

Teaching Animal Molecular Phylogenetics

By C. Leon Harris

PREFACE

To many zoology instructors, the phylogeny of animals is merely a convenience for organizing textbooks and courses, and not to be taken seriously. This approach was reasonable as long as there was no way of testing phylogenies. Moreover, until recently phylogenetics had few practical consequences. The past decade, however, has witnessed the flourishing of evolutionary developmental biology ("Evo Devo"), which promises for the first time to reveal the changes in developmental regulatory genes that led to the evolution of the different body plans that define animal phyla. Discovering the correct sequence of developmental genetic changes obviously depends on having the correct phylogeny to begin with, so animal phylogenetics has suddenly become crucial to a major area of research in zoology. In addition, having the correct phylogeny would permit quantitative tests of a variety of other evolutionary hypotheses regarding such questions as punctuated equilibrium and correlations between environmental and evolutionary changes ([Heulsenbeck and Rannala 1997](#); [Pagel 1999](#)). Molecular phylogenetics does not necessarily provide the correct phylogeny, but it can help eliminate incorrect ones and can suggest alternative hypotheses that otherwise might not have been considered.

Molecular phylogenetics in a broad sense has been around since 1904, when [G. H. F. Nuttall](#) inferred the evolutionary relationships among primates and other mammals from their immunological compatibility. [Libbie H. Hyman](#) (1888-1969) often cited such serological studies favorably, while noting their technical limitations and the continuing need for studies based on traditional embryological and morphological characters. She would have been pleased with how far molecular phylogenetics has advanced, although she would undoubtedly have acerbic comments about some of the conclusions.

The current era of molecular phylogenetics began a few years before Hyman died, with the first comprehensive phylogenies reconstructed from the amino-acid sequences of cytochrome *c* ([Fitch and Margoliash 1967](#)). The availability of nucleic-acid sequences set off another revolution marked by the publication of a paper by [Field et al.](#) in 1988. Since then molecular phylogenetics has crawled, stumbled, and finally learned to stand and walk confidently ([Adoutte et al. 2000](#)). Molecular phylogenetic trees are now common in *Science*, *Nature*, and other journals, and a molecular phylogenetic tree is even the organizing principle of a popular book on diversity ([Tudge 2000](#)). Zoology instructors therefore have to be prepared to explain the discrepancy between the phylogenies common in textbooks and what students are likely to find elsewhere. Rather than being an added burden on the instructor, the introduction of molecular phylogenetics into teaching can be an opportunity to stimulate critical thinking.

Unfortunately, the jargon and methodology of molecular phylogenetics have made it difficult for the average zoology instructor to keep up with the flood of literature. Moreover, the often-conflicting results of molecular phylogenetics have made many wonder whether learning about it is worth the trouble. In the 1990s, however, the main outlines of animal molecular phylogeny have become fairly settled, so the time is now right for a concise introduction to its methods, terminology, and main conclusions.

This introduction has been organized to accommodate instructors with all levels of time and interest. It presupposes a basic understanding of cladistics, which is now the default method of representing phylogenies. Some of the terms of cladistics, as well as specialized terms in molecular phylogenetics, are explained in the [glossary](#) at the end of this document. A fuller treatment of cladis-

tics can be found in [references](#) at the end of this document.

The main concepts and results of molecular phylogenetics can be gleaned in a few minutes by scanning the Table of Contents below. Clicking on a heading links to further discussion, which includes links to references and other sources on the web. Part I introduces the methods of molecular phylogenetics. This section can safely be deferred, or, for more detail, you may refer to books and web sites in the [references](#) or follow the links in this document. Many readers will want to jump to part II, which describes what traditional morphological characters are supported or not, and Part III, which presents molecular-phylogenetic hypotheses as alternatives to the traditional morphology based hypotheses. These sections include historical sketches of traditional phylogenetic concepts, generally using [Hyman's](#) monumental *The Invertebrates* as a starting point. More recent morphological phylogenetic schemes are also outlined, especially the book-length noncladistic study by [Pat Willmer \(1990\)](#) and the equally thorough cladistic study by [Claus Nielsen \(1995\)](#). Parts II and III may convince some zoology instructors that the organization of their syllabus according to traditional phylogenetics needs some revision. Part IV offers suggestions for such revision.

While molecular phylogenetics has developed considerably since its first conception, it is probably still in its larval stage. It will be apparent in Part III that controversies still remain unresolved. This web site will try to keep abreast of developments as they happen. In addition, feel free to email any corrections, suggestions, and questions to c.harris@plattsburgh.edu.

I am indebted to Marge Kemp, the Sponsoring Editor, for her insight in seeing the need for this project, Donna Nemmers, Developmental Editor, for guiding me in its creation, and Mark Christianson, the Media Developer, for its final execution. I am also grateful to Jan Pechenik and Ken Saladin for providing encouragement, stimulating discussions of phylogeny, and a due sense of caution.

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Molecular phylogenetics refers to any method of inferring evolutionary relationships from similarities or differences in molecular structure.

Molecular characters suffer from problems that also afflict morphological characters. For example, neither molecules nor morphology may be able to resolve the phylogeny of evolution that was both ancient and rapid, as in the Cambrian Explosion.

Another problem shared by molecular and morphological characters is homoplasy (nonhomologous characters appearing to be similar in different taxa).

Other problems shared by molecular and morphological phylogenetics arise from polymorphism (homologous characters appearing differently in the same species). Because of polymorphism, the time of divergence may appear to be earlier than it was.

Polymorphism can also result in the incorrect phylogenetic sequence.

Similar problems result from different copies of duplicated genes.

Another problem with molecular phylogenetics is long-branch attraction: the tendency of fast-evolving molecules to appear more closely related than they actually are.

Molecular phylogenetics has gained wide acceptance in spite of these and other problems because it provides a large amount of evidence that is independent of morphology, as well as other advantages.

Several kinds of experiments support the validity of molecular phylogenetics.

Molecular characters can be of two types: discrete (qualitative) differences in molecular sequence and continuous (quantitative) distance between molecules.

The first step in molecular phylogenetics is to select a suitable molecule that is homologous in all the taxa to be included in the phylogeny.

Many molecular characters are much less susceptible to homoplasy and long-branch attraction than are nucleic-acid sequences. These characters include amino-acid sequences, the positions of short and long interspersed elements, and Hox genes.

Elongation factors, actin, and tubulins are among the widely used proteins.

The positions of short and long interspersed elements (SINEs and LINEs) are another increasingly common source of discrete characters.

Hox genes have also been used to infer phylogenetic relationships.

The most commonly used molecular data for higher taxonomic levels are base sequences from genes that encode ribosomal RNA, especially 18S rDNA.

Nucleic-acid sequences must be aligned before they can be compared.

Assumptions may be needed about the probabilities of different molecular changes.

Molecular relationships are represented as trees constructed of branches with nodes at both ends of each branch.

Inferring (reconstructing) a phylogeny consists of creating or selecting one tree out of perhaps millions of possible ones.

The neighbor-joining method (NJ) is an algorithm that generates one tree with the shortest total branch length.

The maximum parsimony method (MP) selects the cladogram with the minimum number of changes in character state.

The maximum likelihood method (ML) begins with an explicit model of evolution and possible trees, then it attempts to find the tree that is most likely with the given data.

With more than a few taxa, any method requires a computer.

To show the temporal sequence of divergence, trees have to be rooted. The root represents the most recent common ancestor of the study group.

For convenience in printing large trees, branches are often represented as horizontal lines joined by vertical lines representing internal nodes. Branches may be unscaled, or they may be scaled according to a distance measure.

Phylogenies reconstructed by different methods are generally similar to each other.

Confidence in an internal branch can be tested by bootstrapping.

A branch with low bootstrap support may be collapsed.

A consensus tree can be created by collapsing branches that are not supported in all trees created by different methods of analysis. A consensus tree can also be produced by comparing molecular and morphological trees.

Molecular and morphological data can be combined to create a "total-evidence tree."

Because of long-branch attraction, differences in sequence alignment, limitations in the size of study groups, and different methods of tree reconstruction, conflicting molecular phylogenies have been proposed. As techniques have improved and more molecules from more species have been sequenced, many of the past conflicts have been resolved.

II. Testing the Validity of Traditional Morphological Characters by Seeing Whether They Are Consistent With Molecular Trees

To be consistent with a given phylogenetic tree, a character must map onto the tree with few changes in character state.

For example, bilateral symmetry is consistent with the traditional morphology-based cladogram for the Big Nine phyla (those with more than 5,000 named species), since it requires only one change in character state.

Segmentation, however, is less consistent with traditional morphology based phylogenetic trees, because it requires at least two changes in character state: one for annelids and arthropods and one for chordates.

Lack of consistency implies that either the character is not synapomorphic (homologous), or the phylogenetic tree is incorrect.

A character that is consistent with both a morphological and a molecular phylogeny is more likely to be phylogenetically informative.

Morphological characters have not led to a consensus phylogeny.

The following morphological characters traditionally used in phylogenetics are also consistent with the widely accepted molecular phylogenetic tree of the Big Nine Phyla: bilateral symmetry and triploblasty, deuterostomy, and spiral cleavage pattern.

Bilateral symmetry and triploblasty are also consistent with a molecular phylogenetic tree that includes all animal phyla.

Deuterostomy in Echinodermata, Hemichordata, and Chordata is also consistent in a molecular phylogenetic tree of all animal phyla.

The spiral-cleavage pattern is somewhat consistent with a molecular phylogenetic tree that includes all animal phyla.

The lophophore by itself is not consistent with a molecular phylogenetic tree, and it may not be a homology.

The occurrence and type of body cavity (whether the animal is acoelomate, pseudocoelomate, or coelomate) is not consistent with the molecular phylogenetic tree and is not homologous.

III. Molecular Phylogenetic Trees as Alternative Hypotheses

Protozoa are not monophyletic.

Metazoans are monophyletic, and choanoflagellates may be their sister group.

Myxozoans appear to be metazoans rather than protozoans.

Mesozoans may be flatworms.

Anthozoa appear to be basal within Cnidaria.

Protostomates appear to be divided into two major clades: Ecdysozoa and Lophotrochozoa. Annelida and Arthropoda belong to Lophotrochozoa and Ecdysozoa, respectively, and are therefore not closely related.

Ecdysozoa, the major protostomate clade that includes Arthropoda, also includes Nematoda and other groups with a cuticle that molts all at once.

Arthropoda is monophyletic; Uniramia is not valid.

Pentastomids are crustaceans.

Lophotrochozoa, the major protostomate clade that includes Mollusca and Annelida, also includes other animals with trochophore larvae, the lophophorates, and all descendants from their most recent common ancestor.

Lophotrochozoa includes spiralian.

Flatworms appear to be lophotrochozoans rather than basal to other Bilateria.

Acoela may or may not be basal to other Bilateria.

Nemertea may be closer to “coelomates” than to flatworms.

Gastrotrichs are not closely related to nematodes.

Acanthocephalans are closely related to rotifers.

Cycliophora appear to be related to rotifers.

Echiura and Pogonophora may be polychaete annelids.

Chaetognatha may not be closely related to other deuterostomates.

Molecular evidence supports the conventional phylogeny of echinoderm classes.

Concentricycloids may be asteroids.

Hemichordata may be closer to Echinodermata than to Chordata.

Vertebrates apparently did not evolve from an echinoderm.

Cephalochordata, rather than Urochordata, may be the sister group of Vertebrata.

Turtles may be the sister group of Crocodylia + Aves rather than basal Reptilia.

Placental mammals may be divided into four superordinal clades.

IV. Incorporating Molecular Phylogenies Into Teaching

The most important conclusions from animal molecular phylogenetics are that Bilateria (triploblasts) and Deuterostomia are each monophyletic, and protostomes comprise the two clades Lophotrochozoa and Ecdysozoa.

The traditional approach of proceeding from the simplest animals to the more complex is pedagogically sound.

The practice of treating the “acoelomates” and the “pseudocoelomates” together as clades outside of coelomates should be abandoned.

More natural groupings would be Lophotrochozoa and Ecdysozoa.

The following proposed sequence of topics is consistent with molecular phylogenetics without departing too radically from the traditional zoology syllabus.

GLOSSARY

REFERENCES

I. The Methods of Molecular Phylogenetics

Molecular phylogenetics refers to any method of inferring evolutionary relationships from similarities or differences in molecular structure.

- The goal of phylogenetics, whether based on molecules or morphology, is to reconstruct the evolutionary history of groups of organisms.
- Molecular phylogenetics is no different in principle from inferring phylogeny from the similarities in morphology. Many of the same methods are applied to both molecules and morphology.
- Since molecular changes underlie all inherited morphological changes, molecular phylogenetics can be viewed as simply a more direct approach to morphological phylogeny.

Molecular characters suffer from problems that also afflict morphological characters. For example, neither molecules nor morphology may be able of resolving the phylogeny of evolution that was both ancient and rapid, as in the Cambrian Explosion.

- Just as a telescope is incapable of producing a clear image of a cell, techniques for looking at remote phylogenetic changes are not able to resolve the small details of what occurred during a short time.
- Molecular phylogenetics has so far proved incapable of resolving branching patterns among some clades such as spiralian.

Another problem shared by molecular and morphological characters is **homoplasy** (nonhomologous characters appearing to be similar in different taxa).

- The same base or amino acid can occur homoplasiously at a position on molecular sequences from two taxa, tending to make the two taxa appear to be more closely related than they really are. Such homoplasy is especially likely for DNA, because it has only four different DNA bases. Adenine (A), for example, could occur at the same position in two sequences either because there had been no change at the position or because there had been two or more changes (for example, A to C to A).
- Two homologous DNA sequences that are saturated with mutations will be identical at one-fourth of their positions merely by homoplasy.
- Some molecular characters are virtually immune to homoplasy. These include mitochondrial gene rearrangements and short and long interspersed elements (**SINEs** and **LINES**).

Other problems shared by molecular and morphological phylogenetics arise from **polymorphism** (homologous characters appearing differently in the same species). Because of polymorphism, the time of divergence may appear to be earlier than it was.

- The occurrence of two or more forms within a species (polymorphism) indicates that evolution has occurred before speciation. If different forms of a molecule were present in two populations that later diverged into species, the time of divergence inferred from the molecule will appear to be earlier than it actually was.
- As the molecules continue to evolve separately, however, the original differences between them will become negligible compared with the changes following speciation. Consequently, this problem can be disregarded at higher taxonomic levels.

Polymorphism can also result in the incorrect phylogenetic sequence.

- Consider hypothetical species A, B, and C', the prime indicating that a character in species C' differs from that in A and B. Whether morphological or molecular, the character difference would tend to suggest that species A and B are closer to each other than either one is to C' (Fig. 1a). In fact, however, B and C' might be sister groups that diverged from a polymorphic ancestor, with B and C' each inheriting a different form of the character (Fig. 1b).



Figure 1. Polymorphism can lead to incorrect molecular or morphological trees. (a) Taxa A and B have inherited one form of a molecule, while C' has inherited a different form of the homologous molecule, leading to the inference that A and B are sister groups to the exclusion of C'. (b) In fact, B and C' might be sister groups that inherited different forms of the molecule from a polymorphic ancestor.

- This kind of problem is thought to be responsible for conflicting molecular phylogenies for humans, chimpanzees, and gorillas (Graur and Li 2000, p. 222). Again, however, this is not likely to be a problem at higher taxonomic levels, since evolution of the character subsequent to speciation will obscure the relatively small differences that existed before speciation.

Similar problems result from different copies of duplicated genes.

- If a gene has been duplicated in an ancestor, the descendants will have two types of homologs of the gene or gene product: **orthologous** (derived from the same ancestral copy) and **paralogous** (derived from different ancestral copies).
- Paralogous copies may cause problems similar to those of polymorphism, because, like polymorphisms, they are different versions of the same gene.

Another problem with molecular phylogenetics is **long-branch attraction**: the tendency of fast-evolving molecules to appear more closely related than they actually are.

- Because of homoplasy, long branches (molecular sequences that have evolved rapidly or for a long time) appear to be more closely related to each other than do sequences that have evolved slowly or for less time. When long branches are mixed with short ones, the long branches tend to join one another during tree reconstruction. This problem is called long-branch attraction.
- Long-branch attraction can be avoided by eliminating from the study group taxa in which molecular sequences have evolved more rapidly than in other taxa, and by eliminating parts of sequences that have evolved more rapidly than other parts of the same molecule.

Molecular phylogenetics has gained wide acceptance in spite of these and other problems because it provides a large amount of evidence that is independent of morphology, as well as other advantages.

- In any two taxa there are many more homologous molecules than there are homologous morphological characters, especially if the taxa are as different as, say, sponges and insects.
- Every difference in a molecule is potentially an independent character, so one gene or protein may provide dozens or hundreds of characters. The gene for the RNA in the smaller subunit of the ribosome, for example, contains more than 1,700 bases.
- In contrast to morphological characters, which can be influenced by environment, molecules are for the most part strictly inherited.
- Many molecular characters, such as the presence of a particular base or amino acid at a given position, are strictly binary. In contrast, many morphological characters vary continuously: one must set an arbitrary criterion for whether, for example, a bird's beak is short or long.
- Some molecules may evolve at a regular rate, so it is sometimes possible to estimate the time of divergence of two groups from their degree of molecular difference.

Several kinds of experiments support the validity of molecular phylogenetics.

- The molecular phylogeny of 10 strains of laboratory mice inferred from chromosomal differences agreed exactly with the known phylogeny ([Fitch and Atchley 1987](#)). In contrast, phylogenies based on morphology (lower jaw structure) or life-history traits (litter size, body mass at different ages, etc.) gave conflicting phylogenies, none of which was correct.
- Molecular phylogenetics correctly reconstructed the branching pattern and branch lengths for a virus serially propagated in the presence of a mutagen ([Hillis et al. 1992](#); [Hillis et al. 1994](#)).
- Phylogenies of birds and mammals based on different molecules were more nearly in agreement with each other than were phylogenies based on different morphological characters ([Bledsoe and Raikow 1990](#)).

Molecular characters can be of two types: discrete (qualitative) differences in molecular sequence and continuous (quantitative) distance between molecules.

- The following are examples of discrete characters: differences in base or amino-acid sequences, gene rearrangements and duplications, and the position of [transposable elements](#) on chromosomes.
- The following kinds of data provide distance measures: degree of immunological compatibility, electrophoresis of proteins, the number of discrete differences, and [DNA-DNA hybridization](#).

The first step in molecular phylogenetics is to select a suitable molecule that is homologous in all the taxa to be included in the phylogeny.

- The molecule must occur in all taxa to be studied (the study group).
- The molecule must be large enough to provide a sufficient number of differences for comparison.
- For a phylogeny of higher taxonomic categories (kingdom, phylum, class), the molecule should have evolved slowly, since these taxa have had more time to evolve. One example of such a highly conserved molecule is rDNA—the DNA that encodes one of the ribosomal RNAs.

- For lower taxonomic categories a fast-evolving molecule is needed to ensure that it is sufficiently different among taxa. Mitochondrial DNA (mtDNA) is an example of a fast-evolving molecule.

Many molecular characters are much less susceptible to homoplasy and long-branch attraction than are nucleic-acid sequences. These characters include amino-acid sequences from proteins, the positions of short and long interspersed elements, and Hox genes.

Elongation factors, actin, and tubulins are among the widely used proteins.

- Since there are so many different amino acids, the problem of homoplasy and long-branch attraction are less troublesome in proteins than in nucleic acids.
- Elongation factors are proteins involved in protein synthesis in all organisms. One of the most widely used proteins in molecular phylogenetics is elongation factor-1 α (EF-1 α).

The position of short and long interspersed elements (SINEs and LINEs) are another increasingly common source of discrete characters.

- SINEs and LINEs are highly repetitive DNA sequences that occupy much of the genomes of animals (more than a third in humans). Their only known function is to make copies of themselves to be inserted into the genome, so they are characterized as “junk DNA” as well as “selfish DNA.”
- Since SINEs and LINEs are transposed to random positions in the genome, the occurrence of a particular SINE or LINE at the same location in two different organisms is likely to be a [synapomorphy](#) rather than a homoplasy.
- SINEs and LINEs do not occur broadly across taxa, however, so they have been used mainly to resolve relationships among lower taxonomic categories.

Hox genes have also been used to infer phylogenetic relationships.

- Hox genes occur in clusters and encode transcription factors that regulate development. They are best characterized in segmented animals such as insects, where they function as [homeotic](#) genes determining the identity of each segment depending on its location along the antero-posterior axis. Mutations in Hox genes may be involved in evolutionary changes in body plans.
- Hox genes occur in Cnidaria and all Bilateria where they have been sought. In most animals there is only one cluster of Hox genes, but in most vertebrates there are four duplicated clusters. [Orthologous](#) and [paralogous](#) Hox genes can be identified from one taxon to another by comparing nucleotide sequences.
- Animals with similar clusters of Hox genes can be inferred to be closely related.

The most commonly used molecular data for higher taxonomic levels are base sequences from genes that encode ribosomal RNA, especially [18S rDNA](#).

- 18S rDNA encodes 18S rRNA, which occurs in the smaller subunit of the ribosome. 18S rDNA is also referred to as SSU rDNA (small-subunit ribosomal DNA).
- The 5S rDNA and 28S rDNA from the larger ribosomal subunit have been used occasionally, with results that are in broad agreement with those from 18S rDNA studies ([Hori and Osawa 1987](#); [Christen et al. 1991](#)). The 5S rDNA is apparently too small and variable, however, to be reliable ([Halanych 1991](#)).
- Like all nucleic-acid sequences, those from rDNA are subject to [homoplasy](#) and [long-branch attraction](#).

by making the length of each branch proportional to some distance measure, such as the number of base differences.

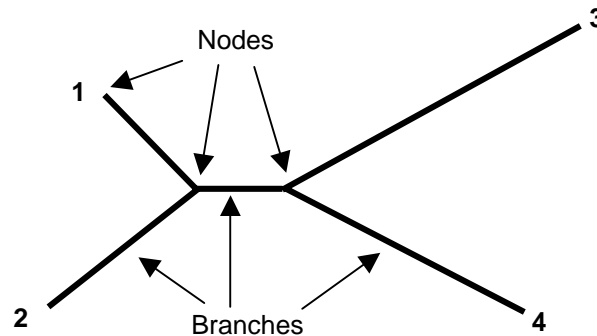


Figure 3. A simple tree with some of the nodes and branches indicated. Terminal nodes represent OTUs (presumptive taxa) 1 through 4. Internal nodes represent hypothetical ancestors. The branches are scaled in this example.

Inferring (reconstructing) a phylogeny consists of generating or selecting one tree out of perhaps millions of possible ones.

- Only three different trees represent all possible relationships of four taxa (Fig. 4).

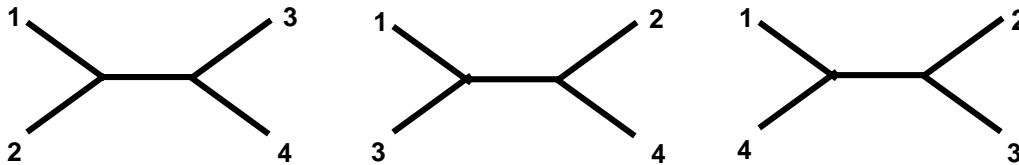


Figure 4. The three different trees possible with four taxa. Branches are unscaled.

- As the number of taxa increases, the possible trees become a forest. The number of possible strictly bifurcating trees with n taxa is $\frac{(2n-5)!}{2^{n-3}(n-3)!}$. Even with only 10 taxa this equals more than 2 million possible trees!
- Phylogenetic reconstruction consists of either generating a single tree according to some algorithm or selecting one tree according to an optimality criterion.
- The most widely used algorithm for generating a single tree is neighbor-joining. The most widely used methods for selecting an optimal tree is maximum parsimony and maximum likelihood.

The neighbor-joining method (NJ) is an algorithm that generates one tree with the shortest total branch length.

- NJ begins by assuming that all taxa are joined at a single node. It then sequentially joins one pair of taxa at a time to find the combination that gives the shortest total branch length (Fig. 5).

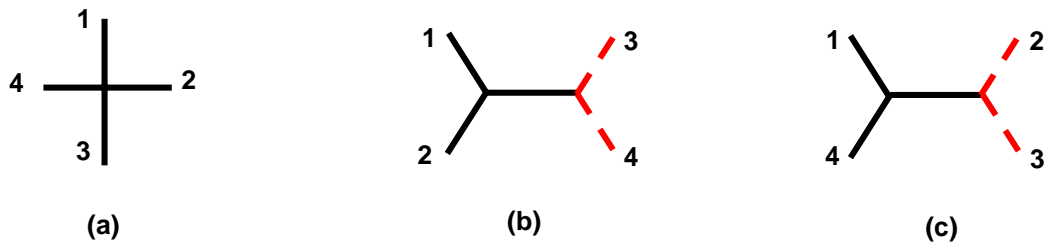


Figure 5. Neighbor-joining applied to the four taxa from Figure 3 illustrated by a graphical procedure called star deconstruction. (a) All the unscaled branches are joined at a single internal node. (b and c) The first (and in this simple case, the only) internal branch is added, with each possible pair of taxa joined as neighbors at one end (dashed, red lines) and the remaining taxa joined at the node at the other end. Only two of the three possibilities are shown here.

- For each of the trees with a different pair joined as neighbors, the two neighbors are combined to form a composite taxon. The length of the branch to that composite taxon is set so that the average distance from the two neighbors to every other taxon is the same as in the original scaled tree (Fig. 6). The neighboring pair of taxa that give the shortest total branch length are assumed to be neighbors in the final tree. In Figure 6, the tree in (a) is shortest, so taxa 3 and 4 would be joined as neighbors. NJ would therefore construct the tree shown in Figure 5b.

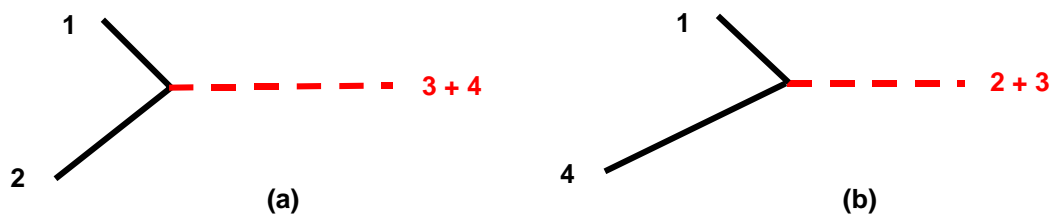


Figure 6. The trees shown in Figure 5b and c after combining the first pair of neighbors into one branch (dashed, red) and rescaling. The tree in (a) has a shorter total branch length than the tree in (b) (as well as the other alternative, not shown).

- After the first pair of neighbors and the first internal branch are found, the procedure is repeated with the first pair of taxa represented by one branch. The second internal branch is then found, and so on. (With only four taxa, of course, there would be only one internal branch.) Finally, the scaled tree is reconstructed using the internal branches that were found.

- ADVANTAGE: NJ takes relatively little computational effort.
- DISADVANTAGES: NJ generates only one tree, which may not be vastly superior to an alternative. If sequences are short, statistical errors increase. Long distances are likely to be underestimated because of multiple substitutions at the same positions. NJ also looks only at the number, not the nature, of changes.

The maximum parsimony method (MP) selects the cladogram with the minimum number of changes in character state.

- When applied to molecular-sequence data, MP begins by identifying **informative sites**. An informative site is one in which there are at least two different character states, at least two of which occur in more than one taxon (Fig. 7).

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1) T T C G A C C G T
2) C T T A A C T G T
3) C T A T G C T G G
4) C T G T G C C G G
           x   y   z

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Figure 7. Aligned homologous DNA sequences from four taxa. Informative sites are indicated by letters x, y, and z. Only positions with two or more different bases, at least two of which occur in more than one taxon, are informative.

- The MP method searches all possible trees to find the one that requires the smallest number of changes for each informative position. The tree requiring the fewest changes is the most parsimonious and therefore preferred, since it requires the fewest hypotheses about evolutionary change (in accordance with Occam's Razor).
- The total number of changes is the length of the tree. For example, based on Figure 7 above, the tree shown in Figure 8a would be shorter than the tree in Figure 8b, requiring four rather than five base substitutions.

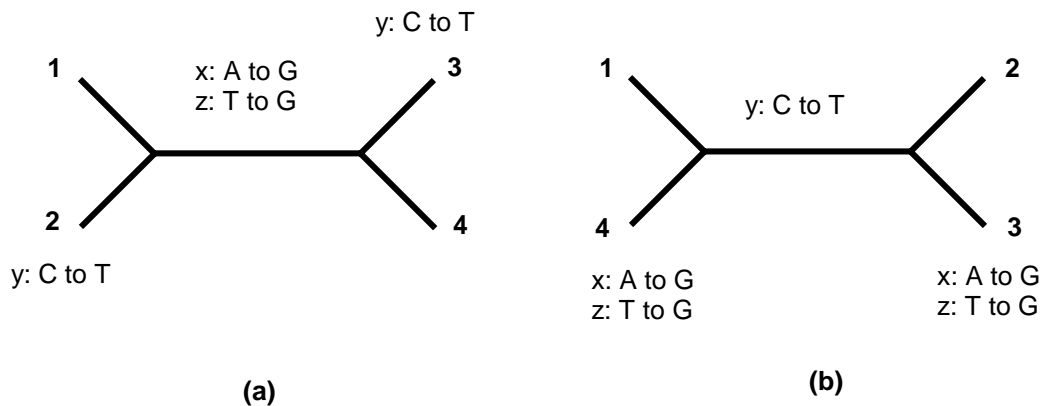


Figure 8. Two of the three possible trees for taxa 1 through 4 with DNA sequences shown in Figure 7. (a) A total of only four base substitutions at the informative sites x, y, and z are required with this tree. (b) Five base substitutions are required with this tree, which is therefore longer and less parsimonious. The remaining tree, grouping 1 with 3 and 2 with 4, also requires five substitutions.

- In the example in Figure 8, all substitutions are assumed to be equally likely. This is called unweighted parsimony. Often more **weight** is given to **transversions**, which are less likely than **transitions**, or only transversions may be counted. If only transversions were counted in Figure 7, only position z would be informative. In that case, the tree in Figure 8a would still be preferred, requiring only one transversion compared with two in b.
- With 12 or more taxa an **exhaustive search** of the more than 13 billion trees is impractical, so the number of trees to be examined for length must first be reduced by some other method. One approach to reducing the number is to first perform what is called a **heuristic search** of the most likely trees. In a heuristic search, NJ or some other method is first used to find a provisional tree. Branches are then rearranged and examined by MP to try to find a shorter tree. If a shorter one is found, all others are ignored, and the process is repeated.
- **ADVANTAGE:** Unlike NJ, MP uses information about the type of change at each informative site and not merely the number of changes.
- **DISADVANTAGES:** By using only informative sites, MP still uses only a small portion of sequence information. MP also has the disadvantage that it often recovers a number of equally parsimonious trees. Both of these problems are minimized by using long sequences with many informative positions. MP produces only cladograms, which are, of course, unscaled phylogenetic trees. The most serious limitation of MP is that with more than 12 taxa an exhaustive search of all possible trees is impractical, so there is no certainty that the most parsimonious tree will be found.

The maximum likelihood method (ML) begins with an explicit model of evolution and possible trees, then it attempts to find the tree that is most likely with the given data.

- With ML, one must first estimate the probability of each kind of change in character state (for example, the probability of no change in a base, a **transition**, or a **transversion**). The likelihood L_n for the bases at each position n and for each tree is then calculated from these probabilities. The logarithm of these values of L are then added to get the **log likelihood** ($\ln L$) of each tree. The tree with the highest (least-negative) value of $\ln L$ is taken to be the most likely.

- Suppose we estimate or assume that the probability of a nucleotide base remaining unchanged is 0.7, the probability of a transition is 0.2, and the probability of a transversion is 0.1. We can now apply these probabilities to calculate the likelihoods of the trees in Figure 8 given the sequences in Figure 7. Figure 9 shows the possible changes in the tree shown in Figure 8a that could have led to the bases at the first position. Table 1 shows how the likelihood is calculated.

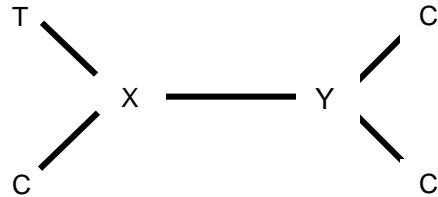


Figure 9. An illustration of ML for the first position in the sequence in Figure 7 and the tree in Figure 8a. For taxon 1 the base at the first position is T, and for the other three taxa the base is C. X and Y represent the bases at the first position for the two ancestral taxa. One explanation for the bases at the position in these four taxa is that both X and Y inherited C from their common ancestor, and there was a transition from C to T in the evolution of taxon 1. Another possibility is that there was a transition in the divergence of X and Y, so that X became T and Y became C, and this was followed by a transition from T to C in the evolution of taxon 2. It is also possible, but less likely, that X was A or G, and there were two transversions in the evolution of taxa 1 and 2. Similarly, Y was most likely C, but it could have been any of the other three bases. Therefore there are 16 (4×4) different ways that the bases at this first position could have occurred with this tree. Each way has a different probability. The likelihood of these bases occurring at this position with this tree is the sum of all these 16 probabilities. Let us assume the probability of no change is 0.7, the probability of a transition is 0.2, and the probability of a transversion is 0.1. If X and Y were both C, then there were four branches with no change and one with a transition at that site, so the probability of each of the four bases being what they are is $0.7^4 \times 0.2 = 0.04802$. If Y had been C and X had been T, A, or G, the probabilities would have been 0.01372, 0.00049, and 0.00049, respectively. These four probabilities with $Y = C$ are shown in the top row of Table 1. Making Y one of the other bases gives the other three rows in the table. Adding all 16 of the probabilities gives the likelihood (L_1) and the log likelihood ($\ln L_1$) for the bases at the first position given the data. This procedure would be repeated for every position in the sequence. Adding all these log likelihoods gives the log likelihood ($\ln L$) for the tree and data. This procedure would be carried out for all trees to find the one with the maximum log likelihood.

Table 1. An application of ML to the first position in the sequence in Fig. 7 and the tree in Fig. 8a. X represents the base at the first position for the ancestor of sister taxa 1 and 2, and Y represents the base for the ancestor of sister taxa 3 and 4. Each row shows the probability of the bases occurring at the first position in the four taxa if the bases at that position in X and Y are as shown, assuming that the probability of no change is 0.7, the probability of transition is 0.2, and the probability of transversion is 0.1. In the first row and first column, for example, if both X and Y had C as the base at the first position in the sequence, then there would have been no change at that site for three of the taxa or for X and Y, and there would have been one transition for taxon 1, giving a probability of 0.04802. If Y had C, and X had A (first row, third column), there would have been no change for two branches, and a transversion for each of the branches X—Y, X—1, and X—2, for a probability of $0.7^2 \times 0.1^3 = 0.00049$. The sum of all 16 probabilities gives the likelihood $L_1 = 0.06858$ and $\ln L_1 = -2.680$ that this tree correctly represents the phylogeny given the bases at this position. This procedure would be repeated for every position in the sequence. Adding all the $\ln L$ values for each position gives the total **log likelihood $\ln L$ for the tree. For the tree in Fig. 8a and the sequences in Fig. 7, the log likelihood is -24.716 . The log likelihood of other trees would be calculated similarly. The log likelihood for the tree in Fig. 8b is -27.732 , and the log likelihood for the third possible tree (not shown) is -28.490 . The tree with the highest $\ln L$ is considered the most likely. Thus, the tree in Fig. 8a is the most likely of the three, as was also shown with MP.**

	X = C	X = T	X = A	X = G	
Y = C	0.04802	0.01372	0.00049	0.00049	
Y = T	0.00112	0.00392	0.00004	0.00004	
Y = A	0.00014	0.00014	0.00007	0.00002	
Y = G	0.00014	0.00014	0.00002	0.00007	$L_1 = 0.06858; \ln L_1 = -2.680$

- **ADVANTAGE:** Unlike NJ and MP, ML uses all the character data and not simply the number of character changes or a few informative positions.
- **DISADVANTAGES:** The main criticism of ML is that the likelihood of each kind of base substitution, and therefore the total likelihood for each tree, depends on explicit assumptions about their probabilities. Another criticism of ML is that, unlike NJ and MP, it cannot be used with morphological characters, since one cannot estimate the probability of changes in character state. ML is also limited by the amount of computer time and memory available to examine every possible tree and calculate the likelihoods for each one. It is often necessary to first perform a heuristic search to narrow the number of trees (as in MP), and thus the tree with the maximum likelihood may be missed. Perhaps the best use of ML is in finding the most likely among several competing hypothetical trees, rather than trying to search all possible ones.

With more than a few taxa, any method requires a computer.

- The computer time and memory required increase rapidly with the number of taxa. The analysis places a large burden on computer resources and limits the number of taxa that can be considered simultaneously. Some analyses, especially with ML, may require months of computer time or may terminate prematurely with a fatal “out of memory” error.
- More than 150 different computer programs are available. For a list and links to many of them, see <http://phylogeny.arizona.edu/tree/programs/programs.html>.

To show the temporal sequence of divergence, trees have to be **rooted**. The root represents the most recent common ancestor of the study group.

- **Figure 3** is an example of an unrooted tree. It represents relationships and distances among the four taxa of the study group, but it does not show the sequence of evolutionary divergences, since it lacks a temporal reference.
- Molecular phylogenetic trees are usually rooted by using molecular information from one or more outgroups that are believed from paleontological or other evidence to be outside the study group (Fig. 10a). Ideally, the outgroup used for rooting is the sister group of the study group.
- Alternatively, the root can be placed at the midpoint of the longest pathway separating two taxa in the study group (Fig. 10b). This assumes that the two most distant taxa diverged earliest from their most recent common ancestor, and each branch thereafter evolved at about the same rate.



Figure 10. Unscaled phylogenetic trees resulting from the rooting of the tree in Figure 3 by two different methods. (a) If an outgroup were thought to be close to 4, the root would have been placed on the branch terminating in 4, resulting in this rooted phylogenetic tree. (b) Without an outgroup, the root would have been placed at the midpoint on the longest pathway between two taxa (between 2 and 3 in Figure 3), resulting in a different phylogenetic tree.

- The number of possible rooted trees is the same as the number of unrooted trees with the number of taxa increased by one, since rooting is equivalent to adding a new taxon to the study group.

For convenience in printing large trees, branches are often represented as horizontal lines joined by vertical lines representing internal nodes. Branches may be unscaled, or they may be scaled according to some distance measure.

- In an unscaled phylogenetic tree, the terminal nodes are aligned, and the positions of internal nodes represent the order of divergence (Fig. 11a). In a scaled phylogenetic tree, the branches are proportional to the degree of molecular difference or some other distance measure (Fig. 11b).

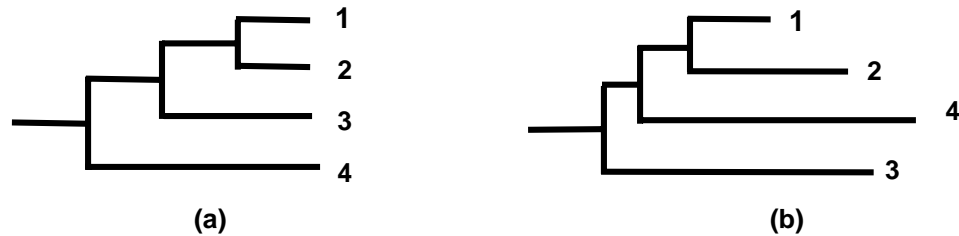
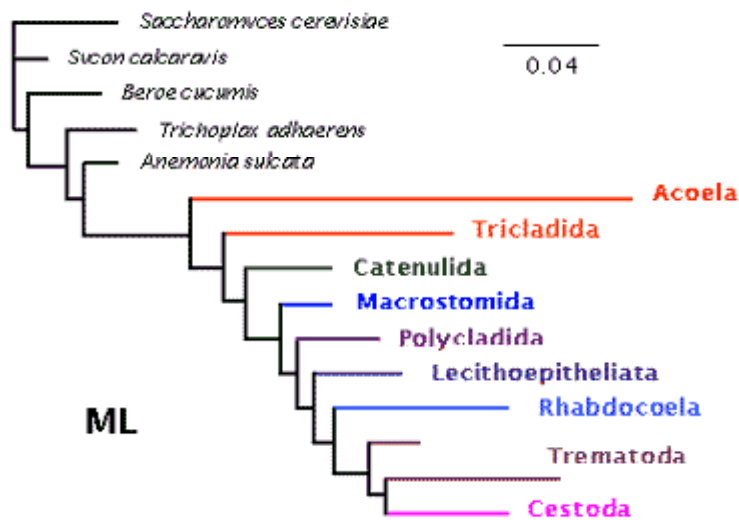
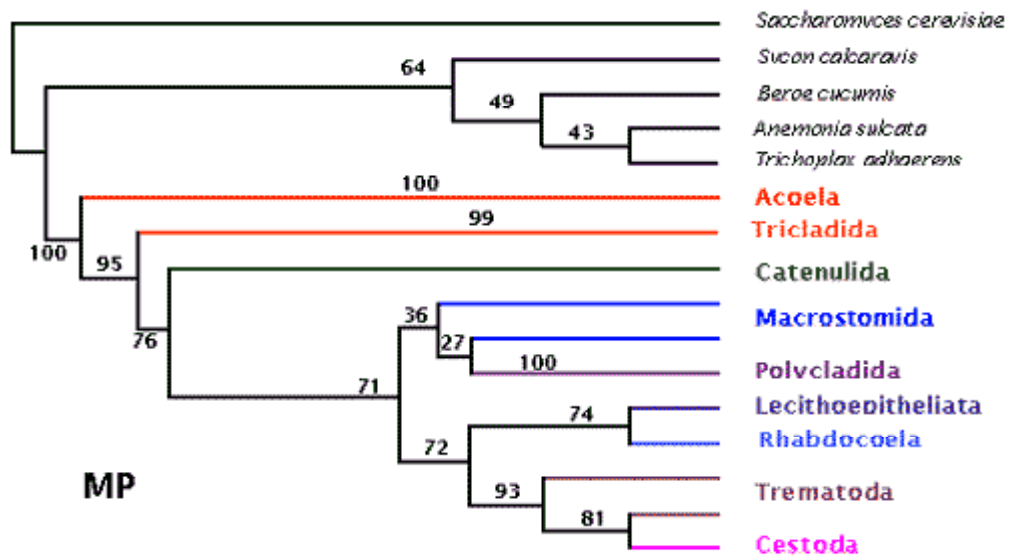
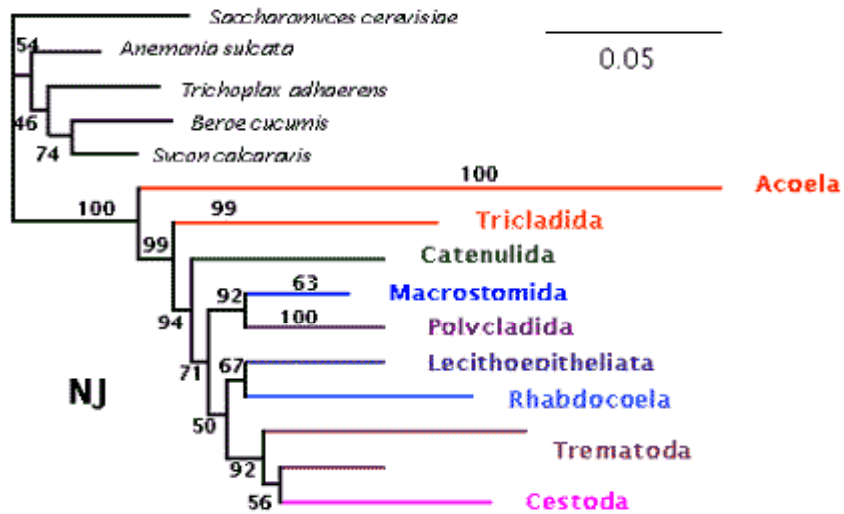


Figure 11. Phylogenetic trees in Figure 3 with horizontal branches. (a) The unscaled tree rooted as in Figure 10a. (b) The scaled tree rooted as in Figure 10b. The distance between two taxa is found by measuring along the horizontal branches connecting them, ignoring the lengths of vertical branches, which represent nodes.

Phylogenies reconstructed by different methods are generally similar to each other.

- Figure 12 shows a comparison of phylogenetic analyses of Platyhelminthes using NJ, MP, and ML.

Figure 12 (next page). Phylogenetic trees for Platyhelminthes using the same 18S rDNA sequences analyzed by NJ, MP, and ML, modified from Figure 2 of [Katayama, Nishioka, and Yamamoto \(1996\)](#). Note that the topologies (branching patterns) for the trees produced by the three methods are all similar. For simplicity, branches for individual species were collapsed to one branch for each order. Yeast (*S. cerevisiae*) was used to root the tree, and four diploblasts were used as outgroups. The scales for NJ and ML show the number of base substitutions per sequence position. Small numbers for NJ and MP are [bootstrap values](#) indicating the reliability of each branch. (See next section.) Bootstrapping was not done for ML because of the large amount of computer time required.



Confidence in an internal branch can be tested by bootstrapping.

- **Bootstrapping** is done by randomly sampling the data and replacing them so that some data are ignored and others represented more than once. A new tree is then reconstructed from the pseudoreplicated data. This is typically done hundreds of times, and the percentage of time an internal branch occurs in the trees is the bootstrap value of the branch. A bootstrap value of more than 90% or 95% is regarded as strong support for the branch. Bootstrap values are shown in Figure 12 on the previous page. Note that branches with high bootstrap values, such as the branch for Acoela, occur by all three methods of tree reconstruction.
- In some situations a method called parametric bootstrapping is more appropriate. In parametric bootstrapping, numerical simulation based on a model of evolution is used to produce the pseudoreplicate samples.
- Bootstrapping tests the precision, not the accuracy, of the branch. That is, it indicates the ability of the data to recover the branch, but not whether the branch is correct.
- A similar but less-used procedure is **jackknifing**, in which data are not replaced after sampling and each datum is therefore used only once.

A branch with low bootstrap support may be collapsed.

- **Collapsing** a branch consists of joining the two nodes of the branch (Fig. 13).

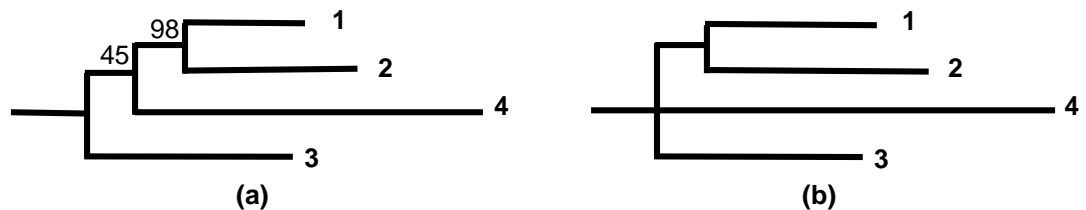


Figure 13. Collapsing branches that are poorly supported. (a) The original tree with one branch having a bootstrap value of only 45%. (b) After collapsing the poorly supported branch there is an unresolved trichotomy for branches (1 + 2), 3, and 4.

A consensus tree can be created by collapsing branches that are not supported in all trees created by different methods of analysis. A consensus tree can also be produced by comparing molecular and morphological trees.

Molecular and morphological data can be combined to create a “total-evidence tree.”

- One difficulty with combining molecular and morphological characters for total-evidence analysis is that the former are typically so abundant that they may overwhelm the morphological characters.

Because of [long-branch attraction](#), differences in sequence [alignment](#), limitations in the size of study groups, and different methods of tree reconstruction, conflicting molecular phylogenies have been proposed. As techniques have improved and more molecules from more species have been sequenced, many of the past conflicts have been resolved.

- Still, one should not accept any phylogenetic tree, whether based on molecules or morphology, at face value. Phylogenetic trees are hypotheses to be tested.
- The advantage of molecular phylogenetics is not that it is infallible, but that it provides a completely independent means of testing morphological hypotheses.
- There is now a broad agreement among molecular phylogeneticists about the main outlines of animal phylogeny.

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II. Testing the Validity of Traditional Morphological Characters by Seeing Whether They Are Consistent With Molecular Trees

To be consistent with a given phylogenetic tree, a character must map onto the tree with few changes in character state.

For example, bilateral symmetry is consistent with the traditional morphology-based cladogram for the Big Nine phyla (those with more than 5,000 named species), since it can be mapped onto the cladogram with only one change in character state.

- The basic phylogenetic outline shown in Figure 14 has been standard since the publication of *The Invertebrates* by Hyman. Hyman's original tree had a "primitive acoel flatworm" as the base of the Bilateria, with Platyhelminthes and Nematoda in the Protostomia. Many authors now make the latter two phyla separate branches basal to the other Bilateria.

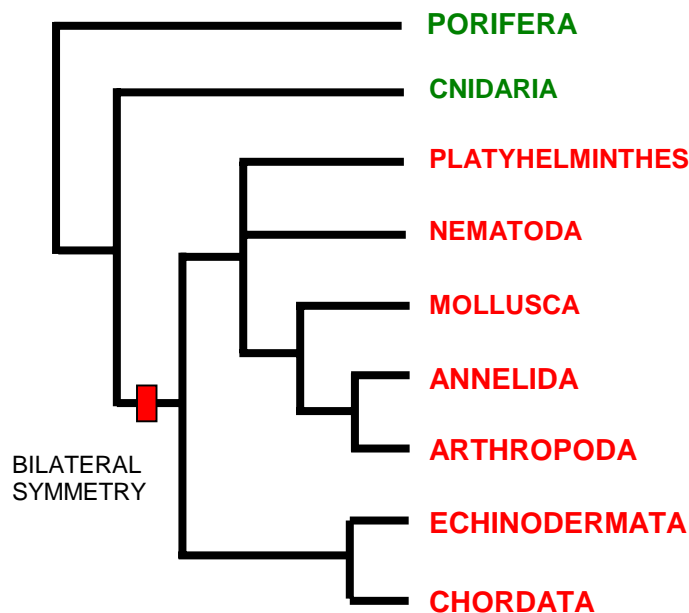


Figure 14. The traditional morphology-based phylogeny of the major animal phyla, based on the "hypothetical diagram" by Hyman (1940, vol. 1, p. 38). Bilateral symmetry is consistent with this phylogeny because it requires only one change in character state to account for its distribution among the phyla.

Segmentation, however, is less consistent with traditional morphology based phylogenetic trees, because it requires at least two changes in character state: one for annelids and arthropods and one for chordates (Fig. 15).

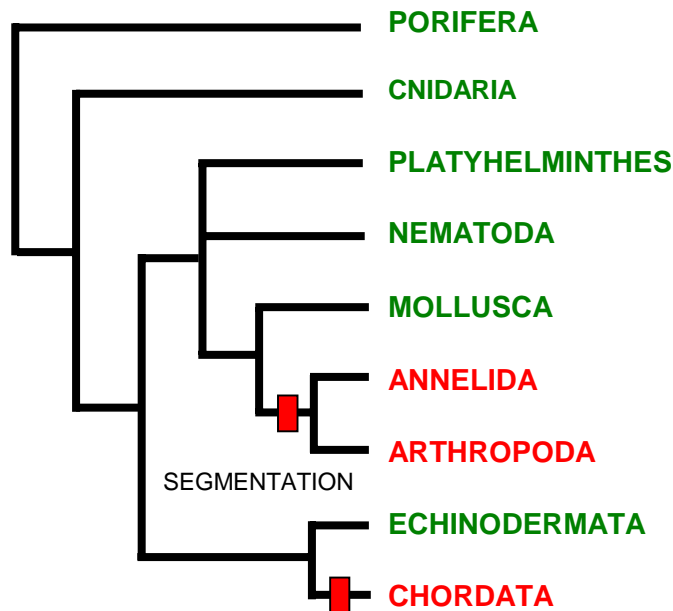


Figure 15. Traditional phylogeny of the Big Nine showing that segmentation is less consistent, because it requires at least two changes in character state to account for its distribution.

Lack of consistency implies that either the character is not synapomorphic (homologous), or the phylogenetic tree is incorrect.

- Similar characters can evolve convergently more than once, as segmentation apparently did in Arthropoda and Chordata. Convergent evolution results in analogous characters that are not indicative of phylogeny. In the language of cladistics, such an evolutionary convergent character is a **homoplasy**. A homoplasy is not a **synapomorphy** (shared, derived, homologous character), which is the only kind of character that is useful in cladistics.
- If analysis indicates that an incharacter is nevertheless a synapomorphy, the tree is less likely to be correct, because it does not provide a parsimonious explanation for the evolution of the character. A different tree should be tried.
- These same principles apply to molecular characters as well as to morphological characters.

A character that is consistent with both a morphological and a molecular phylogeny is more likely to be phylogenetically informative.

- A morphological character is not likely to be useful if it is not consistent with either a tree based on other morphological characters or one based on molecules.

Morphological characters have not led to a consensus phylogeny.

- The use of different characters and methods of analysis have also resulted in numerous different proposed phylogenies (Jenner and Schram 1999). For example, Willmer (1990), like Hyman, used a noncladistic approach in which one or a few striking characters, such as the fate of the blastopore, were heavily weighted. Nielsen (1995) and other cladists give equal weight to a larger number of characters. Pechenik (2000, pp. 18-20) provides a sampling of different trees, and Eernisse et al. (1992) show a dozen variations gleaned from textbooks.
- Hyman's (1940, vol. 1, pp. 37-38) phylogenetic tree of Animalia has a trunk from which Protozoa, Mesozoa, Porifera, and Radiata sprout before giving rise to the Bilateria, as outlined in Figures 14 and 15. She recognized 22 phyla (listed below in bold face with some spellings changed) and arranged them in order of complexity:

Subkingdom Protozoa

Protozoa

Subkingdom Metazoa

Branch A. Mesozoa

Mesozoa

Branch B. Parazoa

Porifera

Branch C. Eumetazoa

Grade I. Radiata

Cnidaria

Ctenophora

Grade II. Bilateria

A. Acoelomata

Platyhelminthes

Nemertea

B. Pseudocoelomata

Aschelminthes (Rotifera, Gastrotricha,
Kinorhyncha, Nematoda, Nematomorpha,
Acanthocephala)

Entoprocta

C. Eucoelomata

1. Schizocoela

Ectoprocta

Phoronida

Brachiopoda

Mollusca

Sipuncula

Priapulida

Echiurida

Annelida

Arthropoda

2. Enterocoela

Chaetognatha

Echinodermata

Hemichordata

Chordata

- [Willmer \(1990, p. 361\)](#) presented a morphology-based tree that she characterized as “undoubtedly wrong” but a useful summary of her conclusions from the morphological evidence. Her summary tree could be shown in a cladogram of the Big Nine phyla that differs from Figures 14 and 15 mainly in that each of four lineages for Nematoda, Mollusca, Annelida, and Echinodermata plus Chordata diverges from a flat-worm-like ancestor. In addition, she divided Arthropoda into three phyla, with only Uniramia allied to Annelida. Her tree for the 36 phyla that she recognized can be summarized as follows:

Descendants of “planulas”

Porifera

Placozoa

Mesozoa

Cnidaria

Ctenophora

“Acoelomate **Platyhelminthes**” (polyphyletic)

Lines of descent from “acoelomate Platyhelminthes”

Gastrotricha, Nematoda, Nematomorpha

Rotifera, Acanthocephala

Entoprocta

Nemertea

Mollusca

Sipuncula

Pentastomida

Tardigrada

Echiura, Pogonophora, Onychophora, Annelida, Uniramia

Pycnogonida

Chelicerata

Crustacea

Loricifera

Kinorhyncha

Priapulida

Ectoprocta, Phoronida, Brachiopoda

Hemichordata, Echinodermata, Chordata

Chaetognatha

Gnathostomulida

- A morphology-based cladogram for the Big Nine phyla derived from [Nielsen's \(1995, p. 6\)](#) summary cladogram is similar to Figures 14 and 15 except that Nematoda is in a separate line from the one in which Platyhelminthes then Mollusca, Annelida, and Arthropoda branch. His cladogram showing the 31 phyla he recognized (in boldface) is summarized in the following slightly simplified indented list:

Animalia

Porifera

Placozoa

Eumetazoa

Cnidaria

Bilateria

Protostomia

Aschelminthes

Rotifera, Acanthocephala

Chaetognatha

Cycloneuralia

Gastrotricha

Nematoda, Nematomorpha

Priapulida

Kinorhyncha

Loricifera

Spiralia

Parenchymia

Platyhelminthes

Nemertea

Bryozoa

Entoprocta

Ectoprocta

Teloblastica

Sipuncula

Mollusca

Annelida

Onychophora

Arthropoda

Tardigrada

Protornaeozoa

Ctenophora

Deuterostomia

Phoronida, Brachiopoda

Pterobranchia, Echinodermata

Cyrtotreta

Enteropneusta

Chordata

Urochordata

Cephalochordata

Vertebrata

The following morphological characters traditionally used in phylogenetics are also consistent with the widely accepted molecular phylogenetic tree of the Big Nine Phyla: bilateral symmetry and triploblasty, deuterostomy, and spiral cleavage pattern.

- Figure 16 summarizes the currently accepted molecular phylogeny for the Big Nine phyla. It differs from the traditional phylogeny in Figures 14 and 15 mainly in dividing the protosomes into two distinct clades, with one including Platyhelminthes, Mollusca, and Annelida, and the other including Nematoda and Arthropoda.

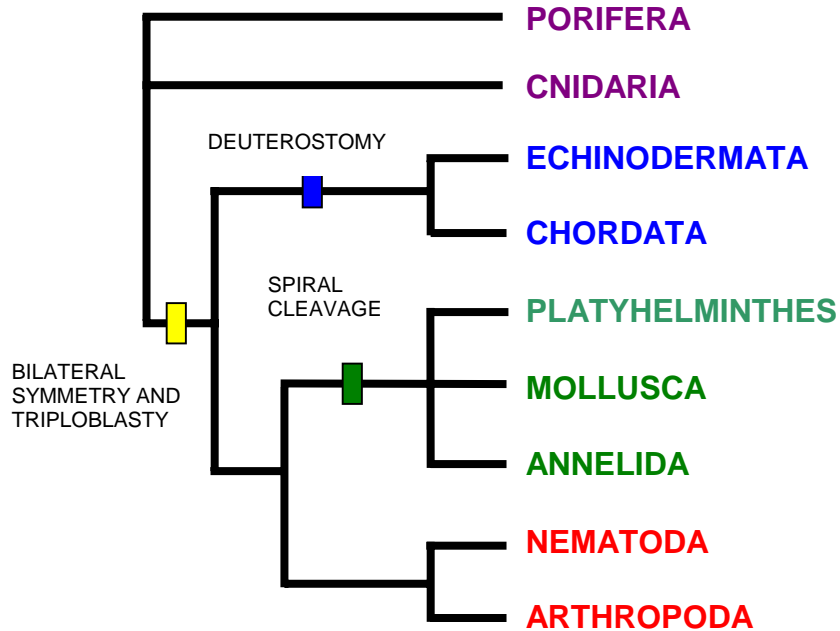


Figure 16. Bilateral symmetry and triploblasty, deuterostomy, and spiral cleavage pattern are consistent with the molecular phylogenetic tree of the Big Nine Phyla.

Bilateral symmetry and triploblasty are also consistent with a molecular phylogenetic tree that includes all animal phyla.

- HISTORY: In the first volume of *The Invertebrates* (1940, pp. 32-39), [Hyman](#) placed the Bilateria in a grade above the Radiata (Cnidaria and Ctenophora), which were in turn above the Porifera. She eschewed the term “diploblast,” noting that sponges do not develop from two germ layers and that all except Hydrozoa have a middle layer of cells that could be called tissue.
- RECENT MORPHOLOGICAL STUDIES: [Willmer \(1990\)](#) rejected not only the diploblast/triploblast dichotomy, but also the Radiata/Bilateria distinction, noting that most sponges and placozoans are asymmetric, and many cnidarians and especially ctenophorans tend toward bilateral symmetry. Nevertheless, she (1990, p. 361) placed Porifera, Cnidaria, and other traditional “Radiata” or “Diploblasts” below a flatworm-like ancestor of the bilaterally symmetric animals. [Nielsen \(1995, p. 64\)](#) also concluded that bilateral symmetry is “a highly questionable synapomorphy of the bilaterians,” but for different reasons he (p. 72) considered it “reasonable to regard the Bilateria as a monophyletic group and as the sister group of the Cnidaria.” His Bilateria includes Ctenophora as the sister group of Deuterostomia (p. 307).
- As shown in Figure 17 (next page), a tree based mainly on 18S rDNA supports the traditional view that “diploblasts” or “radiata” are basal to the bilateria.
- This molecular phylogenetic tree is a composite of many separate studies, some of which will be discussed in Part III. It recognizes 30 phyla. Mesozoa are included within Platyhelminthes, and Echiurida and Pogonophora are included within Annelida. See Figure 1.B of [Adoutte et al. \(2000\)](#) and Figure 2 of [Zrzavý et al. \(1998\)](#) for somewhat different molecular phylogenetic trees of all phyla. It may prove convenient to print a copy of Figure 17 for reference in later discussion.
- The Bilateria are also recovered as a monophyletic clade in molecular phylogenies based on 5S rDNA ([Hori and Osawa 1987](#)) and 28S rDNA ([Christen et al. 1991](#)).

Deuterostomy in Echinodermata, Hemichordata, and Chordata is also consistent in a molecular phylogenetic tree of all animal phyla.

- HISTORY: [Hyman \(1951, vol. 2, p. 5\)](#) accepted the then-prevailing view that deuterostomes (Chaetognatha, Echinodermata, Hemichordata, and Chordata) were a heterologous assemblage of groups that were not closely related. She considered the lophophorates (Ectoprocta, Phoronida, and Brachiopoda) to be intermediate between protostomes and deuterostomes.
- RECENT MORPHOLOGICAL STUDIES: [Willmer \(1990, p. 349\)](#) rejected the protostome/deuterostome dichotomy, but she agreed that Hemichordata, Echinodermata, and Chordata (branching in that order) represented a monophyletic lineage that did not include the lophophorates. [Nielsen \(1995, pp. 76-77\)](#) regarded the fate of the blastopore as an unreliable character, but using other characters, he (p. 62) divided the Bilateria into the two clades Protostomia and Protornaeozoa (Ctenophora plus Deuterostomia). His Deuterostomia clade included hemichordates, echinoderms, chordates, Phoronida, and Brachiopoda, but not Ectoprocta (p. 333).
- If the problematic Chaetognatha are excluded, the molecular tree based on 18S rDNA ([Fig. 17](#)) suggests that the traditional deuterostomes are in fact monophyletic. Chaetognaths, as well as lophophorates, will be discussed in more detail later in Part III.

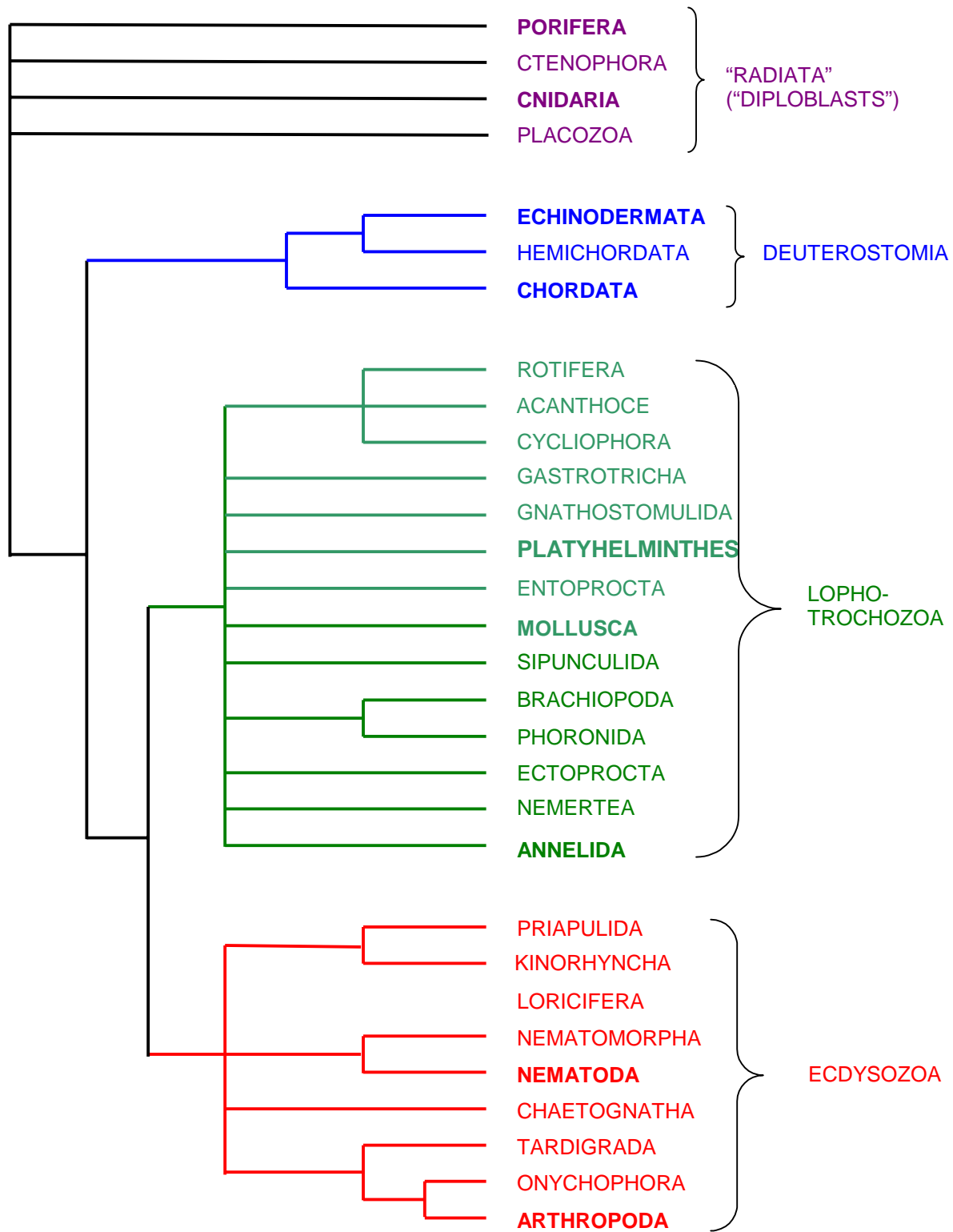


Figure 17. Summary tree from molecular phylogenetic studies of animal phyla. (There are no molecular data for Loricifera.)

The spiral-cleavage pattern is somewhat consistent with a molecular phylogenetic tree that includes all animal phyla.

- HISTORY: Hyman recognized the importance of the cleavage pattern, but she gave more weight to the schizocoelous versus enterocoelous origin of the coelom. She appears not to have regarded “Spiralia” as a distinct group.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, pp. 128-129) cautiously recognized a “core group of spiralian... including certain polyclads, nemerteans, annelids, uniramian arthropods, pogonophorans, echiurans, sipunculans and molluscs.” However, she (p. 268) regarded the molluscs as pseudocoelomates with little in common with other phyla. Nielsen (1995, p. 96) considered it best to treat the Spiralia as the sister clade of Aschelminthes within Protostomia. His Spiralia comprised Sipuncula, Mollusca, Annelida, Onychophora, Arthropoda, Tardigrada, Entoprocta, Platyhelminthes, and Nemertea.
- Molecular phylogenetics (Fig. 17) supports the existence of a clade in which the spiral cleavage pattern may be plesiomorphic (primitive). However, this clade also includes lophophorates, which generally have radial cleavage. It also includes flatworms, which have spiral cleavage but are often not included with coelomate spiralian. It does not include onychophorans, tardigrades, or arthropods. These groups will be discussed in more detail in Part III.

The lophophore by itself is not consistent with the molecular phylogenetic tree, and it may not be a homology.

- HISTORY: Hyman (1959, vol. 5, p. 229) defined the lophophore as “a tentaculated extension of the mesosome that embraces the mouth but not the anus and has a coelomic lumen.” She considered it a homologous character uniting Ectoprocta, Phoronida, and Brachiopoda as lophophorates.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 349) considered the lophophorates to be a “rather close-knit assemblage.” Nielsen (1995, p. 183) found no synapomorphy uniting the ectoprocts with the phoronids and brachiopods. He dismissed the lophophore as nonsynapomorphic, because numerous other characters linked Ectoprocta to Spiralia and Phoronida and Brachiopoda to Deuterostomia.
- Molecular studies (Fig. 17) place the “lophophorates” in the clade of protostomes that includes Platyhelminthes, Mollusca, and Annelida. The lophophorates do not appear to be monophyletic within that clade, however, because Phoronida and Brachiopoda may be more closely related to each other than to Ectoprocta.

The occurrence and type of body cavity (whether the animal is acoelomate, pseudocoelomate, or coelomate) is not consistent with the molecular phylogenetic tree and is not homologous.

- **HISTORY:** Hyman (1940, vol. 1, p. 35), following Schimkevitch (1891), was primarily responsible for the distinction among acoelomates, pseudocoelomates, and coelomates. “Such a division,” she wrote, “stands firmly on a realistic anatomical basis and eschews all theoretical vaporizings....” She dismissed the once-common view that acoelomates and pseudocoelomates originated from coelomates. This “Archeocoelomate Theory” has been revived in recent times, but it is not widely accepted or even familiar among American zoologists. (See [Willmer 1990](#), pp. 33-37 for discussion.) Support for it comes from the fact that the pseudocoel can form by loss of the peritoneum or part of the mesoderm enclosing a coelom, among other ways ([Maggenti 1976](#)). In addition, some nematodes, all leeches, and some other presumptive coelomates and pseudocoelomates have secondarily become acoelomate, showing that a coelomate-to-pseudocoelomate or coelomate-to-acaelomate evolutionary sequence is at least conceivable. All of this casts doubt on the homology of the acoelomate and pseudocoelomate conditions.
- **RECENT MORPHOLOGICAL STUDIES:** Willmer’s (1990, p. 22-38) review of body cavities led her to the conclusion that “it may well be that—contrary to most of the simple invertebrate textbooks—the body cavities of animals are amongst the most misleading of all possible characters.” She (p. 246) concluded that the pseudocoelomates (including molluscs; p. 268) were polyphyletic derivatives of several acoelomate lines. Nielsen (1995) saw “nothing to indicate that the acoelomate condition is ancestral” or “that the various coeloms are homologous” (p. 65), and he opined that the pseudocoelomate versus coelomate distinction had been “strongly overemphasized” (p. 235). He rejected, however, the idea that the acoelomate and pseudocoelomate conditions were derived from coelomate ancestors (p. 236). A cladistic analysis by [Wallace et al. \(1996\)](#) also indicated that pseudocoelomates were polyphyletic, with one clade comprising Rotifera and Acanthocephala and another comprising two lesser clades: (Nematoda + Nematomorpha) and (Kinorhyncha + Loricifera + Priapulida).
- Molecular phylogenetic studies ([Fig. 17](#)) support the nontraditional view that the pseudocoel is apomorphic (derived) with respect to the coelom. The traditional pseudocoelomates (including Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Rotifera, and Entoprocta) are scattered in two distinct clades, each of which also includes coelomates. In addition, the outgroup of these two clades, Deuterostomia, is coelomate. Thus the most parsimonious hypothesis is that the coelom is plesiomorphic in all Bilateria, and the pseudocoel is derived from it.
- Molecular phylogenetic studies also suggest that the acoelomates are derived from coelomates. “Acoelomates” (including Platyhelminthes and Gnathostomulida) occur within a clade that mostly comprises coelomates. The most parsimonious hypothesis is that the acoelomate condition evolved from a coelomate, not that coeloms evolved from acoelomates many times in these other phyla.
- Acoelomates may, however, be monophyletic within this clade ([Giribet et al. 2000](#)).

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III. Molecular Phylogenetic Trees as Alternative Hypotheses

As noted before, if a character is not consistent with one phylogenetic tree, then a molecular phylogenetic tree provides an alternative hypothesis that may better explain the evolution of the character.

Protozoa are not monophyletic.

- HISTORY: [Hyman](#) (1940, vol. 1) considered Protozoa to be a phylum of “acellular” animals. With the widespread acceptance of the Five-Kingdom System, however, Protozoa were moved from the kingdom Animalia to the kingdom Protista, together with algae. This kingdom would be paraphyletic under any hypothesis that animals evolved from a protozoan. It soon became apparent that the protozoa were so diverse that they comprised several, if not dozens, of phyla. In 1980 a committee of the Society of Protozoologists ([Levine et al. 1980](#)) proposed a tentative classification that admittedly did not reflect phylogeny. This scheme divided protozoa into seven phyla, including Apicomplexa, Ciliophora, Sarcomastigophora (sarcodines and flagellates), and Myxozoa.
- Molecular phylogenetics confirms that “protozoa” are polyphyletic, belonging to numerous separate branches within the domain Eucarya (Fig. 18, next page). The flagellates in particular are distributed widely among several clades as follows:
- *Giardia* and *Trichomonas* belong to separate clades near the base of the domain Eucarya ([Baroin et al. 1988](#); [Hasegawa et al. 1993](#); [Sogin et al. 1986](#); [Sogin et al. 1989](#); [Yamamoto et al. 1997](#)).
- Dinoflagellates appear to be closely related to ciliates and apicomplexans ([Wolters 1991](#)). The clade Alveolata that comprises these groups is supported by a variety of molecular data. (See [Baldauf et al. 2000](#), Fig. 2 for a summary of support for this and other eukaryotic clades. See [Patterson 1999](#) for a useful guide to eukaryotic groups.)
- *Volvox* is a green alga more closely related to plants than to animals ([Baldauf et al. 2000](#); [Rausch et al. 1989](#)). (The custom of including *Volvox* in zoology courses is simply a relic of Haeckel’s blastea theory.)
- Choanoflagellates are closer to metazoans than to other protozoans, as will be discussed shortly ([Wainright et al. 1993](#)).
- Plantae (including some algae), Fungi (excluding slime molds and some others), and Animalia (including choanoflagellates) form a monophyletic clade at the tip of the Eucarya. The name Metakaryota has been proposed for this clade.

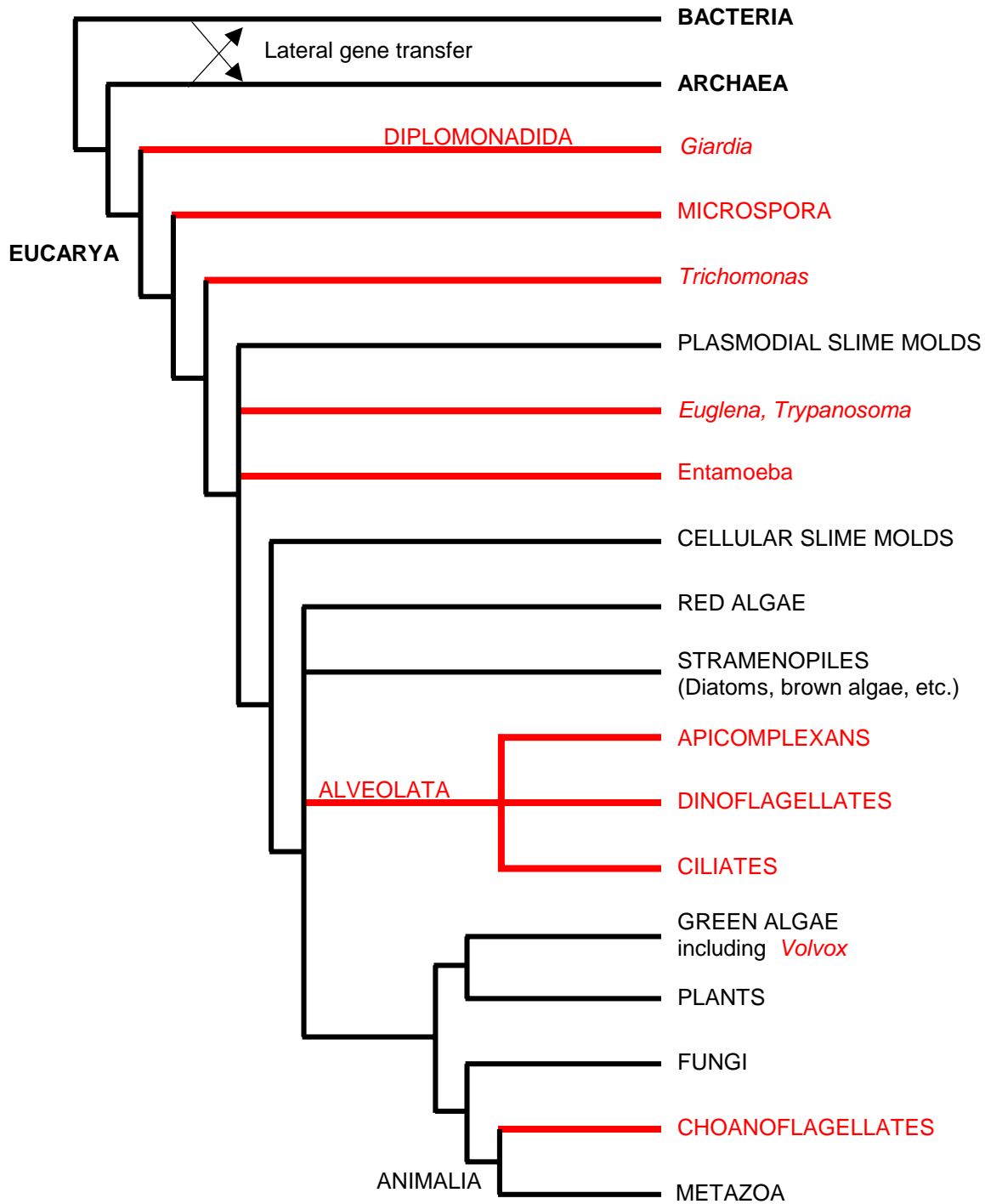


Figure 18. Molecular phylogeny of eukaryotes showing the polyphyly of protozoans. See Baldauf et al. (2000) for a somewhat different cladogram based on protein sequences.

Metazoans are monophyletic, and choanoflagellates may be their sister group.

- HISTORY: Hyman, writing when there were only two or three kingdoms, appears never to have doubted that Animalia, including protozoa, was monophyletic. She noted that “many zoologists believe the presence of choanocytes in sponges can only be interpreted to indicate the direct descent of sponges from Choanoflagellata,” and that the colonial choanoflagellate *Protospongia* is a link between choanoflagellates and sponges (Hyman 1940, vol. 1, pp. 358, 107).
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990), after reviewing the numerous theories on the origin of metazoans (pp. 165-187), concluded (p. 196) that “the best we can do is to remain agnostic, but with suspicions of polyphyly.” Nielsen (1995, p. 27), however, concurred with the traditional view that “the Animalia is a monophyletic group, and the specific characters shared with the choanoflagellates make it natural to consider the two groups as sister groups.”
- The conclusion by Field et al. (1988) that Cnidaria had a separate origin from the other metazoa was shown by (Lake 1990) to be due to long-branch attraction. Subsequent analyses of 18S rDNA indicate that metazoans are monophyletic.
- Analysis of 18S rDNA indicates that choanoflagellates are sister to the monophyletic Metazoa (Wainright et al. 1993). Choanoflagellata are now sometimes included in the clade Animalia (Fig. 18, previous page).

Myxozoans appear to be metazoans rather than protozoans.

- HISTORY: Myxozoan parasites, such as the species responsible for “whirling disease” in salmonids, have long been considered to be protozoans. Although their infective stage is multicellular, they are extremely small and apparently without cellular differentiation, gametes, or blastula. The occurrence of nematocysts in the infective stage, however, has led to speculation for more than a century that they are related to Cnidaria. (See Siddall and Whiting 1999 for references.)
- Analyses of 18S rDNA indicate that Myxozoa is most likely derived from a metazoan near the base of the Bilateria (Smothers et al. 1994). Siddall et al. (1995) concluded from both 18S rDNA and morphology that Myxozoa are cnidarians related to the parasitic narcomedusan *Polypodium*. The myxozoan sequences all have high rates of evolution, however, so it has been argued that the result could have been due to long-branch attraction. Siddall and Whiting (1999) have vigorously defended their conclusion against this charge, showing that the position of Myxozoa remains the same even when *Polypodium* is not included.
- Cavalier-Smith et al. (1996) tentatively accepted the 18S rDNA evidence that Myxozoa were derived from Cnidaria. Unable to decide whether they evolved from a bilaterian intermediate or directly from a cnidarian, they made Myxozoa a separate subkingdom, along with Radiata, Mesozoa, and Bilateria.

Mesozoans may be flatworms.

- HISTORY: Hyman (1959, vol 5, p. 714) lamented that most zoologists persisted in considering Mesozoa to be degenerate flatworms 19 years after she had argued that they were a distinct phylum. Her view appears finally to have triumphed, however. Because of their simple construction, mesozoans are usually considered to be a distinct phylum of a grade somewhere between that of Porifera and Platyhelminthes. Some zoologists, however, point to their complex life cycles as evidence that they derive from flatworms. Many consider mesozoans to be not merely one phylum, but two: Orthonectida and Rhombozoa (= Dicyemida).
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 351) acknowledged that mesozoans “might yet prove to be ‘degenerate flatworms,’” but she thought it more plausible that they evolved in parallel with other bilaterians from a flatworm-like ancestor. Nielsen (1995, p. 436) merely noted the orthonectids and dicyemids as enigmatic groups and did not include them in his cladogram.
- Two studies based on 18S rDNA suggested that orthonectids are not closely related to dicyemids, and that Mesozoa is therefore polyphyletic (Hanelt et al. 1996; Pawlowski et al. 1996). Different analyses, however, suggest that Mesozoa is a monophyletic clade (Siddall and Whiting 1999; Winnepenninckx, Van de Peer, and Backeljau 1998).
- Studies based on 18S rDNA (Katayama et al. 1995; Van de Peer and De Wachter 1997) and Hox-gene sequences (Kobayashi, Furuya, and Holland 1999) support a close relationship between dicyemids and flatworms.

Anthozoa appear to be basal within Cnidaria.

- HISTORY: Hyman (1959, vol. 5, pp. 750-753) scarcely veiled her contempt for the notion that the “advanced” Anthozoa were basal to Scyphozoa and Hydrozoa.
- RECENT MORPHOLOGICAL STUDIES: It is now obvious that one cannot infer the ancestral position of a group from the perceived “grade” of its extant members. Nielsen (1995, p. 58) considered the Anthozoa to be the basal clade of Cnidaria, followed by Scyphozoa then Cubozoa and Hydrozoa.
- Bridge et al. (1992) found that in Anthozoa the mitochondrial DNA is circular, as in Ctenophora and most other organisms, but that in the other classes of Cnidaria mtDNA is linear. From this they concluded that Anthozoa is the most basal class of Cnidaria. Bridge et al. (1995) subsequently found that 18S rDNA sequences and morphological characters also support the placement of Anthozoa at the base of the Cnidaria, with Hydrozoa, Scyphozoa, and Cubozoa in an unresolved trichotomy.
- This result suggests that the polyp-only life cycle is plesiomorphic in Cnidaria, and the medusa is apomorphic.

Protostomes appear to be divided into two major clades: Ecdysozoa and Lophotrochozoa. Annelida and Arthropoda belong to Lophotrochozoa and Ecdysozoa, respectively, and are therefore not closely related.

- HISTORY: Hyman's "hypothetical diagram" (see [Fig. 14](#)) placed all the protostomes except Nemertea, Aschelminthes, and Platyhelminthes in a single lineage with Annelida closer to Arthropoda than to Mollusca. Her list of [schizocoelous eucoelomates](#) did not separate Arthropoda from Mollusca and Annelida. Hyman (vol. 1, 1940, p. 38) and many authors since have assumed a close relationship between Annelida and Arthropoda because of several shared features, including segmentation and a paired ventral nerve cord.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990) divided the protostomes into numerous lines rather than major groups, as shown in the [summary table](#). She (p. 298) concluded that the so-called uniramian arthropods were derived from "a proto-annelid group," but other arthropods were not. A cladistic analysis led [Eernisse, Albert, and Anderson \(1992\)](#) to conclude that Arthropoda are not in the same major clade (Eutrochozoa) with Annelida. Nielsen (1995) grouped Arthropoda, Annelida, and Mollusca together in Teloblastica within his Spiralia, as shown in the [indented list](#) above. He placed the clade Panarthropoda (Arthropoda, Tardigrada, and Onychophora) as the sister group to Annelida.
- Among the earliest and most robust conclusions from 18S rDNA studies is that Arthropoda are in a clade separate from Annelida and Mollusca ([Field et al. 1988](#); [Lake 1990](#)). As sequences from other protostomes were studied, they generally grouped with one or the other of the two clades ([Fig. 17](#)).
- Comparison of [Hox genes](#) supports the conclusion that protostomes divide into these two clades ([De Rosa et al. 1999](#)).

Ecdysozoa, the major protostomate clade that includes Arthropoda, also includes Nematoda and other groups with a cuticle that molts all at once.

- HISTORY: Hyman (1959, vol. 5, p. 745) did not consider Nematoda to be a separate phylum, but a class within the phylum Aschelminthes, along with other pseudocoelomates except Entoprocta. She regarded the aschelminths as being in a separate line of Protostomia from that of Arthropoda. Many authors, following [Hyman's list of phyla](#), consider the Nematoda and other "aschelminths" to be on a branch beneath the coelomates.
- RECENT MORPHOLOGICAL STUDIES: As noted [previously](#), there has been considerable doubt over the usefulness of the pseudocoel as a character. Nematodes have therefore wandered over the phylogenetic tree. Willmer (1990, pp. 245-246) cautiously suggested that Nematoda, Nematomorpha, and Gastrotricha are distinct phyla related to each other in a line that descended from acoelomates independently from the lines of other "aschelminths" and arthropods, as shown in the [summary list](#) above. Nielsen (1995, p. 234) included Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Loricifera, Rotifera, Acanthocephala, Gastrotricha, and Chaetognatha as phyla in the clade Aschelminthes. He included the phylum Arthropoda in the Spiralia, which he made the sister of Aschelminthes in the Protostomia. (See [indented list](#).) Another cladistic analysis by [Wallace et al. \(1996\)](#), however, found that Nematoda, Nematomorpha, Kinorhyncha, Priapulida, and Loricifera were in a clade separate from that of Rotifera and Acanthocephala, and that the "pseudocoelomates" were derived from one or more coelomate ancestors.
- Phylogenies based on 18S rDNA sequences (for example, [Winnepenninckx et al. \(1995\)](#)) generally show "pseudocoelomates" divided between the two protostomate clades, with Rotifera and Acanthocephala in the clade with Platyhelminthes, Annelida, and Mollusca, while Nematomorpha and Priapulida are in the clade with Arthropoda. Nematoda often appears at the base of the other Bilateria.
- However, 18S rDNA has evolved too rapidly in most nematodes to provide a signal free of [long-branch attraction](#). By using only the nematode sequence with the slowest rate of base substitution (from *Trichinella*), [Aguinaldo et al. \(1997\)](#) grouped Nematoda with Arthropoda, Nematomorpha, Kinorhyncha, and Priapulida. Because all these groups share the feature of molting the cuticle all at once, they named the clade Ecdysozoa ([Fig. 17](#)).
- A different study by [Aleshin et al. \(1998\)](#) also found the slowly evolving 18S rDNA sequence from *Enoplus* grouping with Arthropoda. However, there was only weak support for a clade that also includes Nematomorpha, Kinorhyncha, and Priapulida. Two subsequent analyses based on both 18S rDNA sequences and morphology support both the inclusion of Nematoda in the same clade with Arthropoda and the validity of Ecdysozoa as originally defined ([Giribet et al. 2000](#); [Zrzavý et al. 1998](#)).
- Independent support for Ecdysozoa comes from the fact that drosophila, an onychophoran, a priapulidan, and *Caenorhabditis elegans*, but not flatworms, molluscs, or annelids, share the [Hox genes](#) *Ubx* and *Abd-B* ([De Rosa et al. 1999](#)).

Arthropoda is monophyletic; Uniramia is not valid.

- **HISTORY:** The monophyly of Arthropoda was virtually unquestioned until Sydney Manton proposed in the 1960s that arthropods comprise three different phyla: Chelicerata, Crustacea, and Uniramia (Onychophora + Myriapoda + Insecta). Her conclusion was based on the differences among these groups rather than a cladistic analysis of shared, derived homologies. Her anatomical studies led her to conclude that the mandibles of crustaceans develop from the bases of appendages, while those of insects and myriapods develop from entire appendages. She also concluded that the appendages of crustaceans are primitively biramous, while those of insects and myriapods are uniramous. Manton's proposal requires that the overall arthropodan body plan, including a chitinous exoskeleton, segmentation, and ventral nerve cord, would have evolved independently three times. Consequently, few zoologists accepted the proposal of three arthropodan phyla, but many did accept the clade Uniramia (minus Onychophora) as a taxon within Arthropoda.
- **RECENT MORPHOLOGICAL STUDIES:** Willmer (1990, chap. 11) accepted Manton's proposal as follows: "uniramians may be derived from a proto-annelid group, whilst crustaceans probably diverged from the stem spiralian earlier, from a flatworm-like stage; chelicerate origins are still enigmatic." The paleontologist [Jarmila Kukulová-Peck \(1992\)](#) challenged the concept of Uniramia by pointing out that numerous fossil and living crustaceans and insects have polyramous appendages. She also noted that Manton's evidence for "whole-leg mandibles" was from studies of myriapods and onychophorans but not insects. Nielsen (1995, pp. 171, 173) listed a number of synapomorphies that "clearly demonstrate that the Arthropoda are a monophyletic group."
- The expression of [homeotic](#) genes during development shows that insect mandibles develop from only a limb base, as in crustacea, contradicting the whole-leg-mandible hypothesis ([Popadić et al. 1996](#)). Several different molecular-phylogenetic studies (summarized in [Regier and Shultz 1997](#)) also suggest that crustaceans are closer to insects than myriapods are, making Uniramia paraphyletic.
- A study using 12S rDNA sequences supported the monophyly of Arthropoda ([Ballard et al. 1992](#)). Another study combining evidence from 18S rDNA and ubiquitin sequences, as well as morphology, also concluded that Arthropoda are monophyletic ([Wheeler, Cartwright, and Hayashi 1993](#)). Arthropodan monophyly is also supported by evidence from the order of genes in mitochondria ([Boore et al. 1995](#)).
- Although it is clear that Arthropoda are monophyletic, relationships within Arthropoda remain unresolved. Among the disputed conclusions from these studies are the findings that Onychophora belong in Arthropoda, Crustacea are polyphyletic, and Insecta branches from within Crustacea.

Pentastomids are crustaceans.

- HISTORY: The unique body plans of adult tongue worms led to the erection of the phylum Pentastomida. Since the 1970s, however, similarities of sperm morphology, embryology, and cuticle have suggested that pentastomids are crustaceans.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, pp. 298-299) continued to accord Pentastomida the status of a separate phylum derived from “proto-platyhelminthes” in parallel with Crustacea. Nielsen (1995, p. 164), however, considered pentastomids to be crustaceans.
- [Abele, Kim, and Felgenhauer \(1989\)](#) found molecular evidence that Pentastomida are crustaceans. Like rhizocephalans, they appear to be barnacles that are highly adapted for parasitism.

Lophotrochozoa, the major protostomate clade that includes Mollusca and Annelida, also includes other animals with trochophore larvae, the lophophorates, and all descendants from their most recent common ancestor.

- HISTORY: Hyman (1951, vol. 2, p. 16) cautiously accepted the view that “the trochophore is indeed a reminiscence of the common ancestor of the eucoelomate Protostomia and perhaps also of the pseudocoelomate groups.” Many protostomate larvae with little or no resemblance to a trochophore have been said to be modified trochophores, but the phyla in which a trochophore larva is evident are Mollusca, Sipunculida, Annelida, Pogonophora, Echiurida, and perhaps Cyclophora, Entoprocta, and Rotifera. Hyman (1959, vol. 5, p. 600) stated that “the common possession of a lophophore of similar anatomical and histological construction and similar positional relation to the body certainly proves an affinity between Phoronida, Ectoprocta, and Brachiopoda, but it is impossible to define this affinity in specific terms.” She (pp. 603-605) placed the lophophorates among the Protostomia, based on their supposed trochophore larvae. Because of the enterocoelous origin of the coelom in brachiopods and other similarities to deuterostomes, however, Hyman concluded that the lophophorates were “a connecting link between the Protostomia and the Deuterostomia, but the details of this connection cannot be stated.” It is now known that lophophorates have either direct development or larvae that are not trochophores. It is also known that except in Phoronida the mouth does not originate from the blastopore, and most lophophorates have a radial cleavage pattern. As a result of these findings, many authors since the 1970s have considered the lophophorates to be deuterostomes.
- RECENT MORPHOLOGICAL STUDIES: Although Willmer (1990, p. 355) noted nearly as many characters linking lophophorates to protostomes as to deuterostomes, she considered it “sensible to keep the lophophorates as a quite separate super-phylum of tripartite coelomates, linked to deuterostomes just above the acoelomate flatworms... from which they can most readily be derived.” (See [summary list](#).) Willmer (p. 121) noted the flexibility with which the term “trochophore” has been used and concluded that “the point may have been reached where the trochophore seems to be almost totally devalued, and is a useless catch-all term.” Nielsen (1995) found several synapomorphies uniting the Ectoprocta with Entoprocta as a clade in his Spiralia (p. 206), and several synapomorphies placing the Phoronida and Brachiopoda in Deuterostomia (p. 333). Thus he did not regard the lophophorates as a natural (monophyletic) group. (See [indented list](#).) He (p. 86) regarded the trochophore larva as one of the apomorphies defining the Protostomia (Spiralia plus Aschelminthes), even though its occurrence is scattered.
- One of the earliest results of 18S rDNA analyses is that Mollusca, Annelida, Sipunculida, Pogonophora, and Brachiopoda belong to a clade within the protostomates that is distinct from that of the Arthropoda ([Lake 1990](#).) Later studies showed that the clade includes other phyla with trochophore larvae as well as the other two lophophorate phyla, Phoronida and

Ectoprocta. 18S rDNA analyses also suggested that the “lophophorates” were not monophyletic within this clade, since only Brachiopoda and Phoronida appeared to be closely related to each other.

- [Halanych et al. \(1995\)](#) confirmed that the polyphyletic lophophorates are protostomes related to annelids, molluscs, and others with trochophore larvae, and they proposed naming the clade Lophotrochozoa. They formally defined Lophotrochozoa as “the last common ancestor of the three traditional lophophorate taxa, the mollusks, and the annelids, and all of the descendants of that common ancestor.”
- As will be described in the following sections, subsequent molecular studies added Platyhelminthes and other groups with neither a lophophore nor a trochophore larva to the clade.
- The validity of the Lophotrochozoa is independently supported by the finding that annelids, nemertean, flatworms, gastropods, and brachiopods all share unique [Hox genes](#) ([De Rosa et al. 1999](#)).
- Relationships of clades within Lophotrochozoa remain largely unresolved, perhaps because they diverged rapidly at about the same time ([Halanych 1998](#)).

Lophotrochozoa includes spiralian.

- HISTORY: Hyman did not refer to a group “Spiralia,” but she considered the protostomes to be a cohesive group. Since Annelida, Mollusca, and some other coelomate protostomes have spiral cleavage, many authors assume that all protostomes have spiral cleavage as the plesiomorphic condition, even arthropods and other groups where the actual cleavage pattern is usually not spiral. Other authors, however, restrict the term “Spiralia” to phyla in which spiral cleavage is actually and unequivocally observed, though not necessarily in all species. These phyla are Gnathostomulida, Platyhelminthes, Mesozoa, Entoprocta, Mollusca, Sipunculida, Pogonophora, Nemertea, Annelida, and Echiurida. Spiral cleavage is not seen in Nematoda or in Arthropoda except for a few crustaceans.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 222) concluded that the term “Spiralia” should “only be retained with reservations, accepting that we do not know how far it tells us of shared ancestry.” Nielsen (1995, p. 96) divided the Protostomia into two sister groups: Aschelminthes and Spiralia. (See [indented list](#).) His Spiralia comprised Sipunculida, Mollusca, Annelida (including Gnathostomulida, Pogonophora, and Echiurida), Onychophora, Arthropoda, Tardigrada, Entoprocta, Platyhelminthes, and Nemertea.
- 18S rDNA analyses to be discussed later indicate that the phyla that do in fact have a spiral cleavage pattern all belong to Lophotrochozoa, which also includes the lophophorates in which the cleavage pattern is usually radial ([Fig. 17](#)). Other phyla that occur within Lophotrochozoa have a cleavage pattern that may be primitively spiral but is distorted or unobservable because of yolk.

Flatworms appear to be lophotrochozoans rather than basal to other Bilateria.

- HISTORY: Hyman (1940, vol. 1, p. 36) regarded acoelomates (Platyhelminthes and Nemertea) as a distinct branch within the Protostomia. She therefore dismissed the “alleged degradation of flatworms from annelids” as an example of “theoretical vaporizings.” Undaunted, some authors have continued to seriously consider the possibility that flatworms originated from an annelid or some other spiralian coelomate. (See papers by Ax, Ehlers, and especially by Smith and Tyler in [Conway Morris et al. 1985](#).) The morphological evidence for this conclusion includes the fact that, like annelids, flatworms have a classical spiral cleavage, and their serially repeated protonephridia and gonads are reminiscent of segmentation.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 361) concluded that the bilaterally symmetric animals—both pseudocoelmate and coelomate—had diverged along many lines from Platyhelminthes or flatworm-like animals. Nielsen (1995) placed Platyhelminthes within his Spiralia as shown in the [indented list](#).
- Some analyses of 18S rDNA support the traditional position of Platyhelminthes as basal to the other Bilateria. (See, for example, [Van de Peer and De Wachter 1997](#).) When sequences with high rates of base substitution are eliminated to avoid [long-branch attraction](#), however, the majority of flatworms fall within Lophotrochozoa ([Aguinaldo et al. 1997](#); [Carranza, Baguña, and Riutort 1997](#); [Ruiz-Trillo et al. 1999](#)). [Giribet et al. \(2000\)](#) found that Platyhelminthes, as well as other acoelomates, may form a clade (Platyzoa) in Lophotrochozoa.
- The inference from 18S rDNA sequence analyses that flatworms are within Lophotrochozoa is supported by additional evidence from the type of intermediate-filament proteins ([Erber et al. 1998](#)) and the similarity of Hox genes in flatworms to those of annelids ([Balavoine 1998](#); [De Rosa et al. 1999](#)).
- Myzostomids—incompletely segmented animals with trochophore larvae—may represent a link between flatworms and annelids. Myzostomids have generally been considered to be annelids, but evidence from 18S rDNA and [EF-1 \$\alpha\$](#) indicate that they are apparently flatworms ([Eeckhaut et al. 2000](#)).

Acoela may or may not be basal to other Bilateria.

- HISTORY: Identification of a basal bilaterian group has long been a goal of phylogenetics, since it might provide insight into the transition from diploblasts to triploblasts and would provide a more suitable outgroup for cladistic analyses than the usual diploblasts. The Acoela, with their simple, solid bodies and densely ciliated epidermis, fulfilled the expectations of many systematists that the ancestral bilaterian would be planula-like. In her early writings Hyman accepted the view that the Acoela might be basal Bilateria, but in 1967 (vol. 6, p. v) she noted that they did not appear to be as primitive as she and others had formerly thought.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 462) continued to accept the view that Platyhelminthes were basal bilaterians derived from a planula-like ancestor, with acoel-like flatworms being one of several groups from which many lines of bilateria diverged. Nielsen (1995, pp. 221-222), however, considered the idea of a planula-like ancestor of the Bilateria improbable, since, unlike acoels, cnidarian larvae have a permanent gut and lack a syncytial endoderm. He placed Platyhelminthes, including Acoela, within his Spiralia.
- Analyses of 18S rDNA sequences support the basal position of Acoela with respect to the majority of Platyhelminthes (Katayama, Nishioka, and Yamamoto 1996, summarized in Figure 12.) A study by Carranza, Baguña, and Riutort (1997), however, cast doubt on the monophyly of Platyhelminthes, as well as their position as basal Bilateria. The analysis of 18S rDNA data by Zrzavý et al (1998) divided flatworms into several phyla, with most well within the Bilateria. They placed the Acoela at the base of the Bilateria.
- One criticism of these 18S rDNA studies is that all the sequences from Acoela had rates of base substitution several times higher than those for most Metazoa, creating the potential for long-branch attraction. Ruiz-Trillo et al. (1999) undertook a new analysis using only a sequence from an acoel species with a slower rate of substitution. Their much-heralded study found that this acoel nevertheless appeared at the base of the Bilateria, while the bulk of flatworms occurred in Lophotrochozoa. Separating the Acoela from Platyhelminthes can be justified on the basis of the following morphological differences: Acoela have a unique duet-spiral cleavage that lacks the second pair of cells that form in the typical quartet-spiral cleavage, Acoela have a highly regulative development rather than the determinative development of spiralian, and Acoela have only endomesoderm and no ectomesoderm.
- Adoutte et al. (2000) doubted the conclusion of Ruiz-Trillo et al., however, partly because the acoel sequence was saturated with mutations and therefore still liable to long-branch attraction. In addition, they noted research suggesting that Hox-gene sequences in acoels are similar to those of lophotrochozoans. Moreover, in the 18S-rDNA study by Giribet et al. (2000), in which long-branch attraction was avoided by excluding diploblasts, the Acoela did not occur at the base of the bilateria, but close to other flatworms within a clade Platyzoa that was sister to Lophotrochozoa. Analysis of EF-1 α gene sequences suggest that acoels branch within Platyhelminthes (Berney, Pawlowski, and Zaninetti 2000), but the adequacy of EF-1 α for such analyses has been doubted (Littlewood et al. 2001).
- In short, molecular phylogenetics has so far been unable to resolve the position of Acoela.

Nemertea may be closer to “coelomates” than to flatworms.

- HISTORY: Although the nemertean body plan is essentially acoelomate, the rhynchocoel is technically a coelom. Consequently, there has been a long debate about whether nemerteans belong with acoelomates or coelomates. Hyman (1951, vol. 2, pp. 473 and 528) acknowledged that the rhynchocoel is a true coelom, but nevertheless she accepted the prevailing view that nemerteans were acoelomates that had evolved from a flatworm.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, pp. 204-207) referred to nemerteans as “acoelomates that nevertheless possess a coelom” and concluded that they were not closely allied to the line of flatworms that led to coelomate spiralian, but were an “early and specialized independent branch derived from some other group of flatworms.” Nielsen (1995, p. 211) cautiously accepted a sister-group relationship of Nemertea and Platyhelminthes in the clade Parenchymia within Spiralia. (See [indented list](#).) He felt that the rhynchocoel was not homologous with the coeloms of other spiralian (p. 231).
- The molecular-phylogenetic evidence suggesting that acoelomates are derived from coelomates has rendered this issue largely moot. Still, it is interesting that even 18S-rDNA studies that failed to place Platyhelminthes within Lophotrochozoa place Nemertea within Lophotrochozoa, as shown in [Figure 17](#) ([Winnepeninckx, Backeljau, and De Wachter 1995](#)). A study using base sequences for the [EF-1 \$\alpha\$](#) gene also places Nemertea in Lophotrochozoa—in fact, within Mollusca ([McHugh 1997](#)).

Gastrotrichs are not closely related to nematodes.

- HISTORY: Hyman included the gastrotrichs with nematodes in the phylum Aschelminthes on the basis of “slight spaces” between the body wall and viscera, which she characterized as “presumably of the nature of a pseudocoel as they have no definite lining but their embryonic origin is as yet unknown (1951, vol. 3, p. 158).” Most authors continue to ally gastrotrichs with nematodes and other “pseudocoelomates.” [Hummon \(1982](#) and personal communication) has found, however, that this pseudocoel is an artifact of fixation—a pseudo-pseudocoel. In life, gastrotrichs are as acoelomate as flatworms, and there are more morphological characters linking them with flatworms than with nematodes.
- RECENT MORPHOLOGICAL STUDIES: While acknowledging doubts about the existence of pseudocoels in gastrotrichs, Willmer (1990, p. 245) concluded that nematodes and nematomorphs “probably derived from gastrotrich-like ancestors.” (See [summary list](#).) Without using a body cavity as a character, Nielsen (1995, chap. 33) placed Gastrotricha among the aschelminths within his clade Cycloneuralia as the sister group of the clade Introverta (Nematoda plus others). (See [indented list](#).)
- Comparisons of 18S rDNA sequences by [Wirz et al. \(1999\)](#) indicate that Gastrotricha are not closely related to either Nematoda or Rotifera. The exact position of Gastrotricha remains unresolved, but some analyses of 18S rDNA sequences place them within Lophotrochozoa near Platyhelminthes. See, for example, [Garey et al. \(1996\)](#).

Acanthocephalans are closely related to rotifers.

- HISTORY: Hyman (1951, vol. 3, pp. 47-50) elevated Acanthocephala to a separate phylum simply because she could not decide between the conflicting arguments for putting them with Platyhelminthes or with Aschelminthes. Most of the morphological evidence placed them in Aschelminthes with rotifers and other pseudocoelomates. She noted, however, that the pseudocoel in acanthocephalans does not form in the same manner as in other pseudocoelomates, and serological studies suggested that among the intestinal parasites acanthocephalans were closer to cestodes than to nematodes. The argument between flatworm and aschelminth affinities continued for another decade (Hyman, 1959, vol. 5, p. 739), when her most definitive statement on the subject was the following: "Astonishingly, [O. von] Haffner [1950] arrives at the conclusion that the Acanthocephala are closer to the Rotifera than to any other aschelminth group. The author finds the arguments for this strange conclusion very unconvincing."
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 245) concluded that rotifers and acanthocephalans are "probably related." Nielsen (1995, p. 252) considered it "clear that the acanthocephalans and rotifers must be sister groups, and that the acanthocephalans therefore cannot be 'parasitic rotifers'."
- Analyses of 18S rDNA sequences indicate that Acanthocephala are closely related to Rotifera within Lophotrochozoa (Wallace, Ricci, and Melone 1996; Winnepenninckx et al. 1995). Garey et al. (1996) found that Acanthocephala arises from within Rotifera, which would make them derived rotifers presumably highly modified by parasitism. A later study using sequences from more species, however, indicated that Acanthocephala are merely the sister group of the monophyletic Rotifera (García-Varela et al. 2000).

Cycliophora appear to be related to rotifers.

- HISTORY: *Symbion pandora* was first collected in the 1960s from the mouthparts of the Norway lobster, but it was assumed to be a rotifer and stored in a museum drawer. The species was then rediscovered by Peter Funch and Reinhardt Kristensen, who, after studying its many unique features and complex life cycle, erected the new phylum Cycliophora in 1995. Funch and Kristensen proposed that Cycliophora was close to Entoprocta and Ectoprocta.
- Comparisons of 18S rDNA suggest that *Symbion* is in Lophotrochozoa and is closer to Rotifera than to Entoprocta or Ectoprocta (Winnepenninckx, Backeljau, and Kristensen 1998).

Echiura and Pogonophora may be polychaete annelids.

- **HISTORY:** Hyman planned to cover both Echiura and Annelida in the same volume, indicating that she considered them to be closely related. Most authors continue to ally the echiurans (together with sipunculans) to the annelids, as well as to molluscs, on the basis of their trochophore larvae and spiral cleavage. In 1959, when Hyman wrote about them, Pogonophora were still a new and little-known phylum usually allied with the hemichordates. "It is not open to doubt," she wrote (vol. 5, p. 224), "that the Pogonophora belong to the Deuterostomia." Since then, the clearly segmented opithosome has been found, their cleavage has been determined to be spiral, and their larvae have been found to be trochophores. Most authors now therefore consider the pogonophorans to be most closely related to annelids. Some authors have accepted the proposal by [Meredith Jones \(1985\)](#) that the phylum be divided into two: phylum Vestimentifera and phylum Pogonophora (= Frenulata). Most, however, continue to regard both groups as members of the monophyletic Pogonophora.
- **RECENT MORPHOLOGICAL STUDIES:** Willmer (1990, p. 215), after discussing the evidence that echiurans show tentative segmentation during development, concluded that they "should not be placed within the same phylum as segmented annelids, [b]ut the two key features of development to identical trochophores and the presence of identical chaetae (together with a number of other similarities...) should still be enough to keep the two phyla very closely allied in any phylogenetic scheme." She considered it reasonable to place the pogonophorans "not too distant from the main annelid/echiuran lineage" (p. 216). Nielsen (1995, p. 140) concluded that "at present the pogonophorans must thus be regarded as a specialized polychaete group." He also (p. 142) tentatively included the echiurans within Annelida.
- Using base sequences for the [EF-1 \$\alpha\$](#) gene, [McHugh \(1997\)](#) concluded that vestimentiferans and echiurans arose separately from within the polychaete annelids. Using the amino-acid sequences for [EF-1 \$\alpha\$](#) , [Kojima \(1998\)](#) found the same result for vestimentiferans. [Halanych, Lutz, and Vrijenhoek \(1998\)](#), using sequences from genes for mitochondrial cytochrome c oxidase subunit 1, 18S rRNA, and 28S rRNA, found that vestimentiferans and other pogonophorans arose from within the polychaetes relatively recently. These findings, as well as evidence that oligochaetes and leeches also arose from polychaetes, renders Polychaeta paraphyletic.
- Pogonophorans appear to arise from within the order Sabellida and are now often referred to as the family Siboglinida.

Chaetognatha may not be closely related to other deuterostomes.

- HISTORY: Among the many hypotheses for chaetognath affinities was the suggestion first made in the 1860s that they are related to nematodes on the basis of the thick cuticle, similar arrangement of body-wall musculature, and similarities of the grasping spines to the adhesive bristles on the heads of certain marine nematodes (Hyman, 1959, vol. 5, pp. 1, 3). According to Hyman, that view was still current through the 1950s. Hyman acknowledged the similarity of *adult* chaetognaths to “aschelminths,” but she gave more weight to the radial, indeterminate cleavage and deuterostomy. She also noted that in the juvenile the coelom develops enterocoelously, although not in the same way as in Echinodermata, Hemichordata, and Chordata. Her final conclusion was that she could not relate Chaetognatha to any other phylum, and that they were perhaps derived from the early bilateria. Because of “the possibility that Chaetognatha are remotely related to the dipleurula ancestor of the other Deuterostomia,” however, she (1959, vol. 5, p. 66) placed them among the deuterostomes. This practice has generally been followed since.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990, p. 319) regarded the association of Chaetognatha with deuterostomes as “extremely tenuous” and decided that the most probable origin was from “the acoeloid or proto-platyhelminth form that may be at the roots of the Metazoa.” Nielsen (1995, p. 235) tentatively placed the chaetognaths within Aschelminthes in an unresolved trichotomy with (Rotifera + Acanthocephala) and his clade Cycloneuralia, which includes Nematoda and others. (See [indented list](#).)
- Several analyses using the base sequences of 18S rDNA from several species indicate that chaetognaths are not closely related to deuterostomes (Giribet et al. 2000; Telford and Holland 1993; Wada and Satoh 1994b). There is some indication that chaetognaths evolved as a distinct clade from the base of the Bilateria, but this may be a consequence of [long-branch attraction](#). One analysis using 18S rDNA sequences suggests that chaetognaths are related to Nematoda in Ecdysozoa (Halanych 1996).

Molecular evidence supports the conventional phylogeny of echinoderm classes.

- HISTORY: Paleontological evidence as well as morphological characters have long supported the view that crinoids are the oldest extant echinoderms, followed by ophiuroids and asteroids, and finally echinoids and holothuroids.
- Evidence from both 18S rDNA and mitochondrial gene rearrangements support this phylogeny (Smith et al. 1993; Wada and Satoh 1994a).

Concentricycloids may be asteroids.

- HISTORY: Baker, Rowe, and Clark (1986) described a small, disc-shaped animal found in wood collected from kilometer-deep ocean near New Zealand and named it *Xyloplax medusiformes*. A second species of *Xyloplax* was discovered later. Although *Xyloplax* has pentaradial symmetry and podia, it does not have the test of an echiuroid, the cucumber-shape of a holothuroid, or the arms of a crinoid, ophiuroid, or asteroid. For this and other reasons, Baker et al. proposed the new class Concentricycloidea.
- Janies and Mooi (1998) found that both 18S rDNA sequences and morphology placed *Xyloplax* within Asterozoa.

Hemichordata may be closer to Echinodermata than to Chordata.

- HISTORY: Until the 1950s Hemichordata had frequently been included in the phylum Chordata, and Hyman (1959, vol. 5, p. 74) considered it “impossible to deny” that the phylum Hemichordata was related to chordates. On the other hand, the tornaria larva of enteropneusts resembles an asteroid larva so closely that Hyman (1959, vol. 5, pp. 197-199) was moved to write that, “There appears no escape from the conclusion that hemichordates and echinoderms stem from a common ancestor.... In other words, the common ancestral stock gave off the echinoderms as a blind branch, then continued along its main line of evolution to hemichordates and chordates.” Most authors have cited the presence of pharyngeal slits and a dorsal, hollow nerve cord as evidence for a sister-group relationship of Hemichordata to Chordata rather than to Echinodermata.
- RECENT MORPHOLOGICAL STUDIES: Willmer (1990) expressed no strong conviction about the relative position of Hemichordata to Chordata versus Echinodermata, but in her summary figure (p. 361) she placed the Hemichordata as a branch below the Echinodermata on the line terminating with Chordata. Nielsen (1995, pp. 333, 385) divided hemichordates into two phyla, Pterobranchia and Enteropneusta. He placed Enteropneusta as the sister group of Chordata in a clade he named Cyrtotreta, and he placed Pterobranchia in an unresolved trichotomy with Echinodermata and Cyrtotreta. (See [indented list](#).)
- One early study that used 18S rDNA sequences from four deuterostomate species suggested that the acorn worm *Saccoglossus* was closer to vertebrates than to the echinoderm ([Holland, Hacker, and Williams 1991](#)). More recent studies using more 18S rDNA sequences and a variety of analytical methods almost invariably show Hemichordata to be monophyletic and more closely related to Echinodermata than to Chordata ([Cameron, Garey, and Swalla 2000](#); [Halanych 1995](#); [Turbeville, Schulz, and Raff 1994](#)).

Vertebrates apparently did not evolve from an echinoderm.

- HISTORY: Hyman (1959, vol. 5, p. 201) unequivocally rejected the idea, then “widely spread,” that vertebrates originated directly from an echinoderm independently of cephalochordates and urochordates. That view was resurrected, however, by [R. P. S. Jefferies \(1986\)](#), a paleontologist who claims to have identified gill slits, a brain, notochord, dorsal nerve cord, and other chordate features in fossils of extinct “calcichordates,” which are considered by most paleontologists to have been echinoderms. Although most paleontologists do not accept Jefferies identification of these features, the calcichordate theory is still often treated seriously. (Refer to [Gee 1996](#) for further discussion.)
- RECENT MORPHOLOGICAL STUDIES: Nielsen (1995, pp. 377-378) also rejected Jefferies analysis of calcichordate morphology and the calcichordate theory.
- Analysis of 18S rDNA sequences show that Urochordata, Cephalochordata, and Vertebrata all belong to the same clade (Chordata) that is sister to, but clearly separate from, the clade that includes Echinodermata and Hemichordata. (See, for example, [Wada and Satoh 1994b](#); [Zrzavý et al. 1998](#).) This is not the result that would be expected if Chordata or Vertebrata evolved from an echinoderm.

Cephalochordata, rather than Urochordata, may be the sister group of Vertebrata.

- HISTORY: In the first half of the 20th century the amphioxus *Branchiostoma* was considered to be the closest extant relative of vertebrates. Like vertebrates, cephalochordates have myomeres, a ventral pulsating blood vessel that may be homologous with the vertebrate heart, an intestinal diverticulum that resembles the embryonic precursor of the vertebrate liver, and separate dorsal and ventral roots of the spinal cord. One vertebrate feature that cephalochordates apparently lack is a head. Instead the notochord extends to the front of the animal, and there is only an anterior enlargement of the nerve cord in place of a brain. Largely for that reason, many zoologists prefer Garstang's suggestion that vertebrates evolved from a larval urochordate by pedomorphosis, even though there is little direct evidence for the idea. (See [Gee 1996](#) for further discussion.) [Northcutt and Gans \(1983\)](#); [Gans and Northcutt 1983](#)) have attempted to revive the cephalochordate theory by noting that vertebrate cranial structures, such as sense organs and muscles, originate from neural crest, unlike similar structures in the rest of the body. They argue, therefore, that the head is apomorphic in vertebrates and evolved in an amphioxus-like ancestor during the transition from filter feeding to predation.
- RECENT MORPHOLOGICAL STUDIES: Nielsen (1995, p. 396) distinguished between the stiffening rod ("urochord") in the tails of Urochordata and the more-anterior notochords of cephalochordates and vertebrates. This and other characters led him to divide the clade Chordata into the phylum Urochordata and its sister group Notochordata, with the latter comprising the two phyla Cephalochordata and Vertebrata (pp. 385, 418). Thus Vertebrata would have shared a more recent ancestor with Cephalochordata than with Urochordata.
- Analysis of 18S rDNA sequences suggests that *Branchiostoma* is the sister clade of Vertebrata ([Wada and Satoh 1994b](#)). That study also showed that Urochordata, represented by an ascidian, a larvacean, and a salp, form a monophyletic clade outside Cephalochordata + Vertebrata.
- The affinity of Cephalochordata to Vertebrata is further supported by the similarity of the [Hox genes](#) in *Branchiostoma* to those in humans and mice ([Garcia-Fernández and Holland 1994](#)).

Turtles may be the sister group of Crocodylia + Aves rather than basal Reptilia.

- HISTORY: The fossil record for turtles goes back only to the Jurassic period, long after the diversification of the main reptilian lines. It is only because turtles lack skull fenestrations that they have been assumed to be derived from the anapsids of the Permian period, which are assumed to have been basal reptilians. Turtles are therefore generally assumed to be the most basal of extant reptiles.
- Using several unusual features of the mitochondrial genome, as well as sequences from genes for mitochondrial rRNA, [Zardoya and Meyer \(1998\)](#) found that turtles (Testudines) are the sister group of Archosauria (Crocodylia + Aves). Tuataras and lizards form a clade that is basal to Archosauria + Testudines. Turtles are therefore derived diapsids.

Placental mammals may be divided into four superordinal clades.

- HISTORY: Relationships among orders of placental mammals are largely unresolved, partly because of their rapid diversification in the late Cretaceous and early Tertiary periods and partly because of extremes in convergent and divergent adaptation to a wide range of habitats.
- By synthesizing several hundred morphological and molecular phylogenetic trees, Liu et al. (2001) identified nine well-supported clades. Their clades are generally consistent with two purely molecular-phylogenetic trees published by Madsen et al. (2001) and Murphy et al. (2001).
- These molecular studies support four superordinal clades of placental mammals that diverged in the following sequence: Afrotheria, Xenarthra, Glires + Euarchonta (flying lemurs, tree shrews, and primates), and a clade named by Madsen et al. Laurasiatheria. Traditional orders within these four clades are shown in Figure 19.

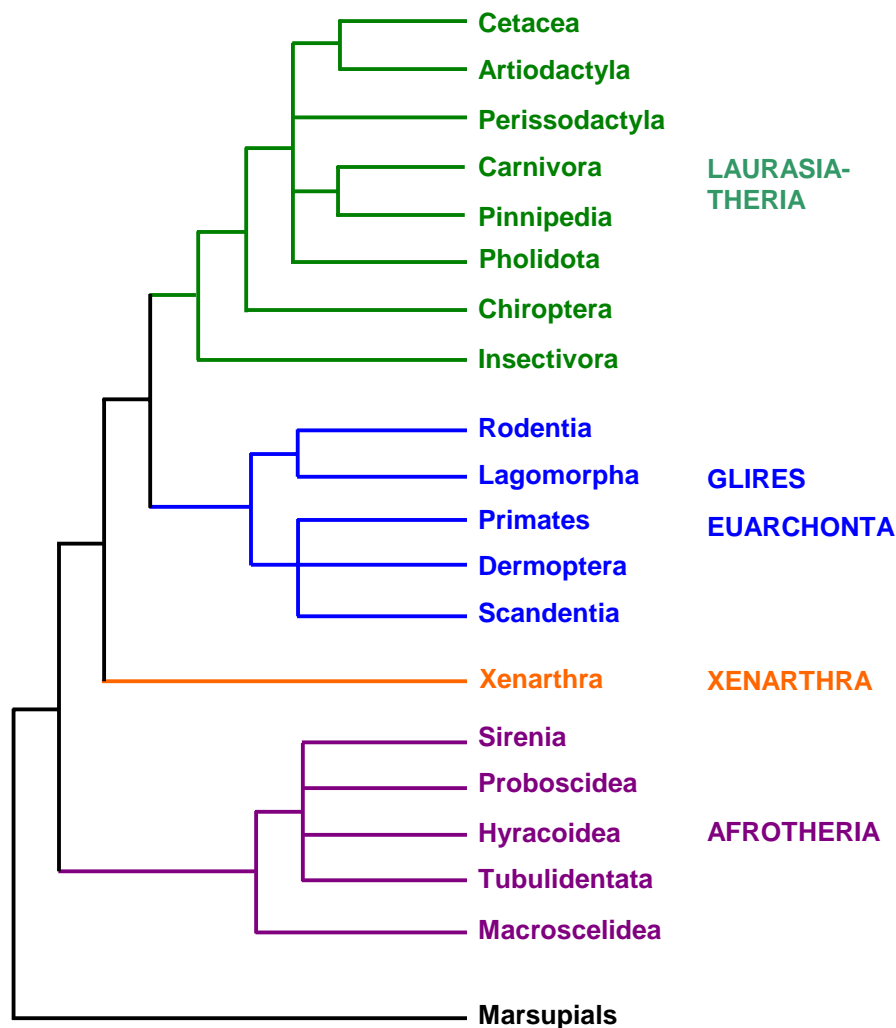


Figure 19. Traditional orders of placental mammals arranged into four major clades based on analysis of gene sequences. Adapted mainly from Murphy et al. (2001).

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IV. Incorporating Molecular Phylogenies Into Teaching

The most important conclusions from animal molecular phylogenetics are that Bilateria (triploblasts) and Deuterostomia (Echinodermata + Hemichordata + Chordata) are each monophyletic, and protostomes comprise the two clades Lophotrochozoa and Ecdysozoa.

- Deuterostomia does not include lophophorates and chaetognaths.
- Pseudocoelomates are not monophyletic, and they are derived from coelomates.
- Acoelomates may be monophyletic, but they are also derived from coelomates.
- Lophotrochozoa comprises lophophorates, groups with trochophore larva, and all descendants of their most recent common ancestor. Lophotrochozoa includes Platyhelminthes and the traditional spiralian.
- Ecdysozoa comprises Arthropoda and other groups of animals that molt the cuticle in one piece, including Nematoda.

The traditional approach of proceeding from the simplest animals to the more complex is pedagogically sound.

- Unlike evolution, most students find it hard to deal with many groups of animals at the same time. Therefore it is probably more practical to cover the major groups in some kind of sequence rather than trigger a Cambrian explosion by attempting to branch out in all directions simultaneously.
- Even though deuterostomates diverged before the protostomate phyla diversified, it might be too jarring for beginning students to jump directly from the relative simplicity of Cnidaria to the complexity of Echinodermata and Chordata.
- It is reasonable, therefore, to follow the presentation of “radiata” with a relatively simple protostomate group, such as Platyhelminthes.

The practice of treating the “acoelomates” and the “pseudocoelomates” as clades outside of coelomates should be abandoned.

More natural groupings would be Lophotrochozoa and Ecdysozoa.

The following proposed sequence of topics is consistent with molecular phylogenetics without departing too radically from the traditional zoology syllabus.

- Topics in the table on the next page are keyed to chapters in the following McGraw-Hill texts:
Integrated Principles of Zoology, 11th ed. Hickman, Roberts, and Larson
Biology of the Invertebrates, 4th ed. Pechenik
Zoology, 5th ed. Miller and Harley
Animal Diversity Hickman and Roberts
Biology of Animals 7th ed., Hickman, Roberts, and Larson

Table 2. A zoology sequence that is consistent with molecular phylogeny.
 Bold numbers refer to chapters; parentheses enclose page numbers; ~ means “except”.

GROUP	<i>Integrated Principles of Zoology 11</i>	<i>Biology of Invertebrates 4</i>	<i>Zoology 5</i>	<i>Animal Diversity</i>	<i>Biology of Animals 7</i>
Protozoans	11	3	8		
Porifera & Placozoa	12 ~(242-243)	4	(122-127)	5	17
Cnidaria & Ctenophora	13	6, 7 (437-438)	(127-139)	6	18
Platyhelminthes	14 (242-243) ~(297-300)	8, 9	10 ~(152-154)	7 ~(121-124)	19 ~(427-429)
Mollusca	16	12	12	9	21
Annelida	17	13 ~(307-311)	13	10	22
Minor Lophotrochozoa	(297-300; 303-310; 318-320; 440-444), 22	10, 11, 18 ~(437-438), 19 (307-311; 448-449)	(152-154; 159-162; 168-169)	(128-129; 135-136; 217-219; 221-225)	(427-429; 434-436; 442-446), 24 ~(533-535; 560)
Nematoda	(311-317)	16	(162-166)	8 ~(128-130)	(437-442)
Arthropoda	18, 19, 20	14	14, 15	11	23
Minor Ecdysozoa	(310-311; 318-319; 444-447; 481-482)	15, 17	(162; 167-170)	(129-130; 135; 219-221; 238-240)	(436-437; 442; 533-535)
Echinodermata & Hemichordata	23 (482-485)	20, 21	16; (256-258)	13 ~(238, 240)	25 ~(560)
Invertebrate Chordata	25	22	17 ~(256-258)	14	26
Vertebrata	26-30		18-22	15-19	27-31

GLOSSARY (click the BACK button on your browser to return)

See also the "Glossary of terms used in Phylogeny Reconstruction" at www.may.ie/academic/biology/james/Glossary.html

18S rDNA. DNA sequence that encodes 18S ribosomal RNA, which is the RNA in the smaller subunit of metazoan ribosomes. Also called SSU rDNA (small subunit ribosomal DNA). 18S indicates the sedimentation factor of the RNA.

Alignment. Adjustment of the position of two or more molecular sequences relative to each other so that homologous positions of the molecule can be compared.

Apomorphic. Derived, as opposed to primitive (plesiomorphic).

Bootstrapping. A technique for estimating the reliability of an internal branch of a tree by resampling the original data set. With DNA sequences the bases at each position are randomly sampled then returned to the pool so that they may be resampled again. The bootstrap value for a branch is the percentage of such resamplings (typically 500 to 1000) that recover the branch. Compare [Jackknifing](#) in which resampling is done without replacement, and [Parametric bootstrapping](#) in which the sequences to be resampled are generated by numerical simulation.

Collapsing. Eliminating a poorly supported branch by merging the nodes at each end of it.

Consensus tree. A tree that is supported by two or more methods, possibly as the result of collapsing internal branches that are not supported by all methods.

DNA-DNA hybridization. A technique, now seldom used, that determines the degree of similarity between DNA sequences from two taxa by separating the DNA into single strands, allowing them to hybridize into a double strand, and measuring the temperature at which the strands separate again.

EF-1 α . Elongation factor-1 α . One of several proteins involved in the synthesis of proteins by ribosomes in eukaryotes. It helps bind aminoacyl-tRNAs to ribosomes during translation.

Elongation factor. A protein involved in translation. See EF-1 α .

Exhaustive search. A search for the optimal tree among all possible ones. Compare Heuristic search.

Heuristic search. A method of reducing the number of trees to be searched when the number is too large for an exhaustive search.

Homeotic. Referring to a gene that directs the appropriate development of a body segment.

Homoplasy. The occurrence of similar characters in two taxa by convergent evolution rather than by inheritance from a shared ancestor. Analogy.

Hox genes. Developmental regulatory genes that occur in clusters. They are best characterized in segmented animals, where they control the identity of each segment depending on anterior-posterior position.

Informative site. A molecular site with two or more character states, at least two of which occur in two or more taxa each.

Jackknifing. A technique for estimating the confidence in an internal branch of a tree by resampling the original data set without replacement, so that no datum is used more than once. With DNA sequences a certain percentage of the positions are sampled and a new tree is reconstructed. The jackknife value for the branch is the percentage of such resamplings (typically 500 to 1000) that recover the branch. Compare [Bootstrapping](#), in which resampled data may be used more than once.

Log likelihood (ln L). A measure of the likelihood of a tree as inferred from the maximum likelihood method.

Long-branch attraction. The tendency of taxa that have evolved rapidly or for long times to appear to be more closely related than they are.

Long interspersed element (LINE). DNA sequence of 3 to 7 kb (kilo-base pairs) that has no known function except producing enzymes that reverse-transcribe other copies of themselves from RNA and introduce the copies into the genome. See also Short interspersed element ([SINE](#)).

Maximum likelihood (ML). An approach to tree construction that begins with the possible trees and deduces which one is most likely given the data and an explicit model of evolution.

Maximum parsimony (MP). A cladistic approach to tree construction that tries to find the tree with the fewest changes among informative sites.

Neighbor-joining (NJ). A method of phylogenetic analysis that constructs a tree with the shortest total branch length.

Operational taxonomic unit (OTU). A group tentatively assumed to be a valid taxon for purposes of phylogenetic analysis.

Orthologous. Homologous as the result of speciation but not gene duplication. Orthologous genes are de-

rived from the same copy of a gene in the most recent common ancestor. Compare Paralogous.

Paralogous. Homologous as the result of gene duplication. Paralogous genes are derived from different copies of a gene in the most recent common ancestor. Compare Orthologous.

Parametric bootstrapping. A technique for estimating the reliability of an internal branch of a tree by resampling sequences that are generated by numerical simulation based on a model of evolution. Compare (non-parametric) [Bootstrapping](#).

Paraphyletic. Referring to a group that does not include all the branches from the ancestral node.

Parsimony. The criterion for selecting a tree because it requires the fewest hypotheses about the evolution of a character.

Plesiomorphic. Primitive, as opposed to derived (apomorphic).

Polymorphism. The occurrence of two or more states of a character in a single species.

Polyphyletic. Referring to an artificial group comprising branches from two or more nodes.

Rooting. Determining the origin of a tree, usually by using an outgroup or by fixing it at the midpoint of the longest path between two taxa.

Scaling. Making the length of a branch in a phylogenetic tree proportional to the degree of evolutionary change.

Short interspersed element (SINE). DNA sequence of 75 to 500 bp (base pairs) with no known function that is reverse-transcribed, usually from tRNA, and inserted into the genome with the aid of long interspersed elements ([LINEs](#)).

Study group. The group of organisms whose phylogeny is being studied.

Symplesiomorphy. Referring to a character that occurs in the outgroup and is therefore assumed to be plesiomorphic (primitive) in the ingroup.

Synapomorphy. A homologous derived character shared by two or more, but not all, taxa in the ingroup.

Total-evidence tree. A tree constructed by using both morphological and molecular evidence.

Transition. Change from one purine to another (adenine to guanine or vice versa) or one pyrimidine to another (thymine to cytosine or vice versa). Compare transversion.

Transposable element. Segments of chromosomes that move to different loci.

Transversion. Change from a purine to a pyrimidine (adenine to thymine, for example) or from a pyrimidine to a purine. Compare transition.

Weighting. Giving more value to some character-state changes than to others, generally because they are less common.

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