WILEY-BLACKWELL

Cladistics 25 (2009) 211–230

Cladistics

10.1111/j.1096-0031.2009.00255.x

Phylogenetic analysis of 73 060 taxa corroborates major eukaryotic groups

Pablo A. Goloboff[a,*], Santiago A. Catalano[b], J. Marcos Mirande[b], Claudia A. Szumik[a], J. Salvador Arias[a], Mari Källersjö[c] and James S. Farris[d]

[a]*INSUE (Instituto Superior de Entomología), CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Instituto Miguel Lillo, Miguel Lillo 205, 4000 S.M.Tucumán, Argentina;* [b]*CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Fundación Miguel Lillo, Miguel Lillo 251, 4000 S.M.Tucumán, Argentina;* [c]*Göteborgs Botaniska Trädgård (Gothenburgh Botanical Garden), Carl Skottbergs Gata 22A, SE-413 19 Göteborg, Sweden;* [d]*Molekylärsystematiska laboratoriet, Naturhistoriska riksmuseet, Box 50007, 104-05 Stockholm, Sweden*

Accepted 21 February 2009

Abstract

Obtaining a well supported schema of phylogenetic relationships among the major groups of living organisms requires considering as much taxonomic diversity as possible, but the computational cost of calculating large phylogenies has so far been a major obstacle. We show here that the parsimony algorithms implemented in TNT can successfully process the largest phylogenetic data set ever analysed, consisting of molecular sequences and morphology for 73 060 eukaryotic taxa. The trees resulting from molecules alone display a high degree of congruence with the major taxonomic groups, with a small proportion of misplaced species; the combined data set retrieves these groups with even higher congruence. This shows that tree-calculation algorithms effectively retrieve phylogenetic history for very large data sets, and at the same time provides strong corroboration for the major eukaryotic lineages long recognized by taxonomists.

After publication of Darwin's theory in 1859, establishing the lines of descent for the major groups of organisms became one of the most important goals in biology. Solving a problem of such magnitude requires consideration of as much relevant evidence as possible, especially in terms of taxonomic diversity, but significant efforts so far have concentrated mostly in assembling data sets with large numbers of genes for reduced numbers of representative taxa (Bapteste et al., 2002; Dunn et al., 2008). Attempts at large taxon samples have been much less common, one of the reasons for that difference being that the complexity of phylogenetic analysis increases linearly with characters or genes, but superexponentially with taxa. Thus data sets beyond a thousand species (e.g. the studies of Källersjö et al., 1999; McMahon and Sanderson, 2006) continue to be exceptional.

Data sets with more than a few thousand taxa had been considered basically intractable until very recently. Some large data sets have been analysed only experimentally, to test specific computer programs and without publication of taxonomic results (e.g. Goloboff and Pol, 2007). The largest phylogenetic data set analysed to date (Smith et al., 2009) used all available rbcL data for about 13 000 plant taxa. This analysis used RAxML (Stamatakis, 2006), a program for rapid maximum likelihood analysis. However, the impressive speed-ups in RAxML come not only from using shortcuts for faster (''lazy'') evaluation of rearrangements (similar to those suggested by Goloboff, 2003[1]), but also from

*Corresponding author:
E-mail address: pablogolo@csnat.unt.edu.ar

[1]Goloboff (2003, p. 95) actually stated that, after regrafting a clade, the branch-length optimization of the three branches around the new node—as in the ''lazy'' optimization used in RAxML—produces too much error, and suggested extending optimization to adjacent branches as well for more accurate calculations. Goloboff (2003) based his observations on his own unpublished maximum-likelihood program.

sacrificing rigour in the tree search—the program evaluates only a reduced number of rearrangements per tree, regrafting every pruned branch at a fixed number of alternative nodes (Stamatakis, 2006, p. 2689). In the case of large and complex data sets (especially those combining multiple genes), it is unknown whether those superficial rearrangement algorithms are actually sufficient to approach optimality.

In this paper, we show that parsimony can be used to analyse data sets with much larger numbers of taxa than those analysed by Smith et al. (2009). Our data set comprises over 73 000 eukaryote taxa, scored for 13 genes and morphology. Our data set is over five times larger than Smith et al.'s, and it includes the 13 000 rbcL sequences they used as a small subset. With the divide-and-conquer techniques and fast branch-swapping algorithms for parsimony implemented in TNT (Goloboff, 1999; Goloboff et al., 2008), it is possible to analyse this data set using search algorithms as thorough and exhaustive as those used for normal sized data sets.

Methods

Sequence retrieval

To retrieve sequences from GenBank, two different strategies were performed. One involved BLAST searches using as query 15 sequences from different lineages of eukaryotes. The maximum allowed by NCBI/BLAST of 20 000 positive results, was accepted in each query. After that, the accession numbers of all the queries were combined to generate a list subsequently used as input file to download these sequences using the batch mode of NCBI-PUBMED (http://www.ncbi.nlm.nih.gov/sites/batchentrez). A second strategy involved searching sequences in NCBI-PUBMED by gene or product name. The sequences were downloaded in GenBank format in all cases; two C scripts (written by P. Goloboff) extracted the genes of interest from the files downloaded, producing FASTA files including the complete taxonomy and the accession number in the name for each sequence. The programs use a Needleman–Wunsch string-comparison algorithm for comparing taxon names, and query the user when finding similar but not identical names, to detect spelling errors; given that this can detect errors only if the taxon name is spelt in different ways for the same gene, the same error-checking routine was incorporated into TNT and used to detect spelling errors for different genes, as TNT read and combined the individual files for all genes. These errors were corrected, so that the spelling for each taxon is identical in each of the input files. The format for taxon names (where AN represents the GenBank accession number) is:

```
Genus_species____AN_@Kingdom_Phylum_Class_Order ...
```

(in some cases, the GenBank taxonomy contains additional subcategories). For the purpose of matching taxon identity, when reading input files, TNT will use only the characters preceding a quadruple underscore (genus and species names). The format used allows the data for each gene to be kept in separate files, and the GenBank accession number for each of the sequences used in the analysis to be preserved, so that the data set is self-documented. Although TNT will ignore all characters beyond a quadruple underscore for the purpose of name-matching, it will store them in the taxon name. The @ symbol is included for easier identification, when using scripts, of the beginning of the taxonomic information contained in the taxon name (see "Evaluation of results").

Gene selection and alignment

To assemble the largest possible data set, we selected from GenBank those genes that have been sequenced for many taxa, filtering for minimum sequence length and level of taxonomic identification. Genes to solve both general eukaryotic relationships and specific groups were included. The sequences included in the matrix are nuclear, plastidial and mitochondrial, totalling 13 genes (sequenced for 750 to $\sim$ 20 000 taxa). We used sequences of nuclear and mitochondrial rRNA (SSU and LSU); nuclear RNAPII; plastid rbcL, matK, and ndhF; mitochondrial COX I-III, CytB and NDI (see Table 1 for details).

All sequences other than LSU and SSU were aligned with Muscle (Edgar, 2004). Nuclear LSU and SSU were aligned with Mafft (Katoh et al., 2005; Katoh, 2008). The alignment of LSU and SSU involved the following steps: (i) separate the complete data set in subsets of approximately 2000 sequences; (ii) align each data set separately using the Mafft option of considering a previously aligned sequence as a "template" for the multiple alignment [17 LSU and 70 SSU sequences, downloaded from the European ribosomal database (http://bioinformatics.psb.ugent.be/webtools/rRNA/ssu and http://bioinformatics.psb.ugent.be/webtools/rRNA/lsu/index.html), which take into account structural considerations]; (iii) find conserved regions common to all the aligned data sets; (iv) subdivide "vertically" each subset of 2000 sequences at the conserved regions identified in step 3, producing data sets of 2000 species per $\sim$ 50–200 bp each; (v) combine each corresponding partial data set obtained in step 4 and generate a data set of 20 000 species per 50–200 bp; (vi) erase the gaps; (vii) perform a multiple alignment with the data set of step 6; (viii) manually adjust the alignments.

Where possible and appropriate, we used amino acid sequences (COX I–III, CytB, RNAPII). The

Table 1
Genes used in the analysis, indicating the number of fragments in which every gene was split for alignment

Gene	Fragments	Taxa	Characters	Scope	Type	Genome
LSU rRNA	11	11700–1267	312–115	Global	DNA	Nuclear
MatK	1	11855	792	Embryophyta	DNA	Plastid
NdhF	1	4864	1209	Embryophyta	DNA	Plastid
RbcL	1	13043	13 043	Embryophyta	DNA	Plastid
COXI	1	7310	1296	Metazoa	PROT	Mitoch
COXII	1	8315	437	Metazoa	PROT	Mitoch
COXIII	1	2309	272	Metazoa	PROT	Mitoch
CytB	1	13766	337	Chordata	PROT	Mitoch
NDI	1	4123	349	Metazoa	PROT	Mitoch
SSU rRNA	6	20462–19336	293–26	Global	DNA	Nuclear
SSU rRNA	1	1314	464	Hexapoda	DNA	Mitoch
RNAPII	2	869–333	515–203	Fungi/Global	PROT	Nuclear
LSU rRNA	1	752	314	Ascomycota	DNA	Mitoch

resulting alignments were inspected visually and, when possible, improved manually; regions that were too gappy were excluded from the final data sets. Given the obviously problematic nature of the alignments and incomplete sequences (many gaps are lack of data, not real deletions), we considered gaps as missing. Multiple sequences for the same species were excluded to maximize taxonomic diversity instead of simply using large numbers of identical sequences.

Morphological characters

In addition to the DNA and protein sequences, 604 (595 informative) morphological characters for high-level groups (with emphasis on unequivocal characters with well known distribution, scorable across all taxa) were included in the matrix. These characters (listed in Appendix 1) were all taken from literature (no new morphological evidence was included). Since most included morphological characters represent major structural conditions, they were slightly upweighted, making them equivalent to three DNA substitutions (i.e. given a weight of 3).

Data set

The full molecular data set includes 73 060 taxa and 9535 characters (7857 of which are parsimony-informative). On average, the taxa in the data set have been sequenced for only a fifth of the genes and, because of low taxonomic overlap between genes, 92% of the entries in the molecular data set (6.4×10^8) correspond to missing entries (gaps, and genes or parts of genes not sequenced). Non-empty matrix cells in the combined data set totalled about 9.9×10^7. The final data set includes all major groups, many of which are represented by a significant proportion of the species described (Table 2).

Computer settings

All time-consuming and RAM-consuming calculations were done on three computers, with 32 GB of RAM each, running under Ubuntu Linux. One of the machines has eight 3.0 GHz Xenon 5472 processors, and the two others have four 3.0 GHz Xenon 5160 processors each.

Tree-search

The combined data set with morphology plus molecules and the molecule-only data set were analysed separately. Tree searches, identical for molecular and combined data sets, ran in parallel on three computers (totalling 16 processors and 96 GB RAM), examining for each data set $\sim 7.5 \times 10^{14}$ rearrangements in ~ 2.5 months' processor-time. To estimate ambiguity, for each data set we used eight independent replicates with tree bisection reconnection (TBR) followed by sectorial searches (see details below). The best tree for each of the two data sets was found by combining the eight independent trees with tree-fusing (Goloboff, 1999; Goloboff and Pol, 2007) and then subjecting the fused tree to sectorial search, as detailed below.

For each starting point, TBR-swapping for the molecule-only trees saved 50 000–56 000 steps from the Wagner trees, and for the combined data set, about 35 000 steps. After concluding TBR, each of the resulting trees was subjected to a sectorial search routine, analysing in parallel 16 sectors (with a size of ~ 4500 each) at the same time. Each sector was analysed for up to 4 h, with the following commands (see documentation of TNT for details):

```
bbreak:
  cluster 20;
timeout 4:00:00;
sectsch:
```

Table 2
Main groups included in the analysis and their level of representation

	Taxonomic accuracy			
	Number of species	Proportion of described spp. (%)	Molec + morph	Molec. only
Nematoda	882	6	>0.99 (>0.99)	0.99 (0.95)
Annelida	942	11	0.91 (0.92)	0.59 (0.66)
Platyhelminthes	1053	10	0.95 (0.95)	0.93 (0.93)
Mollusca	2578	5	Polyphyletic	Polyphyletic
Arthropoda	16 083	2	1.00 (1.00)	0.99 (0.83)
Amphibia	1210	21	1.00 (1.00)	0.97 (0.97)
Lepidosauria	1384	18	1.00 (1.00)	Paraphyletic
Mammalia	2401	54	1.00 (1.00)	0.99 (0.98)
Testudines	189	63	1.00 (1.00)	0.98 (0.96)
Aves	3198	33	1.00 (1.00)	1.00 (0.99)
Teleostei	3801	16	>0.99 (>0.99)	Paraphyletic
Basidiomycota	2691	12	0.99 (>0.99)	0.98 (0.98)
Ascomycota	4827	12	0.99 (>0.99)	0.99 (0.98)
Microsporidia	150	19	1.00 (1.00)	0.97 (0.98)
Coniferales	422	82	0.99 (0.99)	0.98 (0.98)
Liliopsida	5373	10	0.99 (>0.99)	0.99 (0.86)
Eudicotyledons	13 852	8	>0.99 (0.94)	Paraphyletic
Magnoliids	969	22	0.99 (0.97)	0.98 (0.83)
Filicophyta	1724	21	0.99 (>0.99)	0.98 (0.56)
Bryophyta	885	6	0.96 (0.94)	0.94 (0.92)
Marchantiophyta	688	8	0.98 (0.99)	0.94 (0.94)
Chlorophyta	704	5	0.97 (0.90)	0.80 (0.72)
Oomycetes	288	42	0.99 (0.99)	0.99 (0.99)
Phaeophyceae	150	17	0.98 (0.84)	0.97 (0.97)
Bacillariophyta	383	4	0.98 (0.98)	0.97 (0.78)
Rhodophyta	809	20	>0.99 (>0.99)	0.97 (0.97)
Alveolata	943	8	0.99 (0.99)	0.88 (0.77)

The estimated number of species per group was obtained from the list compiled by S.G. Sullivan (http://www.speciesaccounts.org). Of all species included in the analysis, 94% fall within one of the groups shown in the table. Taxonomic accuracy is the proportion of species effectively placed in the group, relative to the GenBank taxonomy (the first number corresponds to the most parsimonious tree found, the number in parentheses indicates the average for searches starting from different starting points).

```
  xss 15-8+6-2 gocomb 50
  combst 5 fuse 4 slack 20 drift 7;
xmult =
  repl 8 rss xss drift 4 hit 10
  dumpfuse keep; tfuse; best;
```

The tree-fusing at the end (tfuse command) guarantees that the final solution for the sector is no worse than the initial one. The results for the sectors were merged, and the resulting tree was subject to TBR in parallel (using three slave processes per machine, the maximum allowed by the RAM available in each machine, for a total of nine slave processes in the virtual machine). This alternation between sectorial search and TBR was repeated in 5–7 cycles, slightly changing the sectors selected, and the random seeds used for searching new solutions for each of the sectors. In the final cycles (as the trees approached optimality), the virtual machine examined about 740×10^6 rearrangements/s, requiring between 0.5 and 2 h to complete TBR.

The trees resulting from each of the eight independent starting points were then subjected to several rounds of tree-fusing, and the resulting tree was subjected to three cycles of alternating sectorial search and TBR in parallel, but in this case breaking the tree into only seven pieces (sectors of about 10 000 taxa), and running each sector for up to 16 h. Each reduced data set was analysed by means of the following commands:

```
bbreak:
  cluster 20;
sectsch:
  xss 32-25 + 5-1 gocomb 50 combst 5
  fuse 4 slack 20 drift 5;
timeout 8:00:00;
xmult =
  repl 8 rss xss drift 4 hit 10
  dumpfuse keep prvmix;
tfuse; tchoose/;
  sectsch = xss5-3 + 1-1
  [ sectsch: xss10 + 3-1;
  xmult =
    xss rss hit 1 rep 8 nofu keep;
  tfuse; best; ] ;
```

The search commands indicated within square brackets are those to be used for analysis of the (five to three) sectors in which the sectorial search command (sectsch) will further partition each reduced tree of ~ 10 000 taxa.

Evaluation of results

Given the magnitude of the present analysis, our comparison with current taxonomy leaves room for a small proportion of misplaced taxa, specially because the sequences available for many of the taxa correspond to just one (or a few) incomplete genes; a small fraction of apparent errors may also correspond to misidentifications and contaminations in the GenBank sequences (estimated in up to 5–10% for some groups; Vilgalys, 2003). Given a group R (of size S_R) in a reference tree, the accuracy with which a tree A recovers the group can be measured by finding the group G_A of tree A (with size S_A) which minimizes the number of taxa that have to be pruned from the trees to make them display the same bipartition (the group G_A which minimizes the number N_I of taxa inside of G_A but not in R, plus the number N_O of taxa outside R but included in G_A). Thus $(S_A—N_I)/S_R$ will take a value of unity when the reference group is monophyletic on the tree being evaluated, and decrease as the tree displays more different groups. In other words, $(S_A—N_I)/S_R$ indicates the proportion of correctly placed species relative to GenBank taxonomy.

Since the taxon names contained the full GenBank taxonomic information for each species, it was possible (by using appropriate scripts) to fully automate the comparison with the taxonomy contained within the names and the colouring of branches for displaying results. The scripts used have been placed at the script repository of TNT (http://www.zmuc.dk/public/phylogeny/TNT/Scripts), and can be used on any data set for which the taxon names contain taxonomic information in the appropriate format. The script *dohi.run* will take as argument the name of a group, so that:

```
dohi _Mammalia_;
```

will find the group in the tree most similar to "Mammalia", and display it on screen (counting the number of non-members included, and of members excluded). The script *mytaxo.run* will perform the same checking as *dohi.run*, but hierarchically, for every taxon up to N levels deep in the taxonomy contained in the taxon names (with level 1 = Kingdom), for the group specified:

```
mxproc 7; mytaxo 7 Mammalia_;
```

note that the underscore preceding the taxon name must be excluded here; if the taxon name is excluded, it uses the whole matrix; if the level is excluded, it checks the taxonomy to the deepest possible level (genus, in our case). The *mxproc* command is needed because the script is recursive and calls itself nestedly. Since Mammalia is a group of level 6 in GenBank taxonomy (Metazoa, Chordata, Craniata, Vertebrata, Euteleostomi, Mammalia), the example (using level 7) will produce as output (in a file called "taxonomy") one subcategory of Mammalia:

```
Mammalia_: ...
Eutheria_: ...
Metatheria_: ...
Monotremata_: ...
```

with each taxon followed by its statistics. Increase the level (first argument) if you wish to descend further into the taxonomic hierarchy. If several trees have been read into memory, the script will calculate for each taxonomic group the minimum, maximum, and average numbers of non-members included, and members excluded, from the most similar group in each tree, as well as the minimum, maximum, and average number of SPR moves needed to make the group monophyletic in the trees. The script *chkone.run* will report the minimum numbers of taxa that have to be pruned (from the set of trees currently in memory) to make a group (given as first argument) monophyletic in all trees, asking the user whether the list of taxa to prune should be saved (saving it to a file called "goodprunings", in append mode). Finally, the script *colorgroups.run* (for Windows only) will take a list of taxa and automatically produce color diagrams displaying the groups. If you wish to use the same color code for several separate groups, you can separate the groups to be given similar color with a plus and a colon, and the groups to be given different colors with blank spaces; for example:

```
colorgroups       _Araneae_+:_Nematoda_
_Diptera_+:_Porifera_
```

will give spiders and nematodes one color, and sponges and flies another. The figures produced with this script can be exported as metafiles.

Results

For each data set, searches from different starting points produced trees with similar scores, suggesting that (despite the immensity of tree-space for this data set, of about $9 \times 10^{345\,593}$ possible trees) the tree-search algorithms used are truly approaching maximum parsimony. For the molecular data set, the eight independent searches converged to trees 0.180–0.007% longer than the best tree found (average 0.067%), and for the combined data set, 0.029–0.073% (average 0.047%). The number of steps for the best molecule-only trees is

725 629, and for the combined tree 730 435. The trees resulting from the two data sets share many similarities, but the combined data set recovers current taxonomic groups more accurately. This is hardly surprising as the bulk of the taxonomy has been established using morphological characters. It must be noted, however, that only a few morphological characters have been included, and that they do not by any means determine the structure of the tree completely: the consistency index of the morphological characters is 0.46, indicating that, on the optimal tree, more than half the transformations in the morphological characters are homoplastic.

The strict consensus of the eight independent searches for the combined data set, excluding those species of most unstable positions and collapsing branches for which the minimum possible length was zero, is shown in Fig. 1 (the species to prune for making the main groups monophyletic were found with the *chkone.run* script described above). One of the trees (the longest of the eight trees, the only one not showing monophyly of Eudicots) was excluded from the consensus. Eudicots may have been missing from that one tree either because the search had simply failed to find a tree short enough to have the group, or because the group is truly unsupported. In any event, as the consensus shown in Fig. 1 is the combination of only a few trees, it may contain some unsupported groups. On the other hand, since the eight independent searches produced trees with a number of additional steps beyond the most parsimonious tree found, they provide a conservative estimation of the consensus tree, which may well be somewhat more resolved. Therefore the consensus of Fig. 1 (with 71 181 taxa and 13 189 nodes) should be viewed only as a rough estimation of the correct consensus.

Although many established taxonomic groups are not recovered exactly by the trees, they have a very close correspondence (Table 2; Figs 2 and 3). The discussion

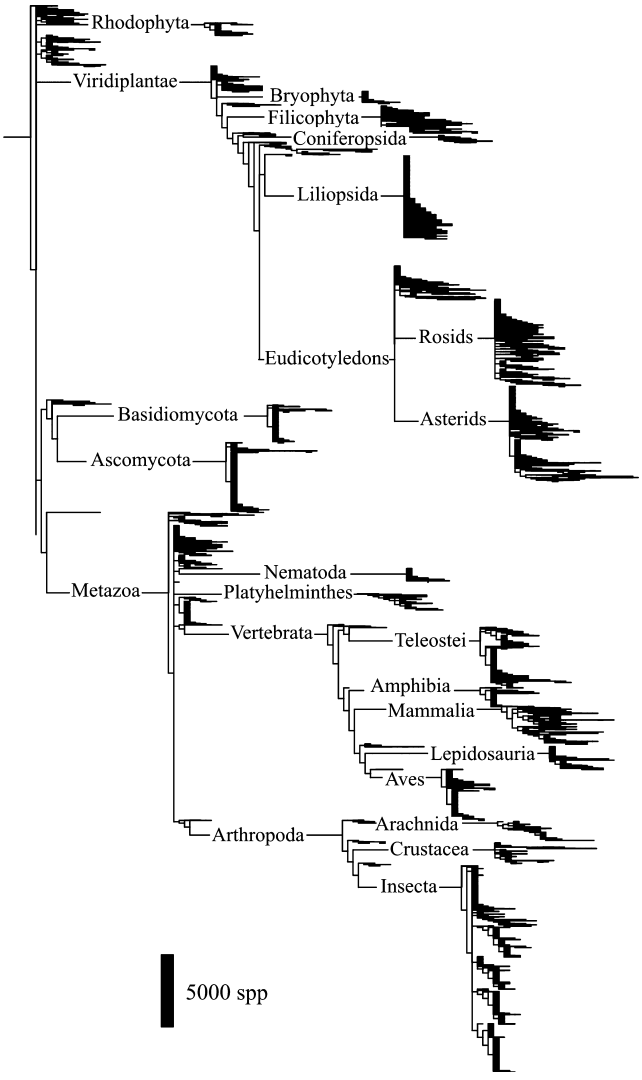

Fig. 1. Pruned strict consensus tree for the combined data set (seven trees, 1879 taxa excluded). The bar shows the span of 5000 species.

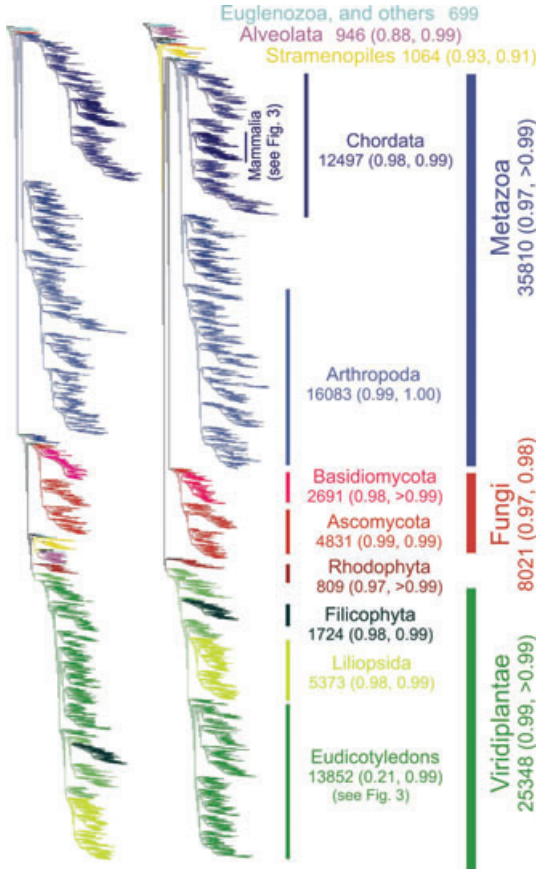

Fig. 2. The full 73 060-taxon trees. The best tree found for the molecule-only data set is on the left; the best tree for the combined data set is on the right. For each group, the number of species included in the analysis is given first, followed by the accuracy of recovery (proportion of correctly placed species relative to GenBank taxonomy) for the molecule-only and the combined data sets, in parentheses.

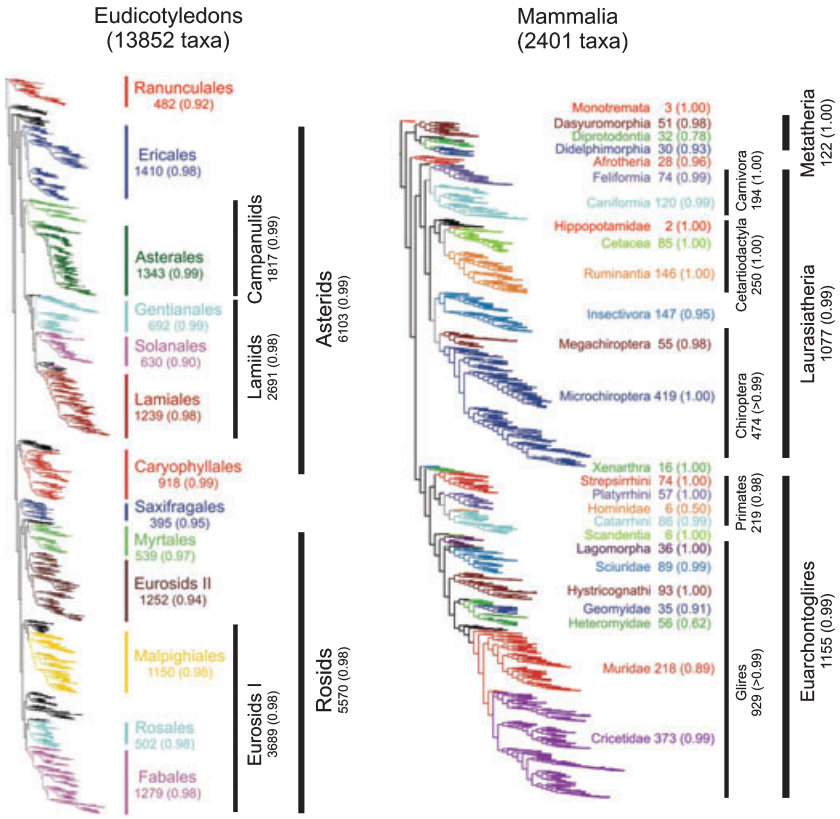

Fig. 3. The sections of the tree for the combined data set corresponding to the Eudicotyledons and the Mammals. For each group, the number of species included in the analysis is given first, followed by the accuracy of recovery.

below uses as a basis the most parsimonious tree found for the combined data set, with comments on the results for the molecule-only data set where appropriate. It must be noted here that the GenBank taxonomy is not authoritative and does not include many recent taxonomic changes. Thus some of the groups not retrieved in our results are not considered as valid groups in current taxonomy, making our evaluation of the accuracy of recovery of current taxonomy an underestimation.

Our analysis literally displays thousands of groupings of potential interest to phylogeneticists, but we concentrate here on only the most significant results. The sections of the trees that agreed most with current taxonomy were in the vascular plants and the mammals. Both of these had the best taxon sampling, with multiple reliable markers. The least accurate parts of the tree were in the invertebrates and protozoans, for which the taxonomic sampling is much less complete, even though it includes many taxa.

Viridiplantae

The tree displays a pattern of relationships within Viridiplantae in general agreement with current ideas on phylogenetic relationships (Fig. 3 shows the Eudicotyledon part of the tree). The main groups within green plants are all present (Chlorophyta, Streptophyta, Embryophyta, Bryophyta, Marchantiophyta, Tracheophyta, Lycopodiophyta, Filicophyta, Spermatophyta, Coniferopsida, Gnetophyta, Cycadophyta, Magnoliophyta, Liliopsida, magnoliids, Eudicotyledons, rosids, and asterids; see Fig. 2), with an average accuracy of recovery of current taxonomy of 0.985. Within vascular plants, 84% of the 62 orders and 65% of the 408 families represented by more than one species are retrieved with an accuracy above 0.850. The accuracy of recovery of current taxonomy is higher in better-sampled groups. The agreement with taxonomy is much lower in Chlorophyta, Bryophyta, and Marchantiophyta, as well as at the generic level. However, half the genera represented by 30 or more species are retrieved with an accuracy above 0.850.

One of the key unresolved issues in green plant phylogeny is the identification of the most basal flowering plants (Frohlich and Chase, 2007). There is now consensus that Monocots are nested within Dicots. Most analyses have placed the group ANA (*Amborella*, Nymphaeales, Austrobaileyales) as a basal grade of angiosperms (Soltis et al., 2000; Hilu et al., 2003). A recent analysis (Goremykin et al., 2003), based on 61

chloroplast genes, resurrected the idea of a Monocot–Dicot early splitting, but that result subsequently has been attributed (Soltis and Soltis, 2004) to a deficient taxon sampling, and has not been upheld by more extensive character and taxon sampling (Jansen et al., 2007; Moore et al., 2007). Our results also reject the basal placement of Monocots, placing *Ceratophyllum* (a rootless aquatic plant) at the base of flowering plants, with the ANA grade at the base of the remaining angiosperms. The basal placement of *Ceratophyllum* in our results agrees with early molecular analyses (Chase et al., 1993), but it should be noted that later studies using additional genes have placed *Ceratophyllum* closer to the Chloranthales, Eudicots, or Monocots. Its position remains uncertain. *Amborella* forms a group with *Nymphaea* and *Hydatella*; although *Hydatella* previously had been considered close to the Poales, recent findings (Saarela, 2007) have also suggested the same basal position as in our trees.

The results for the different runs are ambiguous regarding the interrelationships among Liliopsida, Magnoliids, and Eudicotyledons, although they agree in placing Chloranthales as sister group of Monocots. The relationships among orders within Eudicotyledons are also in close agreement with previous analyses (APG, 2003), with first branchings represented by Ranunculales, Buxales, Proteales, Sabiaceae, and Trochodendrales. Inside the core Eudicots group, the Asterid and Rosid clades are recovered, as well as Euasterids and its division in Lamiids/Campanulids. Within Rosids, Eurosids I and II are recovered, but the placement of Zygophyllales is not well defined.

In the molecule-only results, Viridiplantae is monophyletic, but rooted such that Eudicots are paraphyletic and near the base of green plants (Fig. 3), probably as a result of including sequences only for Embryophyta in the case of chloroplastic *rbcL* and *matK* genes. The few morphological characters included for Viridiplantae (only 41 characters for 25 000 species) are sufficient to correct this rooting problem.

Metazoans

Of the 35 phyla represented by more than one species, the vast majority (including most of their classes) are recovered almost exactly. Placozoa (accuracy 1.0), Porifera (0.96), and Cnidaria (0.99) are basal to Ctenophora (1.0), Myxozoa (1.0), Mesozoa (1.0), and Bilateria (> 0.99). Within Bilateria, internal relationships are similar to recent estimates (Halanych, 2004; Giribet et al., 2007). One of the unexpected results is that Urochordata groups with Entoprocta, Rotifera, Acanthocephala, Platyhelminthes, Nematoda, and Nematomorpha, instead of grouping with other Chordata; alignment of genes for this group was particularly difficult, which may explain its apparently anomalous position. The position of Urochordata aside, Deuterostomata is supported in the shortest trees. Platyhelminthes groups with Nematoda and Nematomorpha, and excludes Acoela and Nemertodermatida (Ruiz-Trillo et al., 1999; Bapteste et al., 2002; Glenner et al., 2004; Giribet et al., 2007). Our study finds a group which is equivalent to Ecdysozoa (Halanych, 2004; Giribet et al., 2007) except that it excludes Nematoda and Nematomorpha. Several recently proposed groups (Platyzoa, Trochozoa, Lophotrochozoa, Spiralia) are not clearly recovered; some may have resulted from poor taxon sampling in previous studies. Arthropoda and all its major groups (including Mandibulata, Myriapoda, and Pancrustacea) are recovered.

Mollusca is the only phylum which, as in other studies, is clearly non-monophyletic in our results. The tree displays molluscs divided in two, with Scaphopoda and Bivalvia as successive sister groups of several other invertebrate phyla, and the rest of the molluscan classes forming a monophyletic group. The most recent analysis (Giribet et al., 2006) displayed Bivalvia and Gastropoda as diphyletic, and Monoplacophora included within Polyplacophora. In our results, Gastropoda, Bivalvia, and Polyplacophora are monophyletic, and Monoplacophora is most closely related to Polyplacophora (instead of nested inside).

The relationships within Chordata are in general agreement with previous analyses. Among major groups, only the basal position of polypteriform fishes relative to a clade composed by Chondrichthyes plus most Dipnoi and Actinopterygii differs from accepted relationships (Nelson, 2006). Many groups of Actinopterygii are recovered exactly or almost exactly (e.g. Chondrostei, Teleostei, Ostariophysi, Euteleostei, Acanthomorpha) and their relationships mostly agree with those proposed in the literature (Nelson, 2006). The low accuracy for Perciformes (0.384) is an example of the problems introduced by the inclusion of non-monophyletic clades in GenBank nomenclature; in our analysis Perciformes contains the Atherinomorpha, Pleuronectiformes, Scorpaeniformes, and Tetraodontiformes, along with other relatively small fish orders, as already proposed in the recent literature (Chen et al., 2003; Nelson, 2006).

Within Tetrapoda, the successive sister groups of Archosauria are Lepidosauria, Testudines, Mammalia, and Amphibia (as currently accepted; Benton, 1990). The high accuracy within Tetrapoda extends to many orders and families; for example, Aves contains a perfectly monophyletic Passeriformes. Within Amphibia, Gymnophiona forms the sister group of Anura and Caudata. Probably the most striking results, however, are those within Mammalia (Fig. 3), which include not only all ordinal groups (except Insectivora, which is not currently considered monophyletic; Asher et al., 2003), but also currently accepted supraordinal groupings

(Geisler, 2001; Murphy et al., 2001; O'Leary and Gatesy, 2007), with a monophyletic Xenarthra, Afrotheria (accuracy 0.93 or higher), a monophyletic Cetartiodactyla contained within an almost perfect Laurasiatheria (0.99), and a monophyletic Euarchontoglires, among others. When all runs are considered, the Hominidae is unsupported, with *Pongo* and Hylobatidae in a trichotomy with a group formed by *Homo*, *Pan* and *Gorilla* (and the latter as sister group of *Homo* and *Pan*). In our trees, *Johnius belangerii* (a percomorph fish) is the sister group of *Homo sapiens*. The *Johnius* sequence (GenBank accession number AY581811) is in fact identical to that of the one species guaranteed to have been present when the sample was taken—man—evidently corresponding to an egregious case of contamination.

Other kingdoms

Among the remaining kingdoms or highest-level groups with 15 or more species, many are recovered with an accuracy above 0.90 (Fungi, Stramenopiles, Alveolata, Rhodophyta, Euglenozoa, Granuloreticulosea, Heterolobosea, Parabasalidea, Haplosporidia, Centroheliozoa, Choanoflagellida, Haptophyceae). Groups that are recovered very ambiguously (or not at all) are Cryptophyta, Lobosea, Acanthamoebiidae, Mycetozoa, and Arcellinida.

The optimal tree displays the group Opisthokonta (Cavalier-Smith, 1987), although some of the runs exclude Eccrinales (12 taxa) and include part of the Arcellinida (18 taxa), making the group ambiguous. As in other previous studies (Yoon et al., 2008), the relationships of eukaryote supergroups are not clearly supported. The group Rhyzaria (Cavalier-Smith, 2002) appears in the optimal trees, but the support is clearly ambiguous, as some of the runs failed to recover it.

Microsporidians are obligatory intracellular parasites, with the smallest eukaryote genome. They are highly modified and have an unusual rRNA (with prokaryotic similarities and many deletions), making any definite placement of the group difficult. Some work has placed them at the base of Eukaryota, but more recent analyses (Baldauf et al., 2000) have proposed that they are related to Fungi. Their position varies significantly across runs—grouping with either Heterolobosea, Parabasalidea, Cryptophyta, Cercozoa, Arcellinida, or Haplosporidia, but never with Fungi.

Our analysis recovers a group which corresponds very closely to Fungi (excluding Microsporidians). The sister group of Fungi is Nucleariidae, in agreement with previous studies (James et al., 2006; Moreira et al., 2007). Five of the six phyla of Fungi (excluding Microsoporidia) are retrieved: Ascomycota, Basidiomycota, Neocallimastigomycota, Blastocladiomycota, Glomeromycota (the latter two with an accuracy of 0.94 or more, the other three with 0.99 or more). The only fungal phylum not obtained here (Chytridiomycota) has already been challenged (James et al., 2006). In our analysis, Chytriomycota is intermingled at the base of Fungi with different basal lineages. Dikarya, one of the most important groups, is retrieved almost exactly in all runs (with 7520 taxa, 7501–7519 of which form a group). Within Basidiomycota, eight of the nine classes with more than one species were recovered with accuracy above 0.85 (average 0.923). The placement of the classes is in agreement with the subphylum divisions, but Agaricomycotina is either the sister clade of the rest of Basidiomycota or a basal grade. Within Ascomycota, the results are worse than in the rest of Fungi, with only four (Saccharomycetes, Sordariomycetes, Lecanoromycetes, and Taphrinomycetes) of the 14 classes recovered with an accuracy of 0.85 or more.

Discussion and implications

No phylogenetic analysis to date has produced an equivalent test for the existence of the main phylogenetic groups. In a small data set where there are only a few representatives of a given group, the probability of the species appearing together is, *a priori*, much larger. In our data set, each representative of a taxonomic group has tens to hundreds of thousands of possible alternative placements. Therefore the fact that a taxonomic group is displayed by our tree, even if imperfectly, is significant to a high degree. For example, the sabre-toothed cat *Smilodon*, one of the fossil taxa included in this analysis, is placed within the felid lineage in both the molecule-only and the combined data sets; given the number of taxa in our analysis (20 cats, 73 040 non-cats), the prior probability of obtaining that placement is about 0.00026. Prior probabilities are much smaller for entire groups, and decrease with the size of the group. To show just three examples: the prior probability of obtaining all three species of Cunninghamellaceae (Fungi) grouped together is about 10^{-10}, but it is 10^{-159} for the 42 species of the genus *Strobus* (Pinaceae), and 10^{-5708} for the 3198 species of Aves. All these groups (and many others) appear in the results simultaneously, for which the joint probability is basically zero. It has often been argued that the congruence between phylogenies obtained independently with morphological characters and molecules is a strong argument for evolution (Zuckerkandl and Pauling, 1965); in this sense, our results provide the strongest test of congruence ever performed.

The fidelity with which the molecular data set recovers hundreds of classic taxonomic groupings is even more surprising in view of the minimal taxonomic overlap between different genes and the enormous proportion of missing entries. This fidelity seems to be a result of the important taxonomic sampling (Soltis

et al., 2004; McMahon and Sanderson, 2006), which produces an intertwined-comb effect. In addition, since the missing entries increase the ambiguity of the data set, they slow down tree-calculation algorithms; should the matrix have contained a smaller proportion of missing entries, it is almost certain that the analyses, in addition to being more reliable, would also have proceeded significantly more quickly. Therefore the present data set constitutes a very stringent test of the parsimony algorithms, in terms both of times needed to complete analyses, and of accuracy. The fact that the alignments used were no doubt grossly suboptimal also makes the observed fidelity even more significant.

Parsimony and likelihood are often considered to be competing methods for phylogenetic inference, and it is often feared that parsimony will show erroneous relationships as the result of long-branch attraction (Felsenstein, 1978). The concordance of our results with previous ideas on phylogeny suggests that these fears are unjustified. Whether because of improved taxon sampling which breaks up long branches (Soltis et al., 2004; Bergsten, 2005), because of multiple genes not evolving under the same homogeneous process (Kolaczkowski and Thornton, 2004; Pickett et al., 2005), or because of the use of characters with more alternative states such as amino acids (Steel and Penny, 2000), the net result is clear: although long-branch attraction may be determining the placement of a few isolated groups, it does not have a significant role in determining the general structure of the tree.

In addition, although programs such as RAxML represent a very significant step forward in model-based phylogenetic inference, it is obvious that the size of data sets that will ever be analysable with some thoroughness by maximum likelihood methods is well below standard parsimony, as demonstrated by Smith et al.'s own discussion:

> Our analysis also demonstrates the limitations of conventional computers for analysing large phylogenies. The matrix manipulation, tree construction, and tree rerooting required at least 8 GB of memory and were conducted on an eight CPU machine. To build even larger matrices, more memory and faster machines will be essential. (Smith et al., 2009)

Although Smith et al. (2009) do not give timings for their runs, it is clear that their eight CPU, 8 GB machine was scarcely capable of dealing with the rbcL data set. In contrast, on a machine such as that described by Smith et al., 100 replicates of jackknife on the complete rbcL data set with TNT will take less than 45 min and 2 GB of memory—but with a full TBR search for each replicate, instead of the more superficial rearrangement algorithms of RAxML. The importance of using more exhaustive search algorithms is plain from the justification offered for the use of a single evolutionary model (GTR) in RAxML:

> The design philosophy of RAxML is based upon the observation that a more thorough topological search has a greater impact on final tree quality than modeling details. Thus, the efficient implementation of rapid search mechanisms is considered to be more important than model details. (Stamatakis, 2006, RAxML manual)

An observation with which we couldn't agree more.

There are programs (e.g. POY; Varón et al., 2008) that can directly analyse unaligned sequences in a parsimony context, providing a better treatment of insertions/deletions (which are problematic for standard parsimony or likelihood). Despite this advantage, however, parsimony methods for unaligned sequences allow exploration of problems with only a few hundred taxa at most. The largest published analysis of unaligned sequences, to our knowledge, is that of Frost et al. (2006) with 532 taxa; this analysis was run in a large computer cluster. This type of method, however desirable, seemed therefore to be beyond reach, given the size of our data set. For the time being, the user is faced with the choice between using either an expanded repertoire of transformations (provided by POY) and a more modest taxon sampling, or a more simplistic approach to sequence analysis (such as TNT's) with a more significant sample for taxa. No doubt, important insights can be gained from both types of analysis.

Prior to the development of the new parsimony algorithms used here, the difficulty of analysing data sets with many taxa had often been considered one of the main obstacles to obtaining comprehensive phylogenies (Moret, 2005; Sanderson, 2007). One of the proposals (Bininda-Emonds et al., 2002) consists of avoiding the computational complexity of large analyses, by breaking them into separate smaller analyses, and combining the resulting trees in a "supertree". The supertree approach has attracted considerable criticism (Goloboff and Pol, 2002; Gatesy et al., 2004); controversy aside, our results clearly show that the intractability of large data sets is no longer a defensible reason to prefer supertrees over the supermatrix approach.

In conclusion, parsimony analysis of entire groups is, computationally, well within current capabilities; a simultaneous phylogenetic analysis of all 58 000 species of vertebrates would be no harder than the present analysis—and separate analyses of all known species of mammals (5400 species) or birds (10 000) would be significantly easier. That is not to say that such analyses are now within reach of the scientific community, for two serious impediments remain. The first serious obstacle is in the complexity of producing acceptable alignments. In our case, none of the individual sequences had more than 20 000 taxa, but it is doubtful that current alignment programs will be effective at handling much larger problems. The second obstacle is that the sequence information required is simply

non-existent, and the morphological information is scanty and fragmentary. Our data set includes basically all the taxa in GenBank that could be combined meaningfully in a single matrix, yet in relative terms the molecular matrix consists of a few observations in a vast sea of missing entries. Filling the millions of missing observations in that matrix, and improving the quality of morphological data available, are the most important tasks to fulfil in working towards reliable and comprehensive phylogenies.

Acknowledgements

The authors thank the Willi Hennig Society for subsidizing the program TNT and making it freely available. Those at the Instituto Lillo thank colleagues (specially N. Giannini, M. Lizarralde, and F. Navarro) for help and discussions. This paper was presented at the 27th International Meeting of the Willi Hennig Society, 28–31 October 2008; input from participants (Lone Aagesen, Jim Carpenter, Gonzalo Giribet, Kevin Nixon, Dennis Stevenson, and Ward Wheeler, among others) was deeply appreciated. Parts of this work were developed with funding from PICT-2007-01314 (to P.A.G.) and PIP-CONICET 6502 (to Mercedes L. de Grosso). The full data set and the trees found (in TNT format), as well as the C scripts used to donwnload GenBank sequences, can be obtained from http://www.zmuc.dk/public/phylogeny/TNT/More/Supp_Data_Set.tgz.

References

APG (Angiosperm Phylogeny Group), 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141, 399–436.

Asher, R., Novacek, M., Geisler, J., 2003. Relationships of endemic African mammals and their fossil relatives based on morphological and molecular evidence. J. Mammal. Evol. 10, 131–194.

Baldauf, S., Roger, A., Wenk-Siefert, I., Doolittle, W., 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290, 972–977.

Bapteste, E., Brinkmann, H., Lee, J.A., Moore, D.V., Sensen, C.W., Gordon, P., Duruflé, L., Gaasterland, T., Lopez, P., Müller, M., Philippe, H., 2002. The analysis of 100 genes supports the grouping of three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*. Proc. Natl Acad. Sci. USA 99, 1414–1419.

Benton, M., 1990. Phylogeny of the major tetrapod groups: morphological data and divergence dates. J. Mol. Evol. 30, 409–422.

Bergsten, J., 2005. A review of long-branch attraction. Cladistics 21, 163–193.

Bininda-Emonds, O., Gittleman, J., Steel, M., 2002. The (super) tree of life: procedures, problems, and prospects. Annu. Rev. Ecol. Syst. 33, 265–289.

Cavalier-Smith, T., 1987. The origin of fungi and pseudofungi. In: Rayner, A.D.M., Brasier, C.M., Moore, D. (Eds.), Evolutionary Biology of the Fungi. Cambridge University Press, Cambridge, pp. 339–353.

Cavalier-Smith, T., 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. Int. J. Syst. Evol. Microbiol. 52, 297–354.

Chase, M., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.G., Qiu, Y.-L., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J.D., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, M., Gaut, B.S., Jansen, R.K., Kim, K.-J., Wimpee, C.F., Smith, J.F., Furnier, G.R., Strauss, S.H., Xiang, Q.-Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguiarte, L.E., Golenberg, E., Learn, G.H., Graham, S.W., Barrett, S.C.H., Dayanandan, S., Albert, V., 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. Ann. Mo. Bot. Gard. 80, 528–580.

Chen, W.-J., Bonillo, C., Lecointre, G., 2003. Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol. Phylogenet. Evol. 26, 262–288.

Dunn, C., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A., Seaver, E., Rouse, G.W., Obst, M., Edgecombe, G.D., Sørensen, M.V., Haddock, S.H.D., Schmidt-Rhaesa, A., Okusu, A., Kristensen, R.M., Wheeler, W.C., Martindale, M.Q., Giribet, G., 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452, 745–749.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 1–19.

Felsenstein, J., 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27, 401–410.

Frohlich, W., Chase, M., 2007. After a dozen years of progress the origin of angiosperms is still a great mystery. Nature 450, 1184–1189.

Frost, D., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., Channing, A., Wilkinson, M., de Sá, R.O., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006. The amphibian tree of life. Bull. Am. Mus. Nat. Hist. 297, 1–370.

Gatesy, J., Baker, R., Hayashi, C., 2004. Inconsistencies in arguments for the supertree approach: supermatrices versus supertrees of Crocodylia. Syst. Biol. 53, 342–355.

Geisler, J., 2001. New morphological evidence for the phylogeny of Artiodactyla, Cetacea, and Mesonychidae. Am. Mus. Novit. 3344, 1–53.

Giribet, G., Okusu, A., Lindgren, A.R., Huff, S.W., Schrödl, M., Nishiguchi, M.K., 2006. Evidence for a clade composed of molluscs with serially repeated structures: Monoplacophorans are related to chitons. Proc. Natl Acad. Sci. USA 103, 7723–7728.

Giribet, G., Dunn, C., Edgecombe, G., Rouse, G., 2007. A modern look at the animal tree of life. Zootaxa 1668, 61–79.

Glenner, H., Hansen, A.J., Sørensen, M.V., Ronquist, F., Huelsenbeck, J.P., Willerslev, E., 2004. Bayesian inference of the metazoan phylogeny: a combined molecular and morphological approach. Curr. Biol. 14, 1644–1649.

Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15, 415–428.

Goloboff, P.A., 2003. Parsimony, likelihood, and simplicity. Cladistics 19, 91–103.

Goloboff, P.A., Pol, D., 2002. Semi-strict supertrees. Cladistics 18, 514–525.

Goloboff, P.A., Pol, D., 2007. On divide-and-conquer strategies for parsimony analysis of large data sets: Rec-I-DCM3 versus TNT. Syst. Biol. 56, 485–495.

Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics 24, 774–786.

Goremykin, V., Hirsch-Ernst, K., Wölfl, S., Hellwig, F., 2003. Analysis of the *Amborella* trichopoda chloroplast genome sequence suggests

that *Amborella* is not a basal angiosperm. Mol. Biol. Evol. 20, 1499–1505.
Halanych, K., 2004. The new view of animal phylogeny. Annu. Rev. Ecol. Evol. Syst. 35, 229–256.
Hilu, K.W., Borsch, T., Müller, K., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M.P., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A.B., Rohwer, J.G., Campbell, C.S., Chatrou, L.W., 2003. Angiosperm phylogeny based on matK sequence information. Am. J. Bot. 90, 1758–1776.
James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-J., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Zheng, W., Wilson, A.W., Schüssler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lücking, R., Büdel, B., Geiser, D.M., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818–822.
Jansen, R., Cai, Z., Raubeson, L.A., Daniell, H., dePamphilis, C.W., Leebens-Mack, J., Müller, K.F., Guisinger-Bellian, M., Haberle, R.C., Hansen, A.K., Chumley, T.W., Lee, S.-B., Peery, R., McNeal, J.R., Kuehl, J.V., Boore, J.L., 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc. Natl Acad. Sci. USA 104, 19369–19374.
Källersjö, M., Farris, J.S., Chase, M.W., Bremer, B., Fay, M.F., Humpries, C.J., Petersen, G., Seberg, O., Bremer, K., 1999. Simultaneous parsimony jackknife analysis of 2538 rbcL DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants. Plant. Syst. Evol. 213, 259–287.
Katoh, T., 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinf. 9, 286–298.
Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33 (2), 511–518.
Kolaczkowski, B., Thornton, J., 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. Nature 431, 980–984.
McMahon, M.M., Sanderson, M.J., 2006. Phylogenetic supermatrix analysis of GenBank sequences from 2228 papilionoid legumes. Syst. Biol. 55, 818–836.
Moore, M., Bell, C., Soltis, P., Soltis, D.E., 2007. Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proc. Natl Acad. Sci. USA 104, 19363–19368.
Moreira, D., von der Heyden, S., Bass, D., López-García, P., Chao, E., Cavalier-Smith, T., 2007. Global eukaryote phylogeny: combined small- and large-subunit ribosomal DNA trees support monophyly of Rhizaria, Retaria and Excavata. Mol. Phylogenet. Evol. 44, 255–266.
Moret, B.M., 2005. Computational challenges from the Tree of Life. Proceedings of the 7th Workshop on Algorithm Engineering and Experiments (ALENEX'05). SIAM Press, Philadelphia, pp. 3–16.
Murphy, W., Eizirk, E., Johnson, W., Zhang, Y., Ryder, O., O'Brien, S., 2001. Molecular phylogenetics and the origin of placental mammals. Nature 409, 614–618.
Nelson, J.S., 2006. Fishes of the World, 4th edn. John Wiley & Sons, Hoboken, NJ, USA.
O'Leary, M., Gatesy, J., 2007. Impact of increased character sampling on the phylogeny of Cetartiodactyla (Mammalia): combined analysis including fossils. Cladistics 23, 1–46.
Pickett, K., Tolman, G., Wheeler, W., Wenzel, J., 2005. Parsimony overcomes statistical inconsistency with the addition of more data from the same gene. Cladistics 21, 438–445.
Ruiz-Trillo, I., Riutort, M., Littlewood, D., Herniou, E., Baguñà, J., 1999. Acoel flatworms: earliest extant bilaterian Metazoans, not members of Platyhelminthes. Science 283, 1919–1923.
Saarela, J., 2007. Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. Nature 446, 312–315.
Sanderson, M.J., 2007. Construction and annotation of large phylogenetic trees. Aust. Syst. Bot. 20, 287–301.
Smith, S.A., Beaulieu, J.M., Donoghue, M.J., 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. BMC Evol. Biol. 2009, 9:37.
Soltis, D.E., Soltis, P.S., 2004. *Amborella* not a basal angiosperm? Not so fast. Am. J. Bot. 91, 997–1001.
Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoop, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C., Farris, J.S., 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. Bot. J. Linn. Soc. 133, 381–461.
Soltis, D.E., Albert, V.A., Savolainen, V., Hilu, K., Qiu, Y.-L., Chase, M.W., Farris, J.S., Stefanović, S., Rice, D.W., Palmer, J.D., Soltis, P.S., 2004. Genome-scale data, angiosperm relationships, and 'ending incongruence': a cautionary tale in phylogenetics. Trends Plant Sci. 9, 477–483.
Stamatakis, A., 2006. RAxML-VI HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Steel, M., Penny, D., 2000. Parsimony, likelihood, and the role of models in molecular phylogenetics. Mol. Biol. Evol. 17, 839–850.
Varón, A., Vinh, L., Bomash, I., Wheeler, W., 2008. POY 4.0.2885. Am. Mus. Nat. Hist. http://research.amnh.org/scicomp/projects/poy.php
Vilgalys, R., 2003. Taxonomic misidentification in public DNA databases. New Phytol. 160, 4–5.
Yoon, H.-S., Grant, Y.I., Tekle, M., Wu, M., Chao, B.C., Cole, J.C., Logsdon, J.M., Patterson, D.J., Bhattacharya, D., Katz, L.A., 2008. A broadly sampled multigene trees of eukaryotes. BMC Evol. Biol. 8, 14.
Zuckerkandl, E., Pauling, L., 1965. Evolutionary divergence and convergence in proteins. in: Bryson, V., Vogel, H.J. (Eds.), Evolving Genes and Proteins. Academic Press, New York, pp. 97–166.

Appendix 1 Character list

List of morphological characters. All characters were taken from the literature. The characters for which no citation is given are classic characters (e.g. mammary glands). The authors cited for each character are not necessarily those who first described it; the citation simply indicates our literature source.

For space reasons, only a very brief description is used for each character. Those characters that were taken from Tree of Life web pages are indicated by the author of the page and the acronym "tol".

Char. 0: Plastid associated with a nucleomorph (Patterson, 1999; Adl et al., 2005). **Char. 1:** C2 Chlorophyll (Adl et al., 2005). **Char. 2:** Flagellar groove with hairs and ridges in two flagelles (Patterson, 1999). **Char. 3:** Intranuclear spindle (Patterson, 1999). **Char. 4:** Inorganic skeleton of 10/20 spicules made of strontium sulphate (Patterson, 1999; Adl et al., 2005). **Char. 5:** Capsule with fusules separating endoplasm and ectoplasm (Patterson, 1999; Adl et al., 2005). **Char. 6:** Kernstab, persistent spindle through interphase (Patterson, 1999). **Char. 7:** Haptonema (Patterson, 1999). **Char. 8:** C1 Chlorophyll (Adl et al., 2005). **Char. 9:** Paranuclear body

(Patterson, 1999). **Char. 10:** Fenestrated organic lorica (Patterson, 1999). **Char. 11:** Inorganic scales and spines (Patterson, 1999). **Char. 12:** Cross-shaped nucleus in vegetative cells mitosis (Patterson, 1999). **Char. 13:** Tripartite tubular hairs (Patterson, 1999). **Char. 14:** Two row mastigonema with anterior direction (Patterson, 1999). **Char. 15:** Branched cristae (Patterson, 1999). **Char. 16:** Intranuclear spindle during mitosis (Lipscomb, 1989; Patterson, 1999; Adl et al., 2005). **Char. 17:** Cruciform mitotic profile (Patterson, 1999; Adl et al., 2005). **Char. 18:** Cortical alveoli forming a continuous layer under plasma membrane (Patterson, 1999; Adl et al., 2005). **Char. 19:** Chromosomes remaining condensed during interphase, dinokaryon (Patterson, 1999; Adl et al., 2005). **Char. 20:** Apical complex consisting of one or more polar rings, rhoptries, micronemes, conoid, and subpelicular microtubules (Adl et al., 2005). **Char. 21:** Polygenomic macronucleous and diploid micronucleus (Patterson, 1999; Adl et al., 2005). **Char. 22:** Cytostome, or 'cell mouth' (Lipscomb et al., 1998). **Char. 23:** Haptonema (Adl et al., 2005). **Char. 24:** Nuclear membrane and endoplasmatic reticulum surrounding chloroplast (Lipscomb, 1989; Williams, 1991; Patterson, 1999; Adl et al., 2005). **Char. 25:** Cell wall of tightly integrated silicified elements, comprised of two valves, at each end of cell, and several girdle bands (Adl et al., 2005). **Char. 26:** Cell wall with alginate (Adl et al., 2005). **Char. 27:** Multilamellate microtubule organizing centre (Patterson, 1999). **Char. 28:** Heteromorphic paraxonemal rods, dorsal flagellum with tubular rod, ventral with lattice structure (Patterson, 1999; Adl et al., 2005). **Char. 29:** Strips of protein material on cell surface (Patterson, 1999; Adl et al., 2005). **Char. 30:** Kinetoplastid, aggregates of DNA, in mitochondrion (Patterson, 1999). **Char. 31:** Parabasal apparatus of dictyosomes anchored to a striate root (Patterson, 1999; Adl et al., 2005). **Char. 32:** Fucoxanthin (Lipscomb, 1989; Daugbjerg and Andersen, 1997; Adl et al., 2005). **Char. 33:** Cyanelle, a plastid with peptidoglycan wall (Patterson, 1999; Adl et al., 2005). **Char. 34:** Cytoplasmic carbohydrate reserve floridean starch (Adl et al., 2005). **Char. 35:** Pit connections (Patterson, 1999; Adl et al., 2005). **Char. 36:** B Chlorophyll (Lipscomb, 1989; Patterson, 1999; Adl et al., 2005). **Char. 37:** Plastids with thylakoids arranged in granae (stacks) (Patterson, 1999; Adl et al., 2005). **Char. 38:** Structure of the ciliary transition (Cavalier-Smith, 1981; Patterson, 1999; Adl et al., 2005). **Char. 39:** Store starch in chloroplasts (Cavalier-Smith, 1981; Patterson, 1999; Adl et al., 2005). **Char. 40:** Plastids with chlorophyll *a* and *b* (Cavalier-Smith, 1981; Patterson, 1999; Adl et al., 2005). **Char. 41:** Vegetative growth from apical cell at end of branches and main axis (Bremer et al., 1987; Adl et al., 2005). **Char. 42:** Meiospores with exine (Doyle, 1998). **Char. 43:** 2n sporophyte (Doyle, 1998). **Char. 44:** Stoma (Bremer et al., 1987; Doyle, 1998). **Char. 45:** True tracheids (Doyle, 1998). **Char. 46:** Independent and branched sporophyte (Bremer et al., 1987; Doyle, 1998). **Char. 47:** Lignine (Bremer et al., 1987). **Char. 48:** Transverse dehiscence (Doyle, 1998). **Char. 49:** Planated frond (Doyle, 1998). **Char. 50:** Leptosporangium (Doyle, 1998). **Char. 51:** Seeds (Bremer et al., 1987; Doyle, 1998). **Char. 52:** Secondary growth (Doyle, 1998). **Char. 53:** Axiliary branching (Doyle, 1998). **Char. 54:** Penetrating pollen tube (Loconte and Stevenson, 1991). **Char. 55:** Embryo maturing before seed releasing (Nixon et al., 1994). **Char. 56:** Girdling leaf traces (Nixon et al., 1994). **Char. 57:** Compound female strobili (Doyle, 1998). **Char. 58:** Cortical secretory canals (Nixon et al., 1994). **Char. 59:** Leptomate aperture of pollen (Nixon et al., 1994). **Char. 60:** Coned scale seed wings (Nixon et al., 1994). **Char. 61:** Lignine in syringal groups (Loconte and Stevenson, 1991; Nixon et al., 1994). **Char. 62:** Syndetocheilic stomates (Nixon et al., 1994). **Char. 63:** Tectum (Nixon et al., 1994). **Char. 64:** Xylema in perforated tubes (Loconte and Stevenson, 1991). **Char. 65:** Cellular early embryogeny (Nixon et al., 1994). **Char. 66:** Tubular micropyle (Loconte and Stevenson, 1991). **Char. 67:** Carpel (Doyle, 1998). **Char. 68:** Sieve-tube element companion cells (Loconte and Stevenson, 1991; Nixon et al., 1994). **Char. 69:** Thick nucellar cuticle (Loconte and Stevenson, 1991; Nixon et al., 1994). **Char. 70:** Swollen nodes (Loconte and Stevenson, 1991). **Char. 71:** Tangentially stratified secondary phloem (Loconte and Stevenson, 1991). **Char. 72:** Filament appendage (Loconte and Stevenson, 1991). **Char. 73:** Spinose exine pollen (Loconte and Stevenson, 1991). **Char. 74:** Homorhizic root system (Loconte and Stevenson, 1991). **Char. 75:** Tricolpate pollen (Doyle, 1998). **Char. 76:** One cotyledon (Doyle, 1998). **Char. 77:** Pre-pollinization final mitotic division of microgametophyte (Loconte and Stevenson, 1991). **Char. 78:** Single posterior cilium without mastigonemes at least in one life-cycle stage or secondarily lost (Adl et al., 2005). **Char. 79:** Collar complexes (Glenner et al., 2004). **Char. 80:** Cell wall with chitin and B-glucan, at least in spores (Tehler, 1988; Patterson, 1999). **Char. 81:** AAA route of lysine (Tehler, 1988). **Char. 82:** Dikaryotic hyphae (Tehler, 1988; Hibbett et al., 2007). **Char. 83:** Ascocarp (Tehler, 1988). **Char. 84:** Woronin bodies (Tehler, 1988). **Char. 85:** Croziers (Tehler, 1988). **Char. 86:** Multilamellar cell wall (Tehler, 1988). **Char. 87:** Basidiocarp and basidiohymenio (Tehler, 1988). **Char. 88:** Tetrapolar sexual compatibility (Tehler, 1988). **Char. 89:** Homeobox-containing genes. **Char. 90:** Sexual reproduction with an egg cell that is fertilized by a smaller, often monociliated, sperm cell (Glenner et al., 2004; Adl et al., 2005). **Char. 91:** A collagen-based extracellular matrix, between two dissimilar epithelia (Adl et al., 2005). **Char. 92:** Outer epithelia with septate or tight junctions (Glenner et al., 2004; Adl et al., 2005). **Char. 93:** Septate junctions (Glenner et al., 2004). **Char. 94:** Nerve cells with chemical synapses (Glenner et al., 2004; Adl et al., 2005). **Char. 95:** Sperm with (single compact) acrosome (Glenner et al., 2004; Adl et al., 2005). **Char. 96:** Basal lamina (Tyler, 2003; Glenner et al., 2004; Adl et al., 2005). **Char. 97:** Striated ciliary rootlets (Glenner et al., 2004). **Char. 98:** Organized gonads (Glenner et al., 2004). **Char. 99:** Definable germ layers (ectoderm, endoderm) (Glenner et al., 2004). **Char. 100:** Mesoderm (Glenner et al., 2004). **Char. 101:** Bilaterian symmetry (Glenner et al., 2004). **Char. 102:** Synapses with acetylcholine (Glenner et al., 2004). **Char. 103:** Gap junctions (Lipscomb et al., 1998; Glenner et al., 2004; Adl et al., 2005). **Char. 104:** Spiral quartet cleavage (Glenner et al., 2004). **Char. 105:** Cnidae (Marques and Collins, 2004). **Char. 106:** Nematocysts (Lipscomb et al., 1998; Marques and Collins, 2004). **Char. 107:** Planula in life cycle (Marques and Collins, 2004). **Char. 108:** Medusoid phase (Marques and Collins, 2004). **Char. 109:** Polypoid phase (Marques and Collins, 2004). **Char. 110:** Location of gonads in epidermis (Marques and Collins, 2004). **Char. 111:** Rhopalia/rhopalioids (Marques and Collins, 2004). **Char. 112:** Type of apical medusa formation "strobilation" (Marques and Collins, 2004). **Char. 113:** Shape of horizontal cross-section quadrate (Marques and Collins, 2004). **Char. 114:** Birhopaloids (Marques and Collins, 2004). **Char. 115:** Rhopalonemes (Marques and Collins, 2004). **Char. 116:** Velarium (Marques and Collins, 2004). **Char. 117:** Nerve rings (Marques and Collins, 2004). **Char. 118:** Strobilation type monodisk (Marques and Collins, 2004). **Char. 119:** Stomodeum, corresponding to the pharynx (Marques and Collins, 2004). **Char. 120:** Adhesive colloblasts (Backeljau et al., 1993). **Char. 121:** Cydippid larval stage. **Char. 122:** Brain ganglion (Backeljau et al., 1993). **Char. 123:** Posterior adhesive organ (Zamparo et al., 2001). **Char. 124:** Mehlis gland (Zamparo et al., 2001). **Char. 125:** Gut as a blind sack without anus (Glenner et al., 2004). **Char. 126:** Posteroventral adhesive organ (Zamparo et al., 2001). **Char. 127:** Posterior adhesive organ with hooks (cercomer; Zamparo et al., 2001). **Char. 128:** Miracidium larva (Zamparo et al., 2001). **Char. 129:** Oncomiracidium larva (Zamparo et al., 2001). **Char. 130:** Tegument covered with microvilli (Zamparo et al., 2001). **Char. 131:** Adults with proglotiids (Zamparo et al., 2001). **Char. 132:** Body wall musculature as U-shape muscles (Hooge and Tyler, 2006). **Char. 133:** Body wall musculature as crossover muscles (Hooge and Tyler, 2006). **Char. 134:** Collagen basal layer under cuticle (Zrzavý, 2002; Nielsen, 2003). **Char. 135:** 6 + 6 + 4 sensilla (Nematoda). **Char. 136:** Rings of scalids on introvert (Kristensen, 2002). **Char. 137:** Large body cavity with amoebocytes and erythrocytes (Priapulida). **Char. 138:** Non-inversible mouth cone with cuticular ridges and spines (Kristensen, 2002). **Char. 139:** Scalids with muscles (Kristensen, 2002). **Char. 140:** Myoepithelial sucking pharynx (Loricifera and Gastrotricha). **Char. 141:** Toes with adhesive glands

(Nielsen, 1995). **Char. 142:** Syncytial germovitellarium (Glenner et al., 2004). **Char. 143:** Trunk with intracellular skeletal lamina (Nielsen, 1995). **Char. 144:** Apical proboscis with intracellular hooks (Nielsen, 1995). **Char. 145:** Radial pharynx (Nielsen, 1995). **Char. 146:** Peripharyngeal brain with three regions (Nielsen, 1995). **Char. 147:** Ectoderm with multilayered cuticle covering all cilia (Rieger and Rieger, 1977; Hochberg and Litvaitis, 2000). **Char. 148:** Unique, cuticle-covered duo-gland adhesive organs (Tyler and Rieger, 1980). **Char. 149:** Myoepithelial sucking pharynx (Loricifera and Gastrotricha). **Char. 150:** Cuticle-covered locomotory and sensory cilia (Rieger and Rieger, 1977; Hochberg and Litvaitis, 2000; Kieneke et al., 2008). **Char. 151:** Visceral helicoidal muscles (Kieneke et al., 2008). **Char. 152:** Cuticle moulted (Nielsen, 2003; Glenner et al., 2004). **Char. 153:** Alpha chitinous cuticle (Nielsen, 2003; Glenner et al., 2004). **Char. 154:** Collagenous cuticle (Glenner et al., 2004). **Char. 155:** Gliointestitial system (Zrzavý et al., 2001). **Char. 156:** Rhynchocoela (Backeljau et al., 1993). **Char. 157:** Body with successively added segments developed from a teloblastic growth zone (Glenner et al., 2004). **Char. 158:** Distinct prostomium (Rouse and Fauchald, 1997). **Char. 159:** Nuchal organs (Rouse and Fauchald, 1997). **Char. 160:** Ventral cirri (Rouse and Fauchald, 1997; Rousset et al., 2007). **Char. 161:** Aciculae (Rouse and Fauchald, 1997; Rousset et al., 2007). **Char. 162:** Dorsal branchiae in a few anterior chaetigers (Rouse and Fauchald, 1997). **Char. 163:** Clitella (Rousset et al., 2007). **Char. 164:** Chambered organ formed as extension of right somatocoel (Littlewood et al., 1997). **Char. 165:** Lophophore (Zrzavý et al., 1998;). **Char. 166:** Ventrolateral appendages (Wheeler et al., 1993). **Char. 167:** Dorsal heart with segmental ostia and pericardial sinus (Giribet et al., 2005). **Char. 168:** Limbs (mostly articulated) with intrinsic muscles (Giribet et al., 2005). **Char. 169:** Telescopic legs (Giribet et al., 2005). **Char. 170:** Sclerotization of cuticle into hard, articulated exoskeleton (Edgecombe et al., 2000; Giribet et al., 2005). **Char. 171:** Tendon cells with tonofilaments penetrating epidermis (Edgecombe et al., 2000). **Char. 172:** Resilin protein (Edgecombe et al., 2000; Giribet et al., 2005). **Char. 173:** Cephalon composed of one pair of pre-oral appendages and three or more pairs of post-oral appendages (Edgecombe et al., 2000; Giribet et al., 2005). **Char. 174:** Cephalic ecdysial glands (Wheeler et al., 1993). **Char. 175:** Fully segmental sclerites (Wheeler et al., 1993). **Char. 176:** Two pairs of maxillae on postacronal somites 4 and 5 (Brusca and Brusca, 1990; Wheeler et al., 1993). **Char. 177:** Appendages of 3rd postacronal somite are mandibles. **Char. 178:** Tripartite brain (Wheeler et al., 1993). **Char. 179:** Mandibulate structure of ommatidia (Wheeler et al., 1993). **Char. 180:** Whole-limb feeding structures (Wheeler et al., 1993). **Char. 181:** Tracheal system (Wheeler et al., 1993). **Char. 182:** Repugnatorial glands (Brusca and Brusca, 1990). **Char. 183:** Organ of Tömösvary (Brusca and Brusca, 1990). **Char. 184:** Gnathochilarium (Brusca and Brusca, 1990). **Char. 185:** Poisonous maxillipeds (Brusca and Brusca, 1990). **Char. 186:** Nauplius larve (Wheeler et al., 1993; Edgecombe et al., 2000). **Char. 187:** Antennules (Edgecombe et al., 2000). **Char. 188:** Fleshy labrum (Edgecombe et al., 2000). **Char. 189:** Sclerotized sternum formed by antennal to maxillulary sternites (Edgecombe et al., 2000). **Char. 190:** Biramous limbs (Wheeler et al., 1993; Edgecombe et al., 2000). **Char. 191:** Two pairs of maxillae (Wheeler et al., 1993). **Char. 192:** Mouthparts specialized for gripping and holding prey and include maxiullules that puncture the prey cuticle and injected a toxic substance (Lange and Schram, 1999). **Char. 193:** Mouthparts in posteriorly directed atrium (Schram, 1986). **Char. 194:** Uropods (Schram, 1986). **Char. 195:** Caridoid thoracic musculature (Schram, 1986). **Char. 196:** Triramus antennules (Edgecombe et al., 2000). **Char. 197:** Unsegmented phyllopodus limbs used in filter feeding (Lange and Schram, 1999). **Char. 198:** Second and fifth thoracopods with subchelate claws, shaped like jackknives(Lange and Schram, 1999). **Char. 199:** Oostergite marsupium (Wheeler et al., 1993). **Char. 200:** Zoea larva (Schram, 1986). **Char. 201:** Thoracic coxal plates (Schram, 1986). **Char. 202:** Respiratory pleopods (Schram, 1986). **Char. 203:** Photophores, special structures to emit light (Lange and Schram, 1999). **Char. 204:** Reduced abdomen held underneath the thorax (Lange and Schram, 1999). **Char. 205:** Soft abdomnen hiden in shell (Lange and Schram, 1999). **Char. 206:** Multisegmental uropodal rami (Schram, 1986). **Char. 207:** Second thoracopod as chelicers (Schram, 1986). **Char. 208:** Maxillipedal siphons (Schram, 1986). **Char. 209:** Thoracopodal expods respiratory (Schram, 1986). **Char. 210:** Foliaceous (crustacean) limbs (Schram, 1986). **Char. 211:** Egg brooding (cephalocaridan) appendages in females (Schram, 1986). **Char. 212:** Caudal rami as paddles (Schram, 1986). **Char. 213:** Short bulbous hearth (Schram, 1986). **Char. 214:** Naupliar carapace (Schram, 1986). **Char. 215:** Cypris larva (Schram, 1986). **Char. 216:** Six naupliar stages (Schram, 1986). **Char. 217:** Male antennules geniculate (Schram, 1986). **Char. 218:** Maxillary plate (Edgecombe et al., 2000). **Char. 219:** Maxillae divided into cardo, stipes, lacinia, and galea (Edgecombe et al., 2000). **Char. 220:** Thorax with three limb-bearing segments (Edgecombe et al., 2000). **Char. 221:** Second maxillae form labrum (Edgecombe et al., 2000). **Char. 222:** Entognathous mouthparts (Wheeler et al., 2001). **Char. 223:** Furcula (Wheeler et al., 2001). **Char. 224:** Collophore (Wheeler et al., 2001). **Char. 225:** Tentorial posterior apodemes (Edgecombe et al., 2000). **Char. 226:** Dicondylic mandibular articulation (Wheeler et al., 2001). **Char. 227:** Five-segmented tarsi (Wheeler et al., 2001). **Char. 228:** Two pairs of thoracic wings (Wheeler et al., 2001). **Char. 229:** Wing flexion derived from a muscle insertion on the third axillary sclerite (Wheeler et al., 2001). **Char. 230:** Prehensile larval labium (Wheeler et al., 2001). **Char. 231:** Pronotal repellant gland (Wheeler et al., 2001). **Char. 232:** Tarsal silk gland (Wheeler et al., 2001). **Char. 233:** Female accessory gland (Ross, 2001; Szumik et al., 2008). **Char. 234:** Female 8st + 1Vfs (Ross, 2001; Szumik et al., 2008). **Char. 235:** Asymmetric 10º tergite, 9º sternite, paraprocts and epiprocts (males) (Ross, 2001; Szumik et al., 2008). **Char. 236:** Asymmetric cerci (Szumik et al., 2008). **Char. 237:** Labium ensheathing maxillary and mandibular stylets (Wheeler et al., 2001). **Char. 238:** Complete holometabolous metamorphosis (Whiting et al., 1997; Wheeler et al., 2001). **Char. 239:** Elytra fully sclerotized (Whiting et al., 1997; Beutel and Haas, 2000; Wheeler et al., 2001). **Char. 240:** Hind-wings folded under elytra (Beutel and Haas, 2000). **Char. 241:** MP1 + 2 bent posteriorly (Beutel and Haas, 2000; Haas, 2006). **Char. 242:** RA34 cut twice by triangular fold (Beutel and Haas, 2000). **Char. 243:** Marginal joint: an elbow-like articulation of the costal margin (Haas, 2006). **Char. 244:** Abdominal sternite II divided by metacoxae (Beutel and Haas, 2000). **Char. 245:** Left mandible with articulate tooth (Beutel and Haas, 2000). **Char. 246:** Halteres formed by hind wings (Whiting et al., 1997; Wheeler et al., 2001). **Char. 247:** Venom production by female accessory gland (Wheeler et al., 2001). **Char. 248:** Volsella (Wheeler et al., 2001). **Char. 249:** Hamuli (Wheeler et al., 2001). **Char. 250:** Clypeus not inflected (Vilhelmsen, 1996, 2001; Schulmeister et al., 2002). **Char. 251:** Petiole (classic character). **Char. 252:** Stinging ovipositor (classic character). **Char. 253:** Vestiture on wings of long setae or scales (Wheeler et al., 2001). **Char. 254:** Larval anal prolegs (Wheeler et al., 2001). **Char. 255:** Abdominal tergum X bilobed (Wheeler et al., 2001). **Char. 256:** Non-functional mandibles (Krenn and Kristensen, 2000; Beutel and Pohl, 2006). **Char. 257:** Galeae forming a proboscis (Krenn and Kristensen, 2000; Beutel and Pohl, 2006). **Char. 258:** Normal type scales on wings (Beutel and Pohl, 2006). **Char. 259:** Adecticous, obtect pupa (Kristensen and Skalski, 1998; Kristensen, 2003; Beutel and Pohl, 2006). **Char. 260:** Frenulum retinaculum wing coupling (Beutel and Pohl, 2006). **Char. 261:** Reduction of Rs system of hind wing (Beutel and Pohl, 2006). **Char. 262:** Intrinsic galeal muscles with both origins and insertions markedly distal from basal region; at least some of them oblique (Krenn and Kristensen, 2004). **Char. 263:** Series of pronouncedly oblique lateral intrinsic muscles (Krenn and Kristensen, 2004). **Char. 264:** Two series of oblique lateral and median intrinsic galeal muscles (Krenn and Kristensen, 2004). **Char. 265:** Haltere location (fore or hind wings) (Wheeler et al., 2001). **Char. 266:** Labial palpi forming labellum (Wheeler et al., 2001). **Char. 267:** Antennae (classic character). **Char. 268:** Larvae with puparium (classic character). **Char. 269:** Antennae with arista (classic character). **Char. 270:** Calypters (classic character).

Char. 271: Male styli IX as claspers (Wheeler et al., 2001). **Char. 272:** Tufted larval tracheal gills (Wheeler et al., 2001). **Char. 273:** Pronotum overlapping propleuron (Kristensen, 1981; Wheeler et al., 2001). **Char. 274:** Saltatorial 'orthopteran' hindlegs (Kristensen, 1981; Wheeler et al., 2001). **Char. 275:** Anterior teeth of proventriculus forming ring of strongly sclerotized teeth (Wheeler et al., 2001). **Char. 276:** Ootheca (Wheeler et al., 2001). **Char. 277:** Clypeofrontal 'blattarian' sulcus (Wheeler et al., 2001). **Char. 278:** Unique complement of sclerotization in cibarium (Kristensen, 1981; Wheeler et al., 2001). **Char. 279:** Wings straplike, fringed (Wheeler et al., 2001). **Char. 280:** Fusion of third valvulae with intrinsic musculature (Kristensen, 1981; Wheeler et al., 2001). **Char. 281:** Prothoracic ctenidium (Wheeler et al., 2001). **Char. 282:** Sclerites firmly connected, without visible membrane (Beutel and Haas, 2000). **Char. 283:** Mesothoracic elytra locking device (Beutel and Haas, 2000). **Char. 284:** Ventrites of meta- and mesothorax connected between and within mesocoxal cavities (Beutel and Haas, 2000). **Char. 285:** Prothoraci trocantin fused with propleura (Beutel and Haas, 2000). **Char. 286:** Propleura concealed and reduced in adults (Beutel and Haas, 2000). **Char. 287:** Cryptonephric Malpighian tubules (Beutel and Haas, 2000). **Char. 288:** Mandibular 'curculionid' pharyngeal process (Marvaldi et al., 2002). **Char. 289:** Lateral lobes 'adephagan' on mentum (Beutel and Haas, 2000). **Char. 290:** Separation of mesothoracic anapisterna (Schulmeister et al., 2002). **Char. 291:** Metanoto-metapleural muscle (Schulmeister et al., 2002). **Char. 292:** ''Fusion'' of the two muscle parts of male genitalia (Schulmeister et al., 2002). **Char. 293:** Distal epipharynx wall sclerotized (Schulmeister et al., 2002). **Char. 294:** Anterior mesofurcal arms fused (Schulmeister et al., 2002). **Char. 295:** Subdivision of mesocoxa by distinct transvese grooves (Schulmeister et al., 2002). **Char. 296:** Accommodation of the mesocoxa in well developed metespisternal depressions (Schulmeister et al., 2002). **Char. 297:** Articulation between first and second abdominal segments with a pair of tooth-like condyli (Ronquist et al., 1999). **Char. 298:** Abdominal tergum 8 of females fully internal and desclerotized (Schulmeister et al., 2002). **Char. 299:** Second abdominal segment forming a node-like petiole (Ronquist et al., 1999). **Char. 300:** Tergum and mesopostnotum abutting strongly enlarged (Ronquist et al., 1999). **Char. 301:** Highly modified mouthparts for filter feeding in larvae (Yeates and Wiegmann, 1999). **Char. 302:** Posterior portion of larva head elongated posteriorly into prothorax (Yeates and Wiegmann, 1999). **Char. 303:** Separate epandrium and hypandrium in males (Yeates and Wiegmann, 1999). **Char. 304:** Larval maxilla reduced to an elongate membraneous lobe (Yeates and Wiegmann, 1999). **Char. 305:** Pupa enclosed in a puparium forming by hardened larva cuticle (Yeates and Wiegmann, 1999). **Char. 306:** Larva with cephalopharyngeal skeleton (Yeates and Wiegmann, 1999). **Char. 307:** Hypogynium circumverted 360° (Yeates and Wiegmann, 1999). **Char. 308:** Adults emerge from puparium by inflation of ptilinum (Yeates and Wiegmann, 1999). **Char. 309:** First abdominal segment with an adventitious ''dipteran'' suture (Yeates and Wiegmann, 1999). **Char. 310:** Dorsolaterally placed cleft or seam in antennal pedicel (Yeates and Wiegmann, 1999). **Char. 311:** Prosternal teeth (Yeates and Wiegmann, 1999). **Char. 312:** Mesophragma with dorsal plates or processes (Wahlberg et al., 2005). **Char. 313:** Parepisternal suture running straight from dorsal end to base of spinasternum (Wahlberg et al., 2005). **Char. 314:** Secondary ''papilionid'' sclerite posterior to metascutellum (Wahlberg et al., 2005). **Char. 315:** Antennal segments with paired lateral grooves (Wahlberg et al., 2005). **Char. 316:** Three raised ventral carinae on antennal flagellum (Wahlberg et al., 2005). **Char. 317:** Chelicerae and pedipals. **Char. 318:** Four pairs of legs. **Char. 319:** Ganglia of post-oral appendages fused into single nerve mass (Edgecombe et al., 2000; Giribet et al., 2005). **Char. 320:** Abdominal appendices reduced, lost or modified. **Char. 321:** Slit sensilla (Wheeler et al., 1993; Wheeler and Hayashi, 1998; Giribet et al., 2005; Shultz, 2007). **Char. 322:** Stomotheca (Shultz, 2007). **Char. 323:** Epistomal lumen spanned by a transverse muscle (Shultz, 2007). **Char. 324:** A pair of large epistomal arms projecting rearward into prosoma and attaching to endosternite (Shultz, 2007). **Char. 325:** Rostrosoma (Shultz, 2007). **Char. 326:** Appendages V and VI with elongate femur-like patellae (Shultz, 2007). **Char. 327:** Cheliceral silk glands (Shultz, 2007). **Char. 328:** Cheliceral serrula interior and exterior (Shultz, 2007). **Char. 329:** A pair of repugnatorial glands in the carapace (Wheeler and Hayashi, 1998; Giribet et al., 2005). **Char. 330:** Penis (Opiliones) (Shultz, 2007). **Char. 331:** Chelicera pivoting on dorsal protuberance of epistome (Shultz, 2007). **Char. 332:** Intercheliceral median organ (Shultz, 2007). **Char. 333:** Pectines (Shultz, 2007). **Char. 334:** Respiratory lamellae on opisthosomal somite 2 (Shultz, 2007). **Char. 335:** Respiratory lamellae on opisthosomal somite 3 (Shultz, 2007). **Char. 336:** Larva 6 legs, adult 8 legs (Wheeler and Hayashi, 1998; Shultz, 2007). **Char. 337:** Raptorial pedipalps (Wheeler and Hayashi, 1998; Shultz, 2007). **Char. 338:** Cheliceral flagellum in male (Shultz, 2007). **Char. 339:** Cheliceral venom gland (Wheeler and Hayashi, 1998; Shultz, 2007). **Char. 340:** Opisthomial silk glands (Wheeler and Hayashi, 1998; Shultz, 2007). **Char. 341:** Palate plate (Shultz, 2007). **Char. 342:** Patella–tibia joint with posterior compression zone (Shultz, 2007). **Char. 343:** Depressor muscle (or homologue) of trochanter femur joint (Shultz, 2007). **Char. 344:** Posterior abdominal somites reduced (= opistothelae) (classic character; Platnick, 1977). **Char. 345:** Abdominal segments fused (classic; Platnick, 1977). **Char. 346:** Diaxial chelicerae (classic character). **Char. 347:** Anterior median spinnerets converted into cribellum (or colulum). **Char. 348:** Tibiae-metatarsi with proprioreceptor bristle/unsclerotized patch (Platnick and Goloboff, 1985). **Char. 349:** Modified eyes (classic character). **Char. 350:** Radular apparatus (Wheeler et al., 1993; Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 351:** Osphradia (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 352:** Mantle covering dorsal surface (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 353:** Calcified outer molluscan shell (Lindgren et al., 2004). **Char. 354:** Gill with filaments of leaflets (ctenidia) (Lindgren et al., 2004). **Char. 355:** Cerebral (pretrochal) eyes (Lindgren et al., 2004). **Char. 356:** Burrowing, foot with anterior enlargement (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 357:** Mantle lobes (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 358:** Pallial lines (Giribet and Wheeler, 2002). **Char. 359:** Posterior pedal gland (juvenile stage) (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 360:** Labial palps (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 361:** Lateral body compression (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 362:** Mantle cavity occupied by gills lateral and posterior to foot (Giribet and Wheeler, 2002). **Char. 363:** Captacula, retractile feeding tentacles (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 364:** Proventriculum (larval hinge apparatus) with differentiated dentition (Giribet and Wheeler, 2002). **Char. 365:** Gasteropod's chelazae connecting the eggs (Ponder and Lindberg, 1997). **Char. 366:** Position of anus near mouth opening at ventral side (Lindgren et al., 2004). **Char. 367:** True pedal ganglia (Lindgren et al., 2004). **Char. 368:** Specific head retractor (Lindgren et al., 2004). **Char. 369:** Hydrostatic muscular system (Lindgren et al., 2004). **Char. 370:** Cephalic tentacles (Lindgren et al., 2004). **Char. 371:** Torsion (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 372:** Tubular protoconch (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 373:** Operculum, in larval stage (Giribet and Wheeler, 2002; Lindgren et al., 2004). **Char. 374:** Cartilaginous ''cranium'' (Lindgren et al., 2004). **Char. 375:** Closed ''circulatory system'' (Lindgren et al., 2004). **Char. 376:** Inner shell sac (Lindgren et al., 2004). **Char. 377:** One pair of cephalopod's fins (Lindgren et al., 2004). **Char. 378:** Enterocoely. **Char. 379:** Mesoderm derived from archenteron (Glenner et al., 2004). **Char. 380:** Coelom tripartite (or derived therefrom). **Char. 381:** Haemal system (Zrzavý, 2002; Glenner et al., 2004). **Char. 382:** Calcified endoskeleton (Backeljau et al., 1993). **Char. 383:** External ciliary grooves for suspension feeding (Littlewood et al., 1997). **Char. 384:** Pentaradial (secondary) symmetry (Littlewood et al., 1997). **Char. 385:** Five open ambulacral grooves (Littlewood et al., 1997). **Char. 386:** Mouth and anus on oral surface (Littlewood et al., 1997). **Char. 387:** Attachment to substratum by aboral face (Littlewood et al., 1997). **Char. 388:** Central body disk sharply marked

off from the arms (Pawson, 2007). **Char. 389:** Odontophore (Pawson, 2007). **Char. 390:** U-shaped pharyngeal gill slits with collagenous skeleton (Zrzavý, 2002; Glenner et al., 2004). **Char. 391:** Epithelia binding iodine and secreting iodothyrosine (Glenner et al., 2004). **Char. 392:** Preoral gut diverticulum (Hemichordata). **Char. 393:** Glomerulus as unique excretory structure (Hemichordata). **Char. 394:** Dorsal nerve concentration/brain behind apical organ/apical pole (Glenner et al., 2004). **Char. 395:** Dorsal longitudinal nerve cord (Glenner et al., 2004). **Char. 396:** Endostyle or thymus (Chordata). **Char. 397:** Postanal tail (Chordata). **Char. 398:** Notochord (Zrzavý, 2002). **Char. 399:** Notochord extended to tip of snout (Lundberg, tol). **Char. 400:** segmentally organized gonads (Lundberg, tol). **Char. 401:** hood-like atrium (Lundberg, tol). **Char. 402:** Body with segmented longitudinal musculature developed from rows of mesodermal pockets from archenteron (Glenner et al., 2004). **Char. 403:** Head skeleton (Lundberg, tol). **Char. 404:** Nephrons (Lundberg, tol). **Char. 405:** Neural crest (Donoghue et al., 2000). **Char. 406:** Adenohypophysis and neurohypophysis (Donoghue et al., 2000). **Char. 407:** Optic tectum (Donoghue et al., 2000). **Char. 408:** Oesophagocutaneous duct (Janvier, tol). **Char. 409:** A series of slime glands (Janvier, tol). **Char. 410:** Mauthner fibres in central nervous system (Donoghue et al., 2000). **Char. 411:** Paired olfactory organ (Donoghue et al., 2000). **Char. 412:** Segmented adenohypophysis (Donoghue et al., 2000). **Char. 413:** Vertebrae (Janvier, tol; Donoghue et al., 2000). **Char. 414:** Extrinsic eye muscles (Janvier, tol). **Char. 415:** Close atrium and ventricle of heart (Janvier, tol; Donoghue et al., 2000). **Char. 416:** Closed pericardium (Donoghue et al., 2000). **Char. 417:** True lymphocytes (Donoghue et al., 2000). **Char. 418:** Nervous regulation of heart (Janvier, tol; Donoghue et al., 2000). **Char. 419:** Spleen (Donoghue et al., 2000). **Char. 420:** Collecting kidney tubules (Donoghue et al., 2000). **Char. 421:** Typhlosole (Janvier, tol). **Char. 422:** Two or three semicircular canals (Janvier, tol). **Char. 423:** True neuromasts (Janvier, tol; Donoghue et al., 2000). **Char. 424:** Sucker mouth with annular cartilage (Janvier, tol). **Char. 425:** Nasohypophyseal opening (Janvier, tol). **Char. 426:** Calcified dermal skeleton (Donoghue et al., 2000). **Char. 427:** Cerebellum (Donoghue et al., 2000). **Char. 428:** Jaws and teeth (Janvier, tol). **Char. 429:** Pelvic girdle and fins/limbs (Janvier, tol). **Char. 430:** Three semicircular canals (Janvier, tol; Donoghue et al., 2000). **Char. 431:** Paired nasal sacs, independent from hypophyseal tube (Janvier, tol). **Char. 432:** Myelinated nerve fibres (Janvier, tol). **Char. 433:** Sperms passing through urinary ducts (Janvier, tol). **Char. 434:** Superior oblique muscle of eye attached anteriorly to eyeball (Janvier, tol). **Char. 435:** Braincase including nasal capsules (Janvier, tol). **Char. 436:** Jaw muscle external to mandibular arch (Janvier, tol). **Char. 437:** Prismatic endoskeletal calcification (Grogan and Lund, 2004). **Char. 438:** Placoid scales (Nelson, 2006). **Char. 439:** Pelvic fins modified as claspers in males (Grogan and Lund, 2004). **Char. 440:** Gill cover over the four gill openings (Nelson, 2006). **Char. 441:** Clasping organ in head (Nelson, 2006). **Char. 442:** Three otholites in ear (Nelson, 2006). **Char. 443:** Laterosensory canal in dentary (Gardiner et al., 2005). **Char. 444:** Two series of pelvic radials (Gardiner et al., 2005). **Char. 445:** Acrodin cap on teeth (Gardiner and Schaeffer, 1989; Gayet et al., 2002). **Char. 446:** Equal number of rays and supporting bones in anal and dorsal fins (Nelson, 2006). **Char. 447:** Premaxilla with internal process lining the anterior part of nasal pit (Nelson, 2006). **Char. 448:** Diural caudal skeleton (de Pinna, 1996). **Char. 449:** Ural neural arches modified as uroneurals (de Pinna, 1996). **Char. 450:** Division of hypurals into dorsal and ventral groups (de Pinna, 1996). **Char. 451:** First two hypurals supported by a single centrum (de Pinna, 1996). **Char. 452:** Urohyal formed by a medial tendon bone (de Pinna, 1996). **Char. 453:** Foramen between hypohyals for passage of hyoidean artery (de Pinna, 1996). **Char. 454:** Ossified basihyal (de Pinna, 1996). **Char. 455:** Posterior myodome extending into basioccipital (de Pinna, 1996). **Char. 456:** Primary bite between parasphenoid and tongue (Greenwood et al., 1966). **Char. 457:** Paired tendon bones on second hypobranchial (Greenwood et al., 1966). **Char. 458:** 18 principal caudal-fin rays (Patterson and Rosen, 1977). **Char. 459:** A complete neural spine on the first preural centrum (Patterson and Rosen, 1977; Hilton, 2003). **Char. 460:** Intestine passing left to stomach (Patterson and Rosen, 1977; Li and Wilson, 1996). **Char. 461:** Fenestra between hyomandibular and metapterygoid for passage of levator arcus palatini (Forey et al., 1996). **Char. 462:** Gill arches free from neurocranium (Forey et al., 1996). **Char. 463:** Symplectic fused with quadrate (Forey et al., 1996). **Char. 464:** Scales not imbricated (Forey et al., 1996). **Char. 465:** Pleurostyle (Lecointre and Nelson, 1996; di Dario, 2004). **Char. 466:** Fusion of hypural two and ural centrum one (Lecointre and Nelson, 1996). **Char. 467:** Fusion of extrascapular and parietal (Lecointre and Nelson, 1996; di Dario, 2004). **Char. 468:** Silvery peritoneum over gas bladder (di Dario, 2004). **Char. 469:** Otophysic connection with otic bullae invading otic capsule (Nelson, 2006). **Char. 470:** Recessus lateralis (Grande, 1985; Nelson, 2006). **Char. 471:** Gasbladder with anterior and posterior chambers (Fink and Fink, 1981, 1996; Nelson, 2006). **Char. 472:** Peritoneal tunic of anterior chamber of gasbladder attached to anterior two pleural ribs (Fink and Fink, 1981, 1996). **Char. 473:** Kinethmoid (Fink and Fink, 1981, 1996). **Char. 474:** Dorsomedial process of palatine contacting mesethmoid (Fink and Fink, 1981, 1996). **Char. 475:** Teeth on fifth ceratobranchial ankylosed to bone (Fink and Fink, 1981, 1996). **Char. 476:** Scaphium on Weberian apparatus (Fink and Fink, 1981, 1996). **Char. 477:** Tripus on Weberian apparatus (Fink and Fink, 1981, 1996). **Char. 478:** Os suspensorium in Weberian apparatus (Fink and Fink, 1981, 1996). **Char. 479:** Mesethmoid articulating anterior to vomer (Fink and Fink, 1981, 1996). **Char. 480:** Haemal spine of PU2 fused with its centrum, and parhypural and haemal spine 1 fused with compound terminal centrum (Fink and Fink, 1981, 1996). **Char. 481:** Auditory foramen on prootic (Fink and Fink, 1981, 1996). **Char. 482:** Deep mediodorsal opening to post-temporal fossa (Fink and Fink, 1981, 1996; di Dario, 2004). **Char. 483:** Lagenar capsules (Fink and Fink, 1981, 1996). **Char. 484:** Replacement dentary and some premaxillary teeth developed in crypts (Fink and Fink, 1981, 1996). **Char. 485:** Baudelot's ligament robust and bifurcated distally (Fink and Fink, 1981, 1996). **Char. 486:** Endopterygoid reduced, not contacting quadrate, metapterygoid or hyomandibula (Fink and Fink, 1981, 1996). **Char. 487:** Third and fourth neural arches fused together and to the complex centrum (Fink and Fink, 1981, 1996). **Char. 488:** Centra 2–4 fused into "complex centrum" (Fink and Fink, 1981, 1996). **Char. 489:** Supraneurals develop in "pattern 2" and first supraneural becoming clearly differentiated from the second (Johnson and Patterson, 1996). **Char. 490:** Crumenal organ (Begle, 1992; Johnson and Patterson, 1996). **Char. 491:** Retractor dorsalis muscle (Rosen, 1973; Nelson, 2006). **Char. 492:** Lobular testis (Parenti and Grier, 2004). **Char. 493:** Elongation of third pharyngobranchial and uncinate process of third epibranchial (Rosen, 1973; Baldwin & Johnson, 1996). **Char. 494:** Well developed median dorsal keel on the mesethmoid (Stiassny, 1996). **Char. 495:** Adipose fin supports (either of hyaline cartilage or chondroid tissue) penetrating the supracarinalis posterior muscle mass (Stiassny, 1996). **Char. 496:** Median chondrified rostral cartilage strongly bound to premaxillary ascending process via well developed rostro-premaxillary ligament (Stiassny, 1986; Johnson and Patterson, 1996). **Char. 497:** Maxilla protrusible together with premaxilla (Olney et al., 1993). **Char. 498:** Larvae with lateral placement of the anus (Endo, 2002). **Char. 499:** X and Y bones in caudal skeleton (Endo, 2002). **Char. 500:** Pince-nez-shaped sulcus and central collicular in otholit (Endo, 2002). **Char. 501:** Spinous dorsal fin primitively with six spines, the anterior-most three of which are cephalic in position, the first modified as a luring apparatus (Nelson, 2006). **Char. 502:** Epiotics separated from parietals and meeting on midline posterior to supraoccipital (Nelson, 2006). **Char. 503:** Restricted lobular testis (Parenti and Grier, 2004; Parenti, 2005). **Char. 504:** Egg demersal, with several chorionic filaments and oil globes that coalesce at vegetal pole (Parenti, 2005). **Char. 505:** Pelvic-rib ligament (Dyer, 1998). **Char. 506:** Caudal skeleton with lower caudal lobe with more principal rays than the upper (Parenti, 2005). **Char. 507:** Caudal fin skeleton symmetrical (Costa, 1998). **Char. 508:**

Dorsal, anal, and pectoral fin rays unbranched (Tyler et al., 2003). **Char. 509:** Three and one-half gills (seven hemibranchs) (Tyler et al., 2003). **Char. 510:** Suborbital stay (Nelson, 2006). **Char. 511:** Bilaterally asymmetrical adults, with one eye migrating during growth (Nelson, 2006). **Char. 512:** Cranium free from pectoral girdle (Laurin, tol). **Char. 513:** Four muscular limbs with discrete digits (Laurin, tol). **Char. 514:** A sacral rib connecting axial skeleton to pelvic girdle (Laurin, tol). **Char. 515:** A layer of dead, horny cells that reduces evaporative water loss (Laurin, tol). **Char. 516:** parathyroid gland (Laurin, tol). **Char. 517:** Harderian gland located anterior to eye (Laurin, tol). **Char. 518:** Pedicellate teeth, crown separated from root by a fibrous tissue (Trueb and Cloutier, 1991; Montero and Autino, 2004; Frost et al., 2006). **Char. 519:** Green rods in the eye (Montero and Autino, 2004). **Char. 520:** Fat bodies associated with gonads (Trueb and Cloutier, 1991; Montero and Autino, 2004; Frost et al., 2006). **Char. 521:** Two types of skin gland (mucous & granular) (Montero and Autino, 2004). **Char. 522:** Papilla amphibiorum present in ear (Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 523:** Paired sensory tentacles on snout (Nussbaum and Wilkinson, 1989; Trueb and Cloutier, 1991; Montero and Autino, 2004; Frost et al., 2006). **Char. 524:** An eversible phallodeum in males formed by a portion of cloacal wall (Nussbaum and Wilkinson, 1989; Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 525:** Papilla neglecta (Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 526:** Carotid labyrinth (Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 527:** Choanal tube opening into archenteron during development (Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 528:** Pronephros modified for sperm transport (Trueb and Cloutier, 1991; Frost et al., 2006). **Char. 529:** Tuberculum interglenoideum (Trueb and Cloutier, 1991; Larson and Dimmick, 1993; Larson et al., 2003; Frost et al., 2006). **Char. 530:** Nine or fewer vertebrae (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 531:** Atlas with a single centrum (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 532:** First spinal nerve exits from spinal nerve canal via intervertebral foramen (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 533:** Fusion of caudal vertebral segments into a urostyle (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 534:** Fusion of radius and ulna, and tibia and fibula (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 535:** Fusion of hyobranchial elements into a hyoid plate (Trueb and Cloutier, 1991; Ford and Cannatella, 1993; Frost et al., 2006). **Char. 536:** Two pairs of sacral vertebrae. **Char. 537:** Amniotic egg (Laurin & Gauthier, tol). **Char. 538:** Astragalus bone (Laurin & Gauthier, tol). **Char. 539:** Paired spinal accessory (11th) and hypoglossal (12th) cranial nerves incorporated into skull (Amniota). **Char. 540:** Carapace formed by costal, neural, and peripheric bones (Ruckes, 1929; Meylan, tol). **Char. 541:** Maxilla, premaxilla, and dentary without teeth but covered by a horny triturating surface (Meylan, tol). **Char. 542:** Quadrate concave posteriorly and exposed laterally on cheek (Meylan, tol). **Char. 543:** Upper and a lower lateral temporal fenestra (Laurin & Gauthier, tol). **Char. 544:** Low concentration of urea in blood plasma (Laurin & Gauthier, tol). **Char. 545:** Suborbital fenestra between palatine, ectopterygoid, and maxilla (Laurin & Gauthier, tol). **Char. 546:** Huxley's foramen in extracolumella (Laurin & Gauthier, tol). **Char. 547:** Transverse cloacal slit (Gauthier et al., 1988). **Char. 548:** Forked tongue (Gauthier et al., 1988). **Char. 549:** Skin shed all at once (Gauthier et al., 1988). **Char. 550:** Femoral and preanal glands (Gauthier et al., 1988). **Char. 551:** Hemipenes (Gauthier et al., 1988). **Char. 552:** Lacrimal duct associated with Jacobson's organ (Gauthier et al., 1988). **Char. 553:** Antorbital fenestra (Benton and Clark, 1988). **Char. 554:** Mandibular fenestra (Montero and Autino, 2004). **Char. 555:** A fourth trochanter on femur (Benton and Clark, 1988). **Char. 556:** Four-chambered heart (Benton and Clark, 1988). **Char. 557:** Feathers (Mindel & Brown, tol). **Char. 558:** Bill (Mindel & Brown, tol). **Char. 559:** Fused digits, carpals and metacarpals (Mindel & Brown, tol). **Char. 560:** Aerial sacs (Montero and Autino, 2004). **Char. 561:** Mesethmoid reaching rostrally markedly beyond naso-frontal hinge (Mayr and Clarke, 2003). **Char. 562:** Two strong grooves on ventral surface of mandibular symphysis (Mayr and Clarke, 2003). **Char. 563:** Dorsal surface of mandibular symphysis essentially flat (Mayr and Clarke, 2003). **Char. 564:** Pterygoid and palatines not fused (Mayr and Clarke, 2003). **Char. 565:** Tubae auditivae paired and close to/adjacent on cranial midline or single anterior opening (Mayr and Clarke, 2003). **Char. 566:** Foramen ilioischiadicum cadually closed (Mayr and Clarke, 2003). **Char. 567:** Facet of basipterygoid for articulation with pterygoid large and ovoid (Mayr and Clarke, 2003). **Char. 568:** Ostia canalis carotici et ophtalmici externi situated in a well marked depression (Mayr and Clarke, 2003). **Char. 569:** Double, and open, incisurae laterales on the sternum (Dyke et al., 2003). **Char. 570:** Incisura capitis of proximal humerus enclosed from crus dorsale fossa by a distinct ridge (Dyke et al., 2003). **Char. 571:** Trochlea metatarsalia III of tarsometatarsus distinctly asymmetric (Dyke et al., 2003). **Char. 572:** "Passerine" tensor propatagialis brevis (Raikow, 1982). **Char. 573:** Bundled spermatozoa with coiled head and large acrosome (Raikow, 1982). **Char. 574:** Type VII deep plantar tendons (Raikow, 1982). **Char. 575:** Mammary glands. **Char. 576:** Postparietals fused (Benton, 1990). **Char. 577:** Lower temporal fenestra only (Benton, 1990). **Char. 578:** Differentiation of cheek teeth in premolars and molars (Benton, 1990). **Char. 579:** Anterior lamina fused to the ventral ramus of alisphenoid, and cranial process of squamosal expanded (Benton, 1990). **Char. 580:** Nipples. **Char. 581:** Vertical tympanic membrane (Benton, 1990). **Char. 582:** Tribosphenic molar teeth (Benton, 1990). **Char. 583:** Modified trophoblast and inner cell mass (Benton, 1990). **Char. 584:** Chorioallantoic placenta (Benton, 1990). **Char. 585:** Seminal vesicle (Benton, 1990). **Char. 586:** Prolonged intrauterine gestation (Benton, 1990). **Char. 587:** Mullerian ducts fused to a median vagina (Benton, 1990). **Char. 588:** Corpus callosus connecting cerebral hemispheres (Benton, 1990). **Char. 589:** Four upper molars (Horovitz and Sánchez-Villagra, 2003). **Char. 590:** "Marsupial" pattern of dental replacement (Horovitz and Sánchez-Villagra, 2003). **Char. 591:** Incisor enamel restricted to labial surface (Meng and Wyss, 2001). **Char. 592:** Paracone and metacone transverse (Meng and Wyss, 2001). **Char. 593:** Lower diastema significant (Meng and Wyss, 2001). **Char. 594:** Prehensile hands (Martin, 1990). **Char. 595:** Modified forelimbs, which support a wing membrane (patagium) (Simmons & Conway, tol). **Char. 596:** Deciduous dentition not resembling adult dentition (Simmons & Conway, tol). **Char. 597:** Posterior laminae present on ribs (Simmons & Conway, tol). **Char. 598:** Echolocation system (Simmons & Conway, tol). **Char. 599:** Double-trochleated astragalus (Luckett and Hong, 1998). **Char. 600:** Stomach with four compartments: rumen, reticulum, omasum and abomasum. **Char. 601:** Paddle-like forelimbs with hyperphalangy and a non-rotational elbow joint (Milinkovitch & Lambert, tol). **Char. 602:** External nares on top of skull (Milinkovitch & Lambert, tol). **Char. 603:** Isolation of earbones related to development of underwater hearing and echolocation abilities (Milinkovitch & Lambert, tol).

References (Appendix only)

Adl, S.M., Simpson, A.G.B., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W., Taylor, M.F.J.R., 2005. The new higher level classification of Eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol. 52, 399–451.

Backeljau, H., Winnepenninckx, B., De Bruyn, L., 1993. Cladistic analysis of Metazoan relationships: a reappraisal. Cladistics 9, 167–181.

Baldwin, C.C., Johnson, G.D. 1996. Interrelationships of Aulopiformes. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D., (Eds.), Interrelationships of Fishes. Academic Press, San Diego, pp. 355–404.

Begle, D.P., 1992. Monophyly and relationships of the argentinoid fishes. Copeia 1992, 350–366.

Benton, M.J., 1990. Phylogeny of the major tetrapod groups: morphological data and divergence dates. J. Mol. Evol. 30, 409–424.

Benton, M.J., Clark, J.M., 1988. Archosaur phylogeny and the relationships of the Crocodylia. In: Benton, M.J. (Ed.), The Phylogeny and Classification of the Tetrapods, Vol. 1. Amphibians, Reptiles, Birds. Clarendon Press, Oxford, UK, pp. 295–338.

Beutel, R.G., Haas, F., 2000. Phylogenetic relationships of the suborders of Coleoptera (Insecta). Cladistics 16, 103–141.

Beutel, R.G., Pohl, H., 2006. Endopterygote systematics – where do we stand and what is the goal (Hexapoda, Arthropoda)? Syst. Entomol. 31, 202–219.

Bremer, K., Humphries, C.J., Mishler, B.D., Churchill, S.P., 1987. On cladistic relationships in green plants. Taxon 36, 339–349.

Brusca, R.C., Brusca, G.J., 1990. Invertebrates. Sinauer Associates, Sunderland, MA, USA.

Cavalier-Smith, T., 1981. Eukaryotic kingdoms, seven or nine? BioSystems 14, 461–481.

Costa, W.J.E.M., 1998. Phylogeny and classification of the Cyprinodontiformes (Euteleostei: Atherinomorpha): a reappraisal. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. EDIPUCRS, Porto Alegre, Brazil, pp. 537–560.

di Dario, F., 2004. Homology between the recessus lateralis and cephalic sensory canals, with the proposition of additional synapomorphies for the Clupeiformes and the Clupeoidei. Zool. J. Linn. Soc. 141, 257–270.

Daugbjerg, N., Andersen, R.A., 1997. A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded rbcl sequence data. J. Phycol. 33, 1031–1041.

Donoghue, P.C.J., Forey, P.L., Aldridge, R.J., 2000. Conodont affinities and chordate phylogeny. Biol. Rev. 75, 191–251.

Doyle, J.A., 1998. Phylogeny of vascular plants. Annu. Rev. Ecol. Syst. 29, 567–599.

Dyer, B.S., 1998. Phylogenetic systematics and historical biogeography of the Neotropical siverside family Atherinopsidae (Teleostei: Atheriniformes). In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. EDIPUCRS, Porto Alegre, Brazil, pp. 519–536.

Dyke, G.J., Gulas, B.E., Crowe, T.M., 2003. Suprageneric relationships of galliform birds (Aves, Galliformes): a cladistic analysis of morphological characters. Zool. J. Linn. Soc. 137, 227–244.

Edgecombe, G.D., Wilson, G.D.F., Colgan, D.J., Gray, M.R., Cassis, G., 2000. Arthropod Cladistics: Combined analysis of Histone H3 and U2 snRNA sequences and morphology. Cladistics 16, 155–203.

Endo, H., 2002. Phylogeny of the order Gadiformes (Teleostei, Paracanthopterygii). Mem. Grad. School Fish. Sci. Hokkaido Univ. 49, 75–149.

Fink, S.V., Fink, W.L., 1981. Interrelationships of the ostariophysan fishes (Teleostei). Zool. J. Linn. Soc. 72, 297–353.

Fink, S.V., Fink, W.L., 1996. Interrelationships of ostariophysan fishes (Teleostei). In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 209–249.

Ford, L.S., Cannatella, D.C., 1993. The major clades of frogs. Herp. Monogr. 7, 94–117.

Forey, P.L., Littlewood, D.T.J., Ritchie, P., Meyer, A., 1996. Interrelationships of elopomorph fishes. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 175–191.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., Channing, A., Wilkinson, M., de Sá, R.O., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006. The amphibian tree of life. Bull. Amer. Mus. Nat. Hist. 297, 1–370.

Gardiner, B.G., Schaeffer, B., 1989. Interrelationships of lower actinopterygian fishes. Zool. J. Linn. Soc. 97, 135–187.

Gardiner, B.G., Schaeffer, B., Masserie, J.A., 2005. A review of the lower actinopterygian phylogeny. Zool. J. Linn. Soc. 144, 511–525.

Gauthier, J., Estes, R., de Queiroz, K., 1988. A phylogenetic analysis of Lepidosauromorpha. In: Estes, R., Pregill, G. (Eds.), Phylogenetic Relationships of the Lizard Families. Stanford University Press, Palo Alto, CA, USA, pp. 15–98.

Gayet, M., Meunier, F.J., Werner, C., 2002. Diversification in Polypteriformes and special comparison with the Lepisosteiformes. Palaeontology 45, 361–376.

Giribet, G., Wheeler, W.C., 2002. On bivalve phylogeny:a high-level analysis of the Bivalvia (Mollusca) based on combined morphology and DNA sequence data. Invertebr. Biol. 121, 271–324.

Giribet, G., Richter, S., Edgecombe, G.D., Wheeler, W.C., 2005. The position of crustaceans within Arthropoda – evidence from nine molecular loci and morphology. In: Koenemann, S., Jenner, R. (Eds.), Crustacea and Arthropod Relationships: Crustac. Issues16, 307–352.

Glenner, H., Hansen, A.J., Sørensen, M.V., Ronquist, F., Huelsenbeck, J.P., Willerslev, E., 2004. Bayesian inference of the metazoan phylogeny: a combined molecular and morphological approach. Curr. Biol. 14, 1644–1649.

Grande, L., 1985. Recent and fossil clupeomorph fishes with materials for revision of the subgroups of clupeoids. Bull. Amer. Mus. Nat. Hist. 181, 231–372.

Greenwood, P.H., Rosen, D.E., Weitzman, S.H., Myers, G.S., 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Amer. Mus. Nat. Hist. 131, 339–456.

Grogan, E.D., Lund, R., 2004. The origin and relationships of early Chondrichthyes. In: Carrier, J., Musick, J.A., Heithaus, M. (Eds.), The Biology of Sharks and their Relatives. CRC Press, Boca Raton, FL, USA, pp. 3–31.

Haas, F., 2006. Relationships in Pterygota. Arthropod Syst Phylogeny 64, 149–158.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., Lumbsch, H.T., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schüßler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., Zhang, N., 2007. A higher-level phylogenetic classification of the Fungi. Mycol. Res. 111, 509–547.

Hilton, E.J., 2003. Comparative osteology and phylogenetic systematics of fossil and living bony-tongue fishes (Actinopterygii, Teleostei, Osteoglossomorpha). Zool. J. Linn. Soc. 137, 1–100.

Hochberg, R., Litvaitis, M.K., 2000. Phylogeny of Gastrotricha: a morphology-based framework of Gastrotrich relationships. Biol. Bull. 198, 299–305.

Hooge, M.D., Tyler, S., 2006. Concordance of molecular and morphological data: the example of the Acoela. Integr. Comp. Biol. 46, 118–124.
Horovitz, I., Sánchez-Villagra, M.R., 2003. A morphological analysis of marsupial mammal higher-level phylogenetic relationships. Cladistics 19, 181–212.
Johnson, G.D., Patterson, C., 1996. Relationships of lower euteleostean fishes. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 251–332.
Kieneke, A., Riemann, O., Ahlrichs, W.H., 2008. Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. Zool. Scr. 37, 429–460.
Krenn, H.W., Kristensen, N.P., 2000. Early evolution of the proboscis of Lepidoptera (Insecta): external morphology of the galea in basal glossatan moth lineages, with remarks on the origin of the pilifers. Zool. Anz. 239, 179–196.
Krenn, H.W., Kristensen, N.P., 2004. Evolution of the proboscis musculature in Lepidotera. Eur. J. Entomol. 101, 565–575.
Kristensen, N.P., 1981. Phylogeny of Insect Orders. Annu. Rev. Entomol. 26, 135–157.
Kristensen, R.M., 2002. An introduction to Loricifera, Cycliophora, and Micrognathozoa. Integr. Comp. Biol. 42, 641–651.
Kristensen, N.P., 2003. Resolving the basal phylogeny of Lepidoptera: morphological evidence. Entomol. Abh. 61, 167–169.
Kristensen, N.P., Skalski, A.W., 1998. Palaeontology and phylogeny. In: Kristensen, N.P. (Ed.), Lepidoptera, Moths and Butterflies 1, Evolution, Systematics and Biogeography, Handbook of Zoology, Vol. IV, Part 35, Walter de Gruyter, Berlin, New York, pp. 7–25.
Lange, S., Schram, F.R., 1999. Evolución y filogenia de los crustaceos. Bol. Soc. Entomol. Aragonesa 26, 235–254.
Larson, A., Dimmick, W., 1993. Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters. Herpetol. Monogr. 7, 77–93.
Larson, A., Weisrock, D.W., Kozak, K.H., 2003. Phylogenetic systematics of salamanders (Amphibia: Urodela), a review. In: Sever, D.M. (Eds.), Reproductive Biology and Phylogeny of Urodela (Amphibia). Science Publishers, Enfield, NH, USA, pp. 31–108.
Lecointre, G., Nelson, G., 1996. Clupeomorpha, sister-group of Ostariophysi. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 193–207.
Li, G.-Q., Wilson, M.V.H., 1996. Phylogeny of Osteoglossomorpha. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 163–174.
Lindgren, A.R., Giribet, G., Nishiguchi, M.K., 2004. A combined approach to the phylogeny of Cephalopoda (Mollusca). Cladistics 20, 454–486.
Lipscomb, D.L., 1989. Relationships among the eukaryotes. In: Fernholm, B., Bremer, K., Jörnvall, H., Rutberg, L., Wanntorp, H.-E. (Eds.), Hierarchy of Life. Exerpta Medica, Amsterdam, pp. 161–178.
Lipscomb, D.L., Farris, J.S., Källersjö, M., Tehler, A., 1998. Support, ribosomal sequences and the phylogeny of the Eukaryotes. Cladistics 14, 303–338.
Littlewood, D.T.J., Smith, A.B., Clough, K.A., Emson, R.H., 1997. The interrelationships of the echinoderm classes: morphological and molecular evidence. Biol. J. Linn. Soc. 61, 409–438.
Loconte, H., Stevenson, D.W., 1991. Cladistics of the Magnoliidae. Cladistics 7, 267–296.
Luckett, W.P., Hong, N., 1998. Phylogenetic relationships between the orders Artiodactyla and Cetacea: a combined assessment of morphological and molecular evidence. J. Mammal. Evol. 5, 127–181.
Marques, A.C., Collins, A.G., 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol. 123, 23–42.
Martin, R.D., 1990. Primate Origins and Evolution: A Phylogenetic Reconstruction. Chapman & Hall, London.
Marvaldi, A.E., Sequeira, A.S., O'Brien, C.W., Farrell, B.D., 2002. Molecular and morphological phylogenetics of weevils (Coleoptera, Curculionoidea): do niche shifts accompany diversification? Syst. Biol. 51, 761–785.
Mayr, G., Clarke, J., 2003. The deep divergences of neornithine birds: a phylogenetic analysis of morphological characters. Cladistics 19, 527–553.
Meng, J., Wyss, A.R., 2001. The morphology of *Tribosphenomys* (Rodentiaformes, Mammalia): phylogenetic implications for basal glires. J. Mammal. Evol. 8, 1–71.
Montero, R., Autino, A.G., 2004. Sistemática y Filogenia de los Vertebrados, con Énfasis en la Fauna Argentina. Universidad Nacional de Tucumán, Argentina.
Nelson, J.S. 2006. Fishes of the World, 4th edn. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.
Nielsen, C., 1995. Animal Evolution: Interrelationships of the Living Phyla. Oxford University Press, Oxford.
Nielsen, C., 2003. Proposing a solution to the Articulata–Ecdysozoa controversy. Zool. Scr. 32, 475–482.
Nixon, K.C., Crepet, W.L., Stevenson, D., Friis, E.M., 1994. A reevaluation of seed plant phylogeny. Ann. Mo. Bot. Gard. 81, 484–533.
Nussbaum, R.A., Wilkinson, M., 1989. On the classification and phylogeny of caecilians (Amphibia: Gymnophiona), a critical review. Herpetol. Monogr. 3, 1–42.
Olney, J.E., Johnson, G.D., Baldwin, C.C., 1993. Phylogeny of lampridiform fishes. Bull. Mar. Sci. 52, 137–169.
Parenti, L.R., 2005. The phylogeny of atherinomorphs: evolution of a novel fish reproductive system. In: Uribe, M.C., Grier, H.J. (Eds.), Viviparous Fishes. New Life Publications, Orlando, FL, USA, pp. 13–30.
Parenti, L.R., Grier, H.J., 2004. Evolution and phylogeny of gonad morphology in bony fishes. Integr. Comp. Biol. 44, 333–348.
Patterson, D.J., 1999. The diversity of Eukaryotes. Amer. Nat. 154, S96–S124.
Patterson, C., Rosen, D.E., 1977. Review of ichthyodectiform and other Mesozoic teleost fishes and the theory and practice of classifying fossils. Bull. Amer. Mus. Nat. Hist. 158, 81–172.
Pawson, D.L., 2007. Phylum Echinodermata. Zootaxa 1668, 746–764.
de Pinna, M.C.C., 1996. Teleostean monophyly. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, CA, USA, pp. 147–162.
Platnick, N.I., 1977. The hypochiloid spiders: a cladistic analysis, with notes on the Atypoidea (Arachnida, Araneae). Amer. Mus. Nov. 2627, 1–23.
Platnick, N.I., Goloboff, P.A., 1985. On the monophyly of the spider suborder Mesothelae (Arachnida: Araneae). J. N.Y. Entomol. Soc. 93, 1265–1270.
Ponder, W.F., Lindberg, D.R., 1997. Towards a phylogeny of gasteropod molluscs: an analysis using morphological characters. Zool. J. Linn. Soc. 119, 83–265.
Raikow, R.J., 1982. Monophyly of the Passeriformes: test of a phylogenetic hypothesis. The Auk 99, 431–445.
Rieger, G.E., Rieger, R.M., 1977. Comparative fine structure study of the gastrotrich cuticle and aspects of cuticle evolution within the aschelminthes. J. zool. Syst. Evol. forsch. 15, 81–124.
Ronquist, F., Rasnitsyn, A.P., Roy, A., Eriksson, K., Lindgren, M., 1999. Phylogeny of the Hymenoptera: a cladistic reanalysis of Rasnitsyns (1988) data. Zool. Scr. 28, 13–50.
Rosen, D.E., 1973. Interrelationships of higher euteleostean fishes. In: Greenwood, P.H., Miles, R.S., Patterson, C. (Eds.), Interrelationships of Fishes. Zool. J. Linn. Soc. 53(Suppl. 1), 397–513.
Ross, E.S., 2001. EMBIA. Contributions to the Biosystematics of the Insect Order Embiidina. Part 1. Origin, Relationships and

Integumental Anatomy of the Insect Order Embiidina. Occasional Papers of the California Academy of Sciences No. 149.
Rouse, G.W., Fauchald, K., 1997. Cladistics and polychaetes. Zool. Scr. 26, 139–204.
Rousset, V., Pleijel, F., Rouse, G.W., Erséus, C., Siddall, M.E., 2007. A molecular phylogeny of annelids. Cladistics 23, 41–63.
Ruckes, H., 1929. Studies in chelonian osteology. Part II. The morpoholgical relationships between the girdles, ribs, and carapace. Ann. NY Acad. Sci. 31, 81–120.
Schram, F.R., 1986. Crustacea. Oxford University Press, Oxford and New York.
Schulmeister, S., Wheeler, W.C., Carpenter, J.M., 2002. Simultaneous analysis of the basal lineages of Hymenoptera (Insecta) using sensitivity analysis. Cladistics 18, 455–484.
Shultz, J.W., 2007. A phylogenetic analysis of the arachnid orders based on morphological characters. Zool. J. Linn. Soc. 150, 221–265.
Stiassny, M.L.J., 1996. Basal ctenosquamate relationships and the intrarelationships of myctophiform (scopelomorph) fishes. In: Stiassny, M.L.J., Parenti, L.R., Johnson, G.D. (Eds.), Interrelationships of Fishes. Academic Press, San Diego, pp. 405–426.
Szumik, C., Edgerly, J., Hayashi, C., 2008. Phylogeny of embiopterans (Insecta). Cladistics 24, 993–1005.
Tehler, A., 1988. A cladistic outline of the Eumycota. Cladistics 4, 227–277.
Trueb, L., Cloutier, R., 1991. A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). In: Schultze, H.P., Trueb, L. (Eds.), Origins of the Higher Groups of Tetrapods: Controversy and Consensus. Cornell University Press, Ithaca, NY, USA, pp. 223–313.
Tyler, S., 2003. Epithelium – the primary building block for Metazoan complexity. Integr. Comp. Biol. 43, 55–63.
Tyler, S., Rieger, R.M., 1980. Adhesive organs in the Gastrotricha I. Duo gland organs. Zoomorphologie 95, 1–15.
Tyler, J.C., O'Toole, B., Winterbottom, R., 2003. Phylogeny of the genera and families of zeiform fishes, with comments on their relationships with tetraodontiforms and caproids. Smithson Contrib. Zool. 618, 1–110.
Vilhelmsen, L., 1996. The preoral cavity of lower Hymenoptera (Insecta). Comparative morphology and phylogenetic significance. Zool. Scr. 25, 143–170.
Vilhelmsen, L., 2001. Phylogeny and classification of the extant basal lineages of the Hymenoptera (Insecta). Zool. J. Linn. Soc. 131, 393–442.
Wahlberg, N., Braby, M.F., Brower, A.V.Z., de Jong, R., Lee, M.-M., Nylin, S., Pierce, N.E., Sperling, F.A.H., Vila, R., Warren, A.D., Zakharov, E., 2005. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. Proc. Biol. Sci. 272, 1577–1586.
Wheeler, W.C., Hayashi, C.Y., 1998. The phylogeny of the extant chelicerate orders. Cladistics 14, 173–192.
Wheeler, W.C., Cartwright, P., Hayashi, C.Y., 1993. Arthropod phylogeny: a combined approach. Cladistics 9, 1–39.
Wheeler, W.C., Whiting, M., Wheeler, Q.D., Carpenter, J.M., 2001. The phylogeny of the extant hexapod orders. Cladistics 17, 113–169.
Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.
Williams, D.M., 1991. Phylogenetic relationships among the Chromista: a review and preliminary analysis. Cladistics 7, 141–156.
Yeates, D.K., Wiegmann, B.M., 1999. Congruence and controversy: toward a higher-level phylogeny of Diptera. Annu. Rev. Entomol. 44, 397–428.
Zamparo, D., Hoberg, E.P., McLennan, D.A., Brooks, D.R., 2001. Phylogenetic analysis of the Rhabdocoela (Platyhelminthes) with emphasis on the Neodermata and relatives. Zool. Scr. 30, 59–77.
Zrzavý, J., 2002. Gastrotricha and metazoan phylogeny. Zool. Scr. 32, 61–82.
Zrzavý, J., Mihulka, S., Kepka, P., Bezdek, A., Tietz, D., 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. Cladistics 14, 249–285.
Zrzavý, J., Hypsa, V., Tietz, D.F., 2001. Myzostomida are not Annelids: molecular and morphological support for a clade of animals with anterior sperm flagella. Cladistics 17, 170–198.