

## Phylogenetic Studies

1. Who's related to whom?
  - Test hypotheses of *evolutionary relationship*.
2. Can diversification in one lineage be correlated with diversification in another lineage?
  - Test for *phylogenetic congruence*.
3. Is a particular feature adaptive or not?
  - Apply *comparative trend analysis*.
4. What is the pattern of character evolution of a group?
  - *Map character states and reconstruct ancestral states*.
5. What is the geographic history of a group?
  - Apply *phylogeographic methods*.

## The importance (and uses) of phylogeny

*Molecular systematics* has revolutionized phylogenetic studies.

- It infers evolutionary relationships among organisms using molecular, statistical, and data-mining techniques.
- It's older than you might think: In 1904, Nuttall used serological cross-reactivity to study the relationships of animals (esp. humans).

The various **methods** include:

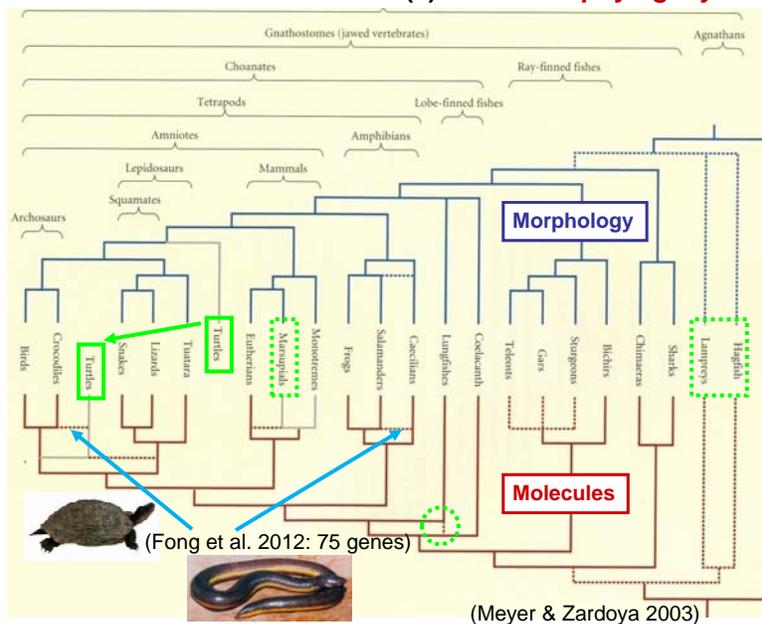
1. Immunological techniques (serological cross-reactivity -- distances)
2. Gel electrophoresis (protein products: allozymes and isozymes)
3. DNA-DNA hybridization (whole-genome distances)
4. Protein sequences (= amino acid sequences; character-based)
5. **DNA sequences** (base-pairs/codons; nucleus & organelles)
6. Various sorts of DNA fragment analysis, e.g. microsatellites, RFLPs, AFLPs, & SNPs from genome-wide scans (RAD-tags, etc.)
7. Whole-genome sequencing (Next-Gen, Rad-seq); Transcriptomics

**DNA data: Advantages over traditional techniques**

1. DNA evolves in a much more *regular* manner than other aspects of the organism's phenotype. The changes tend to be *selectively neutral*.
2. Sequence data are more *amenable to quantitative treatments*, because of the large volume of data generated.
  - Many more "characters" are available, compared to morphology.
  - Allows use of statistical algorithms to generate *dendrograms*.
    - **distance** methods like \*UPGMA and minimum evolution (**ME**)
    - **cladistic** methods such as maximum parsimony (**MP**)
    - **pluralistic** methods like maximum likelihood (**ML**) and **Bayesian** analyses
3. Molecular characters are *always abundant*, and thus can be used to assess relationships even among very small or very simple (reduced, e.g. parasites) organisms.
4. Molecular data provide *new perspectives* on thorny, previously unsolvable problems.
5. **Drawbacks**: rate variation, alignments, low S/N for ancient splits, etc.

\* Unweighted Pair Group Method with Arithmetic Mean

**1. Who's related to whom? (a) Vertebrate phylogeny**



Who's related to whom?  
(b) *Cetacea*

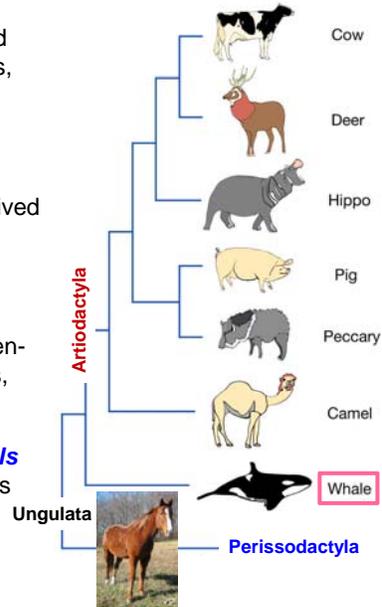


### Cetacea – **Whales** – have always been a puzzle to systematists

- A clade of large mammals that has entered the marine environment.
- The clade is problematical for some classic reasons:
  1. Whales have undergone *rapid* and *major* evolutionary change – within the last 30 million years (the last half of the Tertiary).
  2. Members show *convergence* (homoplasy): the clade has taken on the body form and adaptations of other large marine vertebrates (extinct mosasaurs and ichthyosaurs; living sharks; living bony fishes such as tuna).
  3. Members show *retrograde evolution*, losing useful features found in other mammals. Losses include external ears and noses, hind legs, etc.
- A recurring hypothesis of relationship for Cetacea is the *Ungulata* – the hoofed mammals. This was first proposed in mid 1800s, based on comparative anatomy (e.g., Haeckel 1866).
- An alternative hypothesis proposed that whales were *very ancient*, with ancestry at the very beginning of eutherian mammals in the Cretaceous.

**Early phylogenetic hypotheses based on morphology:**

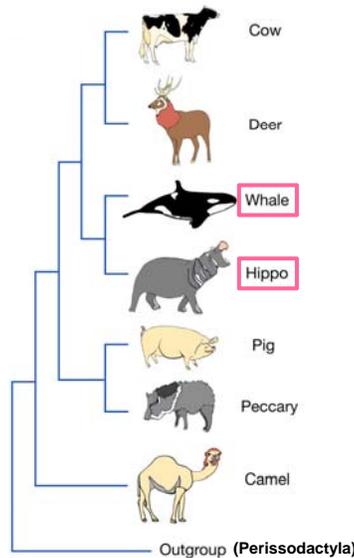
- The non-ungulate hypothesis held sway from the 1940s to the 1960s, because it was promoted by the influential mammalogists, G. G. Simpson and Alfred Romer.
- The ungulate hypothesis was revived in 1966 by Leigh Van Valen, who used dental characters.
- Van Valen’s work narrowed the options to the **artiodactyls** or even-toed ungulates – cows, deer, pigs, peccaries, hippos, and camels.
- Excluded were the **perissodactyls** or odd-toed ungulates, e.g. horses and rhinoceroses.



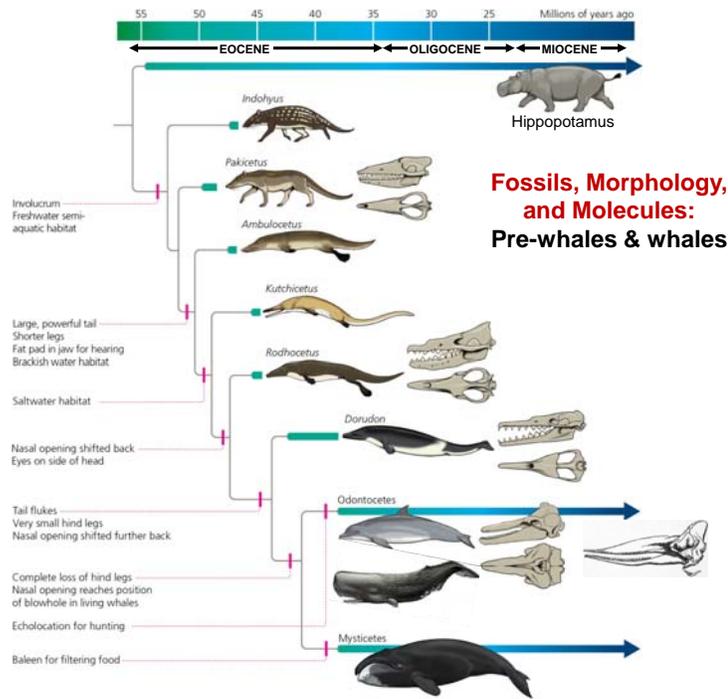
**Phylogeny based on molecules**

*beta*-casein protein gene, \*SINEs or \*LINEs at 20 loci, & 40,000 bp sequences

**Support for “Whippos”**  
(O’Leary & Gatesy 2008)

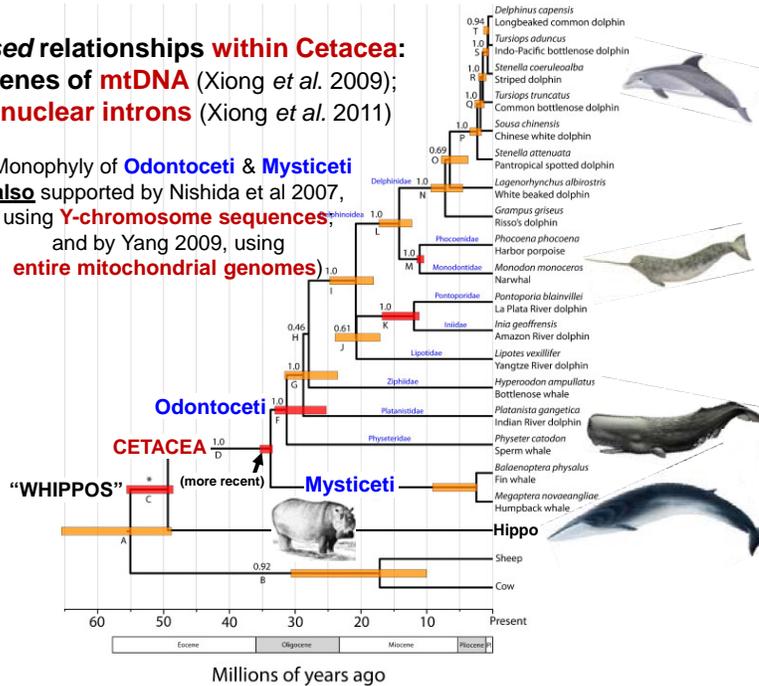


\* short or long interspersed elements: retrotransposons

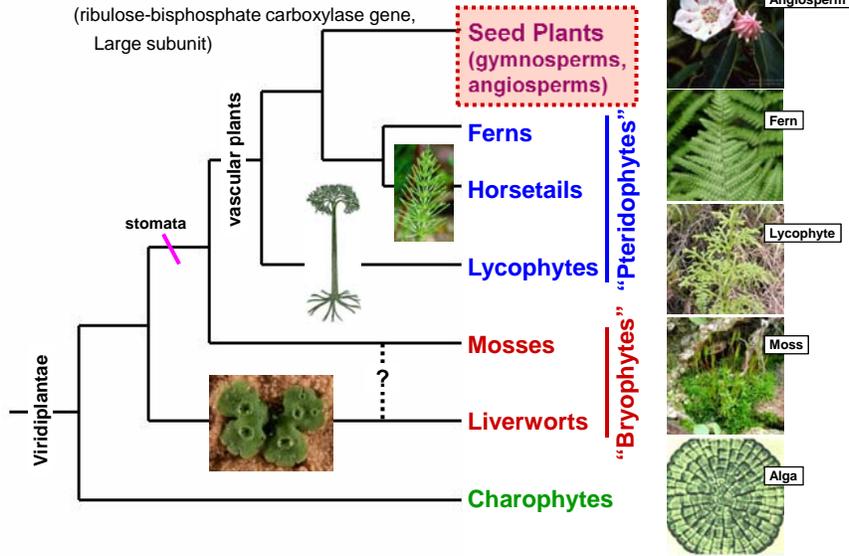


**Revised relationships within Cetacea:**  
 12 genes of **mtDNA** (Xiong *et al.* 2009);  
 also **nuclear introns** (Xiong *et al.* 2011)

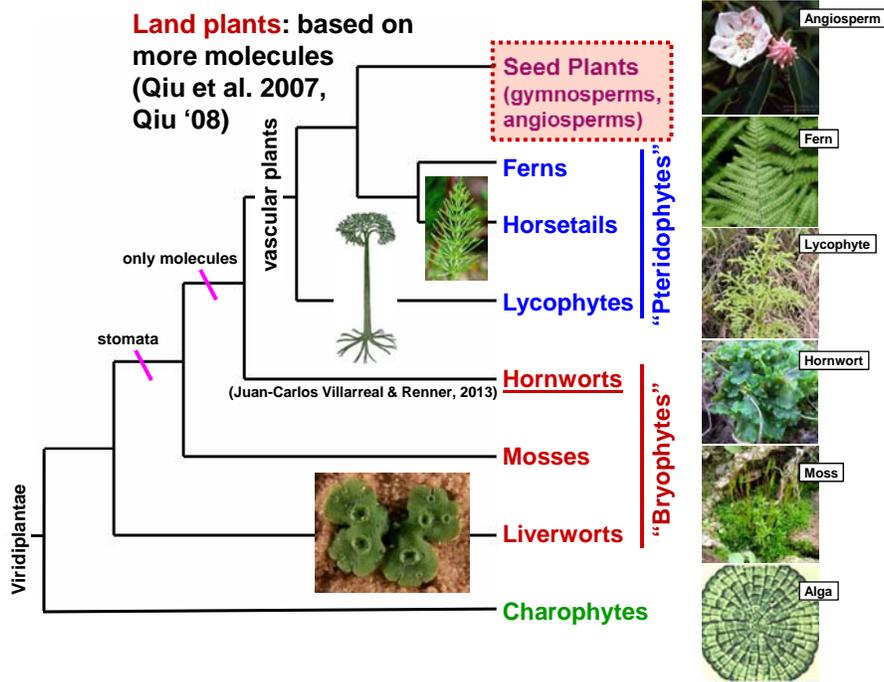
(Monophyly of **Odontoceti** & **Mysticeti**  
 also supported by Nishida *et al.* 2007,  
 using **Y-chromosome sequences**;  
 and by Yang 2009, using  
**entire mitochondrial genomes**)



**Who's related to whom? (c) *Land plants:*  
Not seriously challenged by *rbcL* molecular data**



**Land plants: based on  
more molecules  
(Qiu et al. 2007,  
Qiu '08)**



(d) Basal relationships of seed plants: **Gnetophyta/Gnetales**



*Ephedra* sp.

(globally distributed – dry areas)



*Gnetum gnemon*

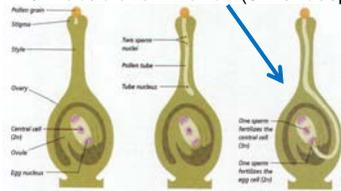
(globally distributed -- tropics)



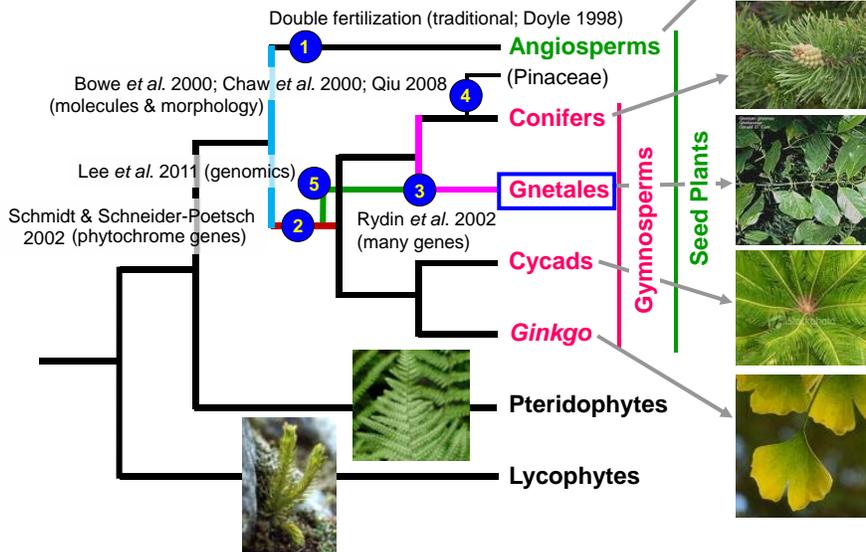
*Welwitschia mirabilis*

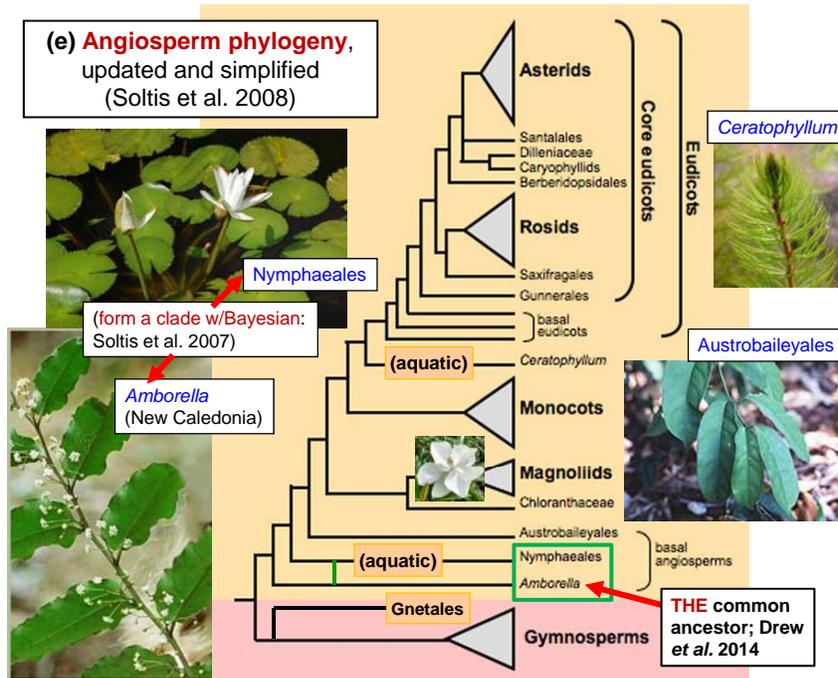
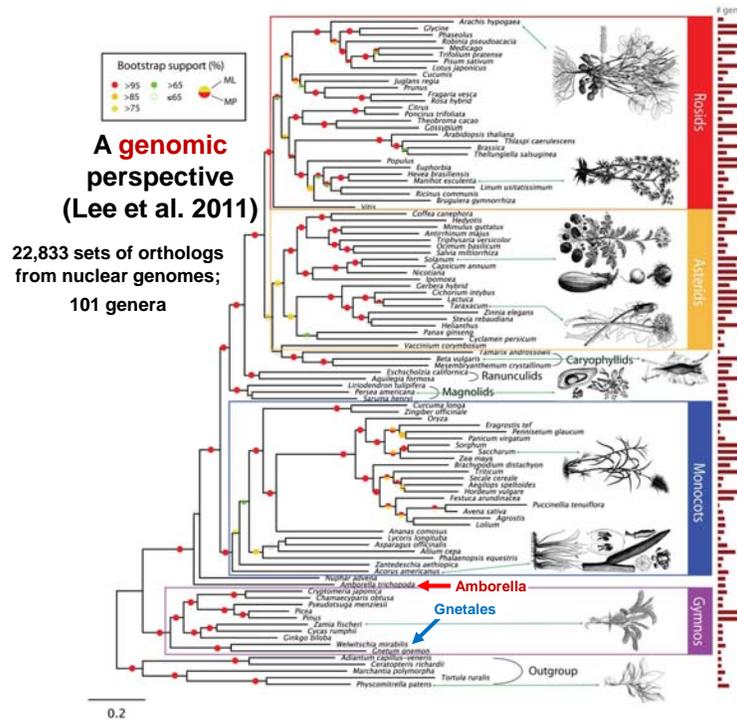
(deserts of Namibia & Angola)

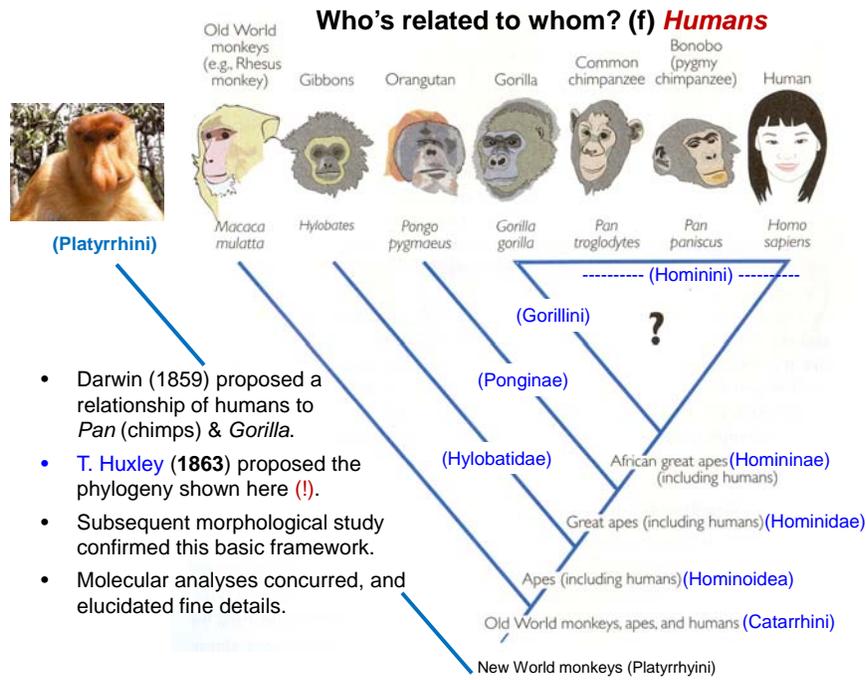
- An odd group of **gymnosperms**
- Sister-group to the **angiosperms**, based on:
  1. presence of vessel elements (in xylem).
  2. flower-like reproductive structure.
  3. **double fertilization** (3N endosperm formed).



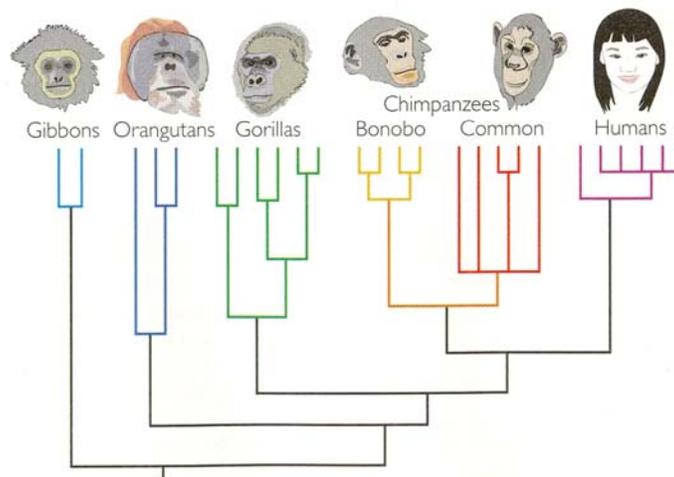
Is Gnetales a **basal angiosperm (1)**, a **derived gymnosperm (2, 3, 4)**, or **sister to gymnosperms (5)**?  
(making the shared features convergent)



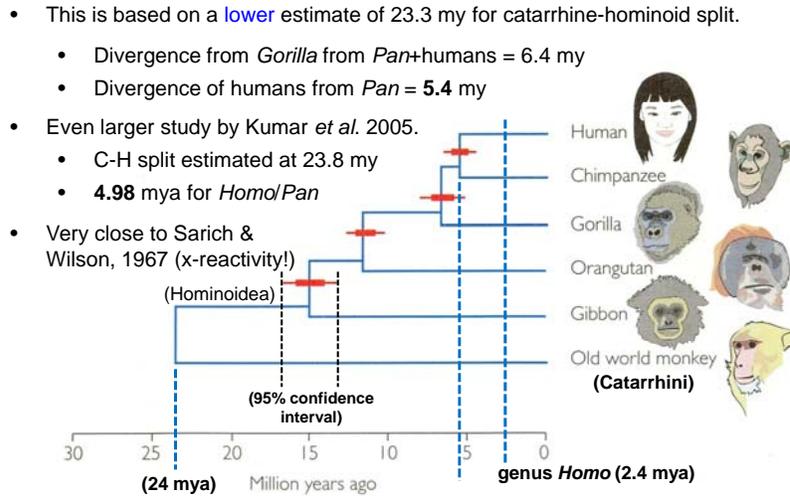




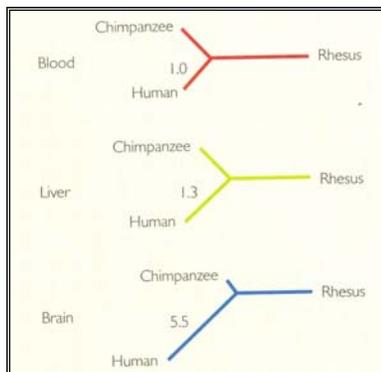
### More molecular analyses: **mtDNA COII** (Ruvolo 1994)



**More support, and time estimates, from sequence data from dozens of proteins used as **molecular clocks** (Stauffer *et al.* 2001)**



**Interesting stuff from **whole-genome analyses** (Enard *et al.* 2002)**

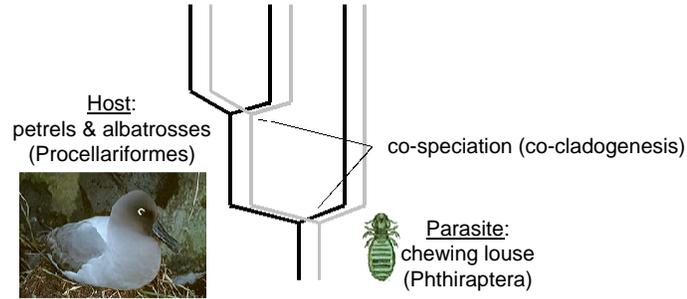


- The diagrams show the divergence in overall patterns of **gene expression** in humans vs. chimpanzees vs. rhesus macaques.
- The number is the ratio of human divergence to chimp divergence.
- In patterns of gene expression in blood and liver, *Homo* & *Pan* are roughly equivalent.
- But there is a major divergence for *Homo* in **brain** gene expression.
- Other work (Gilad *et al.* 2006) confirm King & Wilson's 1975 predictions, that among the genes with elevated expression in humans, a higher than expected number are **transcription factors** (promotors and repressors).

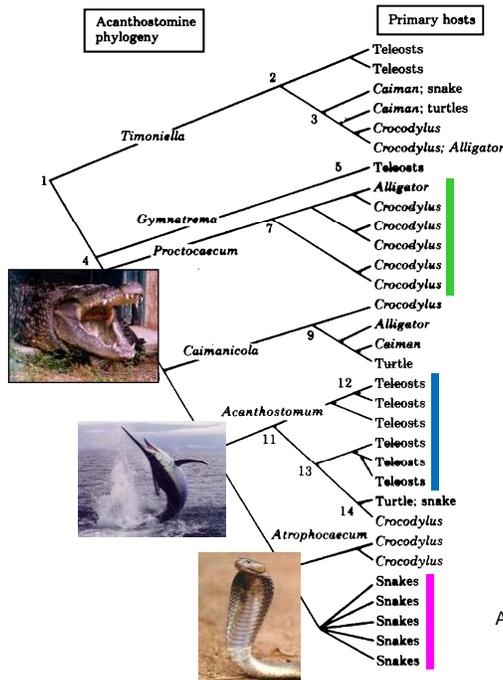
- Whereas chimps and humans are 99% the same with respect to sequences of protein-coding genes, **regulatory** sequences are the regions of concentrated differences (where regulatory proteins bind to influence gene expression).

2. Can the diversification of two (or more) lineages be correlated?

**Host-symbiont or host-parasite co-speciation**



- Phylogeny is especially powerful as a means of **comparison**.
- For example, phylogenies of two *interacting clades* of organisms can be compared with respect to their branching patterns.
- One looks for **congruence** (or matching) of such patterns, as indicative of co-speciation or co-cladogenesis, between...
- hosts and their **parasites, symbionts, mutualists, predators**, etc.

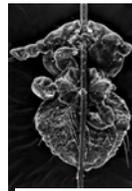
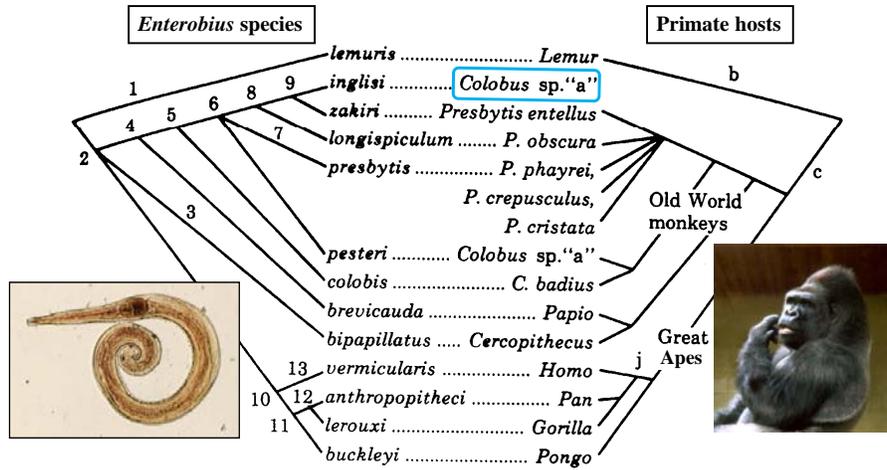


**A. Conservatism of host-parasite associations: Acanthostomine parasites and their vertebrate hosts (Mitter & Brooks 1983)**

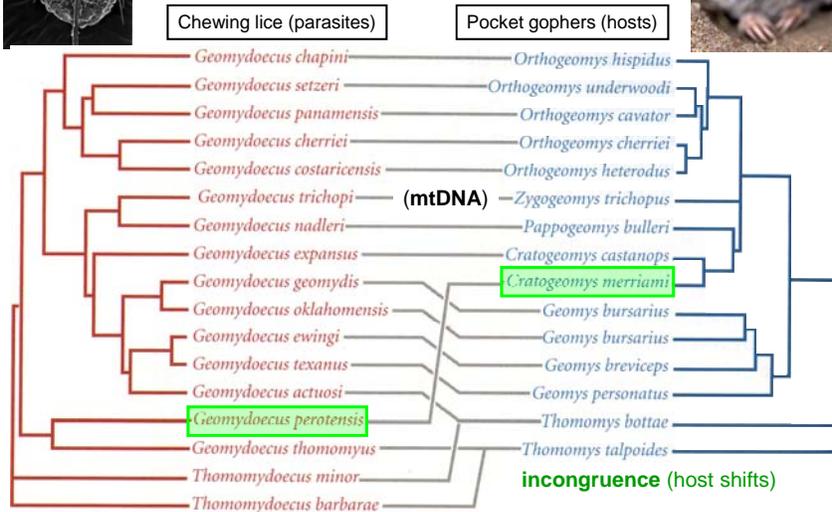


A parasitic flatworm (Digenea), *Acanthostomum*

**B. Phylogenetic congruence of the pinworm genus *Enterobius* (Nematoda) and its primate hosts** (Brooks & Glen 1982)

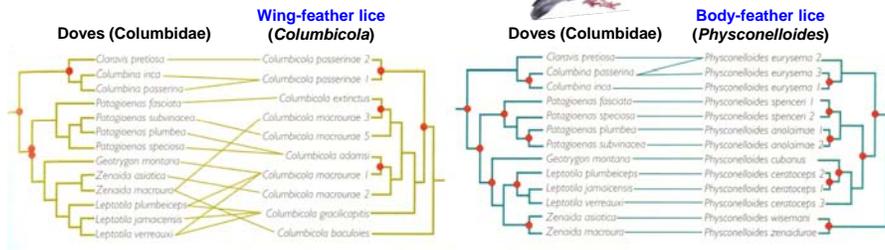


**C. Mirror-image phylogenies of parasites and host: pocket gophers (Geomyidae) & chewing lice (Phthiraptera)** (Hafner *et al.* 2003)



(see also Light & Hafner, 2007, for additional support)

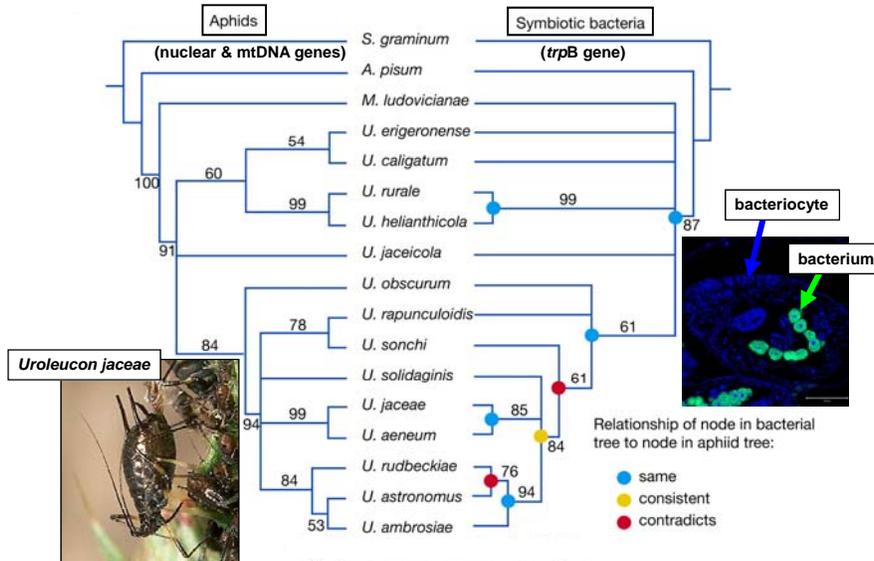
**D. Doves vs. wing- or body-feather lice**  
(Clayton & Johnson 2003)



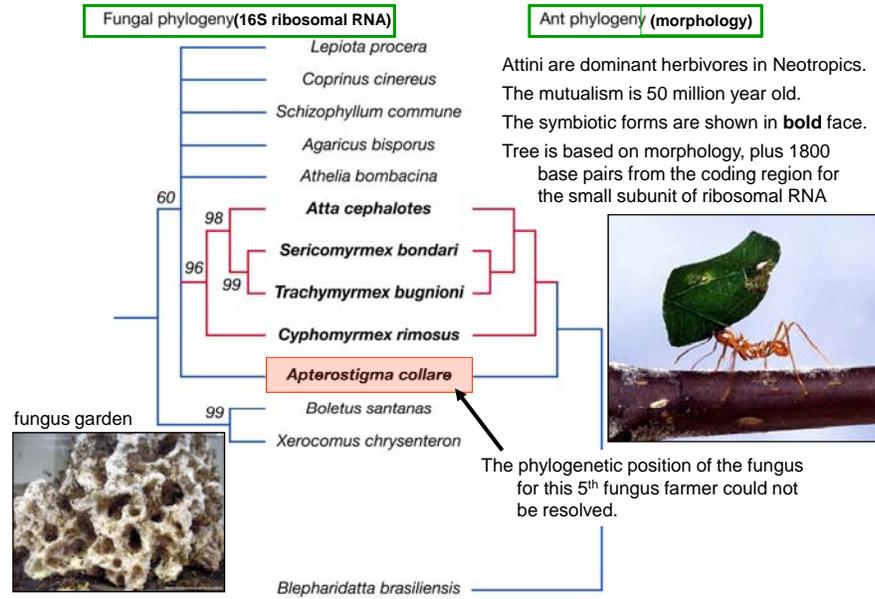
- High host-parasite congruence in body-feather lice, but little evidence of co-speciation for wing-feather lice.
- Not a feeding constraint: artificial host shifts work for both types of lice.
- But there's an ecological constraint: *body-feather lice are poor dispersers* between individual birds.
- Wing-feather lice are *phoretic*, using the legs of hippoboscid (louse) flies to travel between host individuals.



**E. Aphidae have co-specified with their Buchnera endosymbionts**  
(Clark et al. 2000; Moran 2001)



**F. Fungi and leafcutter ants: an obligate mutualism** (Hinkle et al. 1994)



**Fungi and leafcutter ants, continued** (Chapela et al. 1994)

