Simultaneous analysis of basal Hymenoptera (Insecta): introducing robust-choice sensitivity analysis

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Received 6 June 2002; accepted for publication 17 March 2003

Molecular characters are analysed on their own and in combination with morphological data to examine the phylogenetic relationships of the basal lineages of Hymenoptera ('Symphyta'). This study covers 47 sawfly genera and nine apocritan families and includes molecular sequences from five genes – 12S, 16S, 18S and 28S ribosomal genes and cytochrome oxidase 1 – as well as 343 morphological characters. A robust-choice sensitivity analysis is performed with the data. First, the simultaneous analysis is repeated three times, each time employing a different step matrix for weighting the transformations of the molecular characters. Then, the results of all three simultaneous analyses are summarized in a strict consensus in order to avoid basing the conclusions on a narrow set of assumptions. This methodology is discussed in the paper. The relationships among superfamilies largely confirm previous hypotheses, being (Xyeloidea (Tenthredinoidea *s.l.* (Pamphilioidea (Cephoidea (Siricoidea (Xiphydrioidea (Orussoidea Apocrita))))))), where Siricoidea is understood as Siricidae+Anaxyelidae. However, the relationships within Tenthredinoidea *s.s.* proposed here are novel: ({Argidae Pergidae} [*Athalia* {(Diprionidae Cimbicidae) Tenthredinidae minus *Athalia*}]). ©2003 The Linnean Society of London. *Biological Journal of the Linnean Society*, 2003, **79,** 245–275.

ADDITIONAL KEYWORDS: molecular systematics – parsimony – phylogeny – Symphyta – Tenthredinoidea.

INTRODUCTION

Hymenoptera are one of the largest insect orders and their internal phylogenetic relationships remain largely unknown. Naturally, the basal lineages play a key role in the determination of the internal relationships of this group. During the last 50 years of the 20th century, many studies were published on the morphology of basal Hymenoptera (Maxwell, 1955; Lorenz & Kraus, 1957; Oeser, 1961; Rasnitsyn, 1969, 1988; Togashi, 1970; Gibson, 1985, 1993; Johnson, 1988; Whitfield, Johnson & Hamerski, 1989; Heraty, Woolley & Darling, 1994; Basibuyuk & Quicke, 1995, 1997, 1999; Vilhelmsen, 1996, 1997a, 1999, 2000a,b,c). This research included detailed studies of almost all character systems of the adults and larvae. Characters from most of these studies were assembled by Vilhelmsen (2001) and coded for 32 genera of sawflies (plus six outgroup taxa and six Apocrita).

Recently, Schulmeister, Wheeler & Carpenter (2002) added DNA sequences to the morphological data matrix of Vilhelmsen (2001) for the first simultaneous analysis focusing on basal Hymenoptera. They sequenced fragments of the 16S, 18S, and 28S ribosomal DNA and cytochrome oxidase 1 genes for 29 of the sawfly genera used by Vilhelmsen (2001). Schulmeister *et al*. (2002) used the data matrix of Vilhelmsen (2001) without any changes (except for the deletion of a few taxa).

Even more recently, Schulmeister (2003a) revised the morphological data matrix of Vilhelmsen (2001) and extended it to 47 sawfly genera and 9 apocritan taxa (compared to 32 + 6), enlarging the number of sawfly genera by 47%. In that paper, she also added a data matrix with 87 characters from a study of the terminal segments of the male abdomen of basal Hymenoptera including internal and external reproductive organs (Schulmeister, in press) and a matrix with 30 characters from other parts of adult and larval morphology, for example the tarsal plantulae (Schulmeister, 2003b). The morphological analysis of Schulmeister (2003a) included 343 morphological characters in total.

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The purpose of the present study is to add a fifth gene (12S rDNA) to the molecular data matrix of Schulmeister *et al*. (2002), to include sequences of these five genes for most of the taxa coded for morphology, and to analyse this enlarged molecular data set together with the 343 morphological characters of Schulmeister (2003a). The 343 morphological characters used here expand the number of morphological characters employed by Vilhelmsen (2001) and Schulmeister *et al*. (2002) by 45%. This study is the most comprehensive analysis of the basal lineages of Hymenoptera to date; the amount of data included exceeds by far those of previously published papers on this group.

In all analyses presented here, the Tenthredinidae minus *Athalia* come out as monophyletic. But because the tenthredinid taxon sample included in the present study is still rather small, it is too early to propose any nomenclatural changes on this basis. Therefore, a provisional short-hand notation, **Tenthredinidae***, will be used for a potentially monophyletic group that – in the present sample – comprises the Tenthredinidae minus *Athalia*.

MATERIAL AND METHODS

Taxa

The morphological data were taken from Schulmeister (2003a). DNA sequences were obtained for as many of these taxa as time and amplification success permitted. If possible, the same species were sequenced as had been used for the morphology, but in some cases more or less closely related species had to be used instead. For outgroups and within Apocrita, DNA sequences were obtained for more taxa than had been examined morphologically, in order to reduce noise in these areas. Appendix 1 gives the names of the operational taxonomic units (OTUs) and the names of all species studied to code the morphological data (in Schulmeister, 2003a) and those used to obtain the molecular sequences. Additional taxa that were examined (for the discussion), but which were not used to generate the character matrices, are not included in Appendix 1. These taxa are:

Xyelidae: *Xyelecia nearctica* Ross and *Pleroneura bruneicornis* Rohwer

Cimbicidae: *Pachylosticta* sp., *Pseudopachylosticta* sp., *Leptocimbex* sp. and *Pseudoclavellaria* sp.

Argidae: *Sericoceros* sp., *Dielocerus* sp., *Atomacera debilis* Say, *Atomacera decepta* Rohwer and *Zenarge turneri* Rohwer

Pergidae: *Neoeurys* sp., *Cerospastus* sp., *Lagideus* sp., *Parasyzygonia rufosternalis* Mallach, *Ancyloneura* sp., *Pteryperga galla* and *Styracotechys* sp.

Cephidae: *Caenocephus* sp. and *Pachycephus* sp.

Xiphydriidae: *Derecyrta lugubris* (Westwood), *Derecyrta pictipennis* Smith, *Derecyrta variipennis* Rohwer, *Brachyxiphus grandis* Philippi and *Steirocephala* sp.

Morphological data

The morphological data matrices of Schulmeister (2003a: appendices 2–4) were used unaltered for the simultaneous analyses in the present study. The description of the characters is found in Schulmeister (2003a), Schulmeister (in press) and Vilhelmsen (2001). Data matrices and a comprehensive list of all character descriptions can be obtained in electronic form from the author of the present paper.

Molecular data

Most specimens were preserved in 98% ethanol, some were pinned and dried (but had been killed in ethanol). Total genomic DNA was extracted by overnight incubation of a tissue sample (thorax or leg muscles or ovaries) in a solution of guanadium isothyocyanate and 0.14 M beta-mercaptoethanol followed by a standard phenol/chloroform extraction and ethanol precipitation. After drying the DNA, it was resuspended in water.

Target genes were amplified by polymerase chain reaction (PCR), if necessary in several overlapping pieces. Sequences of primers are given in Table 1. A typical PCR procedure for 16S, 18S and 28S was an initial denaturation at 96°C for one minute, and then 30–40 cycles of denaturation (15 s at 96°C), annealing (15 s at 49°C) and extension (15 s at 72°C). For the CO1 gene, the denaturing and annealing temperatures were lowered to 94°C and 46°C, respectively. PCR and Cycle Sequencing were done in a Perkin-Elmer GeneAmp PCR system 9700. PCR products were gene-cleaned with glassmilk (Geneclean II kit; BIO 101, Inc.) Sequencing was performed by the dideoxy termination method with dye-labelled terminators using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase and run on the ABI Prism 377 DNA sequencer and ABI Prism 3700 DNA analyser (Perkin-Elmer). Complementary strands were combined and edited with the computer program Sequencher 3.1 (Gene Codes Corporation). Some sequences were taken from NCBI GenBank. For the origin of all sequences included in the present analysis see Appendix 2.

The 12S sequence corresponds to positions 15114–14808, the 16S sequence to positions 13850–13480, and the CO1 sequence to positions 1863–2903 of the *Apis mellifera* sequence (Crozier & Crozier, 1993). The 18S sequence corresponds to positions 600–1580 and

Table 1. Primer sequences. Positions of primers for mitochondrial genes (16S, CO1) are based on the sequence of *Apis mellifera* (Crozier & Crozier, 1993). Positions of primers for 18S and 28S are based on the sequence of *Drosophila melanogaster* (Tautz *et al*., 1988). Reverse primers are marked by an asterisk. The primer called '18S 9R' is actually situated in the ITS1 gene behind the 18S gene

Primer name	Primer sequence	Position
12S ai	5′ – AAA CTA GGA TTA GAT ACC CTA TTA T – 3′	15179–15155 (*Apis*)
12S bi	5′ – AAG AGC GAC GGG CGA TGT GT – 3′	14788–14807 (*Apis*)
16S A Hym	5′ – TRA CTG TRC AAA GGT AGC – 3′	13859–13842 (*Apis*)
16S B Hym*	5′ – TTA ATT CAA CAT CGA GGT C – 3′	13473–13491 (*Apis*)
18S 1F	5′ – TAC CTG GTT GAT CCT GCC AGT AG – 3′	1–23 (*Dros.*)
18S 3F	5′ – GTT CGA TTC CGG AGA GGG A – 3′	378–396 (*Dros.*)
18S 4F	5′ – CCA GCA GCC GCG CTA ATT C – 3′	573–591 (*Dros.*)
18S a2.0	5′ – ATG GTT GCA AAG CTG AAA C – 3′	1204–1222 (*Dros.*)
18S 5R*	5′ – CTT GGC AAA TGC TTT CGC – 3′	1040–1023 (*Dros.*)
18S bi*	5′ – GAG TCT CGT TCG TTA TCG GA – 3′	1421–1402 (*Dros.*)
18S 7R*	5′ – GCA TCA CAG ACC TGT TAT TGC – 3′	1631–1611 (*Dros.*)
18S 9R*	5′ – GAT CCT TCC GCA GGT TCA CCT AC – 3′	1991–1969 (*Dros.*)
28S A	5′ – GAC CCG TCT TGA AAC ACG GA – 3′	4046–4065 (*Dros.*)
28S B*	5′ – TCG GAA GGA ACC AGC TAC TA – 3′	4413–4394 (*Dros.*)
28S Bout*	5′ – CCC ACA GCG CCA GTT CTG CTT ACC – 3′	4625–4602 (*Dros.*)
CO1 lco hym	5′ – CAA ATC ATA AAG ATA TTG G – 3′	1816–1834 (*Apis*)
CO1 hco extA	5′ – GAA GTT TAT ATT TTA ATT TTA CCT GG – 3′	2511–2536 (*Apis*)
CO1 hco*	5′ – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3′	2518–2493 (*Apis*)
CO1 hco out*	5′ – CCA GGT AAA ATT AAA ATA TAA ACT TC – 3′	2536–2511 (*Apis*)
CO1 hco outout*	5′ – GTA AAT ATA TGR TGD GCT C – 3′	2668–2650 (*Apis*)
CO1 hco extB*	5′ – CCT ATT GAW ARA ACA TAR TGA AAA TG – 3′	2938–2913 (*Apis*)

the 28S sequence to positions 4066–4601 of the *Drosophila melanogaster* sequence (Tautz *et al*., 1988). The number of aligned positions that were included in the present analysis and the number of positions that were found to be parsimony-informative are given for each gene in Appendix 3.

CO1 sequences were aligned by translating them into amino acid sequences. For the alignment and choice of sequence fragments of the rDNA sequences, the strategy of Dowton & Austin (2001) was used. The rDNA sequences were first roughly aligned and split up into clearly homologous regions that showed no or almost no length variation and the highly length-variable regions inbetween. The highly length-variable regions do not align unambiguously, which means that the positional homology of the bases of different species is unclear. These regions were excluded from further analysis, because it is undesirable to base phylogenetic conclusions on homology statements which have a high potential of being incorrect. (Because these regions are usually confined to the loop regions, secondary-structure models do not help with the alignment in these cases, as pointed out by Dowton & Austin (2001).) Highly length-variable regions also usually have a high substitution rate, so that even if the bases were aligned correctly, the signal could be obscured by saturation and noise. Because there are enough readily alignable gene fragments, I decided to confine the analysis to these 'safer' regions. For the DNA sequence fragments chosen to be included in the analysis, alignment was straightforward and could be done manually. CO1 was divided into fragments only to provide more detailed information on the character sampling; no CO1 regions were excluded from the analysis. See Appendix 3 for the position of the included fragments relative to the sequences of *Apis* and *Drosophila* and the alignment of Whiting *et al*. (1997).

Not all fragments were sequenced for each OTU; some OTUs are missing certain fragments. The 12S rDNA gene could be amplified for only very few species of the outgroups and Unicalcarida minus Cephidae. In order to avoid artefacts from missing molecular data, I decided to include 12S sequences only for Xyelidae, Tenthredinoidea *s.l.*, Pamphilioidea and Cephidae, mainly to provide information within Tenthredinoidea *s.l.* Similarly, not all morphological characters were sampled for each OTU. Appendix 4 provides details on the character sampling.

Cladistic analysis

The molecular data were analysed on their own and simultaneously with the morphological data. Of the

morphological data, all characters were treated as unordered, except for characters 20, 23, 27, 30, 35, 36, 41, 42, 46, 48, 59, 78, 79, 99, 112, 117, 134, 146, 157, 171, 188, 191, 192, 193, 224, 228, 229, 237, 263, 266, 288, 296, 299, 304, 343, 347 and 349, which were treated as ordered (see Schulmeister, 2003a). The characters 43, 45, 136, 190, 212 and 216–220 were excluded from all analyses for reasons outlined by Schulmeister (2003a).

The molecular analyses (i.e. combined analyses of all DNA sequences) and the simultaneous analyses (i.e. morphology and molecules) were repeated with three weighting schemes, which are specified in Table 2. In the first weighting scheme, all character transformations were counted as one step. The second and third weighting scheme were created to reflect the approximate frequency of the six types of substitutions in the DNA sequences. The approximate frequency of the substitutions was determined in PAUP by determining the corrected number of mismatches in three pairs of species/OTUs: *Arge nigripes* & *Arge cyanocrocea*, *Orussus abietinus* & Vespidae A, *Macroxyela ferruginea* & Vespidae A. The approximate frequencies of the six types of mismatches are given in Table 2. AT-mismatches (implying transversions) were by far the most frequent.

The second and third weighting scheme are based on the approximate frequencies of the six types of substitutions and are presented in Table 2. It was attempted to determine the widest possible range of weights, within the limits given by the triangle inequality (Wheeler, 1993). In the second weighting scheme, AT-transversions were downweighted by 50% relative to all other transformations (including insertions, deletions and morphological transformations). In the third weighting scheme, AT-transversions were downweighted 50% relative to transitions and transitions were downweighted 30% relative to the remaining transversions and all other transformations.

The results of all three molecular analyses were summarized in a strict consensus cladogram, as were the results of the three simultaneous analyses. This methodological approach is discussed in the section 'Robust-choice sensitivity analysis', below.

The analyses were done with PAUP version 4.0b10 (Swofford, 1998) with default settings (initial tree generated with stepwise addition of taxa, hold = 1, TBR branch swapping, MulTrees = yes, Keep = no, NBest = all, AllSwap = no, ReconLimit = infinity, ChuckScore = no) except that random sequence addition was used (50 replicates). The number of trees resulting from the analyses were reduced by removing branches with a minimum length of zero (condense collapse = MinBrLen).

Bremer support values (= decay indices) were generated for the simultaneous analysis using the equal (= first) weighting scheme. This weighting scheme was chosen because this ensures that the number of steps given by the Bremer values is equal to the number of the implied evolutionary events, be it morphological changes, base substitutions, or deletions/insertions. Otherwise, it would be impossible to tell whether 6 additional steps mean, for example, 2 or 6 evolutionary transformations. The Bremer support values were determined in PAUP one by one, by performing a heuristic search for the shortest tree that does not contain the clade in question (HSearch enforce constr = *name_constrained_clade* converse), thereby ensuring the most accurate estimate.

Table 2. Frequencies of the different types of substitutions and the weights assigned to them in the three weighting schemes. TV = transversion, TI = transition

		1	2	3
(1) AT-mismatches (TV):	41–47%	1	1	1
(2) CT-mismatches (TI):	21–24%	1	2	2
(3) AG-mismatches (TI):	15–16%	1	2	2
(4) GT-mismatches (TV):	7–11%	1	2	3
(5) AC-mismatches (TV):	4–8%	1	2	3
(6) CG-mismatches (TV):	1–3%	1	2	3

RESULTS

The selected regions of the 12S, 16S, 18S, 28S and CO1 genes were analysed together, using three different weighting schemes, as described above. The first weighting scheme resulted in six trees of 9579 steps, the second in two trees of 14 950 steps, and the third in four trees of 16 239 steps. The strict consensus cladograms from the three molecular analyses are very similar; the strict consensus over all three analyses is shown in Figure 1.

The total morphological evidence was analysed simultaneously with the molecular data for the complete taxon sample, using the three different weighting schemes described in Table 2. The analysis using the first weighting scheme resulted in 32 trees of 11 087 steps, the second in two trees of 17 961 steps, and the third in one tree of 20 720 steps. The strict consensus cladograms resulting from the three analyses are almost completely congruent and were summarized in a strict consensus tree (Fig. 2).

Then, the three simultaneous analyses were repeated with the exclusion of those sawfly taxa for which molecular or morphological data were missing, in order to determine potential artefacts from missing molecular data. Those clades of the strict consensus in Figure 2 that were contradicted by the simultaneous analyses with the reduced taxon sample are indicated

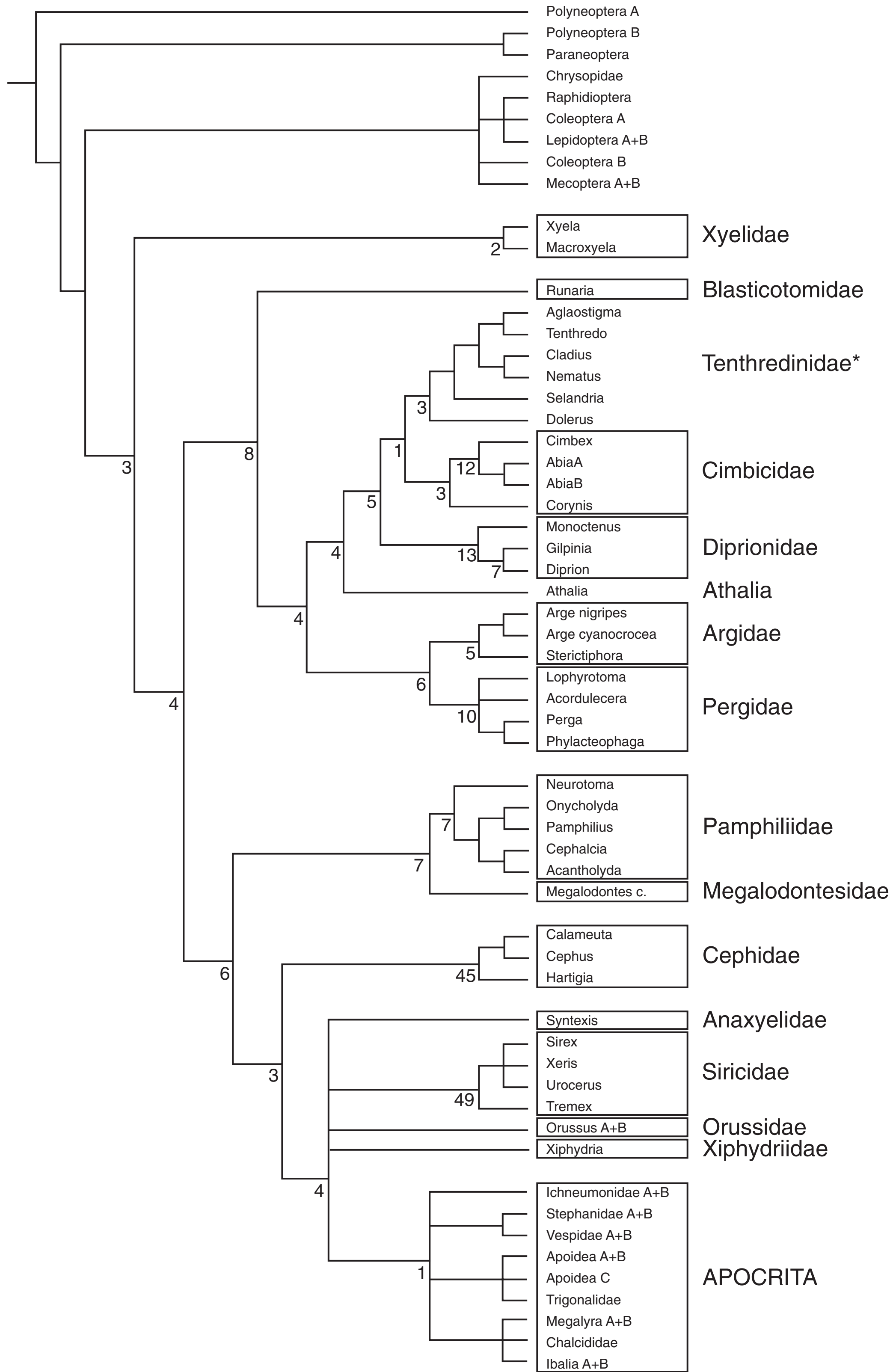

Figure 1. Strict consensus of the cladograms resulting from all three molecular analyses. Numbers below branches are Bremer values determined with the equally weighted analysis.

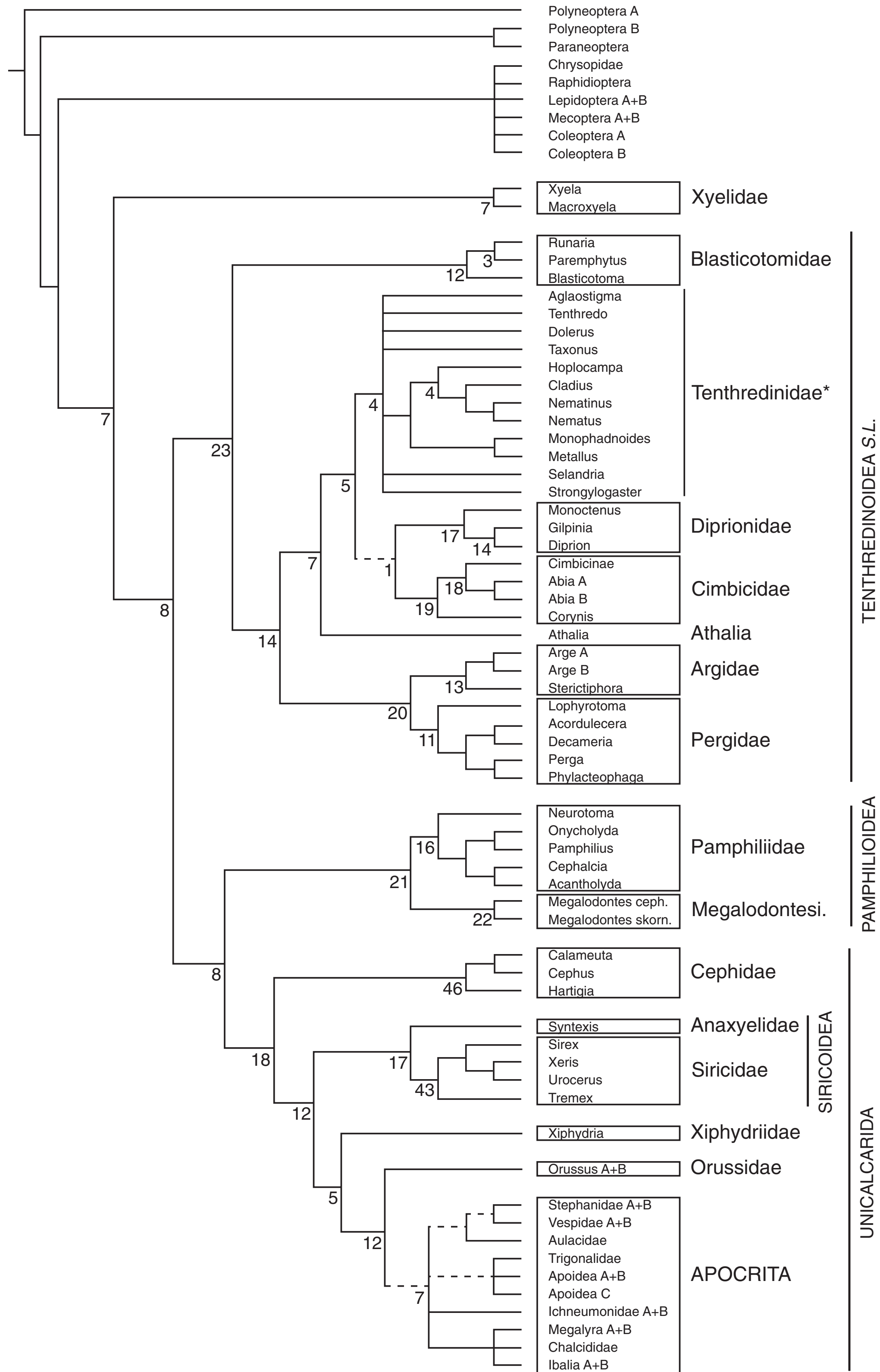

Figure 2. Strict consensus of all three simultaneous analyses. Numbers below branches are Bremer support values determined for the analysis using the equal weighting scheme. Dashed lines indicate the clades which are not found in the results of the analyses from which the taxa lacking either molecular or morphological data have been excluded.

by dashed stem lineages. This concerned Cimbicidae+Diprionidae and Apocrita.

Figure 3 shows the final hypothesis of the relationships on family level, based on the strict consensus of the simultaneous analyses (Fig. 2). In this cladogram, the clades which are contradicted by the morphological tree (Schulmeister, 2003a: fig. 7) or the molecular tree (i.e. those that would not have been found by a 'taxonomic congruence' approach) have been highlighted by dashed lines in order to indicate those areas where more data are particularly needed.

DISCUSSION

Robust-choice sensitivity analysis

Description and defence of robust-choice sensitivity analysis

In phylogenetic analysis of DNA sequences, weights (= costs) have to be assigned to the six types of substitution and insertion-deletion events (indels). These weights can be equal or differential. If unequal weights are assigned, they are specified in a step matrix. (How the values of the weights should be chosen will be discussed below.)

The values of the weights for base substitutions and indels can have a significant influence on the outcome of a cladistic analysis, i.e. phylogenetic hypotheses can vary strongly with the choice of the parameter values. If only a single phylogenetic analysis is performed with one set of parameter values, it remains completely unknown how much the result is dependent on these arbitrarily chosen values. Sensitivity analysis examines the sensitivity of a cladogram to the analytical parameters. It starts by selecting a number of parameter sets (= step matrices) that are to be examined in the sensitivity analysis. (This step is discussed below.) The data are then analysed repeatedly – once with each of the chosen step matrices. This allows examination of the sensitivity of the phylogenetic hypothesis to the analytical parameters. Some nodes might be stable over all analyses, while other parts of the cladogram might differ among analyses.

Having obtained a number of possibly differing hypotheses, one is faced with the problem of how to determine the final hypothesis from them. Wheeler (1995) suggested to choose only one of them, using congruence as an external optimality criterion for the decision process. Some measure of congruence is calculated for each analysis and the respective cladogram resulting from the parameter set analysis that exhibits maximal congruence is chosen as the best phylogenetic hypothesis. However, there are a number of problems with choosing a single step matrix and settling on the hypothesis derived from it. First, there is the problem whether the congruence values used to compare the analyses based on different parameter sets really are comparable in a meaningful way. Second, if only one step matrix is chosen (no matter by which criterion), the problem that the final hypothesis can still be based on very narrow assumptions so that the clades can easily be artefacts of parameter choice is not removed by this approach. Third, there is the problem that in one part of the tree the historically correct result might be obtained by using step matrix A, but not B, whereas in another part of the cladogram, the correct relationships might be obtained only with matrix B, but not A. In short, it is possible that there might not be a single step matrix that leads to the right answer in all parts of the tree. Of course, we would not know which matrix leads to the correct result in which part of the tree, which is why the only 'safe' approach is to accept only those clades obtained by all step matrices. For these three reasons, I do not support the approach to accept all clades obtained by a single analysis, no matter how unstable they might be.

I argue that it is preferable to include only the robust clades in the final hypothesis, which are found in all cladograms resulting from all examined parameter sets. This still does not guarantee that these robust groups are right, but it ensures that the sensitive clades, which are falsified in at least one of the analyses, are excluded from the final hypothesis. Using the strict consensus over all performed analyses is hence not a matter of having *more* confidence in the *robust* clades, but rather a matter of having *less* confidence in the *sensitive* clades which were, after all, falsified by the data at hand. If a clade has been falsified in one or several of the analyses performed with all the available data, how can we have any confidence in it?

This is also the reason why the alternative approach suggested by Wheeler (1995) should be rejected. In this approach, which he did not specify in detail, clades found in a subset of analyses, for example the majority of analyses, should be accepted. This would mean that a clade which was falsified by one or more of the analyses could still be included the final hypothesis. According to the argument given above, this should be avoided.

In a recently submitted manuscript, T. Grant and A. Kluge (unpubl. data) point out that if a group is found in all (or many) of the performed analyses this does not mean that the group has better support than a group that is found in only one (or a few) of the analyses. They are right. And while this can be used as an argument against sensitivity analysis *sensu* Wheeler (1995), it can not be used as an argument against robust-choice sensitivity analysis, because the reason for accepting robust groups is not that these would be better supported than sensitive groups, but that they, the robust groups, have not been falsified in any of the

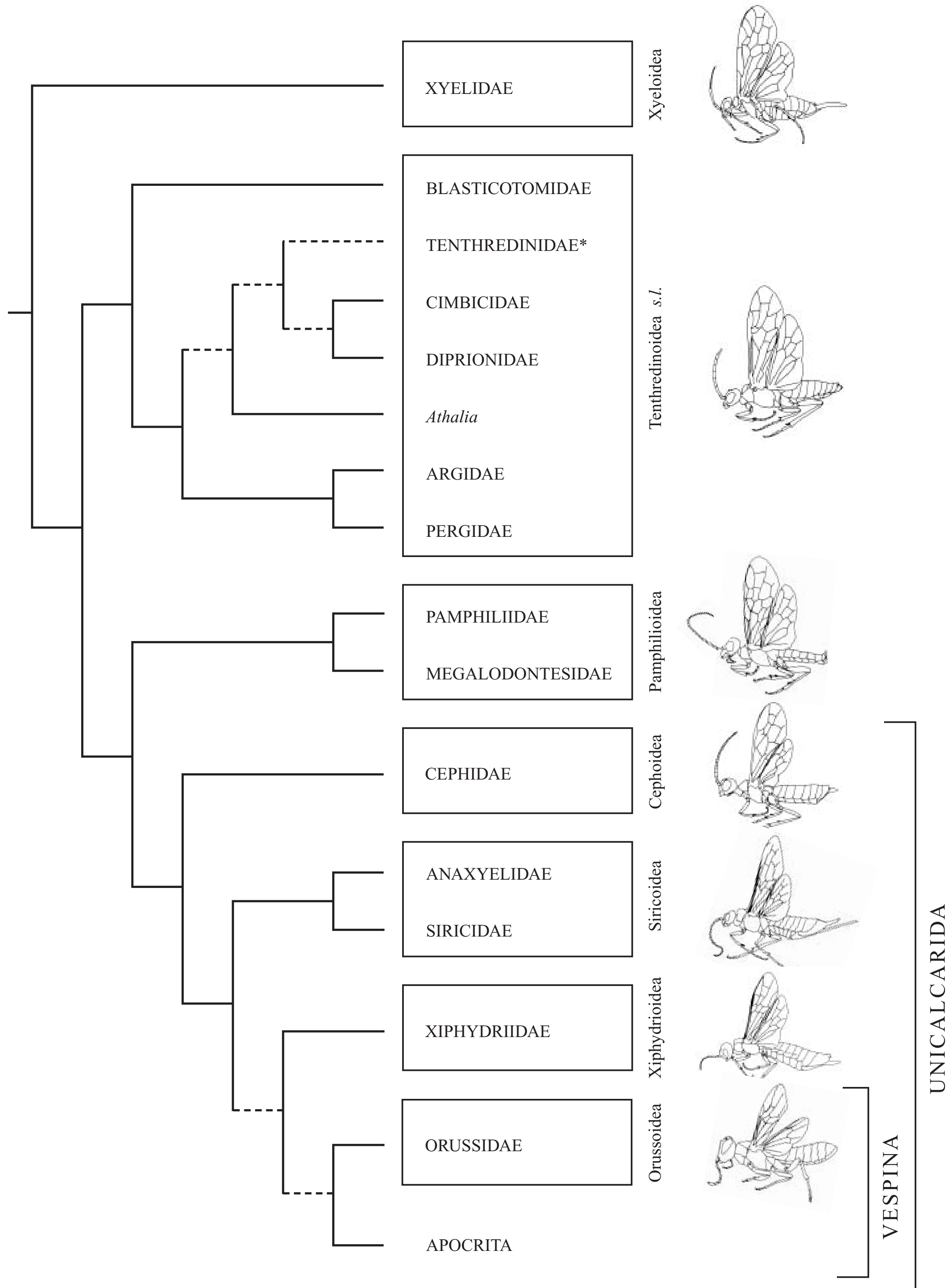

Figure 3. Final hypothesis. It corresponds to the cladogram in Fig. 2, based on simultaneous analyses of all data. Here, broken lines indicate those clades that are contradicted either by the morphological tree (Schulmeister, 2003a) or the molecular tree (Fig. 1), or both. Tenthredinidae* = Tenthredinidae minus *Athalia*. Species illustrations drawn after Goulet (1992).

analyses. The question of support is totally irrelevant to the robust-choice sensitivity analysis proposed here.

From the fact that robust groups do not necessarily have more support than sensitive groups, it also follows that the robust groups are not necessarily more stable to the addition of new data. The argument for excluding instable groups from the final hypothesis is not the amount of support they have by the present data or their potential future stability to the addition of new data, but the fact that they are already contradicted by the data at hand!

The number of theoretically possible step matrices is large, even if the triangle inequality is taken into account (Wheeler, 1993). If the entire range of possible step matrices is examined in a sensitivity analysis, it could happen that no or hardly any groups are found in the results of all performed analyses and the final hypothesis hence be almost completely unresolved. However, there is no reason to explore the entire theoretically possible parameter space. As T. Grant and A. Kluge (unpubl. data) point out, 'a hypothesis is corroborated *by* empirical evidence *in light of* auxiliary assumptions' (e.g. parameter values) and that the critical issue is 'the *validity* of auxiliary assumptions'. In the context of robust-choice sensitivity analysis, this means that only those weighting schemes which are reasonable should be examined, given the data at hand. The step matrices that are to be examined should be determined from the data (with the help of an equally weighted analysis) prior to the analyses. The selection of step matrices is discussed in the following.

Selection of step-matrices

Which combinations of possible step matrices should be chosen for the sensitivity analysis? Above, it has been pointed out that the critical issue is the validity of the auxiliary assumptions – in this case the values of the weights. But what are valid assumptions in this case? Which weighting schemes can be justified? 'Hypothetico-deductivists' argue for equal weighting of all characters and transformations. Contrary to 'hypothetico-deductivists', 'verificationists' argue that the weights should be based on the frequency of the substitution types, as estimated from the data that are to be analysed. They argue that less frequent substitutions are more reliable and that giving them more weight helps to increase the accuracy of the result.

To me, both equal and unequal weighting schemes seem to make sense. Therefore, I suggest employing both in a robust-choice sensitivity analysis. By deriving the final hypothesis from a strict consensus of the results obtained from an equally weighted analysis and the results obtained from analyses using weights according to the frequencies of the substitution, only those clades are retained that would be found in the cladogram of a 'hypothetico-deductivist' as well as the cladogram of a 'verificationist'. Those clades that these two groups of systematists would disagree on are removed in the robust-choice sensitivity analysis.

The equal weighting scheme (in which all substitutions and indels are counted as one step) should always be included in the sensitivity analysis, because it minimizes the number of implied evolutionary events and, in this sense, is the only one that applies parsimony in its strictest sense. This scheme also has the practical advantage that the number of steps of the resulting tree(s) will be identical to the number of hypothesized evolutionary events on the cladogram, which make interpretation of the results and Bremer support values straightforward.

Because transitions are thought to occur more frequently than transversions and hence show more homoplasy, it has often been argued that transitions are less 'reliable' than transversions for the reconstruction of phylogenies and that they ought to be given less weight in cladistic analyses. However, in hymenopteran (ribosomal) DNA sequences, AT-transversions are by far the most frequent substitutions, which was explained by their high A/T content, particularly in the so-called AT-rich regions (Cameron *et al.*, 1992; Derr *et al.*, 1992; Dowton & Austin, 1997, 1999, 2001; Whitfield & Cameron, 1998). In hymenopteran sequences, transitions are only the second and third most frequent substitutions. Therefore, if the weights used in the analysis are to be based on the relative frequencies of the substitutions, generalizing over transitions and transversions would obviously be very inaccurate, at least for hymenopteran DNA sequences. Hence, six-parameter-parsimony, which assigns a separate weight to each of the six substitutions (Williams & Fitch, 1989, 1990), which roughly correspond inversely to their relative frequencies, is preferable in this case. Six-parameter-parsimony has been used previously in a sensitivity analysis of Hymenoptera by Dowton & Austin (2001).

There are two problems inherent to the estimation of the relative frequencies of the substitutions. First, saturation causes hidden changes which makes the estimation difficult. Second, the relative frequencies of the six types of substitutions will differ in different parts of the tree as well as different regions of the genes. Hence, the estimates are only average values and the deviation could be rather large. However, if a range of different step matrices is used, putting much effort into exactly determining the average frequency of each type of substitution over all the internodes of the tree is rather

pointless. It is more important to look at the largest range of values (substitution frequencies) implied by the data.

In creating a range of potential step matrices, there is another limitation. The triangle inequality must be taken into account, as Wheeler (1993) pointed out. This means that the cost of two transversions should not be cheaper than the cost of one transition, otherwise two transversions would be more parsimonious than the transition event implied by the data.

Another parameter (weight) that needs to be specified for a cladistic analysis of molecular data is the indel cost which specifies the weight assigned to an insertion or deletion event. Unfortunately, the frequencies of insertions and deletions cannot be estimated from the data, because indels are unobservable processes. Depending on the alignment parameters, fewer or more gaps are introduced into the alignments, implying a lower or higher frequency of insertion-deletion events. However, for a number of reasons, insertion and deletion events are generally assumed to be much rarer than base substitutions. (This is more pronounced in protein-coding sequences. Ribosomal DNA shows a higher length variability, but this is largely confined to the loop regions of the rRNA.)

For data in which indels are thought to be very rare, for example protein sequences or molecular data sets from which highly length-variable regions have been excluded, it makes sense to assign them a weight which is equal to the highest weight given to any type of base substitution. (There is no justification for assigning a higher weight, because the frequency cannot be determined as for the substitutions.) For data in which indels are thought to be more frequent, different combinations of step matrices for substitutions and weights for indels could be examined in the sensitivity analysis.

Finally, morphological characters need to be assigned a weight relative to the weights assigned to substitutions and indels. This problem is not restricted to sensitivity analysis, but is inherent to all differentially weighted simultaneous analyses. If the weight for the morphological characters is low relative to the weight of substitutions and indel, the result of the simultaneous analysis converges towards the cladogram resulting from the molecular analyses. If the weight of the morphological characters is relatively high, the result of the simultaneous analysis approaches the morphological tree. Because every morphological character evolves with an independent rate, which cannot be determined, there is no justification for downweighting morphological characters relative to molecular transformations. They should hence be given a weight equal to the highest cost assigned to any substitution or indel.

Molecules vs. morphology

The relationships among the superfamilies in the molecular cladogram (Fig. 1) are largely congruent with those implied by the morphological data (Schulmeister, 2003a: fig. 7). The taxonomic congruence between molecules and morphology is much better than in a previous study (Schulmeister *et al*., 2002). The major cause for this improvement is the increased taxon sampling. (If the present molecular data set is analysed with fewer taxa, the resulting cladograms show significant changes.) The exclusion of alignment-ambiguous DNA regions in the present study might also have led to a better agreement with the morphological tree. In spite of this improvement in taxonomic congruence, there are still two points of major disagreement between the morphological tree (Schulmeister, 2003a: fig. 7) and the molecular cladogram (Fig. 1).

Within Tenthredinoidea *s.s.*, there is a significant difference between the molecular and the morphological cladograms. In the molecular trees, Tenthredinidae, Diprionidae and Cimbicidae form a monophyletic group that is the sistergroup to Argidae+Pergidae, whereas in the morphological tree they constitute a basal grade with respect to Argidae+Pergidae. In this aspect, the morphological trees agree – not surprisingly – with the hypothesis of Vilhelmsen (2001), whereas the molecular trees agree with the hypotheses of Rasnitsyn (1988) and Ronquist *et al*. (1999). But in both the molecular and the morphological hypotheses, Argidae+Pergidae are monophyletic and Diprionidae are more basal than Cimbicidae – either with respect to Tenthredinidae* or with respect to Argidae+Pergidae.

The other major difference between the morphological (Schulmeister, 2003a: fig. 7) and molecular trees (Fig. 1) concerns Xiphydriidae and Orussidae. In the morphological trees, Xiphydriidae and Orussidae are closer related to Apocrita than to Siricidae. This is in agreement with recent hypotheses (e.g. Rasnitsyn, 1988; Ronquist *et al*., 1999; Vilhelmsen, 2001). In the molecular trees, however, they are more closely related to *Syntexis* and Siricidae. In the molecular analyses using the first and third weighting scheme, Siricoidea, Orussidae and Xiphydriidae together are monophyletic. In the second molecular analysis, *Xiphydria* is the sistertaxon to *Syntexis*, while Orussidae pair with Siricidae (the relationships between these two groups and Apocrita being unresolved). The monophyly of Siricoidea+Xiphydrioidea+Orussoidea is supported only with a Bremer

support value of 3 (in the equally weighted molecular analysis).

Phylogeny of basal hymenoptera based on total evidence

Figure 2 shows the strict consensus of the results of all three simultaneous analyses. There is some lack of resolution within Tenthredinidae* and Apocrita.

The influence of the ordering of some morphological characters on the simultaneous analysis is very small. If the simultaneous analysis with the equal weighting scheme is repeated with all morphological characters treated as unordered, the only changes within Hymenoptera are found within Tenthredinoidea *s.s.*

The phylogenetic relationships on family and superfamily level presented here are largely congruent with results of recent cladistic analyses by Ronquist *et al.* (1999), Vilhelmsen (2001), and Schulmeister *et al.* (2002). In the following, the phylogeny of the basal lineages of Hymenoptera will be discussed in detail, including prominent synapomorphies (i.e. those that show little homoplasy). (A complete list of synapomorphies can be obtained from the author.) The discussion is based on the phylogenetic hypotheses of Figures 2 and 3. However, in order to determine the morphological synapomorphies, the first analysis was repeated without those taxa for which no morphological data were present, in order to prevent some ambiguous optimizations. Bremer support values (= decay indices) (determined for the analysis using the equal weighting scheme) are given in Figure 2. In the discussion, a value of 1 is considered extremely low, 3–8 are considered low values, 11–14 medium, 16–23 high, and 43–46 extremely high values.

Hymenoptera are clearly and unambiguously supported as monophyletic by the morphological and molecular data. Prominent morphological synapomorphies for members of Hymenoptera are the inflected clypeus (3 : 1, unreversed, also present in *Priacma*), the presence of well developed dorsal tentorial arms (19 : 0, also present in Raphidioptera, reversed in *Orussus* and *Vespula*), the presence of cervical apodemes (46 : 1; unique and unreversed), the fusion of the laterocervicalia and propleura (48 : 1; unique and unreversed), the articulation of the profurcal arms with the propleura (62 : 2; unique and unreversed), the modification of the anterior apical protibial spur into a curved calcar with a velum (72 : 1; unique, but reversed within Tenthredinoidea), the mesolatero-phragmal-mesofurcal muscles being divided into two pairs instead one composite pair (86 : 1; unique and unreversed), the presence of the mesospina (102 : 0; unique, reversed within Tenthredinoidea and Apocrita), the absence of mesothoracic trochantins (104 : 2, unique and unreversed), the basal rings being set off from the rest of the femora (111 : 1; unique and unreversed), the presence of cenchri (116 : 1; unique, reversed in Cephidae and Apocrita), the metathoracic trochantins not being connected to the metapleura and metacoxae (148 : 1; unique and unreversed), the metafurcal arms arising anteriorly on the discriminal lamella of the metathorax (155 : 1; unique and unreversed), the anal cell of the forewing not reaching posterior wing margin (171 : 1; unique and unreversed), the presence of distal hamuli (173 : 0; unique and unreversed) and secondary hamuli (176 : 0; unique; reversed several times), the presence of distinct jugal lobes in the hindwings (184 : 0; also in *Priacma*, reversed in Apocrita), the sclerotization of the pleural parts of the abdominal segments (188 : 1; unique and unreversed), the forewing tegulae being large (241 : 2; unique, reversed in Siricidae), the presence of a cupula of the male genitalia (299 : 0; unique, reversed in Pergidae), and the presence of volsellae (325 : 1; unique and unreversed). The Bremer support for Hymenoptera is relatively low. However, with the large number of unique synapomorphies, the monophyly of this group is quite plausible.

Contrary to the hypotheses of Vilhelmsen (1997b) and Ronquist *et al.* (1999), but in agreement with Vilhelmsen (2001) and Schulmeister *et al.* (2002), **Xyelidae** are monophyletic, albeit with a relatively low Bremer support. Synapomorphies are the presence of a microtrichian brush connected to one of the tormae (8 : 1; convergent in *Micropterix*), the enlarged first flagellomeres (23 : 1, also found in Blasticotomidae), highly asymmetric mandibles (26 : 1; also in Psocoptera and *Micropterix*), a large infrabuccal pouch (29 : 1; also found in *Priacma* and *Micropterix*), free-lying prepecti (90 : 0; also in Raphidioptera, *Hartigia*, and Siricoidea), the presence of internal ridges associated with the mesopseudosternal sulci (96 : 0; also in Argidae+Pergidae, Megalodontesidae, Cephidae and *Syntexis*), the presence of metathoracic paracoxal notches (145 : 0; also in Pamphiliidae), the presence of basal hamuli (172 : 0, also in Pamphiliidae and *Xiphydria*), the reduction of larval abdominal legs to bulges (230 : 1; unique), and the presence of hairs on the valvicepes (318 : 1; also in *Corynis*).

Two of five extant genera of Xyelidae were included in the present study. The enlarged first flagellomeres (23 : 1) are present in all Xyelidae (e.g. Schedl, 1991). The presence of basal hamuli (172 : 0) was not only observed in *Xyela* and *Macroxyela*, but also in *Xyelecia nearctica* and *Pleroneura bruneicornis* (pers. observ.). The presence of hairs on the valvicepes (318 : 1) was also seen in *Megaxyela* (Shinohara, 1992: figs 3 and 5, Smith & Schiff, 1998: figs 3 and 33) and in *Pleroneura* (pers. observ.). These are good indicators for the monophyly of all extant genera of Xyelidae. For a discussion

of the internal phylogeny of Xyelidae, see Schulmeister (2003a).

Prominent synapomorphies for **Hymenoptera minus Xyelidae** are the elongation of the cervical apodemes (46 : 2; unique, but reversed in *Aglaostigma*, *Corynis*, and *Lophyrotoma*), the presence of posterodorsal profurcal apodemes (63 : 1; reversed in Unicalcarida minus Cephidae), the absence of mesofurco-prospinal muscles (68 : 1; also in *Panorpa*, reversed in Pamphiliidae and *Syntexis*), the separation of the mesothoracic anepisterna from the rest of the mesopleura as postspiracular sclerites (92 : 1; unique and unreversed), the absence of metapleural-S2 muscles (141 : 1; unique and unreversed, but unknown for many taxa), the absence of metanototrochanteral muscles (151 : 1; unique), the subcosta of the forewing being fused with R (159 : 2; unique, reversed in Pamphiliidae and Siricidae), Rs of forewings not being furcate apically (164 : 1; unique and unreversed), the absence of a subcosta in the hindwing (178 : 1; also in Psocoptera and *Micropterix*, reversed in Pamphiliidae), the number of maxillary palp segments being increased to six segments (240 : 1; unique, reversed within Unicalcarida minus Cephidae), and the fusion of veins Cu and M of the hindwing at the base of the wing (247 : 1; unique and unreversed).

Synapomorphies of **Tenthredinoidea** *s.l.* are the deeply curved pronotum (39 : 1; unreversed, but also present in Siricidae, Xiphydriidae and Vespina), the posterolateral margins of the pronotum having concavities for the accommodation of the anterior thoracic spiracles (41 : 1; unreversed; also in Xiphydriidae+Vespina), the katepisterna being subdivided (59 : 2; unreversed; also in Siricidae), the posterior thoracic spiracles being covered laterally by the mesepimera (94 : 2 unreversed; also in Xiphydriidae+Vespina), the insertion of the occlusor muscles of the posterior thoracic spiracles on the mesepimera (95 : 1; unreversed, also in *Vespula*, but the character is coded as unknown for most taxa), the elongation of the lateral mesofurcal arms (100 : 1), the absence of mesobasalar-mesocoxal muscles (109 : 1; unreversed; also in Vespina; unknown for most taxa), the separation of the second phragma and the anterior margin of the metanotum by a membranous area of considerable length (113 : 1; unreversed; also in Cephidae and *Syntexis*), the presence of transverse metanotometapleural muscles (115 : 1; unique), the subdivision of the second phragmo-third phragmal muscles (119 : 1; unique, reversed in Cimbicidae), the absence of metathoracic trochantins and attached muscles (148 : 2; also in *Megalodontes* and *Xiphydria*+Vespina), the elongation of the lateral metafurcal arms (157 : 1), the connection of crossvein 2r of forewing to Rs distally of crossvein 2r-m (167 : 1; unique), and the 'fusion' of the two parts of muscle u of the male genitalia (288 : 2; unique and unreversed). There is also a deletion of one basepair in the 12S gene. If character 65 is treated as ordered, the close association of the prospinasternum with the mesothorax (65 : 1) is also a synapomorphy of Tenthredinoidea *s.l.*

Synapomorphies of the **Blasticotomidae** are medially continuous tormae (9 : 1; unique and unreversed), the presence of anterior labral retractor muscles (13 : 0), the enlarged first flagellomere (23 : 1, convergent in Xyelidae and Argidae), the presence of anapleural sclerites (135 : 0; unreversed, also in *Macroxyela* and Tenthredinidae*), close contact of the first tergite and the metepimera along their entire length (139 : 2; unreversed), the absence of the first abcissa of the forewing Rs (161 : 1; unique and unreversed), the absence of the abcissa of the forewing Rs (163 : 1), the presence of a membranous line between the dorsal and pleural parts of the tergites (188 : 2; also in *Xyela*, Cimbicidae, Pamphiliidae and *Xiphydria*), the position of the abdominal spiracles in the dorsal part of the tergite (189 : 1; unique and unreversed, though inapplicable for most taxa), the forewing tip being corrugated (244 : 1), the absence of a basal inflection of the gonostipes (308 : 0), and the tip of the valviceps of the male genitalia being drawn out into a pointed thread-like structure (319 : 1; also in some Nematinae).

The present study found further synapomorphies for the monophyly of **Paremphytus+Runaria**. In addition to the absence of segments distally of the third antennal segment (23 : 2; also in Argidae) mentioned by Vilhelmsen (2001), a notch in the median margin of the parapenis (306 : 1; unique) and a straight distal margin of the ninth sternite (346 : 2; convergently present in *Xiphydria*) also support this group within the Blasticotomidae. Unfortunately, only one blasticotomid species could be sequenced (*Runaria*), so that the molecular evidence can contribute neither to the support of the monophyly of Blasticotomidae nor to that of *Paremphytus*+*Runaria*.

Synapomorphies of the **Tenthredinoidea** *s.s.* are a reversal to the absence of rod-like sensilla on the distal labial palp segment (36 : 0; also in Vespina), the absence of prophragmo-postoccipital muscles (50 : 1; unreversed), the absence of the proximal parts of the subdivided katepisterna (59 : 3; unique), the termination of the mesopseudosternal sulci in the anterior margins of the mesepisterna (97 : 1; reversed in *Phylacteophaga*), slender lateral metanotal processes (114 : 1; reversed in Cimbicidae; also in *Micropterix* and Cephidae), the absence of metanoto-metalaterophragmal muscles (120 : 1; also in Vespina), the anterior metafurcal arms being shorter than the lateral metafurcal arms (156 : 1; unreversed), a banding pat-

tern being present on the first and/or second valvulae (197 : 1; unique and unreversed), the presence of serrulae on the sawteeth of the first valvulae (201 : 1; unique), the strophandry of the male genitalia (294 : 1; also in *Xyela*; unreversed), and the spiculum on the ninth sternite being drawn-out (347 : 1).

The Tenthredinoidea *s.s.* are divided into two groups: Tenthredinidae+Diprionidae+Cimbicidae on one hand and Argidae+Pergidae on the other. This is in agreement with Rasnitsyn (1988) and Ronquist *et al*. (1999: Fig. 3), but not with Vilhelmsen (2001), the recent morphological analysis of Schulmeister (2003a) and the simultaneous analysis of Schulmeister *et al*. (2002). According to the morphological hypotheses of Vilhelmsen (2001) and Schulmeister (2003a), Tenthredinidae form a basal grade with respect to a monophyletic Diprionidae+Cimbicidae+Argidae+Pergidae clade. If a simultaneous analysis (using the equal weighting scheme) is performed with a constrain to retrieve a monophyletic Cimbicidae+Argidae+Pergidae, the resulting 20 trees (of 11 101 steps) are 14 steps longer than the most parsimonious trees. These trees also contain a monophylum Diprionidae+Cimbicidae+Argidae+Pergidae. Interestingly, even in this tree, the Tenthredinidae do not form a paraphyletic grade of several lineages, as suggested by the morphological tree, but only two lineages, the more basal one comprising only *Athalia*, and the Tenthredinidae* still being monophyletic! If, however, a simultaneous analysis is performed that is constrained to retrieve a sistergroup relationship of Nematinae and Diprionidae+Cimbicidae+Argidae+Pergidae, the Tenthredinidae* are broken up into a basal grade of five lineages (including Nematinae). But these trees are 22 steps longer than the most parsimonious cladograms.

A large part of the support for the **Tenthredinidae+Diprionidae+Cimbicidae** clade is provided by characters from the terminal segments of the male abdomen. Synapomorphies are the separation of the dorsal flanges (of the ovipositor) from the median bridge by weakly sclerotized strips (205 : 1; unique, reversed in Diprionidae), the presence of two segments in the larval abdominal legs (231 : 1; reversed in *Metallus*), the absence of mesopseudosternal sulci (251 : 1), the presence of muscle a between the ninth sternite and the male genitalia (267 : 2; unreversed; also in *Cephalcia*), the insertion of muscle k within the valviceps (278 : 1), the parapenis being set off from the rest of the gonostipes (304 : 2; unique, reversed in *Corynis*), the presence of a dorsal flap on the harpe (314 : 1; unique, reversed in Cimbicidae), and the constriction of the eighth sternite (343 : 1/2/3; unreversed). Prominent molecular synapomorphies are an insertion of one base in the 12S gene (which had previously been deleted at the base of the Tenthredinoidea *s.l.*), a unique and unreversed deletion of one base in the 12S gene, and a unique and unreversed deletion of one base in the 16S gene. Rasnitsyn (1988) also proposed the absence of an enlarged third antennal segment (23 : 0) and the absence of preapical metatibial spurs (158 : 1) as synapomorphies of this group. However, the absence of these features is interpreted as plesiomorphic in the present analysis.

Athalia is the sistergroup of Tenthredinidae*+Diprionidae+Cimbicidae. This is also the case in the molecular trees. In the morphological tree (Schulmeister, 2003a: fig. 7), *Athalia* is even the sistergroup of all other Tenthredinoidea *s.s.* In any case, *Athalia* is the most basal tenthredinid taxon included here. Benson (1962), in his treatment of Athaliini, already stated that *Athalia* is 'a genus from near the base of the Tenthredinid stem showing some primitive features'. According to Benson (1962: p. 349), all four genera of the Athaliini (*Hennedyia*, *Hennedyella*, *Hypsathalia* and *Athalia*) usually have a vestigial abscissa of the forewing Rs. This is unique within Tenthredinoidea *s.s.* and corresponds to the condition found in Blasticotomidae. However, because of the variation of this character within species of *Athalia* (some specimens lacking the abcissa) and because of problems with the delimitation of character states (see Schulmeister, 2003a), I decided to replace this character with another one, so that this primitive feature of *Athalia* did not contribute to its basal placement in the present analysis.

The monophyly of **Tenthredinidae*+Diprionidae+Cimbicidae** is supported only by two synapomorphies: the presence of denticles on the plantulae (257 : 1; unreversed; also in *Cephus*+*Calameuta*) and a ventral inflection of the ninth sternite of the male (349 : 2; reversed in some taxa). This clade is divided into Diprionidae+Cimbicidae on one hand and Tenthredinidae* on the other.

The monophyly of **Cimbicidae+Diprionidae** was suggested neither by previous hypotheses nor by any of the molecular analyses presented here. In the partitioned analyses, Diprionidae are always more basal than Cimbicidae, either with respect to Tenthredinidae* (molecules) or with respect to Argidae+Pergidae (morphology). Trees with a monophyletic Tenthredinidae*+Diprionidae are only one step longer than the most parsimonious trees. The Cimbicidae+Diprionidae clade is also quite instable: if the taxa that do not have molecular data are excluded from the analyses, Cimbicidae+Diprionidae are not monophyletic in all most parsimonious trees. Potential synapomorphies for Cimbicidae+Diprionidae are the cenchri being at least twice as broad as long (117 : 1/2; also in Argidae+Pergidae, *Selandria*, Nematinae, Megalodontesidae, and *Syntexis*), the cenchri not being inflected (118 : 1; also in Argidae+Pergidae), the reduction of the larval antennae to three segments or

less (224 : 4; also in Unicalcarida minus Cephidae), and the fusion of the digitus and basivolsella of the male genitalia (326 : 1; also in Megalodontesidae and some Apocrita).

Many synapomorphies support the monophyly of **Cimbicidae**. The antennae are clubshaped (24 : 1; unreversed, also in *Perga*), the prospinasternal apodeme is long (66 : 1; also in *Athalia* and *Arge*), the lateral metanotal processes are blunt or inconspicuous (114 : 0, unreversed), the second phragmo-third phragmal muscles are undivided (119 : 0, unreversed), the metalaterophragmal lobes are weakly developed or absent (126 : 1; unique within Tenthredinoidea), the metalaterophragmo-metafurcal muscles are absent (127 : 1, unreversed, also in *Vespula*), the first tergite is medially undivided (129 : 1; unreversed; also in Pergidae, *Sterictiphora*, Megalodontesidae and Vespina), the first tergite is completely fused to the metepimera (139 : 3; unreversed, also in Argidae+ Pergidae and Apocrita), the apodemes/tendons receiving the posterior metapleuro-metafurcal muscles are absent (143 : 1; unreversed; also in Argidae+Pergidae and Xiphydriidae+Vespina), a membranous line and an angle are present between the dorsal and pleural parts of the tergites (188 : 2; unreversed, also in *Xyela*, Blasticotomidae, Pamphiliidae and *Xiphydria*), the larval antennae have only two segments (224 : 5), the larvae have supraspiracular glands (232 : 1, unique and unreversed), the forewing tip is corrugated (244 : 1; within Tenthredinoidea also in Blasticotomidae, *Lophyrotoma* and *Perga*), the dorsal flap on the harpe is lacking (314 : 0; unreversed), the cranial end of the basivolsella covers the gonostipital arm (327 : 1, unreversed, also in *Lophyrotoma* and *Stephanus*), and the ventral inflection of the basal margin of the ninth sternite is protruding (349 : 3; unique and unreversed).

The present study included members from only three of the four subfamilies of Cimbicidae: Coryninae, Abiinae and Cimbicinae. The fourth subfamily, Pachylostictinae, was not included. However, the clubshaped antennae known from all Cimbicidae (e.g. Schedl, 1991) support the monophyly of the entire family. Moreover, specimens of the Pachylostictinae *Pachylosticta* sp. and *Pseudopachylosticta* sp. (as well as the Cimbicinae *Leptocimbex* sp. and *Pseudoclavellaria* sp.) not only show the clubshaped antennae (24 : 1), but also the undivided first abdominal tergite (129 : 1), the fusion of the first abdominal tergite with the metepimera (139 : 3), the presence of a membranous line and an angle between the dorsal and lateral parts of the abdominal tergites (188 : 2), the absence of a dorsal flap on the harpe (314 : 0), and the cranial part of the basivolsella covering the gonostipital arms (327 : 1) (pers. observ.). Hence, there is good reason to believe that members from all four cimbicid subfamilies form a monophyletic group.

Synapomorphies of the **Diprionidae** are the lateral projections on the antennae (22 : 1; unique (in this form) and unreversed), the absence of crossvein 2r of the forewing (166 : 1; also in Argidae+Pergidae and some Nematinae), the absence of sawteeth on first valvulae (199 : 0), the presence of ctenidia on first valvulae (203 : 1; unreversed; also in *Aglaostigma* and *Hartigia*), the dorsal flanges being continuous with the median bridge and the sites of insertion of the posterior T9–2nd valvifer muscles (205 : 0), the flattened larval antenna (in which the segments lie next to each other) (225 : 1; also in *Nematus*, but not in *Nematinus* or *Hoplocampa*, and in Vespula), the antenna being divided into more than 15 segments (237 : 0; unreversed), the inclination of the parapenis (309 : 1; also in some Tenthredinidae*), and the presence of a row of teeth on the valviceps (321 : 1; also in *Strongylogaster* and *Sceliphron*).

Both subfamilies of Diprionidae were included in the present analysis: Monocteninae with *Monoctenus* and Diprioninae with *Diprion* and *Gilpinia*. The specific shape of the male antennae in all diprionid species (e.g. Schedl, 1991) is at least some support for the monophyly of the entire family, but it would be desirable to examine more members of the family for the presence of the other synapomorphies. Within Diprionidae, the **Diprioninae** (*Gilpinia* and *Diprion*) come out as monophyletic. Synapomorphies are the absence of the muscles s and si in the male genitalia (286 : 3), the ectophallic membrane forming two flat pockets on the ventral side of the male genitalia (298 : 1; unique), the glandula mucosa being U-shaped (339 : 2; also in *Tenthredo*), and the absence of a distinct spiculum on the ninth sternite of the male (347 : 0; within Tenthredinoidea *s.s.* also in Cimbicinae and Abiinae).

The monophyly of **Tenthredinidae*** (i.e. Tenthredinidae minus *Athalia* in the present taxon sample) is supported by the absence of the labral retractor muscles (13 : 0), a reversal to the presence of a velum on the calcar (72 : 1), the presence of anapleural sclerites (135 : 0; unreversed, also present in Blasticotomidae), and the insertion of muscle u on the lateral face of the harpe (289 : 1; also in Blasticotomidae and *Tremex*). These are relatively few synapomorphies and the Bremer support value of 4 is one of the lowest in the entire tree. A future analysis with more tenthredinid species will have to show which other species are part of this monophyletic subclade of Tenthredinidae and which belong elsewhere. Within Tenthredinidae*, the monophyly of the four taxa of **Nematinae** is supported by the narrowness of the tentorial bridge (20 : 1; unreversed; also in *Abia*, *Athalia* and *Cephalcia*), the cenchri being at least twice as broad as long (117 : 1; also in *Selandria*, Cimbicidae+Diprionidae, Argidae+Pergidae, Megalodontesidae, and *Syntexis*), the reduction of the larval antenna to four segments

(224 : 3), the reduction of the legs on the eighth abdominal segment of the larva (266 : 2; unreversed; also in Argidae+Pergidae), and the tip of the valviceps of the male genitalia being drawn out into a pointed thread-like structure (319 : 1; also in Blasticotomidae; reversed in *Nematus*).

The Tenthredinidae (i.e. including *Athalia*) do not come out as monophyletic in the present analyses. Enforcing the monophyly of this family (in a simultaneous analysis with the equal weighting scheme) requires 7 additional steps. Rasnitsyn (1988) suggested that the Tenthredinidae including Diprionidae are monophyletic. This hypothesis was not tested in the reanalysis of his data by Ronquist *et al*. (1999), which did not include Diprionidae as a separate taxon. It was tested, however, by Vilhelmsen (2001) and Schulmeister *et al*. (2002), albeit with a very insufficient taxon sample: Vilhelmsen used four tenthredinids and two diprionids, Schulmeister *et al*. used only four tenthredinids and one diprionid. Rasnitsyn's hypothesis was rejected by the former, but supported by the latter study. The present study more than doubled the taxon sample for these groups to 12 tenthredinids and three diprionids. The hypothesis of a monophyletic Tenthredinidae including Diprionidae is now clearly rejected. (The hypothesis is also rejected by the separate analyses). Enforcing the monophyly of Tenthredinidae *sensu* Rasnitsyn in a simultaneous analysis (using the equal weighting scheme) requires 10 additional steps.

The monophyly of **Argidae+Pergidae** is strongly supported. This is in agreement with previous analyses except for the most parsimonious trees of Ronquist *et al*. (1999: fig. 2) and one of the most parsimonious trees of the initial analysis of Vilhelmsen (1997b), in which the Pergidae came out as the sistergroup of the Tenthredinidae+Diprionidae+Cimbicidae. But this grouping was not found in any of the analyses of the present study—neither in the simultaneous analyses, nor the molecular or morphological analyses. Moreover, trees with a sistergroup relationship between Pergidae and Tenthredinidae+Diprionidae+Cimbicidae are 33 steps longer than the most parsimonious trees, a relatively large amount. With the current data, it would be shorter to have Argidae as the sistergroup to Cimbicidae+Diprionidae+Tenthredinidae, which is still 28 steps longer than the most parsimonious hypotheses. The monophyly of Argidae+Pergidae is thus well supported. They show a large number of synapomorphies: the pronotum is fused with the mesopleura ventrally of the anterior thoracic spiracles (42 : 2; unreversed; also in Cimbicinae+Abiinae, *Selandria* and *Aglaostigma*), the profurco-laterocervical muscles have a single insertion on the cervical apodemes (47 : 1; also in Pamphilioidea+Unicalcarida), the dorsal mesofurco-profurcal muscles are absent (70 : 1; within Hymenoptera found only here and in *Tenthredo*), a distinct projection forms a ring of sclerotized cuticle around the insertion point of the mesoscutello-metanotal muscles (112 : 2; also in *Cimbex*), the cenchri are at least twice as broad as long (117 : 1), the posterior part of the cenchri is not inflected (118 : 1; also in Cimbicidae+Diprionidae), the first tergite and metepimera are completely fused (139 : 3; unreversed, also in Cimbicidae and Apocrita), the apodemes/tendons receiving the posterior metapleuro-metafurcal muscles are absent (143 : 1; unreversed; also in Cimbicidae and Xiphydriidae+Vespina), the vein 2r of the forewing is absent (166 : 1; also in Diprionidae and some Nematinae), the anal cell of the forewing is at least constricted (171 : 2), secondary hamuli are missing (176 : 1), second valvifers and third valvulae are totally fused (204 : 1; unreversed; also in *Ibalia*), second valvulae are fused distally (210 : 1; unreversed; also in *Nematus*, Pamphilioidea, and Vespina), the larval antenna has only one segment (224 : 6; also in Vespina), legs are absent on the eighth larval abdominal segment (266 : 2, also in Nematinae), the phallotrema is closed basally (324 : 1; unique in this particular condition and unreversed), the fibula ducti is very large and situated on the surface of the ductus ejaculatorius (335 : 1; unique, but reversed in *Phylacteophaga*), and the vesiculae seminales are very small, lumped together and squeezed between the glandulae mucosae (342 : 1; unique).

Members of only two argid and five pergid subfamilies were included in the present analysis. But the absence of vein 2r from the forewing (166 : 1) has been reported from all Argidae and Pergidae (e.g. Schedl, 1991) and the synapomorphies 117 : 1, 118 : 1, 139 : 3 and 171 : 2 were seen not only in the exemplars included in the present study, but also in *Sericoceros* sp. (Erigleninae, Argidae), *Dielocerus* sp. (Dielocerinae, Argidae), two species of *Atomacera* (Atomacerinae, Argidae), *Neoeurys* sp. (Euryinae, Pergidae), *Cerospastus* sp. (Philomastiginae, Pergidae), *Lagideus* sp. (Syzygoniinae, Pergidae), *Parasyzygonia rufosternalis* (Parasyzygoniinae, Pergidae), *Pteryperga galla* (Pteryperginae, Pergidae) and *Styracotechys* sp. (Styracotechyinae, Pergidae) (pers. observ.), indicating that a more inclusive sample of these two families would also come out as monophyletic. The apomorphies 117 : 1 and 139 : 3 are also found in *Zenarge turneri* (Zenarginae, Argidae) (author's pers. observ.), but the anal cell of the forewing is complete (and not constricted) in *Zenarge* (171 : 1), and the cenchri seem to be inflected in *Zenarge* (118 : 0) (pers. observ.), which means that the synapomorphies 171 : 2 and 118 : 1 are not present in all Argidae and Pergidae. The fusion of the pronotum with the mesopleura ventrally of the anterior thoracic spiracles (42 : 2) might also not be found in all Argidae and Pergidae; they

seem to be merely abutting (42 : 1) in *Zenarge*, *Dielocerus* and *Sericoceros*, although it is hard to tell in dried museum specimens. Also, there seem to be secondary hamuli in *Atomacera* (176 : 0). In sum, of the seven synapomorphies of Argidae and Pergidae that can be examined externally, only three were found in all examined members (117 : 1, 139 : 3 and 166 : 1). The phylogeny and monophyly of Argidae+Pergidae should hence be reexamined with a larger taxon sample.

The monophyly of **Argidae** (or rather *Arge*+*Sterictiphora*) was weakly supported by past analyses (Vilhelmsen, 2001; Schulmeister *et al*., 2002). Vilhelmsen (2001) found only two synapomorphies for Argidae: the enlarged first flagellomeres (23 : 2, also in Xyelidae and Blasticotomidae), and the presence of expanded lobes anteriorly on the metepimera covering the metapleural arms (133 : 1; unique). With the larger taxon sample used in the present paper, the closeness of the medioventral propleural margins (56 : 1) comes out as a synapomorphy of Argidae, because of the five pergid taxa only *Perga* and *Phylacteophaga* show this condition. With the recoding of character 117 and the current topology, the extreme narrowness of the cenchri is also found to be a synapomorphy of Argidae (117 : 2). Furthermore, the new characters from the male reproductive organs provide four additional synapomorphies: the absence of a basal inflection of the gonostipes (308 : 0; unreversed; also in *Decameria*, Blasticotomidae, Cephidae, *Syntexis* and one ichneumonid), the presence of a ridge on the distal edge of the harpe (315 : 1; unique and unreversed), the glandulae mucosae being more or less on top of each other (338 : 1; unique and unreversed), and the glandula mucosa being sigma-shaped (339 : 3; unreversed; also in *Trichiosoma*, *Strongylogaster*, *Cladius* and *Orussus*). The alternative hypothesis in which *Sterictiphora* is the sistergroup of Pergidae, is 13 steps longer with the present data. The monophyly of *Arge*+*Sterictiphora* is hence well supported.

Only two of the six subfamilies of Argidae were included in the present analysis, Arginae and Sterictiphorinae. But the enlarged first flagellomeres (23 : 1) are present in all Argidae (e.g. Schedl, 1991). The ridge on the harpe (315 : 1) is found not only in Arginae and Sterictiphorinae, but also in members of Dielocerinae (*Dielocerus* sp.) and Erigleninae (*Sericoceros* sp.) (pers. observ.). (However, the ridge seems be absent from *Atomacera* (Atomacerinae), but it was not possible to be certain based on my own observation of dried museum specimens). The extremely narrow cenchri (117 : 2) were also found in *Dielocerus*, *Sericoceros* and *Atomacera*, but not in *Zenarge*, which shows state 1 of this character. Hence, of the three apomorphies of Argidae that could be checked in museum specimens, two were not found in all examined species. The question of the monophyly of Argidae should hence be revisited in the future with a larger sample of alcohol-preserved Argidae. *Zenarge* is a very interesting taxon in that respect. It is the only argid species examined by me that is lacking the vein m-cu in the hindwing (181 : 1) (reported by Benson, 1963), a characteristic that is found in the tenthredinoid sample of the present study only in Pergidae and *Monophadnoides*+*Metallus*. Another derived feature of *Zenarge* is the presence of preapical spines on mid- and hindtibiae (158 : 0), which is found in the present tenthredinoid sample otherwise only in *Arge* and three of the five pergids. (The argids *Atomacera*, *Dielocerus* and *Sericoceros* do not have preapical spines.) Interestingly, in addition to these derived characters, *Zenarge* also shows a plesiomorphic feature that is unique within Argidae+Pergidae: a complete anal cell in the forewing (171 : 1). The position of *Zenarge* is hence quite enigmatic and its inclusion in a phylogenetic analysis of Argidae+Pergidae crucial for the determination of the monophyly of the Argidae.

Synapomorphies for the five members of **Pergidae** (= Pterygophoridae) included in the present analysis are the absence of the prothoracic katepisterna (58 : 1; unreversed), the insertion of the mesofurco-metabasalar muscles on the anterior margin of the metapleura (101 : 1), the absence of the anal cell of forewing (171 : 4; unique within Tenthredinoidea *s.l.*, reversed in *Decameria*), the placement of the distal hamuli in a straight line (174 : 1; unique within Tenthredinoidea *s.l.*; unreversed), the absence of crossvein m-cu of the hindwing (181 : 1; unreversed; within Tenthredinoidea also in *Metallus*+*Monophadnoides*), the absence of the second anal vein of hindwing (249 : 1; unique within Tenthredinoidea *s.l.*, unreversed), the presence of muscle y between the two basivolsellae (282 : 2; unique, reversed in *Phylacteophaga*), and the complete reduction of the cupula (299 : 2; unique and unreversed).

Pergidae are a speciose and morphologically diverse group. Previous analyses using the exemplar approach (Vilhelmsen, 2001; Schulmeister *et al*., 2002) included members of only two of the 14 subfamilies listed by Abe & Smith (1991) – *Perga* and *Phylacteophaga* – which was clearly insufficient to test the monophyly of Pergidae, especially because these two exemplars are more closely related to each other than to some other pergids, according to the present study. The present analysis included members of five pergid subfamilies: Perginae (*Perga*), Phylacteophaginae (*Phylacteophaga*), Pterygophorinae (*Lophyrotoma*), Perreyiinae (*Decameria*) and Acordulecerinae (*Acordulecera*). Because the internal phylogeny of Pergidae is entirely unknown, this sample is still insufficient to make conclusions about the monophyly of all members of Pergidae. However, some

of the characters that were found to be synapomorphies of the five pergid species in the present study are known to be present in other pergid taxa as well. As far as I am aware, Pergidae are the only hymenopterans which completely lack the cupula (basal ring) of the male genitalia. This condition is known not only from the species included here, but (as far as I know) from all pergid species whose genitalia have been examined or depicted in the literature, which in addition to those included in the present study covers the subfamilies Pergulinae, Philomastiginae, Conocoxinae, Parasyzygoniinae, Syzygoniinae and Loboceratinae (Smith, 1990), as well as Euryinae (*Ancyloneura* sp.) and Styracotechyinae (*Styracotechys* sp.) (pers. observ.). This means that for members of 13 of the 14 subfamilies of Pergidae it is known that the males of the examined species are lacking the cupula. For the subfamily Pteryperginae there is no information on the male genitalia available. The crossvein m-cu is lacking in the hindwing (181 : 1) in all members of Pergidae (e.g. Smith, 1990: p. 8; Schedl, 1991: p. 15). This condition is also found in *Zenarge* (Zenarginae, Argidae) (Benson, 1963), but this could be due to convergence. Finally, all described pergid species are missing the anal cell of the hindwing (249 : 1) (e.g. Smith, 1990: p. 8; Schedl, 1991: p. 7). These two synapomorphies are unique within Tenthredinoidea *s.l.* with the present taxon sample. These three synapomorphies – the lack of the vein m-cu and the anal cell in the hindwing and the lack of a cupula – make the monophyly of all Pergidae seem quite plausible.

Pamphilioidea+Unicalcarida are monophyletic. (For circumscription of Unicalcarida see Fig. 3). Schulmeister *et al*. (2002) suggested that Pamphilioidea is the sistergroup to Tenthredinoidea *s.l.* This relationship also showed up in some of the unordered implied weights analyses of Vilhelmsen (2001). It was, however, not found in any of the present analyses, be it molecular, morphological or total evidence. Enforcing the monophyly of Pamphilioidea+Tenthredinoidea *s.l.* (using the equal weighting scheme) requires eight more steps, a relatively small amount (Fig. 2). Pamphilioidea and Unicalcarida share a number of derived features: the distal part of the labrum lies posteriorly of the tips of the mandibles (4 : 1; unique; reversed in Ichneumonidae), a sclerotization separates the occipital and oral foramina (16 : 1; unreversed; also present in *Corynis* and some outgroup taxa), the medioventral propleural margins are closely abutting (56 : 1; unreversed, also in some Tenthredinoidea), the distal hamuli are positioned in a straight line (174 : 1; reversed in *Acantholyda* and Siricidae; also in Pergidae), the larval thoracic legs are reduced and have segments of equal size (228 : 1; unique, further reduced in Unicalcarida), claws are missing on the larval thoracic legs (229 : 2; unique and unreversed), and the two muscles b of the male genitalia are widely separated (267 : 1; also within Pergidae).

Pamphilioidea are monophyletic, which has never been seriously disputed. Pamphilioidea are monophyletic in all molecular and simultaneous analyses presented here. Together with a large number of synapomorphies – most of which are unique for basal Hymenoptera – there can be hardly any doubt about the monophyly of this group. It has one of the highest Bremer support values in the entire tree: the shortest cladogram without a monophyletic Pamphilioidea is 21 steps longer than the most parsimonious trees. (In this alternative, Megalodontesidae come out as the sistergroup to Unicalcarida.) Synapomorphies of the Pamphilioidea are the presence of a sclerotized subgenal bridge separating the mandibular foramina from the oral foramen (14 : 1; unique in basal Hymenoptera and unreversed), the elongation of the mandibles (26 : 2; unique and unreversed), the extension of the sitophore beyond the functional mouth (27 : 2; unique in basal Hymenoptera and unreversed), the posterior margin of first valvifers being closely appressed or partly fused with the anterior margin of tergite 9 (196 : 1; unique and unreversed), the lamnia being reduced in size relative to the radices (198 : 1; unique in Hymenoptera and unreversed), the distal ends of second valvifers and proximal ends of third valvulae being widely separated (207 : 1; unique in Hymenoptera and unreversed), the presence of styli (209 : 1; also present in Raphidioptera and *Xyela*, unreversed), the distal fusion of the second valvulae (210 : 1; unreversed), the larval antenna being divided into seven segments (224 : 0; unique and unreversed), the presence of a larval suranal hook (233 : 1; unique and unreversed), the corrugated structure of the tips of the forewings (244 : 1; reversed in Cephalciinae), the absence of muscles e from the male genitalia (272 : 1; also in *Hoplocampa*, unreversed), the anteromedial corner of parapenis being drawn out (304 : 0; unique in basal Hymenoptera and unreversed), and the median face of the harpe extending much more cranially than the lateral face (316 : 1; unique and unreversed).

Synapomorphies of **Pamphiliidae** are the absence of metapleural apodemes (142 : 0), the presence of metathoracic paracoxal notches (145 : 0; unreversed; also in Xyelidae), the presence of a distinct subcosta in the forewing (159 : 0; unreversed), the presence of basal hamuli (172 : 0; unreversed; also in Xyelidae and Xiphydriidae), the presence of a subcosta in the hindwing (178 : 0; unreversed; unique within non-xyelid Hymenoptera), the longitudinal division of the second abdominal tergite (187 : 1; unique and unreversed), the presence of a membranous line between the dorsal and lateral parts of the tergites (188 : 2;

unreversed; also in *Xyela*, Blasticotomidae, Cimbicidae, and *Xiphydria*), the presence of muscle m in the male genitalia (280 : 0; unreversed; also in Xyelidae and Siricidae), and a prominent insertion of one base in the 28S alignment. Both pamphiliid subfamilies were included in the present study: two of the four genera of Cephalciinae and all three genera of Pamphiliinae listed by Abe & Smith (1991). Benson (1945) reported that the medial division of the second abdominal tergite is present in all species of Pamphiliidae. His study did not cover *Chinolyda*, but this genus has the division as well (A. Taeger, pers. comm.). This character is unique to this family and lends credibility to the monophyly of all Pamphiliidae.

The studies of Vilhelmsen (2001) and Schulmeister *et al*. (2002) found Pamphiliinae to be paraphyletic. Vilhelmsen's (2001) study included *Pamphilius* and *Neurotoma*. The study of Schulmeister *et al*. (2002) combined sequences from *Onycholyda* and *Pamphilius* in one OTU, Pamphiliini, in addition to the OTU Neurotoma. The present study now includes all three genera of Pamphiliinae as separate OTUs: *Neurotoma*, *Onycholyda* and *Pamphilius*. The paraphyly of Pamphiliinae is confirmed with this larger sample. *Onycholyda+Pamphilius* are more closely related to the Cephalciinae than to *Neurotoma*. The two included species of **Cephalciinae** together are monophyletic. They are united by the following apomorphies: laterosternal sclerites are present (60 : 0), the tips of the apical protibial spurs are blunt and membranous (73 : 1; also in Diprionidae and within Cimbicidae), and the tips of the frontwings are smooth = coriaceous (244 : 0). According to Benson (1945), the last two apomorphies are also found in the genus *Caenolyda* (Cephalciinae).

Two species of **Megalodontesidae** were included in the present study, but DNA sequences could be obtained for only one of them. Synapomorphies for these two species are the labrum being several times higher than broad (5 : 1), the presence of flat lateral projections on the antennae (22 : 2; also in *Lophyrotoma*), the termination of the mesopseudosternal sulci in the anterior margins of the mesepisterna (97 : 1), the cenchri being at least three times as broad as long (117 : 2), the first abdominal tergite being continuous medially (129 : 1), the posterior end of the first abcissa of the forewing Rs being more proximal than the anterior end (162 : 1), the absence of muscles l (279 : 1) and muscles p (284 : 1; unique) of the male genitalia, the dorsal reduction of the cupula (299 : 1; also in *Metallus*, *Arge*, *Dolichovespula*, and one ichneumonid), the medial inflection of the parapenis (307 : 1), the fusion of the digitus to the basivolsella (326 : 1), the constriction of the eighth sternite (343 : 1), the spiculum being drawn out (347 : 1), the ventral inflection on the basal margin of ninth sternite (349 : 2), and the eighth tergite of the male being apically extended to cover the anus (350 : 1).

Schulmeister (2001) has already described the peculiar glandulae mucosae of *Megalodontes cephalotes*. Each glandula has three blind ends, which appear to be 'holding' the vesicula seminalis (Schulmeister in press: fig. 13G). Unfortunately, it was impossible to tell from the dried and shrunken internal organs of the examined specimens of *M. skorniakowii* whether they are similar to those of *M. cephalotes*.

The Megalodontesidae are a small and rather uniform family, containing less than 100 species. They were revised by Springate (1994), who proposed to include all of them in the genus *Megalodontes*. All have flabellate antennae (22 : 2), which is unique within Pamphilioidea+Unicalcarida and is hence a good indicator of the monophyly of the entire family. Springate describes the cupula (gonocardo) in Megalodontesidae in general as 'strongly fused dorsally with base of gonostipes, less so ventrally.' This is incorrect, as the cupula is certainly not fused to the gonostipes in the two species that I have examined (the male genitalia of these species were also examined by Springate). In *M. cephalotes* and *M. skorniakowii*, the cupula is clearly separate of the gonostipes (as shown by dissection), very narrow ventrally, broad laterally and absent dorsally. As Springate (1994) did not include the cupula in his drawings and did not notice that it is absent dorsally, it can unfortunately not be deduced from his work whether the dorsal reduction of the cupula (299 : 1), a potential synapomorphy of Megalodontesidae, is present in all species of this family or only in some.

The name **Unicalcarida** was introduced by Schulmeister *et al*. (2002) for the clade comprising Cephidae, Anaxyelidae, Siricidae, Xiphydriidae, Orussidae and Apocrita. The name is based on an externally visible synapomorphy, the reduction of the posterior apical protibial spur, leaving only the anterior spur, which is modified to a calcar. The clade is supported by a high Bremer support value and a good number of apomorphies. The distal epipharyngeal wall is sclerotized (6 : 1; unique in Hymenoptera and unreversed), the labral compressor muscles are absent (12 : 1; unreversed), the rod-like sensillae on the labial palp are situated in an invagination (36 : 2; unique within Hymenoptera; absent in Vespina), the profurco-prospinal muscles are absent (67 : 1; unreversed; unique within Hymenoptera), the posterior apical protibial spurs are missing (71 : 1; unreversed, also in *Runaria* and *Phylacteophaga*), a pair of lobes is present on the second phragma (pseudophragma) (88 : 1; unique, but reversed in *Syntexis*, *Orussus* and *Schlettererius*), the lateral attachment points of the mesopostnotum are invaginated with the mesepimera (89 : 1; unique), the anterior mesofurcal arms are fused (99 : 1; unique and

unreversed), the metapostnotum is medially divided (128 : 1; reversed in Vespina), the larval antennae are reduced to four segments (224 : 3), the larval thoracic legs are unsegmented (228 : 2; unique and unreversed), and a larval suranal process is present (234 : 1; unique; reversed in Vespina).

Synapomorphies of the three genera of **Cephidae** included here are the presence of a postoccipital bridge (17 : 1; unreversed; also in *Cimbex*, *Perga* and *Syntexis*), the presence of a notch on the probasitarsus opposite of the calcar (74 : 1; unreversed; also in Vespina), the presence of slender lateral metanotal processes (114 : 1), the absence of cenchri (116 : 0), the presence of a posterior articulation between the first tergite and the metepimera (139 : 1; unique and unreversed), the presence of a constriction between the first and second abdominal segment (185 : 1; unreversed; also in Apocrita), the crossvein in the anal cell of the forewing being at right angles to the anal veins (245 : 1), the presence of a notch in the anterior margins of abdominal sternites (262 : 1; also in *Metallus* and *Syntexis*), the presence of a long, distally directed muscle k which inserts within the valviceps (278 : 2), the absence of muscles qr of the male genitalia (285 : 1), the absence of the harpes and the muscles t of the male genitalia (287 : 1), the presence of muscle z in the male genitalia (293 : 1), the fusion of the parossiculus with the gonostipes (328 : 1), the presence of the ventral median sclerotized style (336 : 1; unreversed; also in Siricidae), and the vasa deferentia being straight (341 : 2). In addition to these morphological synapomorphies, Schulmeister *et al*. (2002: fig. 9C) found some unique and identical insertions (of several basepairs) in the 28S gene for the three sequenced species of Cephidae. The Bremer support for Cephidae is extremely high.

The present study includes representatives of only two of the three tribes of the Cephinae and no member of the Athetocephinae. However, the absence of cenchri (116 : 0) and a constriction between the first and second abdominal segment (185 : 1) in all described members of Cephidae including *Athetocephus* (e.g. Benson, 1938; Muche, 1981; Schedl, 1991) support the monophyly of the entire family. Moreover, Benson (1935) described the male genitalia of *Athetocephus* as looking like those of *Cephus pygmeus* as depicted by Boulangé (1924: fig. 118), which suggests that the harpe is missing (287 : 1), that the parossiculus is fused to the gonostipes (328 : 1), and that a median sclerotized style is present (336 : 1) in *Athetocephus* as well. These three apomorphies are also found in *Caenocephus* sp. (Hartigiini) and in *Pachycephus* sp. (Pachycephini) (pers. observ.), which covers all three tribes of Cephinae. In addition, the crossvein of the anal cell of the forewing is at right angles to the anal veins (245 : 1) and there is a notch in the anterior margins of abdominal sternites (262 : 1) in *Pachycephus* sp. and *Caenocephus* sp. (pers. observ.), providing an indicator that these characters are synapomorphies at least for the Cephinae, if not for all Cephidae.

Synapomorphies of the **Unicalcarida minus Cephidae** are the absence of posterodorsal profurcal apodemes (63 : 0), the fusion of the anterior mesofurcal arms for most of their length (99 : 2; unique and unreversed), the fusion of the cordate apodemes to the walls of tergite 9 for a distance corresponding to at least half the length of the anterior flanges (193 : 2), the presence of sawteeth only for a short distance distally on the first valvulae (200 : 1, unique and unreversed), the absence of larval eyes (221 : 1, unique and unreversed), the reduction of the larval antenna to only three segments (224 : 4), the reduction of the larval epicranial sulcus (226 : 1; unique within Hymenoptera), and the absence of intrasegmental annulation in the larva (227 : 1; unique within Hymenoptera).

The monophyly of **Siricoidea** = Anaxyelidae+Siricidae was first suggested by Schulmeister *et al*. (2002). It was supported by two prominent morphological synapomorphies: the absence of metapleural apodemes (142 : 0; within non-xyelid Hymenoptera also in *Lophyrotoma* and in Pamphiliidae), and the line of fusion of the cordate apodemes being situated in a depression (194 : 1; also in *Lophyrotoma* and *Orussus*). The present study provides five additional morphological synapomorphies of the Siricoidea (*sensu* Schulmeister *et al*., 2002): the presence of metanototrochanteral muscles (151 : 0; coded within Siricoidea only for *Syntexis* and *Sirex*), the incompleteness of the radial cell of the forewing (242 : 1; unreversed; also present in *Sterictiphora, Orussus* and Stephanidae), the ninth tergite of the female being elongated into a tip which extends beyond the cerci (263 : 1; unique and unreversed), the labrum of the larva being asymmetrical (265 : 1; unreversed; also in *Dolerus*), and the presence of muscle n (281 : 3). In the analyses of Ronquist *et al*. (1999) and Vilhelmsen (2001), as well as the morphological analyses of Schulmeister (2003a), Siricidae are more closely related to Xiphydriidae+Vespina than to *Syntexis*, the Siricoidea paraphyletic. This hypothesis is 17 steps longer with the total evidence and hence is clearly rejected.

Synapomorphies of **Siricidae** are the presence of a postgenal bridge (18 : 1; unreversed; also in Vespina), the fusion of the paraglossae with the glossa (34 : 3; paralleled only in *Panorpa* and unreversed), the presence of prominent projections on the pronotum which separate a smooth anterior surface from distinctly sculptured dorsolateral regions (40 : 1; unique and unreversed), the elongation of the mesospina (102 : 1; unique and unreversed), the termination points of the metathoracic paracoxal sulci not being near the ante-

rior or posterior margin of the metepisterna (147 : 2), the subcosta of the forewing being distinct from the costa (159 : 0; unreversed; also in outgroups, Xyelidae, and Pamphiliidae), the posterior end of the 1st abcissae of forewing Rs ending more proximal than the anterior end (162 : 1; unreversed; also in Megalodontesidae and Apocrita), the distal hamuli being placed in zigzag lines (174 : 0), the forewing tegulae being significantly reduced in size and hidden under the pronotum (241 : 1; unreversed; unique within Hymenoptera), the tip of the radial cell of the forewing not being close to the wing margin (243 : 1; unreversed; also in *Arge* and two pergids), the absence of the second mesotibial apical spur (254 : 1; unreversed; unique for basal Hymenoptera), the apices gonostipites pointing cranially instead of medially (303 : 1; unreversed; also in Sterictiphora and Megalyra), the presence of a ventral median sclerotized style (336 : 1; also in Cephidae), and the ninth sternite of the males being drawn out into a long, pointing tip (346 : 1; unique and unreversed). In addition to these morphological synapomorphies, Schulmeister *et al*. (2002) found unique insertions (their fig. 9 A, B) and a unique deletion (their fig. 9C) in the 28S gene present in all four sequenced species of Siricidae. There is also a deletion of two basepairs in the 16S gene and an insertion of one basepair in the 18S gene. The Bremer support for Siricidae is one of the highest in the entire tree.

There are only two extant subfamilies of Siricidae and members of both are included in the present analysis: *Xeris*, *Sirex* and *Urocerus* of the Siricinae, and *Tremex* of the Tremecinae. In addition to these genera, *Siricosoma* and *Xoanon* of the Siricinae and *Eriotremex* of the Siricinae all have the ninth tergite of the female extended to a hornlike projection (Benson, 1938, 1943), the cornus, which is unique within Hymenoptera and supports the monophyly of the entire family.

The Bremer support value for the **Xiphydriidae+Vespina** is relatively low, the shortest alternative hypothesis is only 5 steps longer; this has *Xiphydria* as sistergroup to the Siricidae, which apparently shows the influence of the molecular data. Enforcing a monophyletic Siricoidea+Xiphydriidae+Orussidae clade (as found in two of the molecular analyses) requires 33 additional steps in the simultaneous analysis (using equal weighting). Synapomorphies of Xiphydriidae+Vespina are the absence of occipital sulci and ridges (15 : 1; also found in *Abia* and some outgroup taxa), the presence of (weakly developed) parapsidal signa on mesoscutum (78 : 1; unique, reversed in *Megalyra*), the presence of a straight transscutal fissure which is less sclerotized than the rest of the mesoscutum (79 : 2; unique, reversed in Ichneumonidae), the mesopostnotum being covered dorsally by the metanotum (87 : 2; unique and unreversed), the elongation of the lateral mesofurcal arms (100 : 1), the absence of hindwing tegulae (123 : 1; unique within Hymenoptera, unreversed), the absence of apodemes/tendons receiving the insertions of the posterior metapleuro-metafurcal muscles (143 : 1; unique within Unicalcarida), the absence of metathoracic trochantins and attached muscles (148 : 2), the elongation of the lateral metafurcal arms (157 : 1), and the gonocondyle being formed into a distally directed loop (302 : 1; unique; reversed in *Orussus* and *Ibalia*).

Externally visible autapomorphies of ***Xiphydria*** are the presence of basal hamuli (172 : 0; also in Xyelidae and Pamphiliidae), the presence of a membranous line between the dorsal and lateral parts of the tergites (188 : 2; also in *Xyela*, Blasticotomidae, Cimbicidae and Pamphiliidae), and the male ninth sternite having a straight distal margin (346 : 2; also in *Runaria+Paremphytus*).

Xiphydria is the only genus of the Xiphydriidae included in the present study. The basal hamuli (172 : 0) are also present in *Derecyrta lugubris*, *D. pictipennis*, *D. variipennis*, *Steirocephala* sp. and *Brachyxiphus grandis* (pers. observ.), which are all members of the Derecyrtinae. This means that they are found in both subfamilies of Xiphydriidae. The line between the dorsal and lateral parts of the tergites (188 : 2) is found in all Xiphydriidae (e.g. Schedl, 1991). The straight distal margin of the male ninth sternite (346 : 2) was also found in *Derecyrta variipennis* (pers. observ.). An apomorphic character (not included in the present analysis) present in all described Xiphydriidae is the elongation of the cervical sclerites (e.g. Schedl, 1991). Taken together, these characters provide good support for the monophyly of the entire family.

For the following clades, the synapomorphies have been determined with the complete taxon sample, because Stephanidae come out as a sistergroup to Orussidae in the reduced taxon sample, which changes the optimization of some characters.

The assumption of the monophyly of Orussidae+Apocrita, i.e. **Vespina**, is generally accepted today. Königsmann (1977) and Wei & Nie (1997) suggested that Cephidae would be the sistergroup of Apocrita. However, this hypothesis is 42 steps longer than the most parsimonious trees, which is an extremely high amount. These trees are congruent with the hypothesis (Pamphilioidea {[Siricoidea (Xiphydriidae Orussidae)] (Cephidae Apocrita)}).

Synapomorphies of Orussidae+Apocrita are the presence of a postgenal bridge (18 : 1; also in Siricidae; reversed in *Ibalia*), the tentorial bridge being narrow and arched (20 : 2), the presence of a corpotendon (21 : 1), the presence of multiporous plate sensilla

(25 : 1), the presence of a hoodlike glossa (33 : 1; also in *Syntexis*, reversed in *Orthogonalys*), the reduction of the scale-bearing part of the paraglossae (34 : 2), the absence of rod-like sensilla from the labial palp (36 : 0), insertion of the ventral premental adductors on a common tendon (37 : 1), the inflection and smoothness of the dorsal parts of the propleura (53 : 1; unique within Hymenoptera and unreversed), the presence of probasitarsal notches and well developed probasitarsal combs (74 : 1, 75 : 2), the presence of well-developed parapsidal signa (78 : 2), the absence of mesothoracic anepisterna (92 : 2; unreversed; also in some pergids and in *Tremex*), the subdivision of mesocoxae by distinct transverse grooves (106 : 1; unique and unreversed), the absence of mesobasalar-mesocoxal muscles (109 : 1), the mesofurco-mesocoxal muscles arising anteriorly of the paracoxal ridges (110 : 1), the metapostnotum being continuous medially (128 : 0), the first abdominal tergite being continuous medially (129 : 1), the apodemal parts of the metabasalares being reduced or absent (132 : 2), the metapleural arms abutting the first tergite (134 : 1; unique and unreversed), the accommodation of the mesocoxae in well-developed metepisternal depressions (144 : 1; unreversed, unique within Hymenoptera), the termination points of the metathoracic paracoxal sulci being in or close to the metapleural sulci (147 : 1), the crossvein 1r of the forewing being incomplete or absent (165 : 2; unique and unreversed), the anal cell of the forewing being petiolate (or absent) (171 : 3), the absence of the hindwing costae (177 : 1; unique within Hymenoptera; reversed within Vespidae and Apoidea), the absence of the hindwing crossveins 1r-m (179 : 1; unique), 3r-m (180 : 1), and m-cu (181 : 1), the distal fusion of second valvulae (210 : 1), the larval antenna being reduced to only one segment (224 : 6), the absence of larval thoracic legs (228 : 3), the absence of the larval suranal process (234 : 0), the absence of the second anal vein of the hindwing (249 : 1), and the absence of the harpes and muscles t of the male genitalia (287 : 1).

Many autapomorphies of *Orussus* were found in the present analysis. However, for most of these it is unknown whether they occur in other orussids as well and so do not help to assess the monophyly of the family. The presence of an ocellar corona (1 : 1; also in Stephanidae), the modification of the female antennae into 'hammers' (238 : 1; unique), the extension of the fore basitarsus beyond the second tarsal segment to form a 'basitarsal spur' (252 : 1; unique), the subdivision of the fore tibia of the female (253 : 1; unique), and the configuration of the female ovipositor (264 : 1; unique) are known from all genera of **Orussidae** (L. Vilhelmsen, pers. comm.). (Most of these characters are described in Vilhelmsen *et al*. (2001).) Unique characters of the male terminalia found in *Orussus* which are potential synapomorphies for Orussidae but for which other Orussidae have yet to be examined are the presence of a unique muscle running across the male external genitalia from the left to the right edge of the ninth sternite (270 : 1), the extension of the volsella well beyond the gonoforceps (333 : 1; unique) and a strengthened line on the ninth sternite parallel to its basal margin (348 : 1; unique). (These characters are described in Schulmeister (in press).)

Synapomorphies of **Apocrita** are the presence of hypopharyngeal pectens (30 : 1; unique), the retraction of the cervical prominences (44 : 2; unique; reversed in one ichneumonid), the absence of laterosternal sclerites (60 : 1; reversed in Aulacidae), the fusion of the prospinasternum with the mesothorax (65 : 2; unique within Unicalcarida and unreversed), the absence of the mesospina (102 : 2; reversed in Stephanidae), the absence of cenchri (116 : 0; unreversed; also in outgroups and Cephidae), the fusion of the metapleural arms with the first tergite (134 : 2; unique and unreversed), the complete fusion of the first tergite with the metepimera (139 : 3), the absence of an anal cell in the forewing (171 : 4; unreversed; also in Pergidae), the absence of jugal lobes from the forewings (184 : 1; unique within Hymenoptera; reversed within Apoidea), and the presence of a constriction between the first and second abdominal segment (185 : 1; also in Cephidae). The Bremer support value for Apocrita is rather low. The clade is quite instable, too. If, for example, the analysis is repeated with the exclusion of those taxa for which the morphological characters have not been coded (which includes many of the apocritans), Stephanidae come out as a sistergroup to Orussidae, making Apocrita paraphyletic.

NOMENCLATURE

Schulmeister *et al*. (2002) have already proposed including Anaxyelidae in the superfamily Siricoidea because of the sistergroup relationship of *Syntexis* and Siricidae. Because this relationship has withstood further scrutiny in the present analysis, this concept of Siricoidea has been used in the present study as well. In addition, Schulmeister *et al*. introduced the name Unicalcarida for the Cephoidea+Siricoidea+Xiphydrioidea+Orussoidea+Apocrita clade. This clade is very well supported in the present analyses, with a relatively high Bremer support and numerous synapomorphies.

No new nomenclatural changes are proposed here. It would be premature to propose nomenclatural changes for the groups within Tenthredinoidea *s.s.*, even though these relationships are stably supported by all three simultaneous analyses. The tenthredinid sample is simply too small to allow predictions as to

which other tenthredinids would also be included in the Tenthredinidae* and which other taxa would appear in the lineage leading to *Athalia*. A larger taxon sample could even change the relationships within Tenthredinoidea *s.s.* completely. But if these relationships were to hold up to further scrutiny, it would probably be the best solution to narrow the family Tenthredinidae down to include only Tenthredinidae* and to assign family status to monophyletic tenthredinid clades outside of Tenthredinidae* (e.g. Athaliidae). This way, the family Tenthredinidae would not get larger than it already is and Cimbicidae and Diprionidae could retain the rank of families.

The present study also suggests that Pamphiliinae – contrary to Cephalciinae – are paraphyletic and that they might have to be split up into Neurotominae and Pamphiliinae *s.s.*

SUMMARY AND CONCLUSIONS

The relationships among the superfamilies presented in the final hypothesis (Fig. 3) largely agree with the hypotheses of Ronquist *et al*. (1999), Vilhelmsen (2001), Schulmeister *et al*. (2002) and Schulmeister (2003a). Contrary to Ronquist *et al*. (1999), Xyelidae are monophyletic. Contrary to Ronquist *et al*. (1999) and Vilhelmsen (2001), Anaxyelidae and Siricidae were found to be sistergroups (and should hence be classified as a single superfamily, following the convention currently used for basal Hymenoptera). Contrary to Schulmeister *et al*. (2002), Pamphilioidea are the sistergroup of Unicalcarida, not Tenthredinoidea *s.l.*

The nomenclatural changes proposed by Schulmeister *et al*. (2002), in which Siricoidea were redefined as Siricidae+Anaxyelidae and the name Unicalcarida had been introduced for Cephoidea+Siricoidea+Xiphydrioidea+Vespina, have been confirmed in the present study.

However, the relationships within Tenthredinoidea *s.s.* proposed here are novel. Tenthredinidae+ Cimbicidae+Diprionidae is monophyletic and the sistergroup to Argidae+Pergidae. Within the first group, *Athalia* (Tenthredinidae) is the sistergroup to the remaining taxa, Tenthredinidae is thus paraphyletic. The rest of the Tenthredinidae (Tenthredinidae minus *Athalia* = Tenthredinidae*) is monophyletic and is the sistergroup to Cimbicidae+Diprionidae. These relationships disagree with the four previous studies as well as the molecular analyses of the present study. Some relationships within Tenthredinoidea *s.s.* are only weakly supported. Trees with a sistergroup relationship between Diprionidae and Tenthredinidae* or between Cimbicidae and Tenthredinidae* are only one step and two steps, respectively, longer than the most parsimonious hypotheses. The sistergroup relationship of Cimbicidae and Diprionidae might hence prove unstable to the addition of taxa and characters.

The disagreement among the simultaneous, molecular and morphological analyses is not very surprising because the taxon sample used here for Tenthredinoidea *s.s.* (and particularly Tenthredinidae) is still very small compared to the number of described species in this group (*c*. 7000, Goulet, 1993). Moreover, for some tenthredinid species included in the present analysis, DNA sequences were not yet available, which might also have contributed to the incongruence between molecules and morphology.

Future research efforts regarding the phylogeny of the basal lineages of Hymenoptera should focus on the following areas. (1) Above all, the phylogeny of Tenthredinoidea needs to be examined with a much larger taxon sample, particularly from the Tenthredinidae, Argidae and Pergidae. (2) The monophyly of Apocrita should be reexamined with a much larger and diverse sample because it has proven to be very unstable to the number and choice of taxa. Additional genera from the Xiphydriidae, Orussidae, Stephanidae and Evanioidea might be particularly helpful in this respect. (3) The analyses from the present study should be repeated with the inclusion of fossil taxa because the optimization of characters can change with the addition of such fossil data. An analysis including fossil taxa is needed most for a reexamination of the monophyly of Siricoidea because the Anaxyelidae have only one extant species.

ACKNOWLEDGEMENTS

I am most grateful to Ward Wheeler, who allowed me to do the molecular part of my work in the lab of the American Museum of Natural History and generously provided for all lab expenses. Without his and James Carpenter's hospitality, enthusiasm, discussions, advice, criticism and support this work would not be the same. My sincere thanks go to Kelly DeMeo, who showed me how to sequence DNA and generated many DNA sequences after I had to leave New York. I also want to thank Mark Dowton, Malte Jänicke, Niels Peder Kristensen, Stefan Schmidt, David R. Smith and Andreas Taeger who gave me valuable specimens. Loans of other specimens were made by Henri Goulet, David R. Smith and Andreas Taeger. Ward Wheeler and James Carpenter allowed me to use some of their sequences before publication. I want to thank James Carpenter, Donald Quicke and two anonymous referees for comments and corrections which helped to improve this paper.

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APPENDIX 1

Species used to generate the morphological data and DNA sequences. The first column gives the names of higher taxa and the second and third those of the exemplars that were used in the present study and that of Vilhelmsen (2001). For the complete taxon names (including author and year of description) of the species used for morphology please refer to Schulmeister (2003a) and Vilhelmsen (2001). For the complete taxon names of species for which sequences were retrieved from GenBank, please see the original papers. The fourth column gives the names of the operational taxonomic units. Higher taxa are separated by long lines, operational taxonomic units (OTUs) by shorter lines.

Taxa	Species used for morphology	Species used for DNA sequence	Name of OTU
Blattaria + Plecoptera	–	*Blaberus* ssp. *Aphanicerca capensis*	Polyneoptera A
Orthoptera	–	*Melanoplus* ssp.	Polyneoptera B
Psocoptera + Hemiptera	*Amphigerontia bifasciata*	*Valenzuela* sp. *Triatoma* ssp. *Lygus lineolaris*	Paraneoptera
Chrysopidae	*Chrysopa perla* / Chrysopidae sp.	Chrysopidae sp. *Anisochrysa carnea*	Chrysopidae
Raphidioptera	*Raphidia xanthostigma* / Raphidioptera sp.	Raphidioptera sp.	Raphidioptera
Coleoptera	*Priacma serrata*	Cantharidae sp.	Coleoptera A
	–	*Carabus* ssp. *Leptocarabus procerulus* *Colpocaccus posticus*	Coleoptera B
Lepidoptera	*Micropterix calthella*	*Micropterix calthella* (Linnaeus 1761)	Lepidoptera A
	–	*Papilio* ssp.	Lepidoptera B
Mecoptera	*Panorpa communis* / *Panorpa* sp.	*Panorpa* ssp.	Mecoptera A
	–	*Panorpodes pulcher* *Boreus coloradensis*	Mecoptera B
Xyelidae	*Xyela julii* / *Xyela* sp.	*Xyela* sp.	Xyela
	Macroxyela ferruginea	*Macroxyela ferruginea* (Say 1824)	Macroxyela
Blasticotomidae	*Runaria reducta*	*Runaria reducta* (Malaise 1931)	Runaria
	Paremphytus flavipes	–	Paremphytus
	Blasticotoma filiceti / *Blasticotoma nipponica*	–	Blasticotoma
Tenthredinidae	*Tenthredo arcuata*, *Tenthredo* sp. / *Tenthredo campestris*	*Tenthredo mesomela* Linnaeus 1758	Tenthredo
	Aglaostigma lichtwardti	*Aglaostigma lichtwardti* (Konow 1892)	Aglaostigma
	Dolerus niger and *Dolerus* sp.	*Dolerus* sp.	Dolerus
	Selandria serva	*Selandria serva* (Fabricius 1793)	Selandria
	Strongylogaster multifasciata	–	Strongylogaster
	Monophadnoides sp.	–	Monophadnoides
	Metallus sp.	–	Metallus
	Athalia rosae and *Athalia* sp.	*Athalia* sp.	Athalia
	Taxonus agrorum	–	Taxonus
	Hoplocampa fulvicornis	–	Hoplocampa
	Nematinus luteus	–	Nematinus
	Nematus ssp.	*Nematus* sp.	Nematus
	Cladius pectinicornis	*Cladius pectinicornis* (Geoffroy 1785)	Cladius
Diprionidae	*Monoctenus juniperi* and sp.	*Monoctenus juniperi* (Linnaeus 1758)	Monoctenus
	Gilpinia sp.	*Gilpinia* sp.	Gilpinia
	Diprion sp. (*pini* or *similis*)	*Diprion* sp. (*pini* or *similis*)	Diprion
Cimbicidae	*Cimbex* sp. / *Trichiosoma* sp.	*Cimbex americana* (Leach 1817)	Cimbicinae
	Abia fasciata / sp.	*Abia fasciata* (Linnaeus 1758)	Abia A
	–	*Abia* sp. (*lonicerae*?)	Abia B
	Corynis sp. / *crassicornis*	*Corynis crassicornis* (Rossi 1790)	Corynis

Appendix 1 *Continued*

Taxa	Species used for morphology	Species used for DNA sequence	Name of OTU
Argidae	*Arge nigripes*, *Arge pullata* *Arge gracilicornis* / –	*Arge nigripes* (Retzius 1783)	Arge A
	– / *Arge cyanocrocea*	*Arge cyanocrocea* (Forster 1771)	Arge B
	Sterictiphora furcata	*Sterictiphora furcata* (Villers 1789)	Sterictiphora
Pergidae	*Perga condei* / *Perga* sp.	*Perga condei* (Benson 1939)	Perga
	Phylacteophaga froggatti	*Phylacteophaga froggatti* (Riek 1955)	Phylacteophaga
	Lophyrotoma analis	*Lophyrotoma analis* (Costa)	Lophyrotoma
	Acordulecera sp.	*Acordulecera* sp.	Acordulecera
	Decameria sp.	–	Decameria
Pamphiliidae	*Neurotoma nemoralis* / *fasciata*	*Neurotoma fasciata* (Norton 1862)	Neurotoma
	Onycholyda amplecta	*Onycholyda amplecta* (Fabricius 1804)	Onycholyda
	Pamphilius sylvaticus, sp., *hortorum, Pamphilius middlekauffi*	*Pamphilius hortorum* (Klug 1808)	Pamphilius
	Cephalcia sp. (*abietis* or *arvensis*)	*Cephalcia* sp. (*abietis* or *arvensis*)	Cephalcia
	Acantholyda erythrocephala and *Acantholyda* ssp.	*Acantholyda posticalis* Matsumura 1912	Acantholyda
Megalodontesi.	*Megalodontes cephalotes*	*Megalodontes cephalotes* (Fabricius 1781)	Megalodontes ceph.
	Megalodontes skorniakowii	–	Megalodontes sk.
Cephidae	*Cephus cultratus*, *nigrinus* / *Cephus pygmeus*	*Cephus pygmeus* (Linnaeus 1767)	Cephus
	Calameuta filiformis and *pallipes*	*Calameuta filiformis* (Eversmann 1847)	Calameuta
	Hartigia linearis and *xanthostoma*/ *Hartigia trimaculata*	*Hartigia trimaculata* (Say 1824)	Hartigia
Anaxyelidae	*Syntexis libocedrii*	*Syntexis libocedrii* (Rohwer 1915)	Syntexis
Siricidae	*Sirex juvencus* / *Sirex* sp.	*Sirex noctilio* Fabricius 1793	Sirex
	Xeris spectrum	*Xeris spectrum* (Linnaeus 1758)	Xeris
	Urocerus gigas	*Urocerus gigas* (Linnaeus 1758)	Urocerus
	Tremex columba	*Tremex columba* (Linnaeus 1763)	Tremex
Xiphydriidae	*Xiphydria camelus*	*Xiphydria prolongata* (Geoffroy 1785)	Xiphydria
Orussidae	*Orussus abietinus* and *occidentalis*	*Orussus abietinus* (Scopoli 1763)	Orussus A
	–	*Orussus minutus* Middlekauff 1983	Orussus B
Stephanidae	*Schlettererius cinctipes* *Stephanus serrator*	*Schlettererius cinctipes* (Cresson 1880)	Stephanidae A
	–	*Neostephanus* sp. *Megischus bicolor* (Westwood 1841)	Stephanidae B
Megalyridae	*Megalyra fasciipennis*	*Megalyra* sp.	Megalyra A
	–	*Megalyra* sp.	Megalyra B
Trigonalidae	*Orthogonalys pulchellus*	*Labidogonalos* sp.	Trigonalidae
Aulacidae	*Aulacus striatus* *Pristaulacus erythrocephalus*	–	Aulacidae
Ichneumonidae	Ichneumonidae sp.1	*Enicospilus* sp.	Ichneumonidae A
	Ichneumonidae sp.2	*Labena grallata*	Ichneumonidae B
Ibaliidae	*Ibalia rufipes*	*Ibalia anceps* Say 1824	Ibalia A
	–	*Ibalia leucospoides* (Hochenwarth 1785)	Ibalia B
Chalcididae	–	*Brachymeria* sp.	Chalcididae
Apoidea	*Sceliphron caementarium*	*Sceliphron caementarium* (Drury 1773)	Apoidea A
	–	*Ammophila* sp.	Apoidea B
	–	*Nomada* sp.	Apoidea C
Vespidae	*Vespula rufa* / *Dolichovespula adulterina*	*Vespula maculifrons* (Buysson 1905) *Apoica* sp.	Vespidae A
	–	*Polistes aurifer* Saussure 1853 *Polistes apachus* Saussure 1857	Vespidae B

APPENDIX 2

Origin of the DNA sequences used in the present study. OTUs are separated by horizontal lines. here = present study; Carp. = Carpenter & Wheeler (1999); Dow. = Dowton & Austin (1994, 1995, 1999 & 2001); Liu = Z. Liu, unpubl. data; Schulm. = Schulmeister *et al*. (2002). The fragments of CO1 sequenced by Dowton & Austin correspond roughly to the second CO1 fragment in the present study (see Appendix 4).

Species used for DNA sequencing	12S	16S	18S	28S	CO1
Blaberus discoidalis, giganteus or sp.	–	U17767	U65112	AF321254	–
Aphanicerca capensis	–	–	–	–	AF429296
Melanoplus bruneri or *lakinus* or sp.	–	AF145557	U65115	U65173	AF229004
Triatoma rubrofasciata or *dimidiata*	–	AY035468	–	–	AF301594
Lygus lineolaris	–	–	–	U65177	–
Valenzuela sp.	–	–	AF423793	–	–
Anisochrysa carnea	–	–	X89482	–	–
Chrysopidae sp. (Germany)	–	here	–	here	–
Raphidioptera sp. (Germany)	–	Schulm.	–	Schulm.	Schulm.
Cantharidae sp.	–	Schulm.	–	Schulm.	Schulm.
Carabus granulatus or *nemoralis*	–	AF219428	AF012507	–	–
Colpocaccus posticus	–	–	–	U65179	–
Leptocarabus procerulus	–	–	–	–	AB047568
Micropterix calthella	–	here	here	here	here
Papilio dardanus, *troilus* or *garamas*	–	AF095451	AF286299	U65199	AF044021
Panorpa sp.	–	Schulm.	–	Schulm.	Schulm.
Panorpa germanica	–	–	X89493	–	–
Panorpodes pulcher	–	AF180062	–	–	AF180101
Boreus coloradensis	–	–	AF286285	U65205	–
Xyela sp.	here	Schulm.	Schulm.	Schulm.	Schulm.
Macroxyela ferruginea	here	Schulm.	Schulm.	Schulm.	Schulm.+Dow.
Runaria reducta	here	Schulm.	Schulm.	Schulm.	Schulm.
Tenthredo mesomela	here	Schulm.	Schulm.	Schulm.	Schulm.
Aglaostigma lichtwardti	here	here	here	here	here
Dolerus sp.	here	Schulm.	Schulm.	Schulm.	Schulm.
Selandria serva	here	here	here	here	here
Athalia sp.	here	Schulm.	Schulm.	Schulm.	Schulm.
Nematus sp.	here	Schulm.	Schulm.	Schulm.	Schulm.
Cladius pectinicornis	here	here	here	here	here
Monoctenus juniperi	here	here	here	here	here
Gilpinia sp.	–	Schulm.	Schulm.	Schulm.	Schulm.
Diprion pini	here	here	here	here	here
Cimbex americana	here	Schulm.	Schulm.	Schulm.	Schulm.
Abia sp. (probably *lonicerae*)	–	Schulm.	Schulm.	Schulm.	Schulm.
Abia fasciata	here	here	here	here	here
Corynis crassicornis	here	Schulm.	Schulm.	Schulm.	Schulm.
Arge nigripes	here	here	here	Carp.+here	Carp.+here
Arge cyanocrocea	here	Schulm.	Schulm.	Schulm.	Schulm.
Sterictiphora furcata	here	Schulm.	Schulm.	Schulm.	Schulm.
Perga condei	here	Dow.	Schulm.	Schulm.	Schulm.+Dow.
Phylacteophaga frog.	here	Dow.	Schulm.	Schulm.	Schulm.+Dow.
Lophyrotoma analis	here	here	here	here	here
Acordulecera sp.	–	–	here	here	–
Neurotoma fasciata	–	Schulm.	Schulm.	Schulm.	Schulm.
Onycholyda amplecta	–	Schulm.	Schulm.	Schulm.	Schulm.
Pamphilius hortorum	–	here	here	here	here
Cephalcia sp.	–	Schulm.	Schulm.	Carp.	Schulm.
Acantholyda posticalis	–	Schulm.	Schulm.	Schulm.	Schulm.

Appendix 2 *Continued*

Species used for DNA sequencing	12S	16S	18S	28S	CO1
Megalodontes cephalotes	–	Schulm.	Schulm.	Schulm.	Schulm.
Cephus pygmeus	–	Schulm.	Schulm.	Schulm.	Schulm.
Calameuta filiformis	–	Schulm.	Schulm.	Schulm.	Schulm.
Hartigia trimaculata	–	Dow.	Schulm.	Schulm.	Schulm.+Dow.
Syntexis libocedrii	–	Schulm.	Schulm.	Schulm.	Schulm.
Sirex noctilio	–	Schulm.	Schulm.	Schulm.	Schulm.+Dow.
Xeris spectrum	–	here	here	here	here
Urocerus gigas	–	Schulm.	Schulm.	Schulm.	Schulm.
Tremex columba	–	Schulm.	Schulm.	Schulm.	Schulm.
Xiphydria prolongata	–	Schulm.	Schulm.	Schulm.	Schulm.
Orussus abietinus	–	Schulm.	Schulm.	Schulm.	Schulm.
Orussus minutus	–	here	here	here	here
Schlettererius cinctipes	–	Dow.	Schulm.	Schulm.	Schulm.+Dow.
Neostephanus sp.	–	here	here	here	here
Megischus bicolor	–	–	–	–	Dow.
Megalyra sp. 1	–	Schulm.	Schulm.	Schulm.	Schulm.
Megalyra sp. 2	–	Dow.	–	–	Dow.
Labidogonalos sp.	–	Schulm.	Schulm.	Carp.	Carp.
Enicospilus sp.	–	here	here	here	here
Labena grallata	–	here	here	here	here
Ibalia anceps	–	Liu	Liu	Liu	Liu
Ibalia leucospoides	–	Dow.	–	–	Dow.
Brachymeria	–	here	Carp.	Carp.	Dow.
Sceliphron caementarium	–	here	here	here	here
Ammophila sp.	–	here	here	Carp.	Carp.+here
Nomada sp.	–	here	here	Carp.	here
Vespula maculifrons JC8	–	here	–	Carp.	Carp.
Apoica sp.	–	–	U65153	–	–
Polistes aurifer JC151	–	here	here	–	here
Polistes apachus JC152	–	–	–	here	–

APPENDIX 3

DNA fragments included in the analysis. The first column gives the name of the fragment. The next three columns give the position of the beginning and end of the fragments based on the sequence of *Apis mellifera* (Crozier & Crozier, 1993), the sequence of *Drosophila melanogaster* (Tautz *et al*., 1988) and the alignment of Whiting *et al*. (1997). A '?' means that the homology to the sequence position of *Drosophila* could not be determined because the sequences of *Drosophila* and Hymenoptera are too different in these regions. The fifth column gives the total number of aligned positions for each gene included in the analysis (which differs from the number of positions in the complete alignment of these sequences). The sixth and seventh columns give the number and percentage of positions that are parsimony-informative (determined with PAUP).

Fragment	*Apis*	*Drosophila*	Whiting *et al.*	Included positions	Parsimony-informative	Percentage
12S A	15114–15028					
12S C	15020–15003					
12S E	14953–14911					
12S G	14882–14852					
12S I	14830–14808			206	98	48%
16S A	13850–13780					
16S C	13766–13703					
16S E	13650–13616					

Appendix 3 *Continued*

Fragment	*Apis*	*Drosophila*	Whiting *et al.*	Included positions	Parsimony-informative	Percentage
16S G	13549–13480			222	129	58%
18S A		600–?	205–333			
18S C		?–1580	insert – –	890	173	19%
28S A		4066–4098	15–46			
28S C		4103–?	62 – –			
28S E		4456–4601		550	129	23%
CO1 A	1863–2022					
CO1 B	2023–2516					
CO1 C	2517–2627					
CO1 D	2628–2903			1044	607	58%
SUM				2912	1136	39%

APPENDIX 4

Morphological characters and DNA sequence fragments that were included in the analysis. The names of the fragments correspond to those in Appendix 3. A '+' means that the fragment/character set is present for that species and that more than 50% of the characters in that fragment/set have been sampled. A '#' means that less than 50% and more than 0% of this fragment/characters set have been sampled for that species. MV: characters from Vilhelmsen (2001); MN: new characters from Schulmeister (2003a: characters 237–266); MT: characters from the terminal segments of the male (Schulmeister, in press; 2003a: characters 267–353).

				12S					16S				18S		28S			CO1			
OTU	MV	MN	MT	A	C	E	G	I	A	C	E	G	A	C	A	C	E	A	B	C	D
Polyneoptera A									+	+	+	#	+	+	+	#		+	+		
Polyneoptera B									+	+	+	+	+	+	+	+			+	+	+
Paraneoptera	+		#						+	+	+	+	+	+	+	+		+	+	+	+
Chrysopidae	+	#							+	+	+	+	+	+	+	+	+				
Raphidioptera	+	#	#						+	+	+	+	+	+	+	+	+	+	+	+	+
Coleoptera A	+	#	#						+	+	+	+	+	+	+	+	+	+	+	+	+
Coleoptera B									+	+	+	+	+	+	+	+		+	+	+	+
Lepidoptera A	+	#	#						+	+	+	+	+	+	+	+	+				
Lepidoptera B									+	+	+	+	+	+	+	+		+	+	+	+
Mecoptera A	+	+	#						+	+	+	+	+	+	+	+	+	+	+	+	+
Mecoptera B									+	+	+	+	+	+	+	+				+	+
Xyela		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Macroxyela	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Runaria	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tenthredo	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Aglaostigma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Dolerus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Selandria		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Athalia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nematus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cladius	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Monoctenus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gilpinia	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	
Diprion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cimbicinae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Abia A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Appendix 4 *Continued*

OTU	M V	M N	M T	12S A	12S C	12S E	12S G	12S I	16S A	16S C	16S E	16S G	18S A	18S C	28S A	28S C	28S E	CO1 A	CO1 B	CO1 C	CO1 D
Abia B										+	+	+	+	+	+	+	+	+	+	+	+
Corynis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arge nigripes	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Arge cyanocroc.		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sterictiphora	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Perga	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phylacteophaga	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lophyrotoma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acordulecera	+	+	+										+	+	+	+	+				
Neurotoma	+	+	+						+	+	+	+	+	+	+	+	+	#	+	+	
Onycholyda	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pamphilius	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cephalcia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acantholyda	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Megalodontes c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cephus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Calameuta	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
Hartigia	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Syntexis	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Sirex	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Xeris	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Urocerus	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Tremex	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	
Xiphydria	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Orussus A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Orussus B									+	+	+	+	+	+	+	+	+	+	+	+	+
Stephanidae A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Stephanidae B									+	+	+	+	+	+	+	+	+	+	+	+	+
Megalyra A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Megalyra B									+	+	+	+								+	+
Trigonalidae	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Ichneumonidae A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Ichneumonidae B	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Ibalia A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Ibalia B									+	+	+	+								+	+
Apoidea A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Apoidea B									+	+	+	+	+	+	+	+	+	+	+	+	+
Apoidea C									+	+	+	+	+	+	+	+	+	+	+	+	+
Vespidae A	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+	+
Vespidae B									+	+	+	+	+	+	+	+	+	+	+	+	+
MORPHOLOGY ONLY																					
Paremphytus	+	#	+																		
Blasticotoma	+	+	+																		
Strongylogaster	+	+	+																		
Monophadnoides	+	+	+																		

Appendix 4 *Continued*

	M V	M N	M T	12S					16S				18S		28S			CO1			
				A	C	E	G	I	A	C	E	G	A	C	A	C	E	A	B	C	D
Metallus	+	+	+																		
Taxonus	+	+	+																		
Hoplocampa	+	+	+																		
Nematinus	+	+	+																		
Decameria	+	+	+																		
Megalodontes sk.	+	+	+																		
Aulacidae	+	+	+																		
MOLECULES ONLY																					
Chalcididae									+	+	+	+	+	+	+	+	+			+	+