respectively). In the combined analysis, using both parsimony under DO and Bayesian analysis (Figs 8 and 7, respectively), the paraphyly of Erigoninae is caused by the placement of *Gongylidiellum vivum*, which "jumps" to a more basal position within a clade of erigonine and micronetine taxa. The morphological matrix supports the monophyly of erigonines (including *Gongylidiellum*) with *Helophora* as its sister group (Fig. 3). The total evidence analysis suggests that the sister group of Erigoninae is *Microneta* (Fig. 8).

The subfamily Micronetinae includes some of the most common and abundant non-erigonine linyphiids in Europe, such as *Lepthyphantes* and its close relatives. During the past decade or so, micronetine taxonomy has been extensively revised by Michael Saaristo and Andrei Tanasevitch. Much of their work, based almost exclusively on genitalic morphology, has focused on recircumscribing the large and poorly circumscribed genus *Lepthyphantes* (e.g. Saaristo and Tanasevitch, 1993, 1996, 1999, 2001). Unfortunately, none of their generic revisions has used an explicit methodology for circumscribing higher taxa, and therefore their grouping hypotheses are difficult to evaluate cladistically. In general, Saaristo and Tanasevitch tend to propose morphologically homogeneous species groups of "*Lepthyphantes*" with similar genitalic structures. All parameter combinations under DO (Fig. 8) group the micronetine representatives in a clade that includes the Erigoninae and *Helophora*; the morphological (Fig. 3) and molecular partitions (Fig. 6) also support the monophyly of this group. Morphology under equal weights (Fig. 3) includes *Labulla thoracica* (as sister to *Floronia bucculenta*) in the same clade.

It seems that an erigonine–micronetine lineage is robustly supported, but as a whole micronetines (including *Helophora*) are at best paraphyletic with respect to erigonines, or polyphyletic. As discussed above, erigonines appear monophyletic (except *Gongylidiellum*, see above) but no analysis supported the monophyly of Micronetinae *sensu* Saaristo and Tanasevitch (1996). Some micronetine taxa (including *Microneta*) are more closely related to the erigonine clade than to other micronetines. The molecular partition supports the monophyly of a group of micronetines that includes *Floronia*, *Tenuiphantes*, *Lepthyphantes*, *Drapetisca*, and *Bolyphantes* (Clade A, Figs 5 and 6). The morphological partition supports the same lineage but including *Labulla* (Fig. 3). Micronetines (including *Helophora*) are paraphyletic in five, and polyphyletic in three, of the eight most parsimonious trees (MPTs) under direct optimization (1111; Fig. 8). In all five trees in which erigonines are monophyletic, *Microneta viaria* is the sister group of the erigonine clade (in the other three trees *M. viaria* is sister to a core clade of erigonines). The genus *Helophora* is the sister group of the *Meioneta* plus *Agyneta* clade. Neither Millidge (1977, 1984, 1993) nor Saaristo and Tanasevitch (1996) included *Helophora* in Micronetinae (or Lepthyphantinae), although Merrett (1963) had suggested that it could possibly belong to a group that included all the micronetine genera that he studied in his monograph on linyphiid palpal morphology (his "Syedruleae" or "Group A"). *Helophora insignis* had been incorrectly reported as having a haplotracheate tracheal system (the putatively plesiomorphic condition for linyphiids; see Blest, 1976 and Discussion in the next section). We should mention that, following Blest (1976), we had initially coded in our character matrix *Helophora* as haplotracheate, but upon finding that the preliminary analyses of the sequence data placed *Helophora* as sister to a clade of desmitracheate taxa, we examined the tracheal system of *H. insignis* and discovered that in fact it has an intermediate tracheal system, with the pair of median tracheae branching within the abdomen.

The recently erected subfamily Ipainae (Saaristo, 2007) has been hypothesized to be the sister group of Micronetinae on the basis of the presence of Fickert's gland in the male palp (character 45), although, as pointed out by Saaristo, not all ipaines have this gland. As circumscribed by Saaristo (2007), Ipainae includes the genera *Ipa*, *Epibellowia*, *Metaleptyphantes*, *Solenysa*, *Uralophantes*, *Wubanoides*, and *Epigytholus*. None of these genera is represented in our character matrix. Although Saaristo does not offer an explicit phylogenetic definition of Ipainae (that is, a conjecture supported in terms of synapomorphies), a number of ipaine features are potentially synapomorphic, including the characteristic movable epigynum. Unfortunately, Saaristo did not consider the somatic morphology (with the exception of the conspicuous metallic shine of the abdomen of some species) and invoked morphological differences between the genitalia of micronetines and ipaines to justify the erection of this new subfamily. Preliminary cladistic work on the position of *Solenysa* (Tu and Hormiga, unpublished), carried out by adding four species of *Solenysa* to the character matrix of Miller and Hormiga (2004), suggests that this presumably ipaine genus is nested within Erigoninae and therefore refutes the monophyly of Ipainae as circumscribed by Saaristo (*Solenysa* lacks Fickert's gland but has the movable epigynum; see Tu and Li, 2006). While it is possible that a core of Ipaine species form a monophyletic group, such lineage may very well fall within a subset of "micronetine" taxa. As discussed above, the data do not support the monophyly of Micronetinae *sensu* Saaristo and Tanasevitch (1996). Future work

should add representative taxa of Ipainae to the matrix to test its monophyly and phylogenetic position.

The monophyly of *Linyphia* and its close relatives (*Neriene*, *Microlinyphia*, *Dubiaranea*, *Orsonwelles*, and *Frontinella*), the "Linyphiini" of some authors, is supported by the combined analyses (Figs 7 and 8) as well as by various data partitions (e.g. "clade D" in Fig. 6), although its sister group remains ambiguous. Candidate taxa include *Notholepthyphantes*, *Pityohyphantes*, and *Pocobletus*, depending on the analysis. In the DO combined analysis, *Bathyphantes* and *Diplostyla* are sister taxa (Fig. 8), but equally weighted morphological data do not group them (Fig. 3; the $k = 1$ tree also recovers *Bathyphantes* plus *Diplostyla*). The former result is surprising, given how similar they are (*Diplostyla concolor*, the only species of the genus, had been often treated as a member of *Bathyphantes*; see Platnick, 2008). Presumably this is caused by the many highly homoplasious characters, as implied weights do recover this clade. The molecular partition strongly supports *Bathyphantes–Diplostyla*, regardless of analytical methods or alignment strategies (Figs 5 and 6). *Bathyphantes–Diplostyla* groups with *Australolinyphia*, *Laetesia*, and mynoglenines (with representatives from New Zealand; clade C in Figs 6 and 7). The monophyly of clade C is supported by the parsimony- and model-based analyses of the combined data under all treatments.

Character evolution

There are two somatic character systems whose evolutionary trends in linyphioids should be reassessed in light of the phylogenetic results reported here: the tracheal system and the cephalothoracic specializations. The evolution of the tracheal system in linyphiids has received a fair amount of attention (Blest, 1976; Millidge, 1984, 1986; Hormiga, 1994b, 2000; Miller and Hormiga, 2004). Within Araneoidea, the most common condition is two pairs of simple (unbranched) trunks extending anteriorly from the tracheal atrium. The trunks are confined to the abdomen, and the atrium opens to the exterior by means of a spiracle located anterior to the spinnerets. This "haplotracheate" (Millidge, 1984) arrangement has been known for more than a century (e.g. Lamy, 1902) and has been hypothesized as primitive for Araneoidea (Purcell, 1909). It has also long been hypothesized that that the haplotracheate condition is modified into a more elaborate tracheal system in some linyphiids and in other araneoid families (Lamy, 1902; Levi, 1967; Ramírez, 2000). Blest (1976) showed that most erigonines have medial tracheal trunks that branch (often extensively) into tracheoles. The tracheoles extend into the prosoma through the pedicel and into the appendages. Millidge (1984) coined the term "desmitracheate" for the taxa with this apomorphic tracheal arrangement, and subsequently (Millidge, 1986) reported the presence of median trunks branching into tracheoles in several members of the Micronetinae, namely *Tennesseellum*, *Meioneta*, and *Agyneta* (see also Hormiga, 1994b). We have allocated interspecific variation in tracheal anatomy among five characters (127–131).

All eight optimal cladograms found with DO (Fig. 8) require two independent origins of the branched median trunks within Linyphiidae: one in the clade composed by *Helophora*, *Meioneta*, and *Agyneta*, and a second one in the clade that includes all erigonines except *Ostearius* and *Gongylidiellum*. Outside the Linyphiidae, there is one additional origin in mysmenids, although several similar and independently derived cases are known in araneoid taxa not present in our matrix (e.g. in Synaphridae; Lopardo and Hormiga, 2007). DO analysis of the molecular partition also requires two origins. Parsimony analysis of the morphology requires only one origin of branched median trunks within Linyphiidae (with a reversal in *Ostearius* and *Gongylidiellum*). The DO total evidence results are congruent with those of Hormiga (2000), who found that erigonines were primitively haplotracheate and that the desmitracheate condition evolved in a more distal clade of erigonines (the intermediate tracheal system of *Tibioploides* and *Laminacauda* arose once from a haplotracheate ancestor and once from a desmitracheate ancestor, respectively). On the other hand, Miller and Hormiga's (2004) equal weights analysis suggested that the desmitracheate system is synapomorphic for Erigoninae, but secondarily and independently reversed to haplotracheate in *Tibioploides* and some *Laminacauda* species. Miller and Hormiga's (2004) successive character-weighting analysis produced a hypothesis of tracheal evolution similar to that of our results and those of Hormiga (2000), with erigonines being primitively haplotracheate.

In sum, our results suggest independent origins of the desmitracheate system in micronetines and erigonines, and that erigonines were primitively haplotracheate. A stricter test of the second hypothesis requires denser taxonomic sampling, particularly of haplotracheate groups such as *Sisicus* or *Neomaso* and intermediates such *Tibioploides* and some *Laminacauda*, none of which was available for sequencing.

The biological significance of the diversity of tracheae in linyphiids is poorly understood. In order to try and assess the nature of this variation in a comparative context, it is useful to review some of the physiological and metabolic implications of tracheal variation. Like most spiders, linyphiids have a dual respiratory system consisting of book lungs that oxygenate the hemolymph, and tracheae that carry oxygen directly to tissues. In spiders, the rates of metabolism are directly proportional to the respiratory surface area, and are positively correlated with book lung volume. The modification of

the respiratory or circulatory variables potentially provides for aerobic scopes of three- to eightfold (Anderson and Prestwich, 1982). It is important to note that the direct relationship between book lung area and metabolic rate is sometimes based on measurements that do not include the area of the fine tracheoles across which oxygen diffuses directly into tissues, and therefore underestimates total respiratory surface of spiders with well developed tracheal systems (Opell, 1990: 215). The walls of the whole tracheal system can serve a gas exchange surfaces, and for that reason they have been considered to be tubular or tracheal ''lungs'' (Schmitz and Perry, 2000, 2001).

Opell (1990, 1998) studied the respiratory system of representatives of several genera of the orbicularian family Uloboridae, and demonstrated the complementarity of book lung and tracheal systems. Uloborids exhibit a wide range of tracheal systems, from fairly simple trunks restricted to the abdomen to highly branching patterns that extend into the prosoma and legs. Opell (1990) comparative analysis shows that there is an inverse relationship between book lung and tracheal system development. This suggests that both systems act in consort to supply a spider's increased oxygen demands, and that their development is influenced not only by the total respiratory demands, but also by the specificity of these demands. Uloborid species that more actively monitor reduced webs have more extensive tracheal systems (and smaller book lung areas) than do orb-weaving species that manipulate their webs less aggressively.

The physiological importance of branching tracheal systems that extend into the prosoma has been studied in some salticid species (Schmitz and Perry, 2001, 2002), in which it has been shown that the tracheae play an important role in gas exchange when there are high metabolic demands. These authors also propose that book lungs and tracheae function in tandem, with both systems serving the entire body, and tracheae serving areas of great metabolic demands (gonads and nervous system). On the other hand, in species with simple tracheae confined to the abdomen, such as the wolf spider *Pardosa lugubris* (L.) (Lycosidae), the tracheae seem to be of no importance for overall oxygen exchange, although they may be of some importance in local oxygen supply or in overall carbon dioxide release (Schmitz and Perry, 2000). The available data support the idea that spider species with a well developed tracheal system have greater aerobic abilities than do those that rely mainly on lung respiration because they have simple tracheae restricted to the abdomen (Schmitz, 2004). Branching tracheae may support the aerobic metabolism only during the most intense physical exercise (such as high-speed running in salticids), playing a role in the local supply to organs at low and medium levels of activity (Schmitz, 2004, 2005).

One possible way of assessing the significance of desmitracheate patterns is to try to find life-history traits that may correlate with the tracheal modifications, using a ''convergence approach'' (Maddison, 1990; Eggleton and Vane-Wright, 1994), although there are no reasons to argue *a priori* that a single hypothesis may explain the multiple origins of highly branching tracheae. In fact, if anything, the implication of the non-homology of desmitracheate patterns across spiders leaves quite open the possibility of diverse evolutionary pathways. The anatomical diversity of spider tracheal systems is much higher than what is represented by the taxonomic context of our study. The multiple occurrences of branching median tracheae across spider lineages (Lamy, 1902; Purcell, 1909; Bromhall, 1987) suggest that any comparative study attempting to unravel its evolutionary origins should be broadly circumscribed taxonomically, which is beyond the goals of the present study. Despite these caveats, one can try to search for common patterns among lineages with branching median tracheae. Within araneoids, similar branching tracheae are often found in groups that are of small size and that live in the leaf litter (and that are not each other's closest relatives), such as mysmenids, synaphrids, some tetragnathids (e.g. *Glenognatha*), and cyatholipids (although most cyatholipids build aerial webs in vegetation or on tree trunks, some are litter dwellers, such as *Matilda* species in Western Australia). It has been suggested (e.g. Levi, 1967 and references therein) that extensive tracheal development may have evolved to help in reducing water loss. If so, this is not necessarily the case across all groups: Opell (1998) has shown how in uloborids, variation in activity patterns, rather than environmental humidity, better predicts differences in tracheal development. At present time there are not enough data available to test the water loss hypothesis in linyphiids.

Hormiga (2007) suggests that there are two main types of sheet web in linyphioids: ''aerial'' and ''substrate'' webs. The distinction between these two types resides in the web perimeter and the richness of attachment points delimiting the web, rather than the substrate on which the web is spun. Aerial webs tend to have fewer attachment points in the perimeter of the web and the sheet is more evident (e.g. Fig. 2b). On the other hand, in substrate webs the sheet is less evident, not necessarily in one plane, it has more attachment points and, as a result, the web seems to have been woven to fit the substrate (e.g. Fig. 2h). Erigonines often spin their webs in the leaf litter or in cavities in the soil, and in the few erigonine species documented, substrate webs seem more common than aerial webs (but exceptions exist; see Fig. 2d for a typical aerial web spun by an erigonine). It is tempting to speculate about a causal connection between the transition from aerial into substrate webs and the modification of the simple

haplotracheate pattern into a desmitracheate system, based on the energetic differences of monitoring these different types of web. Our working phylogenetic hypothesis suggests two origins of highly branched median tracheae. Unfortunately, neither the detailed taxonomic cladistic hypothesis nor the necessary biological data needed to establish in more detail, and ultimately test, such hypothesis are not available. Nonetheless, we would like to suggest this line of enquiry for future comparative work at an ordinal taxonomic level.

One of the most remarkable anatomical features of linyphiids are the cephalic specializations found in several lineages, especially in erigonines, which include a broad diversity of cephalic lobes, turrets, sulci, pits, cuticular pores coupled with glands, and modified setae (Bristowe, 1931; Schlegelmilch, 1974; Blest and Taylor, 1977; Schaible et al., 1986; Blest, 1987; Hormiga, 1994b, 2000; Vanacker et al., 2003a,b). Distinctive behaviours, often involving courtship, mating, and nuptial gifts, are associated with these features, but they remain poorly known ethologically, histologically, and ultrastructurally. Cephalic structures are particularly diverse in erigonines, are restricted to males, and exhibit the classic elements of a system evolving under sexual selection (Eberhard, 1985, 1996; Vanacker et al., 2003a; Huber, 2005). In-depth inquiry on the evolution of this complex system is well beyond the scope of our study, but we can address whether the sulci and pores (and associated glands) found in mynoglenines, erigonines, and some linyphiines have evolved independently or can be traced to a single origin. In mynoglenines the sulci are in subocular position and are lined up with cuticular pores associated with glands. Very similar sulci are found in both sexes and juveniles of Mynogleninae (Blest and Taylor, 1977). Mynoglenine sulci apparently are not involved in courtship (at least in the species studied by Blest and Pomeroy, 1978) and may produce defensive secretions. In erigonines the sulci are post-ocular, limited to adult males and function during courtship, and the glands differ cytologically (Blest and Taylor, 1977; Schaible et al., 1986; Schaible and Gack, 1987). Erigonine females grip male sulci with their fangs, as first noted by Bristowe (1931), although this has been studied in very few species. More recently, Hormiga (1999) described cephalothoracic pits lined with cuticular pores in both sexes of several ''linyphiines'', including *Bathyphantes*, *Diplostyla*, and *Vesicapalpus*; presumably the pores connect to glands, although histological proof is lacking. The cladistic analyses of Hormiga (1994b, 2000) and Miller and Hormiga (2004) implied independent origins of sulci and pores in mynoglenines and erigonines, as well as multiple origins within the latter lineage. No erigonines in the present matrix have lateral sulci or pits, and only *O. gibbosus* has numerous cuticular pores. We coded the subocular sulci of mynoglenines and of *Bathyphantes* and *Diplostyla* (lateral) as separate (characters 83 and 81, respectively; see Appendix 1). Direct optimization analysis of the total evidence grouped mynoglenines with *Bathyphantes* and *Diplostyla*, as well as *Laetesia* and *Australolinyphia* (Fig. 8), but that topology still would require two separate origins, even if the sulci were considered homologous (two origins are also required under the direct optimization analysis of the molecular partition; Fig. 6). On the other hand, both Bayesian topologies (molecular partition and all combined data, Figs 5 and 7, respectively) would support a single origin if optimized with parsimony (we present this observation for comparative purposes only since it is unclear to us why anyone, epistemologically speaking, would choose to use parsimony to optimize a character on a Bayesian tree). The preferred cladogram requires five origins of the proliferation of pores in males, including one in the clade that groups mynoglenines, *Bathyphantes–Diplostyla*, *Laetesia*, and *Australolinyphia* (Figs 8 and 9). Thus, these cephalothoracic specializations evolved independently in erigonines and mynoglenines. Although none is included in our character matrix, erigonines with lateral sulci belong to the ''Distal Erigonines'' clade (Hormiga, 2000; Miller and Hormiga, 2004), represented here by *Oedothorax* and *Gonatium*, suggesting independent origins in erigonines and ''non-erigonine'' linyphiids. These results also suggest that while the subocular mynoglenine sulci and lateral linyphiine sulci (e.g. *Bathyphantes*) may have evolved independently, the proliferation of glandular pores in the prosoma has a single origin in ''clade C''.

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Appendix 1

Most of the characters presented here are discussed in detail and illustrated in Hormiga (1994a,b, 2000, 2002, 2003), Miller and Hormiga (2004), Hormiga and Scharff (2005), and Hormiga et al. (2005). References to those characters are abbreviated as H94a, H94b, H00, H02, H03, MH03, HS05 and HBS05, respectively. The bibliographic abbreviation is followed by the character number in the cited reference. For example, HS05-23 refers to 'character 23' in the matrix of Hormiga and Scharff (2005). For simplicity, we have cross-referenced most of the characters to the matrices of Hormiga (2000) and/or Miller and Hormiga (2004). Only those characters that are new or that have been recoded are discussed in this section (they are indicated with an asterisk after the character number). When necessary for clarity, an exemplar taxon exhibiting the character state being described is given in parentheses, and if possible reference to an illustration is made. In general, when the same character has been used in several matrices, we cite only the most recent usage as an example of its definition. Citation does not necessarily imply authorship of the homology hypothesis. For example, the number of retrolateral cheliceral teeth (character 97 in this matrix) has traditionally been used in spider taxonomy since the nineteenth century. Consequently, this character often appears in spider cladistic matrices, including all the above-cited. We refer to it as H03-57, but Hormiga (2003) is not the author of this character. The scoring of these characters for the study taxa is presented in Appendix 2. All multistate characters were treated as non-additive (Fitch, 1971).

Male palp

Character 1. Alveolar sclerite: 0, absent; 1, present (*Pimoa breuili*, Hormiga, 1994a: fig. 54) (H03-1).

Character 2*. Ectal marginal cymbial process: 0, absent; 1, present (*Pimoa rupicola*, Hormiga, 1994a: fig. 16).

This character refers to the denticulate process in the genus *Pimoa* (the process is edenticulate in *Weintrauboa*). We do not consider it homologous to the ectomarginal process in linyphiids (e.g. *Labicymbium—contra* Miller and Hormiga, 2004). The linyphiid cymbial process is also present in pimoids (e.g. in *Pimoa rupicola*, Hormiga (1994b, fig. 16), to the right of alveolar sclerite; *Weintrauboa contortipes*, Hormiga (2003: fig. 1C), between the tegulum and the distal part of the pimoid cymbial sclerite). Both position and conjunction refute primary homology of these cymbial processes in linyphiids and pimoids.

Character 3. Cymbial cuspules: 0, absent; 1, present (H03-3).

Character 4. Cymbial cuspules location: 0, on dorsal surface of cymbium, but not on process itself (*Weintrauboa contortipes*, Hormiga, 2003: fig. 1E); 1, on cymbial process itself (*Pimoa rupicola*, Hormiga, 1994a: fig. 16) (H03-4).

Character 5. Cymbial orifice: 0, absent; 1, present (H02-45).

Character 6*. Ecto basal cymbial process: 0, absent; 1, present (*Floronia bucculenta*, Roberts, 1993, p. 143). This character refers to a basal process in the ectal margin of the cymbium.

Character 7. Pimoid cymbial sclerite: 0, absent; 1, present (*Pimoa*, Hormiga, 1994a: fig. 11; H03-5).

Character 8*. Mesobasal cymbial process: 0, absent; 1, present (*Tapinopa longidens*, Roberts, 1993, p. 143). This character has been modified from Miller and Hormiga (2004; their ''character 4: Cymbial retrobasal process'') to better accommodate the morphological variation found in our taxonomic sample.

Character 9. Cymbium retrolateral groove: 0, absent; 1, present (MH04-7).

Character 10. Paracymbium: 0, present; 1, absent (MH04-10).

Character 11*. Paracymbium attachment: 0, intersegmental (*Linyphia triangularis*, Hormiga et al. (1995: fig. 5A); 1, integral (*Pimoa altioculata*, Hormiga, 1994a: fig. 303); 2, articulated (*Tetragnatha versicolor*, Hormiga et al., 1995; figs 7C–E); 3, *Stemonyphantes* type (Hormiga, 1994b: fig. 2). The coding of this character is discussed in Hormiga (1994b) and Hormiga et al. (1995).

Character 12*. Paracymbium morphology: 0, straight and narrow (*Tetragnatha versicolor*, Hormiga et al., 1995: fig. 7C, D); 1, finger-like hook, not flat (e.g., *Theridiosoma gemmosum*, Coddington, 1986, fig. 147); 2, linguiform and fused basally to pimoid cymbial sclerite (Hormiga, 1994a: figs. 15–17); 3, triangular (*Pimoa altioculata*, Hormiga, 1994b: figs. 303–304); 4, straight hook, one flat plane (two dimensions) *Neocautinella*, Miller and Hormiga, 2004: fig. 15A; *Stemonyphantes*, Hormiga, 2000; plate 12A); 5, spiral, one folded plane (two dimensions) (*Valdiviella*, Miller and Hormiga, 2004: fig. 15D; *Ceratinops*, Hormiga, 2000; : plate 15A); 6, cup-shaped (*Pahora* and *Synotaxus*; Griswold et al., 1998: fig. 18, 19); 7, *Nesticus* type (Griswold et al., 1998: fig. 14); 8, square (*Pseudafroneta perplexa*; Blest, 1979: fig. 498); 9, *Frontinella* type (*Frontinella communis* and *Orsonwelles polites*; Hormiga, 2002: fig. 23). Coding of this character follows Hormiga (2000) and Miller and Hormiga (2004) with minor modifications.

Character 13. Paracymbium apophyses. 0, absent; 1, present (H94a-13).

Character 14. Paracymbium basal setae. 0, absent; 1, present (MH04-14).

Character 15. Bulb–cymbium lock mechanism: 0, absent; 1, present (Griswold et al., 1998, character 12).

Character 16. Protegulum: 0, absent; 1, present (H00-8).

Character 17. Protegular papillae: 0, absent; 1, present (H00-9).

Character 18. Protegular papillae form: 0, scale-like; 1, rod-like (MH04-18).

Character 19. Tegular sac: 0, absent; 1, present (H00-10).

Character 20. Papillae on tegulum: 0, absent; 1, present (MH04-20).

Character 21. Tegulum to subtegulum orientation in unexpanded palp: 0, tegulum distal to subtegulum; 1, tegulum mesal to subtegulum; 2, tegulum ventral to subtegulum (MH04-21). State 1 ''tegulum mesal to subtegulum'' includes some taxa that have a meso-ventral tegulum.

Character 22*. Sperm duct switchback: 0, absent; 1, present (Hormiga, 1994a: fig. 9). We have combined into one character characters 21 and 22 from MH04. This character codes for the presence of a switchback (or a conspicuous loop) of the spermduct in the tegulum, often before entering the embolic division, but sometimes it is further removed from embolic division (e.g. in *Neriene* spp.). Some taxa (e.g. *Clitaetra*, *Azilia*) may have additional loops. A potential character, not included in this analysis, would be the contrast between a gradual change on the diameter of the sperm duct (e.g. *Hylyphantes graminicola*, in Hormiga, 2000: fig. 16A) versus a more sudden change in diameter (e.g. *Hilaira excise*, Hormiga, 2000: fig. 14D).

Character 23. Suprategulum: 0, absent or vestigial; 1, present (MH04-24).

Character 24. Suprategular base: 0, approximately the same width as the rest of the suprategulum; 1, wider (H02-8).

Character 25. Suprategulum junction with tegulum: 0, continuous with tegulum; 1, with partial or complete membranous division (MH04-24).

Character 26. Foramen: 0, in suprategulum; 1, in tegulum (MH04-28). This character is non applicable to taxa without a suprategulum.

Character 27. Suprategular distal apophysis initial orientation: 0, extends distally beyond suprategulum; 1, extends ventrally from suprategulum (MH04-31).

Character 28. Suprategular marginal apophysis: 0, absent; 1, present (H00-14).

Character 29. Median apophysis: 0, absent; 1, present (MH04-35).

Character 30. Conductor: 0, absent; 1, present (MH04-36). See also discussion in Griswold et al. (1998) for the latter two characters.

Character 31. Pimoid embolic process: 0, absent; 1, present (H03-26).

Character 32. Theridiid tegular apophysis: 0, absent; 1, present (Griswold et al., 1998, character 18).

Character 33. Column: 0, absent; 1, present (H00-24). The column is treated here *sensu lato*, as membranous connection between tegulum and embolus base (or radix).

Character 34. Embolic membrane: 0, absent; 1, present (H00-18).

Character 35. Embolic membrane texture: 0, without papillae; 1, with papillae (MH04-41).

Character 36. Embolus base: 0, broad; 1, narrow (H02-11).

Character 37. Embolus shape: 0, straight to curved; 1, spiral (MH04-44).

Character 38. Embolus apical half: 0, filiform; 1, wider, not thread-like (H02-2).

Character 39. Embolic papillae: 0, absent; 1, present (MH04-46).

Character 40. Radix: 0, absent; 1, present (H00-20).

Character 41. Radix–embolus connection: 0, continuous; 1, membranous (MH04-51).

Character 42. Radical tail piece: 0, absent; 1, present (H00-21).

Character 43. Radical anterior process: 0, absent; 1, present (H00-23).

Character 44. Radical mesal tooth: 0, absent; 1, present (MH04-58).

Character 45. Fickert's gland: 0, absent; 1, present (H00-25).

Character 46. Terminal apophysis: 0, absent; 1, present (H00-26).

Character 47. Terminal apophysis position: 0, apical–ectoventral; 1, mesal; 2, ectal (H02-16).

Character 48. Terminal apophysis coiling: 0, not coiled; 1, spirally coiled (H02-19).

Character 49. Terminal sclerite: 0, absent; 1, present (H02-21).

Character 50. Transversal sclerite: 0, absent; 1, present (H02-22).

Character 51. Lamella characteristica: 0, absent; 1, present (H00-27).

Character 52. Lamella characteristica size: 0, small; 1, large (H02-23).

Character 53. Mesal tooth of lamella characteristica: 0, absent; 1, present (H02-26).

Character 54. Mesal tooth of lamella characteristica size: 0, small; 1, large (H02-27).

Character 55. Mesal tooth of lamella characteristica position: 0, medial (open curve at lamella characteristica base); 1, basal (more closed curve) (H02-28).

Character 56*. Palpal tibia of male, dorsal apophysis: 0, absent; 1, present (*Labulla thoracica*; Hormiga and Scharff, 2005: fig. 5A).

Character 57. Palpal tibia of male, prolateral apophysis: 0, absent; 1, present (H00-28).

Character 58*. Palpal tibia of male, prolateral apophysis orientation: 0, initially directed distally (*Diplocephalus*, Hormiga, 2000: fig. 6C); 1, initially directed dorsally (*Drepanotylus*, Hormiga, fig. 8B)(MH04-68).

Character 59. Palpal tibia of male, retrolateral apophysis: 0, absent; 1, present (MH04-70).

Character 60. Palpal tibia of male, ventral tibial process: 0, absent; 1, present (MH04-72).

Character 61. Palpal tibia of male, prolateral trichobothria: 0, two; 1, one; 2, zero; 3, four or more; 4, three (H00-30).

Character 62. Palpal tibia of male, retrolateral trichobothria: 0, four; 1, three; 2, two; 3, one (H00-31).

Character 63. Palpal patella length in male: 0, short (more than 2.1 times longer than high); 1, long (less than twice as long as high)(MH04-76).

Character 64. Palpal patella distal dorsal macroseta strength: 0, weak to moderate; 1, very strong (MH04-78).

Epigynum

Character 65. Epigynum dorsal plate scape: 0, absent; 1, present (H00-32).

Character 66. Epigynum ventral plate scape: 0, absent; 1, present (H00-33).

Character 67*. Ventral plate scape socket: 0, absent; 1, present (*Tenuiphantes tenuis*, Hormiga, 2000: plate 6D). See comments under character 70.

Character 68. Epigynum ventral plate scape form: 0, straight; 1, sigmoid (H00-34).

Character 69. Epigynum protrusion: 0, protruding less than its width; 1, protruding more than its width (H03-40).

Character 70*. Epigynum ventral plate socket: 0, absent; 1, present (MH04-89).

Character 70 codes whether there is a cuticular socket on the ventral plate which is independent of the presence of a scape; e.g. in *Bathyphantes gracilis* there is a socket on the ventral plate but it lacks a scape (see Wiehle, 1956: figs 418A, B-419). Character 67 codes whether the socket is in a scape (e.g. *Tenuiphantes tenuis*, Hormiga, 2000: plate 6D) or directly on the plate itself. In *Helophora insignis* the entire epigynum is projected (it is laterally made of ventral plate, but it envelops a projected dorsal plate), and in addition it carries a distal socket on the ventral plate, but we do not consider this epigynum to have a scape *sensu strict*o.

Character 71. Epigynum dorsal plate socket. 0, absent; 1, present (MH04-90).

Character 72. Atrium: 0, absent; 1, present (H00-36).

Character 73. Atrium texture: 0, spiral grooves; 1, spiral folds; 2, smooth (Hormiga et al., 2003, character 50).

Character 74. Copulatory duct: 0, separate from fertilization duct; 1, spirals fertilization duct (H00-37).

Character 75. Copulatory duct encapsulation: 0, absent; 1, present (H00-38).

Character 76. Copulatory duct turning point: 0, absent; 1, present (H02-40).

Character 77. Spermathecae: 0, two; 1, four (H00-39).

Character 78. Spermathecae shape: 0, round to slightly oblong; 1, strongly oblong (MH04-98).

Character 79. Fertilization duct orientation: 0, posterior; 1, mesal; 2, anterior; 3, dorsal (MH04-99).

Somatic morphology

Character 80. Cephalic region in male: 0, not raised; 1, raised (H00-41).

Character 81*. Cephalic sulci on margin of prosoma: 0, absent; 1, present (*Bathyphantes pallidus*, 1999: figs. 6-11). These sulci are located in the margin of the prosoma, close to the pedipalpal trochanter and were first described by Hormiga (1999). The cuticle of these pits is lined up with multiple pores which presumably are glandular openings.

Character 82. Cephalic post-PME lobe in male: 0, absent; 1, present (H00-43).

Character 83. Cephalic sulci of subocular clypeus in male: 0, absent; 1, present (H00-47).

Character 84. Cephalic cuticular pores in male: 0, rare; 1, common (H00-50).

Character 85. Prosoma setae in female: 0, hirsute; 1, setae absent or very rare; 2, Setae sparse, restricted to radii and lateral margins of thorax (MH04-111).

Character 86. Clypeus texture in male: 0, nearly smooth; 1, squamate (MH04-112).

Character 87*. Clypeal setae in female: 0, More than one (*Bolyphantes luteolus*, Hormiga, 2000; : pl. 2a); 1, only one seta below the anterior median eyes (*Laminacauda plagiata*, Hormiga, 2000: pl. 44a, b); 2, none (e.g., *Mysmena* sp., Guyana). Modified from MH04-113 to accommodate the variation found in the present taxonomic sample.

Character 88. Thoracic furrow: 0, nearly smooth, often recognizable only from pigment, not invagination; 1, thoracic furrow a distinct invagination (MH04-114).

Character 89. Chelicerae size: 0, equal in males and females; 1, larger in males (MH04-115).

Character 90. Cheliceral lateral face in male: 0, smooth; 1, stridulatory striae (H00-55).

Character 91. Cheliceral stridulatory striae: 0, ridged; 1, scaly; 2, imbricated (H00-56).

Character 92. Cheliceral stridulatory striae rows in male: 0, widely and evenly spaced; 1, compressed proximally; 2, compressed distally; 3, compressed and evenly spaced; 4 compressed proximally and distally, widely spaced centrally (MH04-118).

Character 93. Cheliceral setal bases on front-lateral face in male: 0, nearly flush with chelicerae to small bumps; 1, formed into distinct bumps; 2, greatly enlarged (MH04-120).

Character 94. Cheliceral dorsal spur in male: 0, absent; 1, present (H00-57).

Character 95. Cheliceral fang furrow in male: 0, tapered; 1, wide and flat to concave (MH04-122).

Character 96. Cheliceral teeth, prolateral margin of fang furrow in female: 0, 7 or less; 1, 9-13 (H02-45).

Character 97. Cheliceral teeth, retrolateral margin of fang furrow in female: 0, zero; 1, one; 2, two; 3, three; 4 four or more (H00-58).

Character 98. Sternum–labium attachment: 0, separate; 1, fused (MH04-124).

Character 99. Endites of male: 0, smooth; 1, tuberculate (MH04-125).

Character 100. Palpal tarsus claw in female: 0, present; 1, absent (H00-59).

Character 101. Palpal tarsus proximal dorsomesal macrosetae in female: 0, absent; 1, present (MH04-127).

Character 102. Palpal tarsus distal dorsomesal macrosetae in female: 0, absent; 1, present (MH04-128).

Character 103. Palpal tarsus proximal dorsoectal macrosetae in female: 0, absent; 1, present (MH04-129).

Character 104. Palpal tarsus distal dorsoectal macrosetae in female: 0, absent; 1, present (MH04-130).

Character 105. Palpal tarsus ventromesal macrosetae in female: 0, zero; 1, two; 2, three; 3, four; 4, five to ten; 5, 11–12 (MH04-131).

Character 106*. Palpal tarsus ventroectal macrosetae in female: 0, zero; 1, one; 2, two; 3, three; 4, four; 5, five; 6, six or more (MH04-132).

Character 107*. Autospasy at patella–tibia joint. 0, absent; 1, present (H00-60).

Character 108. Femur III + IV Trichobothria: 0, absent; 1, present (H02-53/54).

Character 109. Femoral macrosetae: 0, present; 1, absent (Griswold et al., 1998, character 59).

Character 110. Femur I dorsal macroseta(ae): 0, absent; 1, present (MH04-134).

Character 111. Femur I prolateral macroseta(ae): 0, absent; 1, present (MH04-134).

Character 112. Tibia I distal dorsal macroseta: 0, absent; 1, present (MH04-137).

Character 113. Tibia II distal dorsal macroseta: 0, absent; 1, present (MH04-139).

Character 114. Tibia III proximal dorsal macroseta: 0, absent; 1, present (MH04-142).

Character 115. Tibia III distal dorsal macroseta: 0, absent; 1, present (MH04-141).

Character 116. Tibia IV distal dorsal macroseta: 0, absent; 1, present(MH04-143).

Character 117. Tibia I prolateral macroseta(ae): 0, absent; 1, present (MH04-144).

Character 118. Tibia I retrolateral macroseta(ae): 0, absent; 1, present (MH04-145).

Character 119. Tibia I ventral macroseta(ae): 0, absent; 1, present (MH04-146).

Character 120. Metatarsus I dorsal macroseta(ae): 0, absent; 1, present (MH04-147).

Character 121. Metatarsus I prolateral macroseta(ae): 0, absent; 1, present (MH04-148).

Character 122. Metatarsus I retrolateral macroseta(ae): 0, absent; 1, present (MH04-149).

Character 123. Metatarsus I ventral macroseta(ae): 0, absent; 1, present (MH04-150).

Character 124. Metatarsus IV trichobothrium: 0, present; 1, absent (H00-65).

Character 125. Tarsus IV ventral setae; 0, smooth; 1, serrated (theridioid tarsal comb) (Griswold et al., 1998, character 62).

Character 126. Pedicel sternite and pleurites in male: 0, separated; 1, juxtaposed or fused (MH04-153).

Character 127. Median tracheal trunks: 0, unbranched; 1, branched (MH04-157).

Character 128. Median tracheal trunks branching: 0, median tracheae with few branches; 1, median tracheae highly branched (MH04-158).

Character 129. Median tracheal trunks width: 0, about as wide as laterals or thinner; 1, much wider than laterals (MH04-159).

Character 130. Median tracheal trunk length: 0, restricted to abdomen; 1, pass through pedicel into prosoma (MH04-160).

Character 131. Tracheole taenidia: 0, absent; 1, present (H00-52).

Character 132. ALS piriform spigot bases: 0, normal; 1, reduced (Griswold et al., 1998, character 69).

Character 133. Aciniform spigots in female posterior median spinneret: 0, absent; 1, one; 2, two; 3, three; 4 four; 5 more than four (modified from H00-66).

Character 134. Minor ampullate nubbin in female posterior median spinneret: 0, absent; 1, present (H00-67).

Character 135. Mesal cylindrical gland spigot base on female posterior lateral spinneret: 0, same size as other cylindrical; 1, enlarged (H00-68).

Character 136. Aciniform spigots in female posterior lateral spinneret: 0, absent; 1, one or more (H00-69).

Character 137. Aggregate–flagelliform triplet in male posterior lateral spinneret: 0, absent; 1, present (at least in part)(MH04-166).

Character 138. PLS aggregate spigot size: 0, circumference subequal or less than distal cylindrical spigot base; 1, circumference distinctly greater than distal cylindrical spigot base (modified from Agnarsson, 2004, character 214).

Character 139. Epiandrous gland spigots: 0, absent; 1, present (MH04-169).

Character 140. Epiandrous gland spigot arrangement: 0, singles; 1, grouped (MH04-170).

Behaviour

Character 141. Mating behaviour, male during spermweb construction: 0, above spermweb; 1, below spermweb (H00-72).

Character 142. Mating behaviour, male during ejaculation: 0, above spermweb; 1, below spermweb (H00-73).

Character 143. Web type: 0, orb web; 1, sheet web; 2, Chicken-wire (*Synotaxus* type); 3, cob web (Griswold et al., 1998, character 80).

Character 144. Orb frame: 0, two-dimensional; 1, three-dimensional (Griswold et al., 1998, character 81).

Character 145. Radii lengthened: 0, absent; 1, present (Griswold et al., 1998, character 84).

Character 146. Accessory radii: 0, absent; 1, present (Griswold et al., 1998, character 85).

Character 147. Post-SS hubs: 0, absent; 1, present (Griswold et al., 1998, character 88).

Character 148. Web placement: 0, aerial; 1, close to substrate (MH04-174).

Character 149. Web knock-down lines: 0, few or absent; 1, numerous (MH04-175).

Appendix 2

Argiope argentata	000-000000110000--00110-----110010-00001-00000--000----00-00010000--0000-00000000000000100--00004000111145000111111111111111000-00-05101001**01**	??000000 -
Tetragnatha versicolor	000-000000001100--00010-----010010-01000----00--000----00-000011----------011-0000?000110--01104000101044010111110111011011000-00-031010011	?1000000 -
Theridiosoma gemmosum	000-000000110000--01110-----11000--0-000----00--000----00-00130100--0000-00000000000?1?0000--000?1001000000000011100011000001000-00-13101002?	??011110 0
Mysmena sp.	?00-000001----?0--??0?0-----0000????10??????00--000----00-00??0010--000???????0000?212000--?0?04001??????0?1--00100????????0?11?1?120011011	??011111 -
Alaranea merina	010-000000160100--01210-----01000--01000----00--000----00-00130001000000-00000000000?0?0100--00003101111??0010000100000000101?????120-11011	??1----0 ?
Theridion varians	00--000001----10--00010-----110110-00100----00--000----00-002?0000--0000-00000000000?210100--0000-000?????001--11101000000011?????120010111	?13----? ?
Steatoda grossa	000-000001----10--00010-----110110-01000----00--000----00-00130100--0000-00000000000?0?0100--00000000000012001--11111000000011110-00-140010111	??3----1 0
Nesticus cellulanus	000-000000171100--00110-----11010--10100----00--000----00-00120000--0000-00000000000?0?0100--00004100000011001--11111000000011?0-00-131010112	?13----1 -
Synotaxus waiwai	000-000000160000--00200-----00010--01000----00--000----00-001201-0--00-0-1000000000?1??000--00004000000000001--00000000010101?????131010110	??2----0 -
Weintrauboa contortipes	0110001000020100--00200-----11100--01000----00--000----00-00030100--0000-00000100002?2?0100--00004000111142001111111111111111000?????120110011	??1----? ?
Pimoa altioculata	1111001000130100--00210-----11100--11000----00--000----10-00010100--1000-00000200000?2?0101030000200011105310011111111111111110000-00-100100110	??1----1 1
Pimoa rupicola	1111001000120100--0-210-----11100--11000----00--000----10-00010100--0000-00000200000?2?0101-00000200010105310011111111111111110000-00-10011?0??	???????? ?
Stemonyphantes blauveltae	000-000000040100--0111101000000010-11001111000--000----00-01121100--0000-10000000002101010?0000020001111441001111111111000010000-00-100101011	??1----1 0
Tenuiphantes tenuis	000-010000051100--0001100000000001100-1110000110000100--00-00120101110100-00001000000?0001000000410111112210001111111110100010000-00-120110011	001----1 0
Lepthyphantes minutus	000-010100051100--0?1?1000000000110001111000110000100--00-00120101110100-00001000012101010300004100111132100011111111111101000-00-120110011	??1----0 1
Bolyphantes luteolus	000-010000051100--0001100000000001100-1110000110000100--00-00120101110100-0000101?00120000104000041001111321000111111111111111000-00-120110011	???????? ?
Microneta viaria	000-0100000511010-0011100000000001110011111010010000100-000-00120101110100-000000000?2100110300104101110101310000111111100000000?0-00-13011100-	???????? ?
Meioneta rurestris	000-010100051100--0010100000000011100111101?110000100--0100012000111010-0000020000?2100012110104101100??1000011111000000010?1111?12011100-	???????? ?
Agyneta ramosa	000-0101000511???0?1?1000000000111001111?0?110000100--00-00130001110100-00000?00000?1???12000000?0?0000441????11111000????00?1111?1201?0??	??1????? ?
Bathyphantes gracilis	00--000000040100--0010111000000011-01001000000--001110100-001200101-0110-0??00?01001210001000000410111114210011111111110000000000-00-120110011	??1-?--1 0
Diplostyla concolor	000-0000000511010-0010100000000011001101101000--00110--00-00120011100101200000010002100012300104100111133100111111111000001000-00-120111011	??1----1 ?
Drapetisca socialis	000-010100051100--0010100000000011000?11010110000110--00-00130000--1000-00001100000210101032000410011013410001111111101000000-00-120110011	??1----1 0
Floronia bucculenta	010-010000051100--0011110000000011110001101001000000----10-10120101110100-00001110000210111000010410011113310011111111111111010?0-00-120110011	??1----1 0
Microlinyphia dana	000-000000040100--0010110000000010-11001000001--001110000-00120110--00112101010000002100111310004100111143100111111111111111000-00-120110011	001----0 1
Linyphia triangularis	000-000000040100--0011111000000011011001000001010011110000-00110010--00111001010000002101111310104100111143100111111111111111000-00-130110011	001----0 1
Neriene variabilis	000-000000040100--0011111000000001111001?1100001010111100000-00110010--00110001000000?2000111300104100111143100111111111111111100?????120110011	??1----**01** 1
Neriene radiata	000-000000040100--001111000000001110001?1100001011111100000-00110010--00110001000000?2000111301104100111143100111111111111111?0-00-11011?011	001----0 1
Orsonwelles polites	010-100000090100--0010110100000011100101000001101011111100-00400000--0000-00000100002101 00--10114100111146110111111110111010000-00-140110010	??1----0 1
Orsonwelles malus	010-100000090100--0010110100000011100101000001101011111100-00300000--0010-00000100002?2?0100--10114100111146110111111110111010000-00-130111010	??1----0 1
Pityohyphantes costatus	000-000000040100--0010110000000011000001000001001011100000-00120011100011000001000002?00111132010310011111331001111111111111110?0-00-130110011	??1----0 1
Labulla thoracica	000-000000051100--0021100001000010-01001100001200011 0--10-00120101000001000101210012001012300003100111124100111111111111101000-00-120111011	001----1 0
Helophora insignis	000-0000000511010-0010100001000011111000100000-000110--00-10120000--1100-00000200000?2101010100004101101032101--11111111100100010000120111100-	??1----? ?
Frontinella communis	000-000000190100--0000100100000011110101000000--101110000-00120100--0000-00001200000210101130000310011114410000111111110000100 0-00-12011001**01**	??1----0 1
Dubiaranea distincta	000-0000000401010-0010110000000011010001000000--10110--00-00120010--0-10-00101000000200101132010410011114410011111111111111110?0-00-12011001**01**	??1----0 1
Laetesia sp.	000-0000000401010-0011111000000001110010100000--00110--00-00120110--0-11000001000012100110310104100111133100111111111111100000?0-00-120110011	??1----1 0
Australolinyphia remota	000-000000040100--0010110100000010-11001001000--0010---00-00120001100100-00100000001210012??101041001111331001111111111111000102-00-11011?0??	??1----1 0
Notholepthyphantes australis	000-0000000401010-0010110001000011100101011000--00110--10-10120010--0010-01000000000100011000003100111114100111111111111000102-00-120110010	??1----**01** 0
Pocobletus sp.	000-0100000401012??0?1011000000011?110?1000001010010000--00-00120000--0000-00000200002?2?000???000041011114310001111111110000010?0-00-12011?0??	??1----0 1
Haplinis diloris	000-000000040100--0010110100000011011001000000--000----00-00010101100100-01100000011201001031000410011104210001111111000000010-00-120111010	11?????? ?
Novafroneta vulgaris	000-000000040100--0000110100000011010101000000--000----00-00120100--0000-00000000011201101031000410011004310011111111000000010-00-130111010	???????? ?
Pseudafroneta perplexa	000-000000080000--0010111010000001101000100000--000----00-00010110--0010-01100000011?1101131000410011104310011111111101000000102-00-110111002	???????? ?
Hilaira excisa	000-000010050100--0000010001000010-0-101111000--000----01100121000--0000-00000100100211001031000410110002210000111111000000000111111201100-	???????? ?
Ostearius melanopygius	000-0000100501011001101000100000110001010100000--000----01010120000--0000-00010100000?2100100011041111010321000011111000000001000-00-12011100-	11?????1 0
Gongylidiellum vivum	000-0000100501011100101010100000101-101111100--000----01100130000--0000-00000000002110012101104111100022100001110000000001000-00-120111100-	???????? ?
Erigone psychrophila	000-000000050101 0-000010101000001110-101011100--000----01111121000--0000-01000110000201011232000411110002210000111000000000001111012011100-	111--??1 0
Oedothorax gibbosus	000-0000000501011111101100011000011001001111000--000----11010121000--0000-01000000101211001100100410110002210000111000000000001111012011100-	???????? ?
Gonatium rubens	000-000100050000--10101000100000111010010110000--000----01010130000--0000-00000110000200001100000410110003210000001000000000001111101201110??	???????? ?