CSIRO PUBLISHING

Invertebrate Systematics, 2013, **27**, 38–52
http://dx.doi.org/10.1071/IS12014

Pedipalp sclerite homologies and phylogenetic placement of the spider genus *Stemonyphantes* (Linyphiidae, Araneae) and its implications for linyphiid phylogeny

Efrat Gavish-Regev[A,D,E], *Gustavo Hormiga*[B] *and Nikolaj Scharff*[A,C]

[A]Department of Entomology, Natural History Museum of Denmark, Zoological Museum, University of Copenhagen, Universitetsparken 15, Copenhagen DK 2100, Denmark.
[B]Department of Biological Sciences, The George Washington University, Washington, DC 20052, USA.
[C]Center for Macroecology, Evolution and Climate, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark.
[D]Current address: Department of Zoology and The National Collections of Natural History, Tel Aviv University, Tel Aviv 69978, Israel.
[E]Corresponding author. Email: efrat.gavish@gmail.com

Abstract. Male secondary genitalia (pedipalps) are useful characters for species discrimination in most spider families. Although efforts have been made to establish pedipalp sclerite homologies, there are still many inconsistencies in their use. The majority of the morphological characters used to reconstruct the linyphiid phylogeny address male genitalic variation; these inconsistencies may affect the phylogeny and our understanding of linyphiid evolution. *Stemonyphantes* Menge, 1866, has been hypothesised to be sister to all remaining Linyphiidae. However, despite the basal position of *Stemonyphantes*, its pedipalp sclerite homologies are not well understood and, along with its monophyly, have never been thoroughly tested in a phylogenetic context. We tested the homology of tegular and radical structures of five *Stemonyphantes* species to the known linyphioid and araneoid sclerites. All minimum-length trees found under all analytical methods used support *Stemonyphantes* monophyly and its placement as the sister group to all other Linyphiidae. Our study suggests that *Stemonyphantes*, unlike any other linyphiids, does have homologues of the araneoid median apophysis and conductor. As *Stemonyphantes* is the sister group of all other linyphiids, resolving its pedipalp sclerite homologies is critical for understanding sclerite homologies and the phylogeny of the entire family.

Additional keywords: cladistics, genitalia, homology, morphology, systematics, taxonomy.

Received 27 March 2012, accepted 22 August 2012, published online 13 March 2013

Introduction

Linyphiidae is the second richest family level lineage of spiders, currently with 4419 species in 589 genera (Platnick 2012). Linyphiids are medium to small sheet-web weavers with a worldwide distribution, yet are most diverse in the northern temperate regions where they account for a large fraction of spider species richness and abundance (Scharff *et al*. 2003; Scharff and Gudik-Sørensen 2006; Arnedo *et al*. 2009). They build sheet webs without a retreat and run upside down on the underside of the sheet. Like many other spiders, most linyphiids are generalist predators. This, together with their abundance in arable land, makes them an important component of the assemblage of natural enemies in many agroecosystems (Nyffeler and Sunderland 2003).

Recently, linyphiid higher-level phylogenetic relationships were tested using both molecular and morphological data; however, the different data partitions and the combined analysis suggested different phylogenies (Arnedo *et al*. 2009). The monophyly of linyphioids (Pimoidae + Linyphiidae) and Linyphiidae itself is well supported by morphology-based phylogenies (Hormiga 1994*a*, 1994*b*, 2000; Hormiga and Tu 2008) and by combined molecular and morphological data, but not recovered when using molecular data only (Arnedo *et al*. 2009; Dimitrov *et al*. 2012). Currently, the monophyly of the family Linyphiidae is unambiguously supported by four morphological synapomorphies, all from the male pedipalp: the presence of a suprategulum, the presence of a linyphiid radix and the absence of a median apophysis and conductor (Hormiga 1994*b*; Miller and Hormiga 2004; Hormiga *et al*. 2005; Arnedo *et al*. 2009). Within Linyphiidae the monophyly of the subfamilies Mynogleninae and Erigoninae are generally well supported by morphological as well as molecular data (Hormiga 1994*b*, 2000; Miller and Hormiga 2004; Arnedo *et al*. 2009). The monophyly and validity of the monotypic subfamily Stemonyphantinae Wunderlich, 1986, have not yet been tested. However, *Stemonyphantes* Menge,

1866 has been suggested as the sister group to all other linyphiids (Wunderlich 1986; Hormiga 1994*b*, 2000; Miller and Hormiga 2004; Hormiga *et al*. 2005; Hormiga and Scharff 2005).

Arnedo *et al*. (2009), in their recent phylogenetic analysis of combined morphological and molecular data from 35 linyphiids (representing all currently used subfamilies – Stemonyphantinae, Mynogleninae, Erigoninae and Linyphiinae (Micronetini plus Linyphiini)) and 12 outgroup species (representing nine araneoid families), found support for the basal placement of *Stemonyphantes* within Linyphiidae. However, different analyses and data partitions produced different hypotheses for the position of *Stemonyphantes* within linyphioids: as a sister group of the family Pimoidae or in an unresolved trichotomy with Pimoidae and the remaining Linyphiidae (combined analysis under direct optimisation), or as a sister group to all other linyphiids (all other parameter sets, Bayesian combined analysis and morphological data alone).

The type species of *Stemonyphantes* was described about two and a half centuries ago by Linnaeus (1758) as *Aranea lineata*, but the genus *Stemonyphantes* was first erected almost a century later by Menge (1866), and included only three species until the end of the 19th century: *Stemonyphantes lineatus* (Linnaeus, 1758), *S. conspersus* (L. Koch, 1879) and *S. sibiricus* (Grube, 1861). Today, *Stemonyphantes* includes 18 species, all from the northern hemisphere (Tanasevitch 2011, 2012; Platnick 2012). *Stemonyphantes* are relatively large linyphiids (~4–6 mm), usually found on the ground level and near the base of vegetation in grasslands and gardens, under stones and in burrows, and may be found also in open areas of forests and along seashores. Their sheet webs are not conspicuous and usually only a few threads are visible. In Britain and other parts of northern Europe, adults are found year-round, but with peaks in autumn–winter and mid-summer (Roberts 1995; Harvey *et al*. 2002). A few attempts have been made to homologise the tegular sclerites of *Stemonyphantes* to those of other linyphiids without much success (Blauvelt 1936; Merrett 1963; van Helsdingen 1968; Millidge 1977), probably due to its unusual pedipalp morphology (Hormiga 1994*b*), but this has never been done in an explicitly phylogenetic context.

In many animal orders, including spiders, the male external genitalia have evolved rapidly and divergently and are species specific (Eberhard 1985). In linyphiids, like in the majority of spider families, the male secondary genital organs (hereafter: palps) and female genitalia are the most useful morphological characters for discrimination between species and genera (Comstock 1910; Eberhard 1985; Eberhard and Huber 2010). Although palp homologies are important for spider family-level phylogeny and considerable efforts have been made to establish palp homologies within genera and beyond the genus level, there are still many inconsistencies in the use of the names of homologues palp sclerites (Comstock 1910; Blauvelt 1936; Merrett 1963; van Helsdingen 1968; Saaristo 1971; Millidge 1977, 1980; Coddington 1990; Hormiga 1994*a*, 1994*b*; Agnarsson *et al*. 2007). As more than half of the morphological characters used to reconstruct linyphiid phylogenetic relationships code features of the male palp, inconsistencies in the use of palp homology names may affect the results of the phylogenetic analysis and the tree topology, and thus our understanding of the evolution of linyphiids. *Stemonyphantes* has been hypothesised to be the sister clade to the rest of linyphiids, and therefore resolving its palp sclerite homologies is needed to address the homologies of palp sclerites within the entire family.

Merrett (1963) described and illustrated the palp morphology of more than 100 linyphiid species from Great Britain in detail and suggested two basic generalised linyphiid palp types: ‘simple’ and ‘complex’ palp types, which predominantly differ in embolic division and suprategulum morphology. All linyphiid male palps consist of a paracymbium attached to the cymbium, basal haematodocha, subtegulum, tegulum, suprategulum and an embolic division connected to the suprategulum by the membranous column (Comstock 1910; Merrett 1963; Saaristo 1971; Millidge 1977, 1980). In the ‘complex’ type, the embolic division consists of a radix, which bears the embolus, the terminal apophysis and the lamella. The ‘simple’ type consists of a single sclerite with a radical part and an embolic part that carries the embolus (Merrett 1963). As noted above, two of the linyphiid synapomorphies are provided by the absence of the araneoid median apophysis and conductor (Coddington 1990; Hormiga 1994*a*, 1994*b*, 2000; Miller and Hormiga 2004; Arnedo *et al*. 2009). The presence of a median apophysis and a conductor is plesiomorphic for araneoids, and these two sclerites are present in many species of Pimoidae, the putative sister group of Linyphiidae (Hormiga 1994*a*, 1994*b*, 2000; Miller and Hormiga 2004; Hormiga and Tu 2008; Arnedo *et al*. 2009). All described pimoids have a conductor, but the median apophysis is absent in several species. In pimoids with a median apophysis this sclerite is usually a small hook that arises on the tegulum and may share its base with the membranous conductor that also arises from the tegulum (Hormiga 1994*a*; Hormiga *et al*. 2005).

To investigate the phylogenetic placement of *Stemonyphantes* within linyphioids and the monophyly, validity and circumscription of the subfamily Stemonyphantinae, we tested various competing primary hypotheses (see de Pinna 1991 for discussion of primary versus secondary homology hypotheses) of palp sclerite homologies between *Stemonyphantes* and other linyphioids, by adding four *Stemonyphantes* representatives to the morphological data matrix of Arnedo *et al*. (2009). The aforementioned morphological matrix included only one *Stemonyphantes* species, *S. blauveltae* Gertsch, 1951. We specifically addressed the homology of tegular and embolic apophyses of the five *Stemonyphantes* species (*S. lineatus*, *S. conspersus*, *S. agnatus* Tanasevitch, 1990; *S. altaicus* Tanasevitch, 2000; and *S. blauveltae*) within the context provided by a sample of linyphioid and araneoid taxa. These five species represent the variation of palp sclerite morphology in *Stemonyphantes*.

The initial conjecture of *Stemonyphantes* palp sclerite primary homologies (H0) follows the Arnedo *et al*. (2009) morphological phylogeny: i.e. the tegulum, as in the rest of the linyphiids, bears neither a median apophysis nor a conductor, and the embolic division has both a radical tail-piece and anterior radical processes. This hypothesis suggests that linyphiids (including *Stemonyphantes*) are monophyletic, and that the absence of both a median apophysis and a conductor, and the presence of a suprategulum and a linyphiid radix are synapomorphies for this family. We tested this null hypothesis against various alternative hypotheses of palp sclerite primary

homologies where the median apophysis, conductor, radical tail-piece and anterior radical processes were scored as absent or present in various combinations (H1–H3; Table 1). The competing hypotheses may suggest that linyphiids, excluding *Stemonyphantes*, are monophyletic (as recent molecular analysis suggests; Arnedo *et al*. 2009; Dimitrov *et al*. 2012), and that in *Stemonyphantes* the median apophysis and conductor are present and symplesiomorphic (H1, H2).

Materials and methods

Morphology

Specimens were studied in 70% ethanol, and methyl salicylate (Holm 1979). Soft tissues were digested with SIGMA Pancreatin LP1750 enzyme complex (Álvarez-Padilla and Hormiga 2007) or CIBA Vision Unizyme enzymatic eye lens cleaner diluted with distilled water to study internal structures such as tracheae, copulatory ducts and spermathecae of the epigynum. Male palps were expanded through transfers between 10% KOH and distilled water. Specimens were examined and illustrated using Leica MZApo and Leica M205C (Heerbrugg, Switzerland) stereo-microscopes with a camera lucida. Further details were studied using a Leica DMRXE (Heerbrugg, Switzerland) compound microscope with a drawing tube. Digital microscope images were taken using two different imaging systems: a BK Plus Laboratory System from Visionary Digital (Palmyra, PA, USA) equipped with a Canon EOS 7D camera (http://www.visionarydigital.com; verified December 2012), and a Leica M205AC stereo-microscope equipped with a Leica DFC420 camera. Multi-layer pictures were combined using Helicon Focus software ver. 5.0 (Kharkov, Ukraine). All figures were edited using Adobe Photoshop ver. CS3 or GIMP ver. 2.6.10 and Inkscape ver. 0.48. Left structures (palps) are illustrated unless otherwise stated. Where sufficient material was available, one female and one male specimen were examined using scanning electron microscopy (SEM). Specimens were prepared for SEM by first placing them into a series of ethanol concentrations from 75% to absolute ethanol with 5% differences between consecutive concentrations and for 10–15 min in each concentration then overnight in absolute ethanol. Specimens were then cleaned ultrasonically for 30 s using a Bransonic 2000 sonicator (Danbury, CT, USA). Subsequently, the cephalothorax, abdomen, left legs and pedipalps of both the female and male were detached and critical-point dried using a Baltec CPD-030 dryer (Balzers, Liechtenstein). Dried parts were attached to round-headed rivets using aluminium tape with conductive adhesive and coated with platinum-palladium in a JEOL (Tokyo, Japan) JFC-2300HR high resolution coater for 140 s. Scanning electron micrographs were taken with a JEOL JSM-6335F scanning electron microscope. All work was carried out at the Zoological Museum, University of Copenhagen.

The following anatomical abbreviations are used in the text and figures: a, the connection of the column to the embolic division; ARP, anterior radical processes; BH, basal haematodocha; C, conductor; CB, cymbium; CL, column; DTA, dorsal tibial apophysis; dp, process on the dorsal tibial apophysis; E, embolus; E tip, the tip of the embolus; EBCP, ecto-basal cymbial process; EMCP, ectal marginal cymbial process; EP, embolic part; EPr, embolic process; m, membrane; MA, median apophysis; mTP, median tibial process; MH, median haematodocha; P, paracymbium; P1, radical process 1; P2, radical process 2; P3, radical process 3; P4, radical process 4; PLS, posterior lateral spinnerets; Pt, palpal patella; RMT, radical mesal tooth; RP, radical part; RTP, radical tail-piece; SPT, suprategulum; SPTA, suprategulum distal apophysis; SPTA1, suprategulum distal apophysis 1; SPTA2, suprategulum distal apophysis 2; SPTR, suprategulum ring; ST, subtegulum; T, tegulum; TA1, tegular apophysis 1; TA2, tegular apophysis 2; TA3, tegular apophysis 3; TB, tibia; TC, tegular cavity; TR, tegular ridge; VTP, ventral tibial process.

Taxa

In order to test the monophyly, validity and circumscription of the subfamily Stemonyphantinae, and to infer its phylogenetic

Table 1. Primary hypotheses of palp sclerite homology including the null hypothesis (H0, following Arnedo *et al.* 2009) and three alternative hypotheses

The hypotheses specifically test the homologies of tegular projections (characters 29 and 30) and radical processes on the embolic division (characters 42–44) of the five *Stemonyphantes* species, and differ among them in the scoring of the five relevant binary characters (all five characters with states: 0, absence; 1, presence): character 29 (MA, median apophysis), character 30 (C, conductor), character 42 (RTP, radical tail-piece), character 43 (ARP, anterior radical processes) and character 44 (RMT, radical mesal tooth). The most right-hand column shows the differences in scoring relative to the null hypothesis (H0), following Arnedo *et al*. 2009. *All hypotheses except H3 include in their matrices only the original 149 characters from Arnedo *et al*. 2009. The matrix of hypothesis H3 includes two additional characters, a total of 151 characters

Hypothesis*	Character 29: MA	Character 30: C	Character 42: RTP	Character 43: ARP	Character 44: RMT	Changes from H0/ Arnedo *et al.* 2009
H0	Absent in all**	Absent in all	Present in all	Absent in *S. agnatus* Present in all the rest	Absent in all	No changes
H1	Absent in *S. altaicus* Present in all the rest	Present in all	Absent in all	Absent in all	Absent in all	+MA, +C –RTP, –ARP
H2	Absent in *S. altaicus* Present in all the rest	Present in all	Present in all	Absent in *S. agnatus* Present in all the rest	Absent in *S. agnatus* Present in all the rest	+MA, +C, +RMT
H3*	Absent in all	Absent in all	Present in all	Absent in *S. agnatus* Present in all the rest	Absent in *S. agnatus* Present in all the rest	+RMT, +2 novel tegular structures

**'all' refers to the five *Stemonyphantes* species studied (see text for details).

placement we used the morphological matrix of Arnedo *et al*. (2009). This matrix scored 35 linyphiid species representing six subfamilies (Micronetinae, Linyphiinae, Erigoninae, Mynogleninae, Dubiaraneinae, Stemonyphantinae (*Stemonyphantes blauveltae*) and some of Millidge's (1993) 'miscellaneous genera' together with 12 outgroup species representing nine araneoid families (Araneidae, Theridiosomatidae, Mysmenidae, Tetragnathidae, Nesticidae, Theridiidae, Synotaxidae, Cyatholipidae and Pimoidae) (see Arnedo *et al*. 2009 for the complete list of taxa).

To this matrix we added four *Stemonyphantes* species: *S. lineatus* (the type species); *S. conspersus*; *S. altaicus*; and *S. agnatus*, although we have studied specimens of 13 of the 18 described *Stemonyphantes* species (see Appendix 1 for a list of voucher specimens). In addition, we edited and changed the scoring of 12 of the existing characters scored for *S. blauveltae* in Arnedo *et al*. 2009 (see Appendix 2) and scored the 149 characters in that matrix for the additional four *Stemonyphantes* species we added (see below and Table 1 for the differences in scoring for each of the hypotheses H0–H2); for one further analysis (hypothesis H3), two additional characters were added (see below and Table 1; see Arnedo *et al*. 2009 for the complete list of the 149 characters). Mesquite ver. 2.74 (Maddison and Maddison 2007) was used to edit the character matrices.

We here present illustrations of only three *Stemonyphantes* species: *S. lineatus*, *S. agnatus*, both representing two extremes of variation of male palp morphology within the genus, and *S. altaicus*, which represents an intermediate palp morphology.

Characters and hypotheses of palp sclerite homologies

The different primary hypotheses of palp sclerite homologies addressed the tegular projections and radical processes on the embolic division in *Stemonyphantes* (five binary characters in the matrix) relative to the araneoid tegular and radical structures.

Tegular projections

Male *Stemonyphantes* species have two to four apophyses on the tegulum. We tested the hypothesis that one of these structures is the median apophysis (MA) (Figs 1*A*, *C*, 3: TA1) and another is the conductor (C) (Figs 1*A*, *C*, 2*C*, *D*, 3: TR) (Table 1: hypotheses H1, H2; 149 characters; *Stemonyphantes* spp. were scored as having both MA and C), versus the null hypothesis that *Stemonyphantes* species, like other linyphiids, lack both the median apophysis and the conductor (Table 1: hypothesis H0; 149 characters; *Stemonyphantes* spp. were scored as lacking both MA and C). As there are no other suitable characters for *Stemonyphantes* tegular apophyses in the matrix of Arnedo *et al*. 2009, we also tested the hypothesis that *Stemonyphantes* have neither a median apophysis nor a conductor but instead have two unique and novel tegular apophyses (Table 1: hypothesis H3; 151 characters; *Stemonyphantes* spp. were scored as lacking both MA and C but gained two novel tegular apophyses).

Radical processes

Stemonyphantes species have two to five processes on the radical part of their embolic division. Hormiga (2000) coded *Stemonyphantes blauveltae* as lacking a radical tail-piece (RTP) but left the anterior radical processes (ARP) scored as '?' because he considered its presence or absence to be uncertain at the primary level. More recently, *Stemonyphantes* was conservatively scored as having both a radical tail-piece (character 42, Arnedo *et al*. 2009) and anterior radical processes (character 43, Arnedo *et al*. 2009), but both Miller and Hormiga's (2004) and Arnedo *et al*.'s (2009) results suggested that these structures had evolved independently in *Stemonyphantes* (and thus were not homologous to those of other linyphiids; see also Hormiga (2000) under characters 22 and 23). We further tested the hypothesis that *Stemonyphantes* have neither anterior radical processes (ARP), nor a radical tail-piece (RTP) or a radical mesal tooth (RMT) (Table 1: hypothesis H1; 149 characters; *Stemonyphantes* spp. were scored as lacking ARP, RTP and RMT) versus the null hypothesis that one of the processes is one of the anterior radical processes and that another process is the radical tail-piece (Table 1: hypothesis H0; 149 characters; *Stemonyphantes* spp. were scored as having both RTP (Fig. 4: P1) and ARP (Fig. 4: P2)). We also tested the hypothesis that *Stemonyphantes* have anterior radical processes, a radical tail-piece and a radical mesal tooth (character 44, Arnedo *et al*. 2009) (Table 1: hypotheses H2 and H3; 149 and 151 characters respectively; *Stemonyphantes* spp. were scored as having both RTP (Fig. 4: P1), ARP (Fig. 4: P2) and RMT (Fig. 4: P3)). Table 1 summarises the four hypotheses; however, more combinations of the scoring for the five characters were tested (see Supplementary material for the Nexus file of hypothesis H1).

Phylogenetic analyses

To assess the phylogenetic implications of the different competing primary hypotheses of palp sclerite homologies (hypotheses H0–H3; see Table 1) for the five *Stemonyphantes* species, we carried out parsimony analyses using the computer program TNT ver. 1.1 (Goloboff *et al*. 2003, 2008), with separated weighted analyses executed for each of the four matrices corresponding to hypotheses H0–H3.

Heuristic (traditional) searches with tree-bisection-reconnection (TBR) swapping algorithm were carried out using maximum length as the collapsing rule (collapsing rule 3 in TNT), under equal weights, and with 1000 replicates while holding 100 trees per replication (different combinations of the number of replicates and number of trees holding per replication were tested). Analyses under implied weights were also carried out, with the same search parameters as mentioned above, and with concavity constant values from $k = 1$ up to the first k value that gave the same tree (length and topology) as in the most parsimonious tree (MPT) from the equal weights analyses. In this morphological dataset, k values of 15–17 (depending on the different sclerite homology hypotheses) gave the same trees (length and topology) as those of the equal weights analyses. Bremer support (BS), retention index (RI) and consistency index (CI) were calculated with TNT. For the BS values (Bremer 1994) a rough precedent search setting suboptimal to 20 was made to find the upper limit of supports (hold 10 000; sub 20; bb = fillonly tbr; bsupport). The

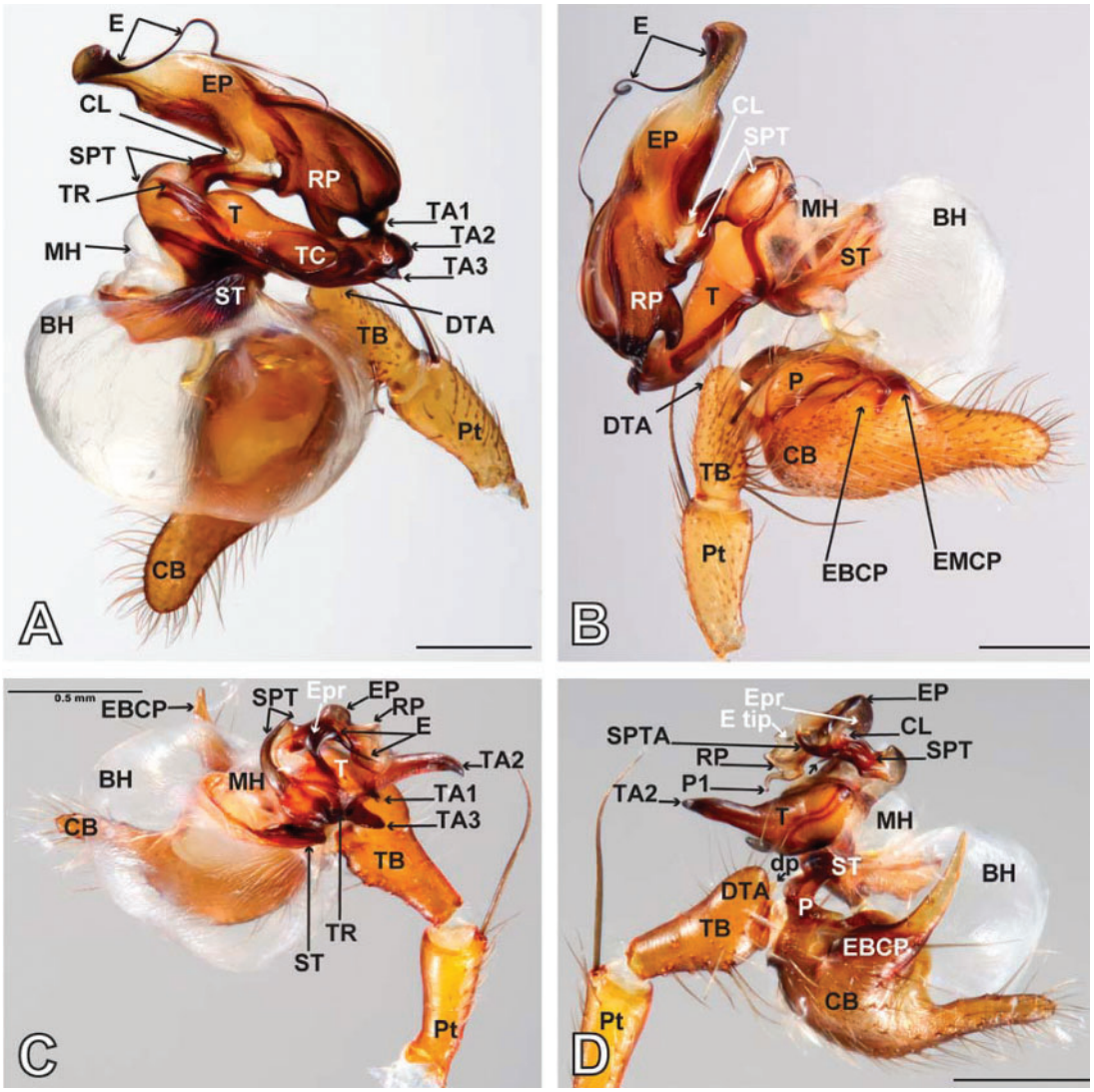

Fig. 1. *A–D. Stemonyphantes* male left palp, expanded. (*A*, *B*) *S. lineatus*. (*A*) Cymbium and tegulum in ventro-ectal view; embolic division in dorso-ectal view. (*B*) Cymbium and tegulum in dorso-mesal view; embolic division in ventro-mesal view. (*C*, *D*) *S. agnatus*. (*C*) Ventro-ectal view. (*D*) Dorso-mesal view; small black arrow pointing to the tip of the embolic process. Abbreviations: BH, basal haematodocha; CB, cymbium; CL, column; DTA, dorsal tibial apophysis; dp, process on the dorsal tibial apophysis; E, embolus; E tip, the tip of the embolus; EBCP, ecto-basal cymbial process; EMCP, ectal marginal cymbial process; EP, embolic part; EPr, embolic process; MH, median haematodocha; P, paracymbium; P1, radical process 1; Pt, palpal patella; RP, radical part; SPT, suprategulum; SPTA, suprategulum distal apophysis; ST, subtegulum; T, tegulum; TA1, tegular apophysis 1; TA2, tegular apophysis 2; TA3, tegular apophysis 3; TB, tibia; TC, tegular cavity; TR, tegular ridge. Scale bars 0.5 mm.

more thorough search was based on the original equal weighted trees. Subsequently, the suboptimal was increased stepwise by 1 up to 20 and so was the tree buffer by 1000 for 20 cycles (commands: sub 1; hold 1000; bb = fillonly tbr; sub 2; hold 2000; bb = fillonly tbr; sub 3; hold 3000; bb = fillonly tbr; sub 4; hold 4000; bb = fillonly tbr; etc. until sub 20; hold 20000; bb = fillonly tbr; bsupport). Mesquite ver. 2.74 (Maddison and Maddison 2007) and WinClada ver. 1.00.08 (Nixon 2002) were used to study character optimisations and ACCTRAN optimisations were preferred for ambiguous character optimisations.

Results

Morphology

General male palp morphology in Stemonyphantes

We focussed on the two apical segments of the palp: the tibia and the modified tarsus (the bulb). The palp is relatively large with a tibia + cymbium length that is approximately half of the length of the cephalothorax. The bulb includes cymbium with paracymbium, basal haematodocha, subtegulum, median haematodocha, tegulum, suprategulum and embolic division connected to the suprategulum by a membranous column.

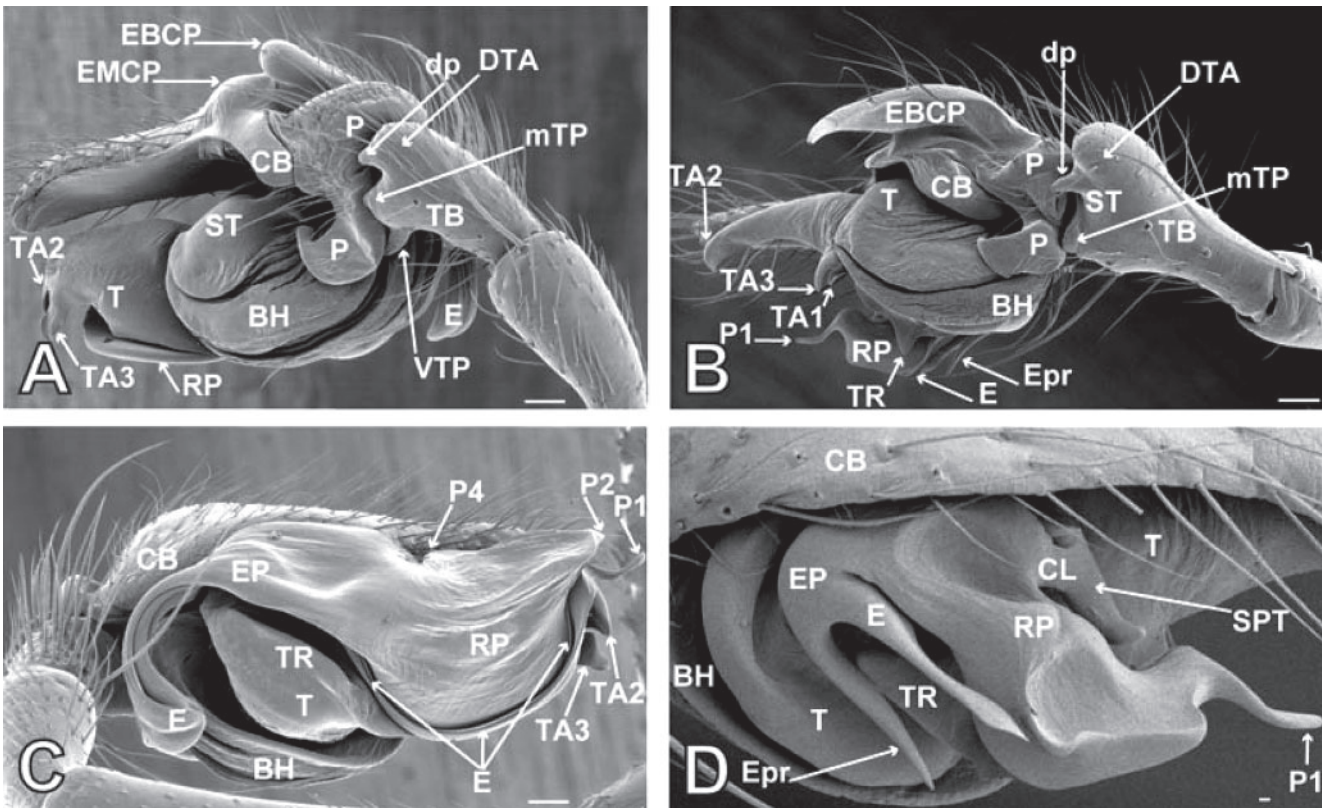

Fig. 2. *A–D*. Scanning electron micrographs of *Stemonyphantes* male left palp. (*A*, *B*) Ectal view. (*A*) *S. lineatus*. (*B*) *S. agnatus*. (*C*, *D*) Ventral view. (*C*) *S. lineatus*. (*D*) *S. agnatus* embolic division. Abbreviations: BH, basal haematodocha; CB, cymbium; CL, column; DTA, dorsal tibial apophysis; dp, process on the dorsal tibial apophysis; E, embolus; EBCP, ecto-basal cymbial process; EMCP, ectal marginal cymbial process; EP, embolic part; EPr, embolic process; mTP, median tibial process; P, paracymbium; P1, radical process 1; P2, radical process 2; P4, radical process 4; RP, radical part; SPT, suprategulum; ST, subtegulum; T, tegulum; TA1, tegular apophysis 1; TA2, tegular apophysis 2; TA3, tegular apophysis 3; TR, tegular ridge; VTP, ventral tibial process. Scale bars 100 µm (*A–C*), 10 µm (*D*).

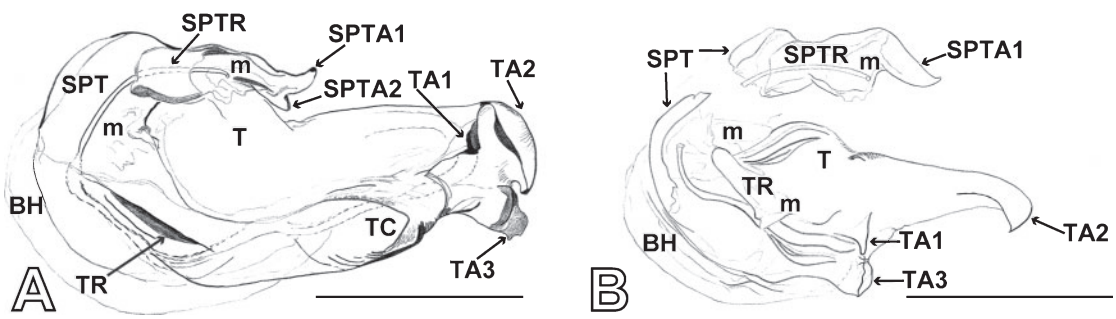

Fig. 3. *A*, *B*. *Stemonyphantes* male left tegulum and suprategulum, ventral views. (*A*) *S. lineatus*. (*B*) *S. agnatus* (the suprategulum was broken in the membranous hinge). Abbreviations: BH, basal haematodocha; m, membrane; SPT, suprategulum; SPTA1, suprategulum distal apophysis 1; SPTA2, suprategulum distal apophysis 2; SPTR, suprategulum ring; T, tegulum; TA1, tegular apophysis 1; TA2, tegular apophysis 2; TA3, tegular apophysis 3; TC, tegular cavity; TR, tegular ridge. Scale bars 0.5 mm.

Expanded palps of *S. lineatus* and *S. agnatus* are shown in Fig. 1. The palpal patella is relatively long, as long as the tibia or longer. Tibia with two ectal and one mesal trichobothria, and two to three apophyses, variable in size across the species: a dorsal apophysis (Fig. 2*A*, *B*: DTA) with a process (dp); a median process (Fig. 2*A*, *B*: mTP); and in some species an additional ventral process (Fig. 2*A*: VTP). Cymbium with ecto-basal cymbial process, variable in size across species, and with an ecto-marginal cymbial process in some species (Fig. 2*A*, *B*: EBCP, EMCP respectively). Although the paracymbium attachment to the cymbium is integral in some species (e.g. *S. agnatus* and *S. altaicus*), in most *Stemonyphantes* species it is membranous with an integral connection on the mesal side (e.g. *S. lineatus*, *S. blauveltae* and *S. conspersus*) and varies in size across species.

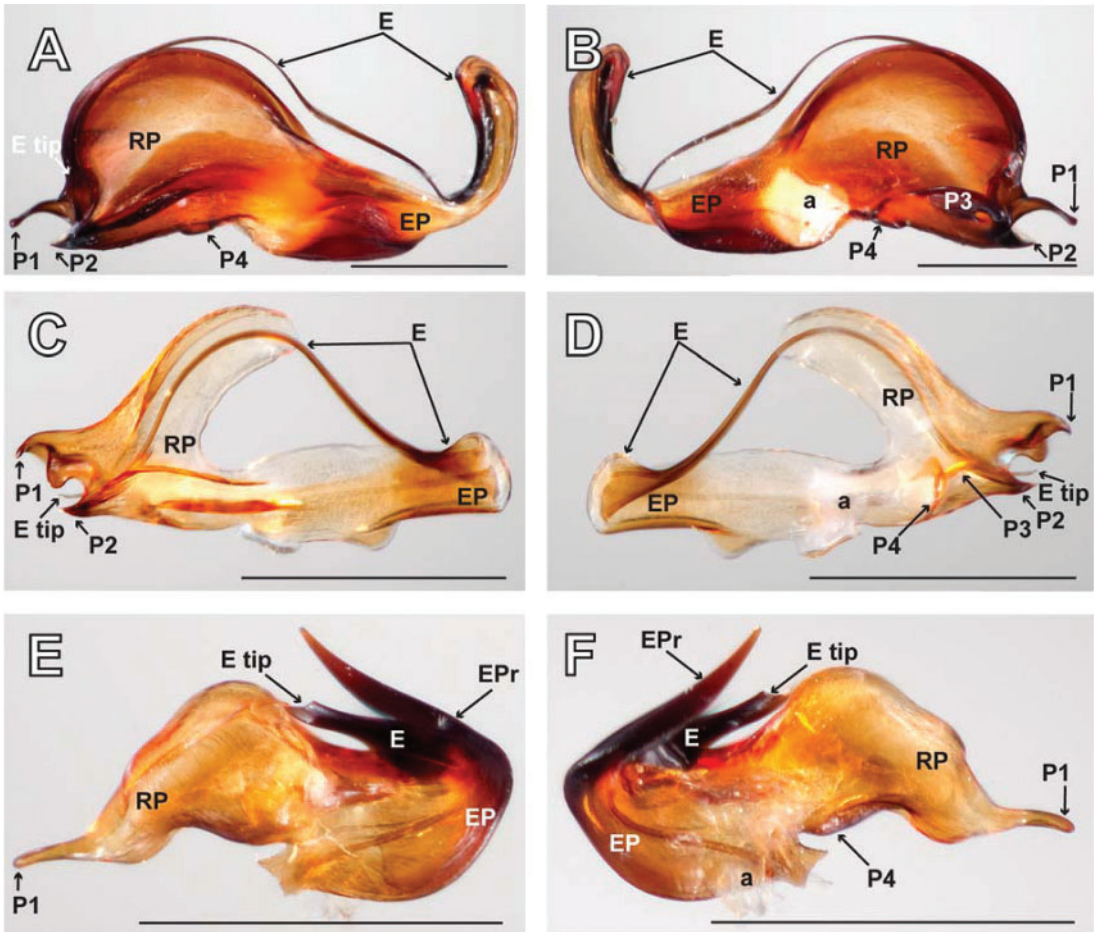

Fig. 4. *A–F. Stemonyphantes* male embolic divisions (ED). (*A*, *B*) *S. lineatus* left ED. (*A*) Ventral view. (*B*) Dorsal view. (*C*, *D*) *S. altaicus* right ED (mirror). (*C*) Ventral view. (*D*) Dorsal view. (*E*, *F*) *S. agnatus* left ED. (*E*) Ventral view. (*F*) Dorsal view. Abbreviations: a, the connection of the column to the embolic division; E, embolus; E tip, the tip of the embolus; EP, embolic part; EPr, embolic process; P1, radical process 1; P2, radical process 2; P3, radical process 3; P4, radical process 4; RP, radical part. Scale bars 0.5 mm.

All *Stemonyphantes* species have a hook-shaped paracymbium with a raised (swollen) base with setae (Fig. 2*A*, *B*). Most of the genitalic differences between *Stemonyphantes* species and the rest of the linyphiids are in the morphology of the paracymbium, tegulum, suprategulum and embolic division.

Tegulum and suprategulum

Stemonyphantes species have an elongated (oval) tegulum (Fig. 2: T) bearing up to four apophyses or processes (Fig. 3: TR, TA1, TA2, TA3) and a suprategulum articulated by a membrane (Fig. 3: SPT, m) with a ventrally membranous hinge (the dorsal side of the hinge is not fully membranous), and with a fully sclerotised median ring (Fig. 3: SPTR) that is unique to *Stemonyphantes*. The distal part of the suprategulum bears apophyses (Fig. 3*A*, *B*: SPTA1, SPTA2). The proximal part of the tegulum bears one long and narrow ridge (Fig. 2*C*, *D*; 3: TR) that is found in all species but varies in shape and sclerotisation or membranous level across species. In some species, the proximal part of the tegulum is divided ventrally by penetration of the membranous hinge of the suprategulum, which creates the mesal wall of a distal cavity (Fig. 3*A*: TC). The distal part of the tegulum bears up to three apophyses, one apical (medial in *S. lineatus*, *S. blauveltae*, *S. conspersus* and *S. agnatus*; Fig. 3: TA2) and two lateral (Fig. 3: TA1 and TA3; both missing in *S. altaicus*). The turning point of the sperm duct is between the two lateral apophyses. In most of the species the apophyses are ventrally concave. Some species have only the apical apophysis (TA2, e.g. *S. altaicus*). The embolic division is connected to the apical membranous part of the suprategulum (Fig. 1: SPT) through a membranous column (Fig. 1: CL) distal to the suprategulum ring.

Embolic division

The embolic division is a flat sclerite that consists of a radical part (RP) and an embolic part (EP) (Fig. 4). In some species (e.g. *S. agnatus*) the embolic division is not flat. The radical part bears one to four or five projections (Fig. 4: P1–P4) in addition to ridges and furrows, and the embolic part bears the embolus and an embolic process in some species (e.g. *S. agnatus*; Fig. 4*E*, *F*: EPr). In the unexpanded palp of all *Stemonyphantes* species (Fig. 2*C*, *D*), the embolic division is positioned with the

radical part pointing towards the distal part of the palp, while the embolic part points towards the proximal part of the palp. The sperm duct and the embolus have a strong turning point, and therefore the tip of the embolus usually points to the distal part of the palp, parallel to the radical part. In most species the embolus, after its turning point, is a curved long and slender filiform structure (e.g. *S. lineatus*, *S. blauveltea*, *S. conspersus* and *S. altaicus*), yet some species (e.g. *S. agnatus*) have a short and stout embolus. In the species with the long filiform embolus, the distal part of the embolus usually rests in a marginal ectal furrow (Fig. 2*C*), and the tip of the embolus rests between the distal projections of the radical part (Fig. 4*A*, *C*: P1–P2). Furthermore, the embolus of the unexpanded palp, is always closely associated with the tegular ridge (Fig. 2*C*, *D*: TR), which seems to keep the embolus in place.

Phylogenetic analyses, characters and hypotheses of homology

All minimum length trees found under all hypotheses explored (H0–H3) and all analytical methods used (equal weights and implied weighting from $k = 3$) have a monophyletic *Stemonyphantes* as sister group to all other linyphiids (Figs 5–7).

Heuristic searches in TNT under equal weights resulted in 12 minimal length trees of length 677 (RI = 0.61; CI = 0.29; H0), 679 (RI = 0.62; CI = 0.29; H2) and 681 (RI = 0.62; CI = 0.29; H3) for each of the three analyses respectively. All 12 trees, in each of the three analyses, were fully resolved, with the Pimoidae clade as the sister group to Linyphiidae, *Stemonyphantes* as the sister to all other linyphiids and disagreed only in three areas on the cladogram (internal relationships of micronetines, erigonines

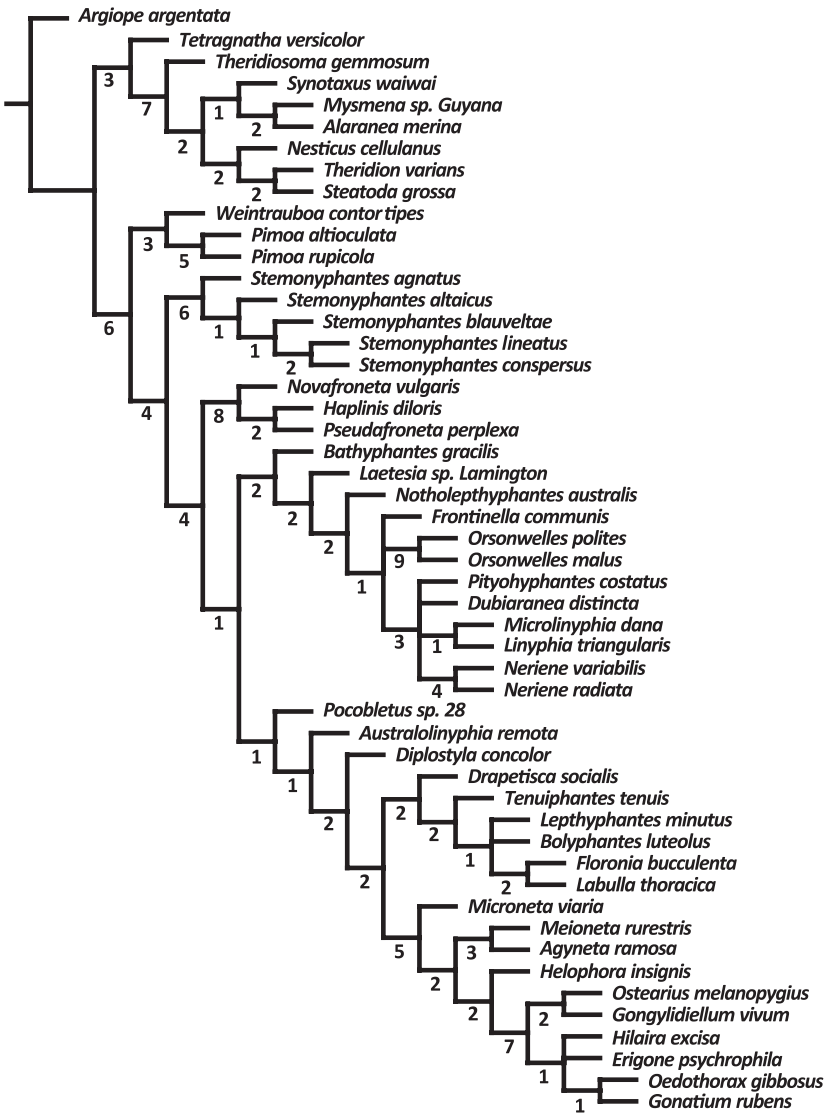

Fig. 5. Strict consensus of 12 most parsimonious trees (all 12 trees 677 steps long under equal weights; RI = 0.61; CI = 0.29) based on the null hypothesis (H0, following Arnedo *et al*. 2009; 149 characters; with five *Stemonyphantes* species scored as lacking MA, C and RMT, and having ARP and RTP) and with Bremer supports (20 000 trees, cut 0).

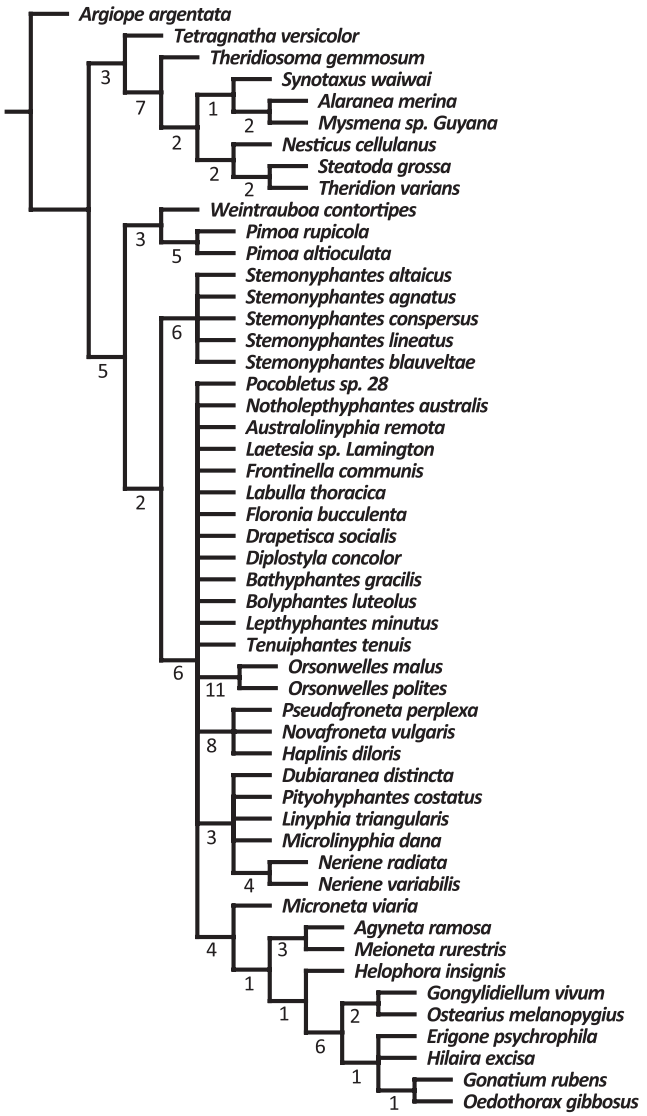

Fig. 6. Strict consensus of 82 most parsimonious trees (all 82 trees 676 steps long under equal weights; RI = 0.61; CI = 0.29), based on hypothesis H1 (149 characters; with five *Stemonyphantes* species scored as having MA and C, and lacking ARP, RTP and RMT) and with Bremer supports (20 000 trees, cut 0).

and Linyphiini). Fig. 5 shows the strict consensus of the 12 most parsimonious trees of length 677 under equal weights for the null hypothesis (H0, following Arnedo *et al*. 2009) with Bremer supports. Hypothesis H1 resulted in 82 minimal length trees of length 676 (RI = 0.61; CI = 0.29; H1). All 82 trees were fully resolved, with Pimoidae as the sister group to Linyphiidae and *Stemonyphantes* as the sister to all other linyphiids. However, they disagreed on the internal relationships of the rest of the linyphiids and on the internal relationships of *Stemonyphantes* (four possible topologies for *Stemonyphantes*). Fig. 6 shows the strict consensus of the 82 most parsimonious trees of length 676 under equal weights for hypothesis H1 with Bremer supports.

Heuristic searches in TNT under implied weighting resulted in one fully resolved tree for each of the *k* values tested (3–15/17) and for each of the four hypotheses H0–H3. These trees had different topologies but they all placed Pimoidae as the sister group to Linyphiidae and *Stemonyphantes* as the sister group to all other linyphiids. For hypotheses H0 and H3 and for hypotheses H1 and H2 the trees inferred under $k = 15$ and $k = 17$ respectively were the same (length and topology) as one of the 12 trees (or 82 trees for hypothesis H1) inferred under equal weights of each of the hypotheses H0–H3. Therefore, we used the implied weighted analyses as criteria to choose one tree among equally weighted trees for each hypothesis. Fig. 7 shows the preferred tree for hypothesis H1 (i.e. five *Stemonyphantes* species scored as having MA, C and lacking RTP, ARP and RMT), which was found under equal weights (one of 82 trees) and under implied weighting ($k = 17$) with character optimisations mapped on the tree (ACCTRAN).

Discussion

Our results support the basal placement of *Stemonyphantes* within Linyphiidae, as the sister group to all other linyphiids, and the monophyly of Stemonyphantinae. Although our study added more species to the morphological matrix of Arnedo *et al*. (2009), it did not add characters (except for those of hypothesis H3). The monophyly of *Stemonyphantes* is supported by just one unambiguous synapomorphy, the morphology of the paracymbium (character 12), but during the study of this genus we found other potential synapomorphies that could support *Stemonyphantes* monophyly. The special intermediate stage between integral and intersegmental paracymbium attachment in *Stemonyphantes* has been suggested as a synapomorphy for the genus (Millidge 1988; Hormiga 1994*b*; Arnedo *et al*. 2009). However, our examination of additional *Stemonyphantes* species revealed species with integral paracymbium attachment (*S. altaicus* and *S. agnatus*; Fig. 2*B*), similar to some pimoids, as well as different morphological variations of the '*Stemonyphantes* intermediate paracymbium attachment', i.e. membranous with an integral connection on the mesal side (*S. lineatus*, *S. blauveltae* and *S. conspersus*; Fig. 2*A*). Therefore, paracymbium attachment is not a synapomorphy for *Stemonyphantes*. One example of a potential synapomorphy for *Stemonyphantes* is the set of unique characters to be found in the suprategulum. All *Stemonyphantes* species have their suprategulum articulated with a membranous hinge (Fig. 3; van Helsdingen 1968; Hormiga 1994*b*) in contrast to all other linyphiids, in which the suprategulum is continuous with the tegulum or with a complete membranous division (Blauvelt 1936; Hormiga 2000; Miller and Hormiga 2004). The suprategulum junction with the tegulum (character 25) does not appear to unambiguously support *Stemonyphantes* in this analysis, due to the merging of two character states into a single state that codes for all non-continuous connections of the suprategulum to the tegulum (all *Stemonyphantes* species, *Linyphia triangularis*, *Gongylidiellum vivum* and *Erigone psychrophila* in this matrix; Miller and Hormiga 2004; Arnedo *et al*. 2009; but see Hormiga 2000). The suprategulum articulation to the tegulum in *Stemonyphantes* is ventrally membranous and dorsally highly sclerotised. An additional potential synapomorphy for *Stemonyphantes* is the fully sclerotised median ring in the

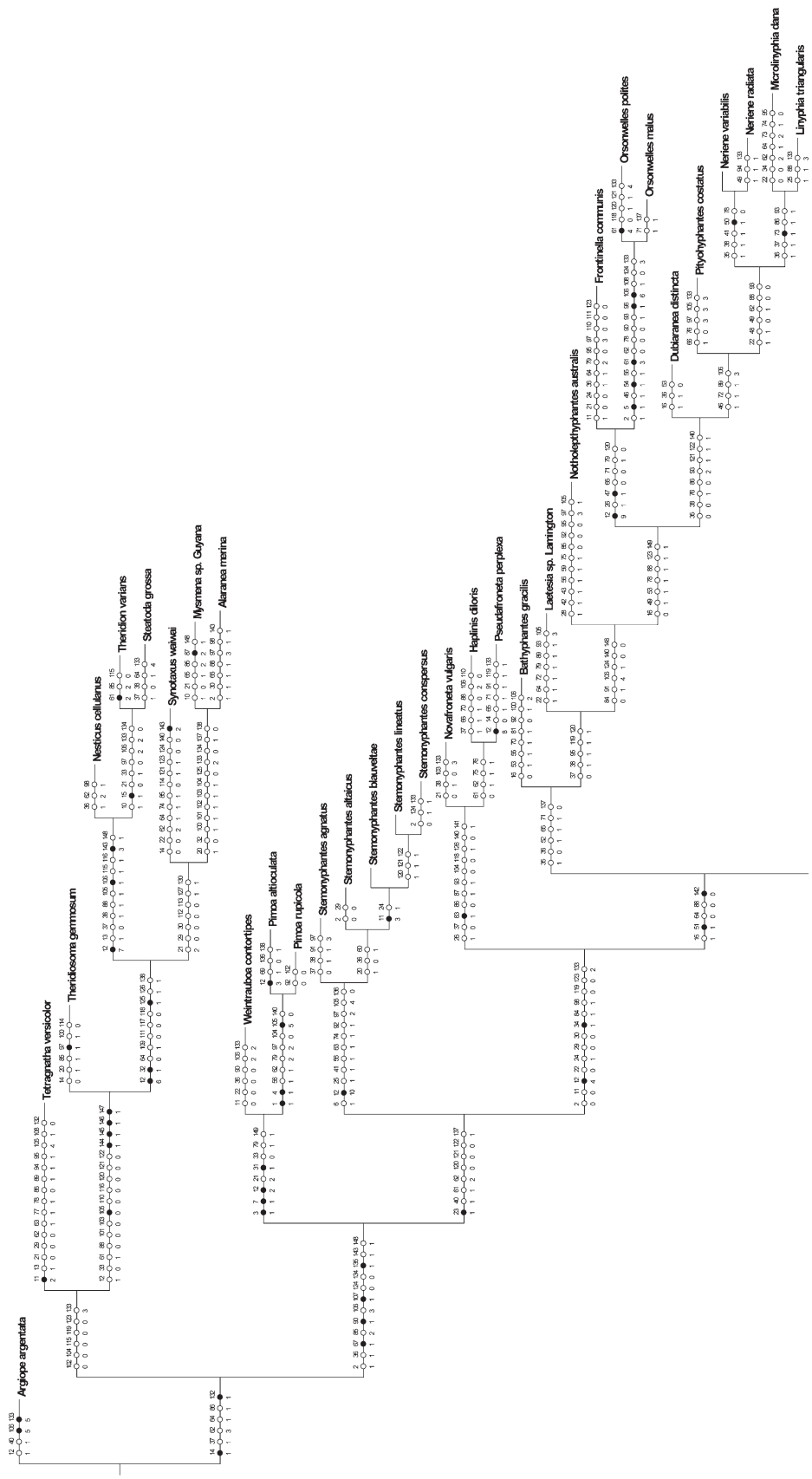

Fig. 7. One tree of length 676 under implied weighting ($k = 17$) based on hypothesis H1 (149 characters; with five *Stemonyphantes* species scored as having MA and C, and lacking ARP, RTP and RMT; morphological character changes optimised using ACCTRAN optimisation (RI = 0.61; CI = 0.29). This tree is identical to one of the 82 trees found with equal weights.

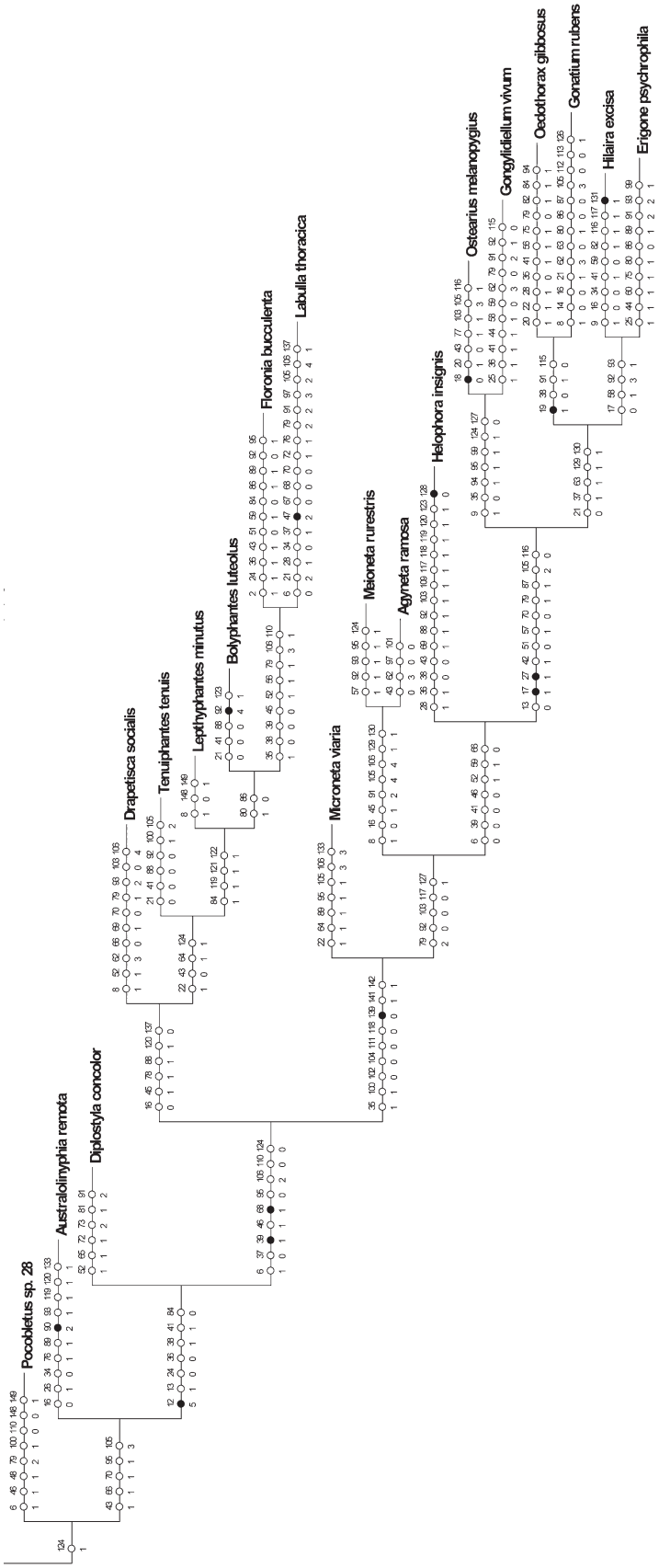

Fig. 7. (*Continued*)

middle of its suprategulum (Fig. 3: SPTR), which is unique to *Stemonyphantes*.

Characters and hypotheses of palp sclerite homology

The tegulum and especially the embolic division of *Stemonyphantes* are very different from that of other linyphiids. We explored various primary hypotheses of palp sclerite homologies and their effect on the monophyly and phylogenetic placement of *Stemonyphantes* in the trees by using different scorings for five binary characters (Table 1). We tested the presence of two tegular apophyses, the median apophysis and the conductor, and the presence of three different radical apophyses: the radical tail-piece, the anterior radical processes and the radical mesal tooth, using four separate matrices to test four different hypotheses (H0–H3) (see Table 1). Scoring these characters as present or absent affected the tree length by a maximum of five steps – hypothesis H3 (tree length 681) versus hypothesis H1 (tree length 676; Fig. 6) – yet had no topological effect on the monophyly and basal placement of *Stemonyphantes* as the sister group to all other linyphiids.

Our results suggest that it is more parsimonious to hypothesise that the two *Stemonyphantes* tegular apophyses, TA1 (Figs 1, 3) and TR (Figs 1, 2*C*, *D*, 3) are homologous to the araneoid median apophysis and conductor. The radical apophyses that we tested (Fig. 4: P1–P3) were not found to be homologous to the erigonine radical structures: the radical tail-piece, the anterior radical processes and the radical mesal tooth. Therefore we rejected the null hypothesis (H0) and the alternative competing hypotheses H2 and H3. Nonetheless, with the current modified matrix, we failed to reject the primary homology hypothesis H1, which suggests that *Stemonyphantes* spp. have a median apophysis and a conductor and lack the radical tail-piece, the anterior radical processes and the radical mesal tooth. The proposition of a single origin for the radical structures, with concomitant multiple losses of these radical structures requires more steps than the hypothesis of several independent origins. We should note that under H1 the ARP and the RTP are coded as absent in *Stemonyphantes*, but are not coded as any other structures in the matrix instead (relative to H0). Therefore, the total number of steps of the MPTs of H0 relative to those under H1 are not directly comparable (as in H1 the two character steps are not 'placed' anywhere else in the matrix).

The conventional view is that the absence of a median apophysis and a conductor are synapomorphies for linyphiids (Coddington 1990; Hormiga 1994*b*; Griswold *et al*. 1998). In pimoids the median apophysis is a small hook, which may share its base with the membranous conductor (Hormiga 1994*a*; 1994*b*, 2003; Hormiga *et al*. 2005; Hormiga 2008). Examination of the palps of *Stemonyphantes lineatus* or *S. blauveltae* does not give a hint to the possible homology of their tegular apophyses to the median apophysis and conductor of pimoids and other araneoids (Figs 1*A*, 2*C*, 3*A*). However, a careful examination of the male palp of *S. agnatus* (Figs 1*C*, 3*B*: TR and TA1) reveals similarities to the pimoid median apophysis (TR in *Stemonyphantes*) and conductor (TA1 in *Stemonyphantes*). *Stemonyphantes agnatus* has on the ventral proximal part of the tegulum a ridge-like sclerite (TR) with a membranous base. The distal part of this tegular ridge is also membranous, while its proximal part, pointing to the suprategular membranous hinge, is more sclerotised. Beyond the membranous part of the tegular ridge there is a highly sclerotised hook (TA1). In the unexpanded palp the embolus is closely related to this tegular ridge, which keeps it in place (Fig. 2*D*), but the tip of the embolus rests in a furrow of the radical part and not on this tegular ridge (Fig. 2*D*). The tegular ridge is found in all *Stemonyphantes* species and with similar relation to the embolus, but varies across species in the level of sclerotisation and the inclusion of the membranous part. The tegular hook (TA1) is found in most of *Stemonyphantes* species but varies in size.

The homologies of the tegular sclerites in linyphiids have been reviewed by several workers, including Blauvelt (1936), Merrett (1963), Millidge (1977), Saaristo (1973, 1975) and Hormiga (1994*b*, 2000). Coddington (1990) compared the palpal morphology of linyphiids to that of other araneoids and suggested that linyphiids and araneids share some characters such as a complex embolic division, the membranous articulation of the embolic division to the tegulum and the radix. In later studies Coddington (1991), Hormiga (1994*a*; 1994*b*) and Scharff and Coddington (1997) suggested independent origin of the araneid and linyphiid radices; however, if Araneidae is sister to 'linyphioids' (Linyphiidae + Pimoidae) their radices may be homologous (Hormiga 1994*b*; see also Griswold *et al*. 1998 for further discussion). In light of the above, it would be interesting to compare the palp of *Stemonyphantes lineatus* (Figs 1*A*, *B*, 3*A*) with the palp conformation of an araneid (see Grasshoff 1968: fig. 38) with a primary homology hypothesis as follows: the *Stemonyphantes* suprategulum ring (SPTR; Fig. 3) may be homologous to the araneid radix; the column may be homologous to the membrane between the araneid radix and stipes; and *Stemonyphantes* fused embolic division (Merrett's 'simple' type of ED) may be homologous to the stipes. This primary homology hypothesis was not tested by us and is suggested here as another possible conjecture to explore. Finally, based on our cladistics results (see Fig. 7), the monophyly of the family Linyphiidae is supported by the following eight synapomorphies: suprategulum (character 23), radix (40), one prolateral and two retrolateral tibial trichobothria in the male palp (61, 62), absence of metatarsus I dorsal, prolateral and retrolateral macrosetae (120–122) and the presence (at least in part) of the posterior lateral spinnerets (PLS) triplet in adult males (137).

In this study we aimed to test the phylogenetic placement of *Stemonyphantes* within linyphioids and the monophyly, validity and circumscription of the subfamily Stemonyphantinae by testing various competing primary hypotheses of palp sclerite homologies between *Stemonyphantes* and other linyphioids. Our results confirm the monophyly and validity of the monotypic subfamily Stemonyphantinae, and the basal placement of *Stemonyphantes* as a sister to the rest of the linyphiids. *Stemonyphantes* spp. have tegular structures that can be homologised to the araneoid median apophysis and conductor. Therefore, the absence of these two tegular sclerites can be hypothesised as synapomorphies for a large Linyphiidae clade that includes all the species in the family except those in the genus *Stemonyphantes*, rather than linyphiid synapomorphies, as has

been proposed in past studies. A species level phylogeny of all known *Stemonyphantes* species (in preparation) will help us determine the basal character sclerite ground-plan for the genus, which will be important for future phylogenetic studies of the family Linyphiidae.

Acknowledgements

We thank Miquel Arnedo, Cor J. Vink, Mike Rix and one anonymous reviewer for comments on an earlier version of the manuscript, and the following colleagues and institutions for making specimens available for study (collection abbreviations used in the text are given in bold in parentheses): Janet Beccaloni (The Natural History Museum, London, UK; **BMNH**), Angelo Bolzern (Naturhistorisches Museum, Basel, Switzerland; **NMBA**), Don Buckle (Saskatoon, Canada), Gonzalo Giribet (Museum of Comparative Zoology, Harvard University, Cambridge, USA; **MCZ**), Valeri Gnelitsa (Sumy State Teacher's Training University, Ukraine), Charles Griswold (California Academy of Sciences, San Francisco, USA; **CAS**), Peter Jäger (Senckenberg Museum Frankfurt, Germany; **SMF**), Jolanta Jurkowska and Beata Pokryszko (Museum of Natural History, Wroclaw University, Poland; **MNHWU**), Mykola Kovblyuk (V.I. Vernadsky Taurida National University, Crimea, Ukraine), Christian Kropf and Holger Frick (Natural History Museum Bern, Switzerland; **NMBE**), Kadir Bogac Kunt (Turkish Arachnological Society), Gunvi Lindberg (Swedish Museum of Natural History, Stockholm, Sweden; **NRM**), Yuri Marusik (Magadan, Russia), Kirill Mikhailov (Zoological Museum Moscow University, Russia; **ZMMU**), Norman Platnick (American Museum of Natural History, New York, USA; **AMNH**), Vlastimil Ruzicka (Institute of Entomology, Ceske Budejovice, Czech Republic), Martin Schmidt-Entling (University of Bern, Switzerland, and University of Koblenz-Landau, Germany), Andrei Tanasevitch (Center for Forest Ecology and Production, Russia), Sergei Zonstein (Department of Zoology and the National Collections of Natural History at Tel Aviv University, Israel; **TAUZM**), Zoological Museum, University of Copenhagen, Denmark (**ZMUC**). The program TNT is available thanks to sponsorship from the Willi Hennig Society. This research was supported by fellowships and grants to EGR from: the Israeli Taxonomy Initiative, the Danish Ministry of Science, Technology and Innovation (CIRIUS, Danish Government Scholarship), the Danish-Israeli fund in memory of Josef and Regine Nachemsohn, Carlsberg Foundation research grant (Carlsbergfondet), SYNTHESYS European Commission's FPVI research grant and the Natural History Museum of Denmark, Zoological Museum, University of Copenhagen (ZMUC). EGR thanks the Israeli ministry of Science, Culture and Sport for supporting the national collections of natural history at Tel Aviv University as a biodiversity, environment and agriculture research knowledge centre. GH was supported by US NSF grants DEB 1144492 and DEB 114417 (to GH and Gonzalo Giribet) and by a Selective Excellence grant from The George Washington University. GH's work at the University of Copenhagen (Zoological Museum) was supported by a scholarship from Danmarks Nationalbank. NS acknowledges the Danish National Research Foundation for support to the Center for Macroecology, Evolution and Climate. Funding for this research was also provided by a grant from the Danish Agency for Science, Technology and Innovation (project 272–08–0480) to NS.

References

Agnarsson, I., Coddington, J. A., and Knoflach, B. (2007). Morphology and evolution of cobweb spider male genitalia (Araneae, Theridiidae). *The Journal of Arachnology* **35**, 334–395. doi:10.1636/SH-06-36.1

Álvarez-Padilla, F., and Hormiga, G. (2007). A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. *The Journal of Arachnology* **35**, 538–542. doi:10.1636/Sh06-55.1

Arnedo, M. A., Hormiga, G., and Scharff, N. (2009). Higher-level phylogenetics of linyphiid spiders (Araneae, Linyphiidae) based on morphological and molecular evidence. *Cladistics* **25**, 231–262. doi:10.1111/j.1096-0031.2009.00249.x

Blauvelt, H. H. (1936). The comparative morphology of the secondary sexual organs of *Linyphia* and some related genera, including a revision of the group. *Festschrift zum 60 Geburtstage von Professor Dr Embrik Strand* **2**, 81–171.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**, 295–304. doi:10.1111/j.1096-0031.1994.tb00179.x

Coddington, J. A. (1990). Ontogeny and homology in the male palpus of orb-weaving spiders and their relatives with comments on phylogeny (Araneoclada: Araneoidea, Deinopoidea). *Smithsonian Contributions to Zoology* **496**, 1–52. doi:10.5479/si.00810282.496

Coddington, J. A. (1991). Cladistics and spider classification: araneomorph phylogeny and the monophyly of orbweavers (Araneae: Araneomorphae; Orbiculariae). *Acta Zoologica Fennica* **190**, 75–87.

Comstock, J. H. (1910). The palpi of male spiders. *Annals of the Entomological Society of America* **3**, 161–186.

de Pinna, M. C. C. (1991). Concepts and tests of homology in the cladistics paradigm. *Cladistics* **7**, 367–394. doi:10.1111/j.1096-0031.1991.tb00045.x

Dimitrov, D., Lopardo, L., Giribet, G., Arnedo, M. A., Alvarez-Padilla, F., and Hormiga, G. (2012). Tangled in a sparse spider web: single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. *Proceedings of the Royal Society Biological Sciences* **279**, 1341–1350. doi:10.1098/rspb.2011.2011

Eberhard, W. G. (1985). 'Sexual Selection and Animal Genitalia.' (Harvard University Press: Cambridge, MA.)

Eberhard, W. G., and Huber, B. A. (2010). Spider genitalia: precise manoeuvres with a numb structure in a complex lock. In 'Evolution of Primary Sexual Characters in Animals'. (Eds J. L. Leonard and A. Córdoba-Aguilar.) pp. 249–284. (Oxford University Press: Oxford, UK.)

Goloboff, P. A., Farris, J. S., and Nixon, K. C. (2003). 'TNT – Tree Analysis Using New Technologi. Willi Hennig Society edition 1.' Available at http://www.zmuc.dk/public/phylogeny/TNT

Goloboff, P. A., Farris, J. S., and Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics* **24**, 774–786. doi:10.1111/j.1096-0031.2008.00217.x

Grasshoff, M. (1968). Morphologische Kriterien als Ausdruck von Artgrenzen bei Radnetzspinnen der Subfamilie Araneinae. *Abhandlungen von der Senckenbergischen Naturforschenden Gesellschaft* **516**, 1–100.

Griswold, C. E., Coddington, J. A., Hormiga, G., and Scharff, N. (1998). Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). *Zoological Journal of the Linnean Society* **123**, 1–99. doi:10.1111/j.1096-3642.1998.tb01290.x

Grube, E. (1861). Beschreibungen neuer, von den Herren L. v. Schrenk, Maack, C. v. Ditmar u.a. im Amurlande und in Ostsiberien gesammelter Araneiden. *Bulletin de l'Academie Imperiale des Sciences de St-Petersbourg* **4**, 161–180.

Harvey, P. R., Nellist, D. R., and Telfer, M. G. (Eds) (2002). 'Provisional Atlas of British Spiders (Arachnida, Araneae).' (Biological Records Centre: Huntingdon, UK.).

Holm, Å. (1979). A taxonomic study of European and East African species of the genera *Pelecopsis* and *Trichopterna* (Araneae, Linyphiidae), with descriptions of a new genus and two new species of *Pelecopsis* from Kenya. *Zoologica Scripta* **8**, 255–278. doi:10.1111/j.1463-6409.1979.tb00638.x

Hormiga, G. (1994*a*). A revision and cladistic analysis of the spider family Pimoidae (Araneoidea: Araneae). *Smithsonian Contributions to Zoology* **549**, 1–104. doi:10.5479/si.00810282.549

Hormiga, G. (1994*b*). Cladistics and the comparative morphology of linyphiid spiders and their relatives (Araneae, Araneoidea, Linyphiidae). *Zoological Journal of the Linnean Society* **111**, 1–71. doi:10.1111/j.1096-3642.1994.tb01491.x

Hormiga, G. (2000). Higher level phylogenetics of erigonine spiders (Araneae, Linyphiidae, Erigoninae). *Smithsonian Contributions to Zoology* **609**, 1–160. doi:10.5479/si.00810282.609

Hormiga, G. (2003). *Weintrauboa*, a new genus of pimoid spiders from Japan and adjacent islands, with comments on the monophyly and diagnosis of the family Pimoidae and the genus *Pimoa* (Araneoidea, Araneae). *Zoological Journal of the Linnean Society* **139**, 261–281. doi:10.1046/j.1096-3642.2003.00072.x

Hormiga, G. (2008). On the spider genus *Weintrauboa* (Araneae, Pimoidae), with a description of a new species from China and comments on its phylogenetic relationships. *Zootaxa* **1814**, 1–20.

Hormiga, G., and Scharff, N. (2005). Monophyly and phylogenetic placement of the spider genus *Labulla* Simon, 1884 (Araneae, Linyphiidae) and description of the new genus *Pecado*. *Zoological Journal of the Linnean Society* **143**, 359–404. doi:10.1111/j.1096-3642.2005.00147.x

Hormiga, G., and Tu, L. (2008). On *Putaoa*, a new genus of the spider family Pimoidae (Araneae) from southern China, with a cladistic test of its monophyly and phylogenetic placement. *Zootaxa* **1792**, 1–21.

Hormiga, G., Buckle, D. J., and Scharff, N. (2005). *Nanoa*, an enigmatic new genus of pimoid spiders from western North America (Pimoidae, Araneae). *Zoological Journal of the Linnean Society* **145**, 249–262. doi:10.1111/j.1096-3642.2005.00192.x

Koch, L. (1879). Arachniden aus Siberien und Novaja Semlja. *Kungliga Svenska Vetenskapsakademiens Handlingar* **16**, 1–136.

Linnaeus, C. (1758). 'Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species cum Characteribus Differentiis, Synonymis, Locis. Tomus I. Editio Decima, Reformata.' (Holmiae.)

Maddison, W. P., and Maddison, D. R. (2007). 'Mesquite – a modular system for evolutionary analysis. Ver. 2.74.' Available at http://mesquiteproject.org./mesquite/mesquite.html

Menge, A. (1866). 'Preussische Spinnen.' (Erste Abtheilung. Schrift. naturf. Ges. Danzig.)

Merrett, P. (1963). The palpus of male spiders of the family Linyphiidae. *Proceedings of the Zoological Society of London* **140**, 347–467. doi:10.1111/j.1469-7998.1963.tb01867.x

Miller, J. A., and Hormiga, G. (2004). Clade stability and the addition of data: a case study from erigonine spiders (Araneae: Linyphiidae, Erigoninae). *Cladistics* **20**, 385–442. doi:10.1111/j.1096-0031.2004.00033.x

Millidge, A. F. (1977). The conformation of the male palpal organs of linyphiid spiders, and its application to the taxonomic and phylogenetic analysis of the family (Araneae: Linyphiidae). *Bulletin of the British Arachnological Society* **4**, 1–60.

Millidge, A. F. (1980). The erigonine spiders of North America. Part 1. Introduction and taxonomic background (Araneae: Linyphiidae). *The Journal of Arachnology* **8**, 97–107.

Millidge, A. F. (1988). The relatives of the Linyphiidae: phylogenetic problems at the family level (Araneae). *Bulletin of the British Arachnological Society* **7**, 253–268.

Millidge, A. F. (1993). Further remarks on the taxonomy and relationships of the Linyphiidae, based on the epigynal duct confirmations and other characters (Araneae). *Bulletin of the British Arachnological Society* **9**, 145–156.

Nixon, K. C. (2002). 'WinClada.' Ithaca, New York: Published by the author. Available at http://www.cladistics.com

Nyffeler, M., and Sunderland, K. D. (2003). Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. *Agriculture Ecosystems & Environment* **95**, 579–612. doi:10.1016/S0167-8809(02)00181-0

Platnick, N. I. (2012). 'The World Spider Catalog, Version 13.0. American Museum of Natural History.' Available at http://research.amnh.org/entomology/spiders/catalog/index.html

Roberts, M. J. (1995). 'Spiders of Britain and Northern Europe.' (Harper-Collins Publishers: London.)

Saaristo, M. I. (1971). Revision of the genus *Maro* O. P.- Cambridge (Araneae, Linyphiidae). *Annales Zoologici Fennici* **8**, 463–482.

Saaristo, M. I. (1973). Taxonomical analysis of the type-species of *Agyneta, Anomalaria, Meioneta, Aprolagus*, and *Syedrula* (Araneae, Linyphiidae). *Annales Zoologici Fennici* **10**, 451–466.

Saaristo, M. I. (1975). On the evolution of the secondary genital organs of Lepthyphantinae (Araneae, Linyphiidae). In 'Proceedings of the 6th International Arachnological Congress'. pp. 21–25.

Scharff, N., and Coddington, J. A. (1997). A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). *Zoological Journal of the Linnean Society* **120**, 355–434. doi:10.1111/j.1096-3642.1997.tb01281.x

Scharff, N., and Gudik-Sørensen, O. (2006). Catalogue of the spiders of Denmark (Araneae). *Entomologiske Meddelelser* **74**, 3–71.

Scharff, N., Coddington, J. A., Griswold, C. E., Hormiga, G., and Bjørn, P. D. P. (2003). When to quit? Estimating spider species richness in a northern European deciduous forest. *The Journal of Arachnology* **31**, 246–273. doi:10.1636/0161-8202(2003)031[0246:WTQESS]2.0.CO;2

Tanasevitch, A. (1990). The spider family Linyphiidae in the fauna of the Caucasus (Arachnida, Aranei). In 'Fauna Nazemnykh Bespozvonochnykh Kavkaza'. (Ed. B. R. Striganova.) pp. 5–114. (Akaedemia Nauk: Moscow.)

Tanasevitch, A. (2000). New species of the family Linyphiidae from South Siberia, Russia (Arachnida: Araneae). *Reichenbachia Staatliches Museum fur Tierkunde Dresden* **33**(31), 243–253.

Tanasevitch, A. (2011). On linyphiid spiders (Araneae) from the Eastern and Central Mediterranean kept at the Muséum d'histoire naturelle, Geneva. *Revue Suisse de Zoologie* **118**, 49–91.

Tanasevitch, A. (2012). Two new *Stemonyphantes* Menge 1866 from Kazakhstan (Aranei: Linyphiidae: Stemonyphantinae). *Arthropoda Selecta* **21**, 363–368.

van Helsdingen, P. J. (1968). Comparative notes on the species of the holarctic genus *Stemonyphantes* Menge (Araneida, Linyphiidae). *Zoologische Mededelingen* **43**, 117–139.

Wunderlich, J. (1986). 'Spinnenfauna Gestern und Heute: Fossile Spinnen in Bernstein und Ihre Heute Lebenden Verwandten.' (Wiesbaden, Erich Bauer Verlag bei Quelle and Meyer: Wiesbaden, Germany).

Appendix 1. List of described species in the genus *Stemonyphantes* Menge, 1866 and known localities (Platnick 2012)
Species examined are underlined and species used for the current analyses are in bold and underlined

Species	voucher specimens	Localities	Remarks
Stemonyphantes abantensis Wunderlich, 1978	SMF-29625; SMF-29626	Turkey	
***Stemonyphantes agnatus* Tanasevitch, 1990**	**ZMMU-TA7144; ZMMU-TA7145**	Russia, Georgia, Azerbaijan	
***Stemonyphantes altaicus* Tanasevitch, 2000**	**ZMMU-TA7139; ZMMU-TA7140**	Russia	
***Stemonyphantes blauveltae* Gertsch, 1951**	**AMNH-Holotype & Allotype series**	USA, Canada	
***Stemonyphantes conspersus* (L. Koch, 1879)**	**NMBE-AR5609; NMBE-AR5612; ZMUC-6090; NHRS-lectotype & paralectotype**	Central Europe to Kazakhstan	
Stemonyphantes curvipes Tanasevitch, 1989	ZMMU-TA5619	Kyrgyzstan	
Stemonyphantes griseus (Schenkel, 1936)	NHRS-type	Kyrgyzstan, China	
Stemonyphantes grossus Tanasevitch, 1985	ZMMU-TA7141; ZMMU-TA7142	Kyrgyzstan	
***Stemonyphantes lineatus* (Linnaeus, 1758)**	**ZMUC-7755; ZMUC-9696**	Palearctic	
Stemonyphantes karatau Tanasevitch, Esyunin & Stepina, 2012	–	Kazakhstan	
Stemonyphantes menyuanensis Hu, 2001	–	China	Not available
Stemonyphantes montanus Wunderlich, 1978	SMF-29623; SMF-29624	Turkey	
Stemonyphantes parvipalpus Tanasevitch, 2007	ZMMU-TA7152; ZMMU-TA7153	Russia	
Stemonyphantes serratus Tanasevitch, 2011	–	Turkey	
Stemonyphantes sibiricus (Grube, 1861)	MNHWU-566; ZMMU (from Magadan)	Russia, Kazakhstan, Mongolia, Kurile Is.	
Stemonyphantes solitudus Tanasevitch, 1994	ZMMU-TA7143	Turkmenistan	
Stemonyphantes taiganus (Ermolajev, 1930)	–	Russia	Not available
Stemonyphantes taiganoides Tanasevitch, Esyunin & Stepina, 2012	–	Russia, Kazakhstan	

Appendix 2. List of character changes (from the original scoring of *Stemonyphantes blauveltae* Gertsch, 1951 by Arnedo *et al*. 2009)
**changed only in some of the hypotheses

Character	Arnedo *et al*. 2009 scoring	New scoring (the changes)
Character 2. Ectal marginal cymbial process	Absent (0)	Present (1)
Character 6. Ecto basal cymbial process	Absent (0)	Present (1)
Character 11. Paracymbium attachment	Intersegmental (0)	membranous with an integral connection (3) ('Stemonyphantes type' of Arnedo et al. 2009)
Character 12. Paracymbium morphology	Straight hook, one flat plan (4)	hook with a raised base with setae (10) ('Stemonyphantes type' of Arnedo et al. 2009)
Character 24. Suprategular base	Approximately the same width as the rest of the suprategulum (0)	Wider (1)
Character 29. Median apophysis**	Absent (0)	Present (1)**
Character 30. Conductor**	Absent (0)	Present (1)**
Character 36. Embolus base	Narrow (1)	Broad (0)
Character 42. Radical tail piece**	Present (1)	Absent (0)**
Character 43. Radical anterior process**	Present (1)	Absent (0)**
Character 44. Radical mesal tooth**	Absent (0)	Present (1)**
Character 56. Palpal tibia of male, dorsal apophysis	Absent (0)	Present (1)