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Phylogeny of Gymnosperms, Coniferales, Pinaceae, Taxaceae etc.

REVIEW. Based on own research.

Version 1. (Not refereed).



Phylogeny of Gymnosperms, Coniferales, Pinaceae, Taxaceae etc.

A review, based on own research.

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Abstract

A review of the phylogeny of the Gymnosperms, in particular Coniferales (family level): Pinaceae (genera level), Araucariaceae, Podocarpaceae, Sciadopityaceae, Taxaceae s.l. (incl. Cephalotaxaceae) and Cupressaceae s.l. (Taxodiaceae + Cupressaceae s.s.). Gnetales belongs to the Gymnosperms. Cycadales and Ginkgoaceae are not investigated in details. The cladograms are mainly based on molecularcladistics, and the results are inspected using morphology, chemistry (including aroma of needles), paleobotanics and others, which may contribute to confirm the results. The reliability of the different ways of the molecularcladistic procedures is discussed and controlled by own investigations and cladograms using the basesequences of the genes rbcL, 18S rRNA and 28S rRNA from GenBank. In rbcL the choice of outgroup turn out to be essential. Gnetales is situated in the middle of the Gymnosperms only if Cycadales/Ginkgo is used as outgroup. The best outgroup for all conifers seems to be Ginkgo, a fact which is deducted from both cladistics and from constructing the hypothetical ancestors "standard Coniferales II" and "standard Pinaceae". Modifications to almost all earlier published Coniferales rbcL-cladograms are suggested, which include smaller or bigger changes within most conifer families.

Key words: Phylogeny, Gymnosperms, Gnetales, Coniferales, Pinaceae, Pinus, Abies, Araucariaceae, Podocarpaceae, Sciadopitys, Taxaceae, Cupressaceae s.l. incl. genus Xanthocyparis. rbcL, 18S rRNA, 28S rRNA, molecularcladistics, chemistry, aroma of needles.

Introduction

Phylogeny. The understanding of the interdependence of geology, climatology and the development of organic life during earlier periods has by now improved enormously. The phylogeny of the seed plants is of increasing importance in order to understand the extant life on earth.

Although it seems that the early ancestors of Gymnosperms and Angiosperms are about the same age, the Gymnosperms were dominating in the Mesozoic, whereas the Angiosperms took over in the Tertiary. The phylogeny of the extant Gymnosperms is of great importance for the paleobotanists in order to state the development during earlier periods.

The problems, which have puzzled the botanics within the phylogeny of the Gymnosperms, are among others the positions of Gnetales, Phyllocladaceae, Sciadopitys, Taxaceae/Taxales, Cephalotaxaceae etc. and whether Pinus is the ancestor within Pinaceae.

Molecularcladistics. In relation to morphology moleculargenetics has a nearly unlimited amount of information, which just waits to be revealed.

Publishing one or a few cladistic trees (different for each gene) with numbers showing more or less certainty (Bootstrap, Decay analysis, Branch-length) is rather confusing for ordinary botanists, who fail to see moleculargenetics as the answer to all the problems concerning family relationships.

According to Hillis et al. (1996) there are no fixed methods for using the many different algorithms and possible manipulations within moleculargenetics, and different authors may reach rather different results from the same set of data. Therefore the method seems even more uncertain and confusing.

In the following there are dealt with these problems, by analysing the methods, and if possible no cladogram is stated, unless it is supported by more than one factor, including moleculargenetics, morphology, chemistry, paleobotany and others, and no factor of significance is contradictory.

Choice of genes.

The genes *rbcL*, 18S rRNA and 28S rRNA were chosen here, because in 1999 they were the only genes for which many species of Gymnosperm were available in GenBank.

Choice of algorithm.

Morphology: Maximum parsimony, MP, is the obvious choice in order to compare characters, which may have evolved due to possible convergent evolution.

Moleculargenetics is different. If the mutations are thought to be random, comparing of similarities must be the most suitable way of constructing cladograms, which indicates the use of the Neighbour Joining algorithm, NJ. However, the NJ-algorithm is rather simple, and it yields only one single result, which makes it difficult to judge, whether there are other trees of almost equal quality.

From the later mentioned "handcounting"-results it is concluded that although the *rbcL*-mutations are absolutely not random, nevertheless comparison using similarities seems a very reliable method. Even if similarities are thought to give the best result within moleculargenetics the parsimonial principle is still valid. Therefore it is obvious to compare the results using both MP and NJ, using only Maximum Likelihood algorithm, ML, or using all three algorithms, the latter is done in this investigation.

Manipulations.

Theoretical considerations have brought forward that transversions should be more reliable than transitions, which is not confirmed for *rbcL*-mutations in the later mentioned "handcounting". Besides it does not make much sense that the results are getting more reliable by excluding more than half of the mutations, which is done if only transversions are considered.

It is assumed by other theoretical considerations that third base mutations are so frequent that it is better to omit them, because they else may be more misleading than directive. This is not the case for *psbA* and *psbB* according to Sanderson et al. (2000) and for *rbcL* according to my "handcounting". Both Källersjö et al. (1998) and Rydin et al. (2000) state that the results from *rbcL* investigations are getting poorer using manipulations.

Multigenetic analysis.

Some authors add different genes into a multigenetic analysis. Mixing genes with different rate of mutation is like adding cherries, apples and melons and then evaluate the average after weight. Among others Sanderson et al. (2000) state the problems caused by mixing fast mutating genes with "long branch attraction" together with slow mutating genes.

Using genes from all three genomes.

Trusting the common genetics it is difficult to find any advantage in the use of genes from all three genomes. Using just three different and more reliable genes would be preferable.

The heritability of chloroplasts and mitochondria are to a great extent uniparental, although the opinion of the pattern of heredity has changed due to the development of the methods of investigation. There are the following three methods: (1) Phenotype markers, (2) Molecular techniques (RFLP) and (3) Cytology (EM + light microscope) according to Reboud & Zeyl (1994). Each method has its limitation, and it is not possible to conclude from the small numbers of species still investigated. According to Gillham (1994) uniparental heritability has with great certainty been invented several times during the evolution. Maternal heredity is common for both within the Angiosperms, and paternal heredity is predominant for chloroplasts within Coniferales according to Szmida et al. (1987), Dong et al. (1992) and others, whereas the reports vary for the mitochondria due to reviews in Mogensen (1996) and Reboud & Zeyl (1994). Particularly Gnetales, Cycadales and Ginkgo are sparingly investigated and only by means of microscope according to Mogensen (1996).

Some authors e.g. Wang et al. (2000) within Pinaceae classify the genomes as paternal, maternal and biparental. This classification is of no influence neither to the mutationrate of the genes according to Otto et al. (1996) nor to the heredity of the mutations, unless the heredity is sex determined, which do not seem to be the case. However Page (1990) states that Pinaceae has developed due to hybridizing of genera, but this statement is contradicted by the *rbcL*-mutations in the "handcounting". On the contrary, even closely related species, which are growing close to each other, and which are able to hybridize, do not normally do so in nature according to Christensen & Dar (1997) and Filppula et al. (1992).

MADS-box genes.

The MADS-box genes are controlling the development of the essential reproductive structures, and thus they become extremely important in the question whether Gnetales belong to the Gymnosperms or is a close sister to the Angiosperms as pointed out by Frohlich (1999).

Outgroup.

The choice of outgroup may influence very much on the result, and therefore it is a necessity to choose the outgroup very carefully. It seems that many wrong conclusions are due to the choice of an inappropriate outgroup.

Material and methods

The molecular genetic investigations are almost exclusively based on the base sequences of the genes *rbcL*, 18S rRNA and 28S rRNA, which were listed in GenBank 1999 with a few later *rbcL* additions. The GenBank identification numbers are listed in appendix (page 21).

The alignment is done manually and the trees are constructed using PAUP v. 4.0b2, Swofford (1998). In order to examine the reliability of the mutations for the *rbcL* gene a great deal of "handcounting" was done mainly within Pinaceae, see below under *rbcL*-results.

rbcL-"handcounting" within Pinaceae:

Although most scientists seem to look down on my method it is still very simple to carry out, it only demands that you are able to tell the difference between the four letters A,G,C and T, that you are able to colour transitions red and transversions blue and green, and that you are able to count to about 30.

In order to state a hypothetically Pinaceae ancestor = "standard Pinaceae" the base sequences of all the Pinaceae species was compared with Ginkgo (and in case of doubt a few Cycadales, Araucariaceae, Podocarpaceae and Sciadopitys were taken in consideration). But as Ginkgo had extremely few mutations according to Pearson (1995) and others it was very easy to construct a very reliable "standard Pinaceae", and it was easy to state the mutations, which separate Ginkgo and Pinaceae.

Likewise it was easy to state the mutations, which were identical for all the *Pinus* species in question as well as splitting the *Pinus* genus into haploxyton and diploxyton. Within the other genera only the number of species of *Abies* were sufficient to proceed in a similar manner.

rbcL-alignment:

The base sequences from the *rbcL* chloroplast gene seemed very reliable. There were no conspicuous errors or problems except for *Selaginella*, which is not dealt with here. *Taxodium* is corrected earlier by Gadek et al. (2000).

18S rRNA-alignment:

Contrary to *rbcL* the nuclear ribosomal 18S rRNA gene is not coding for proteins which leads to complicating frameshift mutations.

All 18S rRNA base sequences except the Coniferales sequences done by Chaw et al. (1993, 1995, 1997A) seemed rather unreliable, and some of them seemed to be not only dependent of the plant but of the person, who did the sequencing, as well. Only very few of seemingly unreliable sequences are included in this investigation, and they were treated the same way, as done by Manhart (1995) concerning the alignment of 16S.

The alignment of the 18S rRNA sequences from Coniferales gave no trouble, they seemed almost aligned in advance.

In spite of two different requests Chaw denied to release his 18S rRNA Pinaceae-sequences from Chaw et al. (1997B), by which reason it was not possible to do Pinaceae in 18S rRNA.

28S rRNA-alignment:

The 28S rRNA base sequences were only about half the length of the 18S rRNA sequences, they were similar to the more difficult of the latter, and they were aligned in the same manner.

Chemistry, needle aroma and others:

The only chemistry mentioned here, which is not based on other papers, is the aroma of the leaves (conifer-needles) by biting in them. The aromas are not listed as "results", because they are only used as broad outlines to judge if they agree with the resulting tree.

Multifactorial analysis:

The main principle for this whole investigation is not only to state the conclusions on the basis of many different analyses showing the same result, but moreover the existence of essential contradictory results is not accepted unless there are good reasons to ignore those results. This principle includes both the molecular genetic results done by myself (using NJ, ML, MP and some "handcounting") and the molecular genetic results done by others, and it includes also morphology and all other information of any significance as well.

My own resulting cladograms are based on a large number of cladograms, both large trees including green algae, pteridophytes, monocots and dicots besides the Gymnosperms plus more limited trees only including a single family with outgroup, or including rather few species from different families. Besides I have used different outgroups. This has been done for all the three genes *rbcL*, 18S rRNA and 28S rRNA and using both NJ, MP and ML algorithms.

Results

rbcL-"handcounting" within Pinaceae:

Compared to the abovementioned "standard Pinaceae" the following results were obtained:

Cedrus:	14 mutations (7 of which were unique at the time). 1 species.
Larix:	10 mutations (2 of which were unique at the time). 1 species.
Pseudotsuga:	14 mutations (5 of which were unique at the time). 1 species.
Pseudolarix:	17 mutations (4 of which were unique at the time). 1 species.
Tsuga heteroph.:	19 mutations (10 of which were unique at the time). 1 species.
Keteleeria:	22 mutations (3 of which were unique at the time). 1 species.
Cathaya:	25 mutations (9 of which were unique at the time). 1 species. Rather unique.
Pinus, haploxyton:	21 specific mutations (15 define Pinus + 6 defining haploxyton). 5 species,
Pinus, diploxyton:	21 specific mutations (15 define Pinus + 6 defining diploxyton). 2 species, (+ a variable number from about 2-10 define the specific Pinus species).
Abies, "Japanese":	19 specific mutations (14 define Abies + 5 defining "Japanese"). 4 species,
Abies, "Californian":	18 specific mutations (14 define Abies + 4 defining "Californian"). 2 species, one of which, <i>A. mariesii</i> , is living in Japan. (+ a variable number from about 2-6 defines the specific Abies species).
I. <i>Picea sitchensis</i> :	14 specific mutations define <i>Picea</i> + 1 defining <i>sitchensis</i> .
II. <i>Picea pungens</i> :	14 specific mutations define <i>Picea</i> + 7 defining the <i>Picea</i> II group + 4 others.
II. <i>Picea abies</i> :	14 specific mutations define <i>Picea</i> + 7 defining the <i>Picea</i> II group + 4 others.

OBS: The *Picea* II group has a row of 7 mutations, which are unique within Pinaceae.

Picea asperata and *P. breweriana* are not listed, because they were not sequenced, when I did my handcounting. Besides the end of the sequence containing the row of mutations, which separates *Picea abies* and *P. pungens* from the other Pinaceae, is not included in the *P. asperata* and *P. breweriana* sequences, and therefore further investigation is not interesting.

The "handcounting" was carried out on the relatively few species, which were sequenced at the time, and it was neither based on any knowledge of the phylogeny nor attributed to the phylogeny (except for some new information about *Abies* and *Picea*). For most genera the mutations were predominantly transitions, but occasionally transversions dominated.

rbcL-cladograms: (Summary page 9 + detailed examples in appendix pages 22, 23 and 24).

The cladograms here are of course identical with the the earlier investigations, when identical algorithms and outgroups are used. These are Chase et al. (1993), Price et al. (1993), Brunsfeld et al. (1994), Källersjö et al. (1998) and Rydin et al. (2002). Only few of the Araucariaceae species done by Setoguchi et al. (1998) and few of the Podocarpaceae species done by Conran et al. (2000) are included in this investigation.

No manipulations, but different outgroups:

According to the examination and the "handcounting" of the Pinaceae sequences there was no reason to change the weighting of transitions/transversions or third position bases, which seemed to be reliable as attempts to manipulate caused less reliable results. The same conclusion is obtained by Källersjö et al. (1998) and Rydin et al. (2002).

The choice of outgroup, however, had great influence on the result as shown below.

Gymnosperm cladograms:

1. Using one Pteridophyte + one Angiosperm as outgroup Gnetales was sister to the Pteridophyte in MP, Gnetales was sister to the Gymnosperms in NJ, and Gnetales was sister to Pinaceae (inside Coniferales) in ML.

Apart from Gnetales Cycadales/Ginkgo were the first of the Gymnosperms in both MP and ML, but in NJ they showed up inside the Coniferales as sister to Pinaceae.

2. Using a green alga as outgroup and including several Pteridophytes Gnetales was sister to the Angiosperms in some of the cladograms.

3. Omitting the Pteridophytes and Angiosperms and using Gnetales as outgroup Cycadales/Ginkgo went inside the Coniferales as sister to Pinaceae using NJ, and as sister to Coniferales II using MP and ML.

4. Omitting the Pteridophytes and Angiosperms and using Cycadales/Ginkgo as outgroup Gnetales went inside the Coniferales as sister to Pinaceae using MP and ML, and as sister to Coniferales II using NJ.

5. Apart from the positions of Gnetales and Cycadales/Ginkgo the families of Coniferales showed the same topology in all the cladograms, and thus it did not depend on neither outgroup nor algorithm (except for minor details concerning Taxaceae s.l.).

Besides the topology of the Pinaceae genera showed some variation in the different cladograms.

The position of Gnetales according to the abovementioned cladograms:

It is a known fact that the evolution of Gnetales is far from the development of the other Gymnosperms, and Gnetales has far more mutations than the other Gymnosperms, a phenomenon which usually gives cladistic complications known as "long branch attraction".

According to the cladograms using Algae as outgroup and including many Pteridophytes the theoretical possibility exists that Gnetales is not a Gymnosperm, but it must be taken in consideration that the Algae-Pteridophytes is a very specific outgroup, which is absolutely not suited to give the right answer to this very complicated problem.

If, however, Gnetales is supposed to be a Gymnosperm, the most convenient outgroup is Cycadales/Ginkgo. Using Cycadales/Ginkgo as outgroup all cladograms of the different algorithms show Gnetales in near connexion with Coniferales. If Gnetales had not been a Gymnosperm it would definitely have gone in the outgroup together with Cycadales/Ginkgo, which it does not. From the fact that Gnetales remain inside the Gymnosperms must be concluded, that Gnetales without any reasonable doubt seems to be a Gymnosperm according to the rbcL base sequences.

Assuming that Gnetales belong to the Gymnosperms it seems that Gnetales has arisen within Coniferales because MP and ML shows it as a sister to Pinaceae, whereas NJ shows it as a sister to Coniferales II.

The two results are contradictory, and therefore the result is not obvious; the problem will be discussed later.

The position of Cycadales/Ginkgo according to the Gymnosperm cladograms:

Using Pteridophytes as outgroup MP and ML show Cycadales/Ginkgo as a sister to Coniferales, whereas NJ shows them as sister to Pinaceae.

Using Gnetales and Cycadales/Ginkgo as outgroup the cladograms are closely related, which means that when Cycadales/Ginkgo is situated within Coniferales with Gnetales as outgroup, then Gnetales is situated within Coniferales with Cycadales/Ginkgo as outgroup. Besides some algorithms show Cycadales/Ginkgo as sister to Pinaceae, and others as sister to Coniferales II.

Cladograms concerning Taxaceae s.l.

Constructing cladograms with *Torreya*, *Amentotaxus* and *Taxus* without *Cephalotaxus* only the NJ tree showed Taxaceae s.s. to be monophyletic, whereas ML and MP showed the family to be paraphyletic. By adding *Cephalotaxus* this problem disappeared totally, and *Cephalotaxus* placed itself right in the middle of *Torreya/Amentotaxus* and *Taxus* and thus Taxaceae s.l. became monophyletic. Also by "handcounting" almost every third mutation of *Cephalotaxus* was identical with a similar mutation in *Torreya*, and every third was identical with a mutation in *Taxus*, and every last third was a mutation of its own.

Relationship within the genera of Pinaceae.

The result depends on the chosen outgroup.

If Podocarpaceae/Araucariaceae/Sciadopitys, which at first may seem to be the nearest relatives, are chosen as outgroup, one of the genera from the very weak *Pinus/Cathaya/Picea*-clade will turn up as ancestor.

If Cycadales/Ginkgo, which is the natural choice, if you execute the Gymnosperms, are chosen as outgroup, *Cedrus* will show to be the ancestor. Although the cladogram is not completely certain down to the last detail, it gives quite a good impression of the relationship within Pinaceae.

The results from the "handcounting" concerning *Pinus* (haplo- and diploxylon) as well as the *Abies* ("Japanese" and "Californian") species are also evident from the cladograms.

18S rRNA-cladograms. (Summary page 9 + one detailed example in appendix page 25)
The resulting cladograms are rather similar to the trees stated in Chaw (1995, 1997A) [the sequences from Chaw (1997B) are not available], Doyle et al. (1994), Hamby & Zimmer (1992), Qui et al. (1999), Troitsky et al. (1991) and for the position of Podocarpaceae within the Gymnosperms with Kelch (1998).

28S rRNA-cladograms. (Summary page 9 + one detailed example in appendix page 25)
The various cladograms (MP, NJ and ML) with a Pteridophyte and an Angiosperm as outgroup are not in agreement with each other.
With Gnetales as outgroup the trees are identical with the resulting tree obtained using rbcL except for Ginkgo joining Pinaceae in 28S rRNA.
With Cycadales/ Ginkgo as outgroup the Coniferales trees are identical with the rbcL tree.
In almost all abovementioned cladograms Cedrus is ancestor within Pinaceae.
In many of the abovementioned cladograms Gnetales is sister to Cycadales.
The resulting MP consensus cladogram (fig 4) in Stefanovic et al. (1998) is due to manipulation (only using transversions) identical with the resulting rbcL tree. (A NJ-cladogram without manipulation only using Pteridophytes as outgroup is almost identical with the Stefanovic et al. tree.)

The reliability of the different cladograms.

An extensive work has been done in order to evaluate the reliabilities of the different cladograms, but as the result was absolutely consistent with the intuitive impression only a few results will be mentioned. The gene rbcL is "old faithful", indeed.

Using Pteridophyte + Angiosperm as outgroup rbcL had in approximate value $RI = 0,765$, which was 14% higher than 18S ($RI = 0,678$) and 27% higher than 28S ($RI = 0,601$) for all three algorithms, where RI using MP was 0 - 0,003 higher than for ML and 0,005 - 0,016 higher than for NJ.

Not surprisingly the cladograms using Gnetales and Cycadales/Ginkgo as outgroup gave higher RI-values. The approximate value for rbcL was $RI = 0,794$, which was 11% higher than for 18S ($RI = 0,714$) and 20% higher than for 28S ($RI = 0,663$).

On the basis of the abovementioned RI numbers rbcL is the most reliable and 28S the least reliable, and Gnetales or Cycadales/Ginkgo is equal reliable outgroups, which are more reliable than the Pteridophyte + Angiosperm outgroup, whereas the difference between the different algorithms (MP, ML and NJ) is almost negligible.

Chemistry: Aroma of needles by biting in them.

The non-metabolic, specific, chemical substances mainly in the essential oils of the conifer-needles are usually considered as secondary metabolites without real importance, although they act not only as insecticides and fungicides, but they also deter higher animals from eating the leaves.

Some of those chemical substances have a rather strong and specific aroma to humans, when you bite in the needles.

Aroma tasting:

The flavour of the needles from *Picea abies* by biting in them is called: Picea-taste. No systematic investigation is carried out, the flavours have been compared from memory by biting in needles of different species, when passing them occasionally.

Pinaceae:

Pinaceae with distinct Picea-taste:	<i>Picea</i> , <i>Abies</i> , <i>Tsuga</i> , <i>Cedrus</i> .
Pinaceae almost without aromatic taste:	<i>Larix</i> .
Pinaceae with a taste different but not very far from the Picea-taste:	<i>Pinus</i> .
Pinaceae with a taste which is rather different from the Picea-taste:	<i>Pseudotsuga</i> (orange-like).

Podocarpaceae:

Podocarpaceae with distinct Picea-taste: Podocarpus nivalis, P. alpinus, P. cunninghamii (=P. hallii).

Podocarpaceae with weaker but still distinct Picea-taste: Saxegothaea conspicua.

Podocarpaceae almost without aromatic taste: Podocarpus totara, P. henkelii, P. latifolius,
P. macrophyllus, Decussocarpus mannii.

Podocarpaceae with a taste different from the Picea-taste: Afrocarpus gracilior, A. falcatus.

Sciadopitys:

The taste of Sciadopitys is somewhat like a mixing of Picea-taste and Pseudotsuga-taste (both recognizable).

Taxaceae, genus Torreya:

The taste of Torreya (T. nucifera, T. californica, T. taxifolium) is near to (or similar to) the taste of Pseudotsuga.

Taxaceae, genus Cephalotaxus:

Cephalotaxus harringtonii has no aromatic taste.

C. fortunei has an aromatic taste very different from the Picea-taste.

Taxaceae, genus Amentotaxus:

Amentotaxus argotaenia has no aromatic taste.

Taxaceae, genus Taxus:

Taxus baccata has no aromatic taste.

"Taxodiaceae":

Sequoia sempervirens has a faint aromatic Picea-like taste.

Cunninghamia lanceolata, Taxodium distichum, Sequoiadendron giganteum, Metasequoia and Cryptomeria japonica has no marked aromatic taste.

Cupressaceae s.s.:

For instance Chamaecyparis, Thuja and Juniperus are well-known for the aromatic taste, very different from the Picea-taste.

Chemistry and Aroma of needles. Whereas the chemistry of the essential oils and other major secondary metabolites are known to some extent, it is not known which of the many chemical substances that causes the aroma. Therefore the content of the different compounds may vary considerable for species with the same taste, and the flavour may vary without no significant change in other major components.

The lack of taste in Taxus may be explained by the lack of mono- and sesquiterpenes as stated in Gottlieb et al. (1990): "*A very different situation is observed for mono and sesquiterpenes which abound in types and numbers in most conifers. Only Taxaceae (Taxus, but not Torreya) and Ginkgoaceae are exceptional in this respect, both characterized by highly oxidized diterpenoids, respectively taxanes and ginkgolides. The oxidative power of their biosynthetic machinery seems hardly compatible with the presence of easily oxidizable, simple monoterpenes.*"

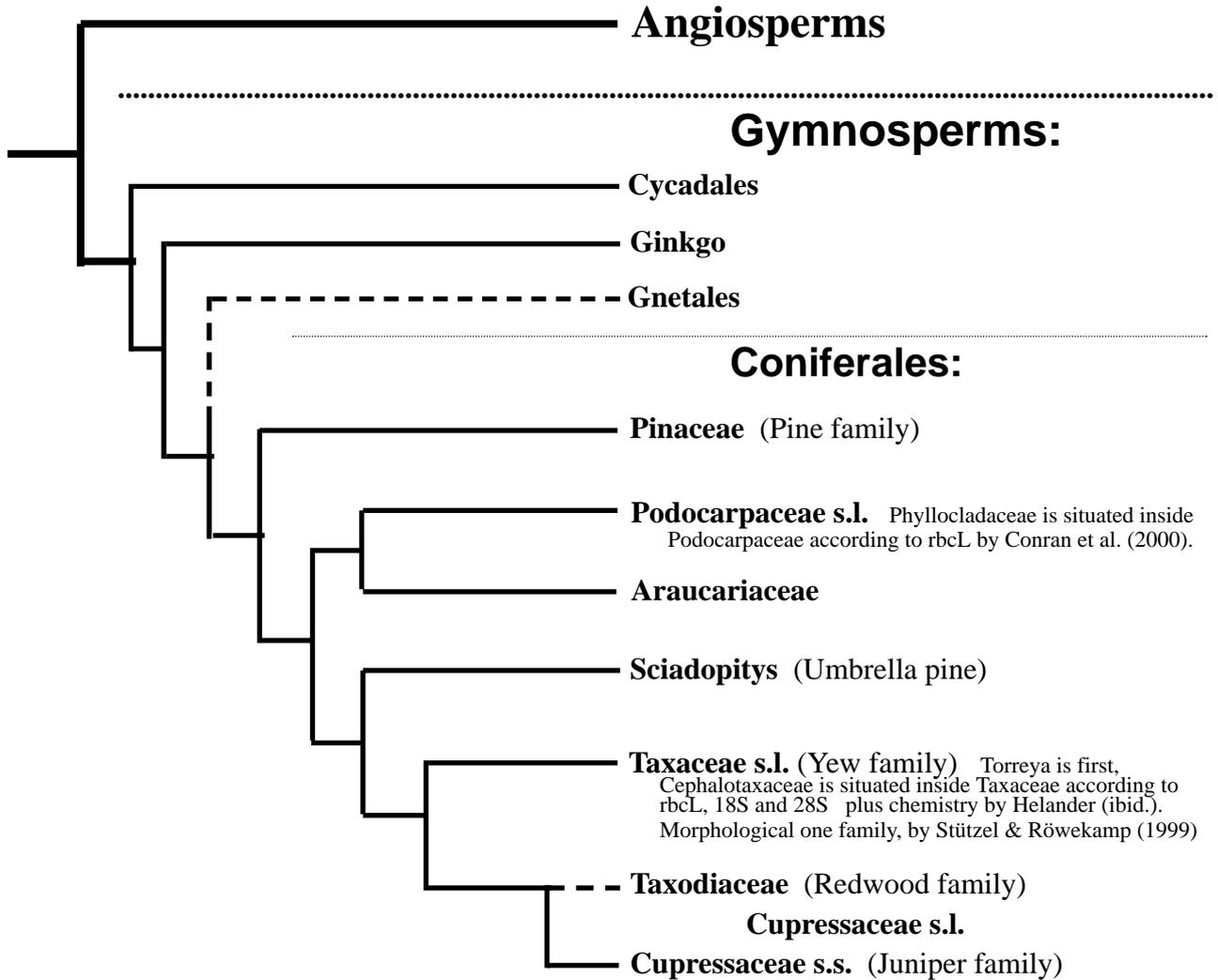
In Gottlieb et al. (1990) three tables are showing flavonoides, terpenes, and other substances, but it is mainly a review of Hegnauer (1962 and 1985), not supplemented from Chemical Abstracts. Besides only the positive findings are stated, which means that the informations are of limited value.

Terpenes: A review of the content of sesqui-, di- and triterpenoids as chemosystematic markers in conifers is given by Otto & Wilde (2001). The aroma of the needles is probably mainly due to monoterpenoids, and from the abovementioned investigation only the sesquiterpenoids may have a boiling point low enough to add a little aroma to the needles. Whereas the conclusion of the review offers some deductions, which may confirm parts of the main tree page 9, it offers as anticipated no information of the aroma of the needles.

Flavonoides: According to Harborne & Baxter (1999) some Podocarpaceae and some Zamia species contain the same flavonoides. Other flavonoides, which is common found in Coniferales II is not mentioned for Pinaceae. Again, like mentioned above, only the positive findings are stated, which means that the informations are of limited value.

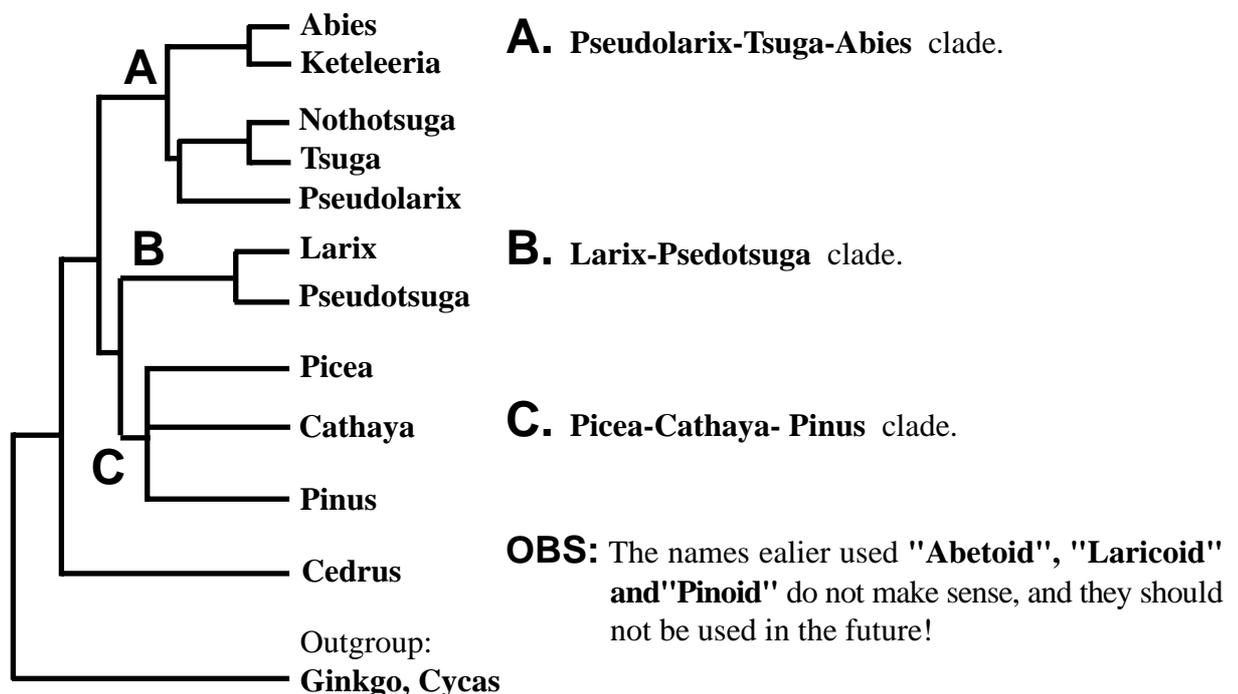
Phylogeny of Gymnosperms

Tree based on rbcL, 18S, 28S and others. By Jenny Helander (ibid.).



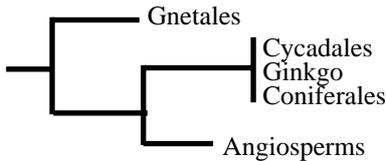
Phylogeny of Pinaceae

Tree based on matK, nad5, 4CL by Wang et al. (2000), plus rbcL and some morphology by Helander (ibid.).



Review: Phylogeny of Gnetales

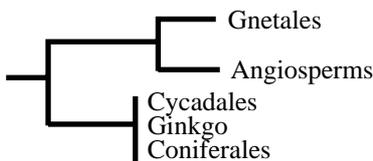
A. Gnetales



18S + 26S rRNA: Hamby & Zimmer (1992).
 rbcL: Albert et al.* (1994); Källersjö et al. (1998).
 rbcL + atpB + (18S + 26S): Rydin et al. (2002)

Marked* is according to Doyle (1998)

B. Anthophyte



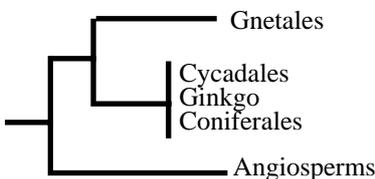
Morph.: Crane* (1985); Doyle & Donoghue* (1986, 1992);
 Laconte & Stevenson* (1990); Nixon et al.* (1994); Doyle (1996).
 18S + 26S rRNA: Hamby & Zimmer (1992).
 rbcL: Chase et al. (1993); Price (1996).
 28S rRNA: Stefanovic et al. (1998).

C1. Gymnosp. Uknown



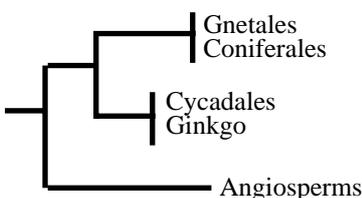
Morph.: Donoghue & Doyle (2000); [Friedman & Carmichael (1995) +
 Friedman & Floyd (2001), this is a possibility.]
 Kemi: Gottlieb & Kubitzki (1984); Carnachan & Harris (2000).
 MADS-box: Becker et al. (2000); Theissen et al. (2000); Winter et al. (1999);
 LEAFY: Frohlich & Meyerowitz (1997).
 rbcL + 18S + 28S: Jenny Helander (ibid.).
 4,5S + 5S + 5,8S: Troitsky et al. (1991).
 9149 bases from different genes: Hansen et al. (1999).
 cpDNA-ligheder: Raubeson (1998) + personal communication.

C2. Gymnosp. First



Legumin: Shutov et al. (1998).
 rbcL: Haseby et al.* (1992).
 chlB: Boivin et al. (1996) - **OBS**: Excl. Angiosperms.
 cpITS1 + cpITS2: Goremykin et al. (1996).
 rpoC1: Samigullin et al. (1999).
 psbA + psbB: Sanderson et al. (2000).

C3. Gymnosp. Conif.-sist.



cpITS1 + cpITS2: Goremykin et al. (1996).
 18S rRNA: Chaw et al. (1995, 1997, 2000)
 rbcL (manipulated): Chaw et al. (2000).
 mtSSUrDNA: Chaw et al. (2000).
 cox3: Malek et al. (1996).
 cox1: Bowe & DePamphilis (1997).
 3 + 4 genes together (only mt-atpA is new): Bowe et al. (2000).
 psbA + psbB: Sanderson et al. (2000).
 5 gener (incl. rbcL + 18S rRNA): Qui et al. (1999).
 rpoC1: Samigullin et al. (1999).
 rbcL + cox1 + nad1 + 18S + m-SSU (7705 bases): Gugerli et al. (2001).

Review. Discussion including all results.

I. Gnetales belongs to the Gymnosperms.

A review of the most important papers concerning the familiar position of Gnetales is given page 10.

Morphologically Gnetales has some features, which do not exist in the rest of the Gymnosperms, and which are rather similar to the Angiosperms, but there are still so many small differences that they may as well be interpreted as parallel development rather than homologies.

Donoghue & Doyle (2000) revise their earlier support to the anthophyte theory, and they state that all the similarities between Gnetales and the Angiosperms might as well be parallel developments. Among others they mention "flowers", double fertilization, vessels, dicot-like leaves besides megaspore and air sacs on the pollen.

According to Carlquist (1996) the vessels are due to parallel development.

Double fertilization by Gnetales may be parallel development to the Angiosperms according to Friedmann & Carmichael (1995), and according to Friedmann & Floyd (2000) the double fertilization by Gnetales may be homolog to similar (but rare) instances found among the Gymnosperms.

Chemistry stated in 1982 finally that Gnetales belong to the Gymnosperms. Hegnauer (1962) mentions a lot of chemical similarities between Gnetales and different Angiosperms from which he concludes a near family relation and confirmation of the anthophyte theory. But Gottlieb and Kubitzki (1982) state that all those apparently similarities were connected to various distant Angiosperms whereas Gnetales has a close relationship with the Gymnosperms in essential chemical pathways.

The Gymnosperms is monophyletic owing to their content of Ferulic acid in the cell walls according to Carnahan & Harris (2000).

Moleculargenetically Gnetales has given great troubles caused by the following three main facts:

(1) The evolution of Gnetales is very different from the evolution of the other Gymnosperms, which has caused many mutations in Gnetales, which is not found elsewhere.

(2) The nearest ancestors to as well the Angiosperms and the Gymnosperms are extinct, which leaves us without proper outgroup.

(3) The splitting up between Gnetales and other Gymnosperms has happened very, very long ago.

Conserved regulatory genes controlling morphology and reproduction like MADS-box and LEAFY are the most obvious choice if you really want to distinguish the relationship.

MADS-box genes show according to Becker et al. (2000), Theissen et al. (2000) and Winter et al. (1999) unambiguous that Gnetales belongs to the Gymnosperms. The same goes for LEAFY-genes according to Frohlich & Meyerowitz (1997). The problem is that only very few taxa are investigated.

rbcL may be used in very many different ways (using different outgroups, manipulations and algorithms) and may thus show whatever you want. By choosing the nearest outgroup (Cycadales/Ginkgo) and without manipulations the three algorithms MP, ML and NJ all show that Gnetales belongs to the Gymnosperms as Gnetales occurs inside Coniferales for every one of the algorithms.

18S rRNA shows Gnetales as a sister to Coniferales with all three algorithms and both Pteridophyte/Angiosperm and Cycadales/Ginkgo as outgroup.

28S rRNA likewise shows that Gnetales belongs to the Gymnosperms (usually as sister to Cycadales) with both Pteridophytes/Angiosperms and Cycadales/Ginkgo as outgroup.

The results for the many other genes, not examined in this paper, are listed in the review page 9.

II. Gnetales may be sister to all other Gymnosperms or may be sister to Coniferales.

Although it is very evident that Gnetales belongs to the Gymnosperms, the position of Gnetales within the Gymnosperms is very uncertain. Theoretically the following four positions is possible:

(1) As sister to the rest of the Gymnosperms, (2) Between Cycadales and Ginkgo, (3) As sister to Coniferales, (4) Inside Coniferales.

Morphologically the reproductive structures of Gnetales are more similar to Coniferales than to Cycadales/Ginkgo, which strongly indicates a near connexion to Coniferales.

Moleculargenetically only the investigations of the three genes rbcL, 18S rRNA and 28S rRNA included the sufficient number of species for conclusions before the work of Rydin et al. (2002).

Using the right outgroup (Cycadales/Ginkgo) *rbcL* shows a strong connection between Gnetales and Coniferales. The less reliable 18S rRNA shows the same connection in the cladograms used here, which are slightly different from the cladograms in Chaw et al. (1997, 2000), probably due to a small difference in the alignment. Only the least reliable 28S rRNA shows Gnetales as sister to the rest of the Gymnosperms. Investigations including other genes (page 10) do not agree in their results. On the basis of a summary of all genes done so far it is concluded that also the molecular genetics indicate a near connection between Gnetales and Coniferales.

Bowe et al. (2000) and particularly Chaw et al. (2000) claim that Gnetales is situated inside Coniferales using 4 respectively 3 genes, one of which is *rbcL*. The same result is obtained using *rbcL* alone as mentioned above using all the three algorithms MP, ML and NJ. But this result may easily be an artifact. Both Gnetales and Cycadales/Ginkgo are not constant situated at the same position in the different cladograms, but they have a tendency of moving around.

Raubeson & Jansen (1992) have proven that a rare chloroplast-DNA mutation is shared by all conifers, whereas it is not present in Gnetales, Cycadales/Ginkgo and Pteridophytes. This fact strongly indicates that Coniferales is monophyletic. As long as not proven otherwise the evidence from Raubeson & Jansen by far surpasses the very weak possibility shown by some of the many contradictory cladograms that Gnetales is a sister to Pinaceae. Furthermore the close morphological relationship between Pinaceae and Coniferales II (see below) contradicts the hypothesis of Gnetales inside the Coniferales, even although it does not exclude the possibility of Gnetales as a Pinaceae sister.

III. The Gymnosperms (including Gnetales) is a monophyletic group.

According to most cladograms and the other abovementioned reasons the Gymnosperms is a monophyletic group.

IV. The Angiosperms is a monophyletic group and sister to the extant Gymnosperms.

As a consequence of the monophyly of the Gymnosperms the Angiosperms is a monophyletic group as well.

An earlier theory suppose that the Angiosperms have developed from the Gymnosperms, which partly was supported by the fact that fossils of Gymnosperms were known from the beginning of the Mesozoic, whereas fossils of certain Angiosperms until recently was unknown before Cretaceous. This conclusion was completely wrong, because no fossil of something between a Gymnosperm and an Angiosperm has ever turned up. Luckily an older certain Angiosperm fossil (from the Jurassic) has recently been discovered according to Sun et al. (1998, 2000).

V. Cycadales and Ginkgo are closely related, and they are probably sister to all other Gymnosperms.

Morphologically the reproductive structures and their evolution are closely related for Cycadales and Ginkgo according to Bhatnagar & Moitra (1997) and others.

Molecular genetic they are not placed with certainty within the cladograms, but when they show up inside Coniferales it must be an artifact (like Gnetales and with same evidence). Assuming that Gnetales is sister to Coniferales, Cycadales and Ginkgo are the first of the Gymnosperms. Most cladograms show Cycadales prior to Ginkgo, many show them as sisters and only a few show Ginkgo before Cycadales. It is most likely to suppose that Cycadales is before Ginkgo and that Cycadales thus is sister to all other Gymnosperms.

Knowing the paleobotanic evidence the abovementioned relationship for Cycadales and Ginkgo is very easy to accept as they both have developed very early according to the fossils, where both have been known from the beginning of the Mesozoic i.e. for more than 200 million years according to Stewart and Rothwell (1993).

VI. Coniferales is monophyletic.

As mentioned above the evidence of conifer-monophyly in Raubeson & Jansen (1992) showing a rare chloroplast-DNA mutation only within all conifers, but not within Gnetales, Cycadales/Ginkgo and Pteridophytes by far surpasses the very weak possibility of Gnetales derived from within Coniferales, which some of the many contradictory cladograms show. This implies that Coniferales until further evidence of the opposite must be considered monophyletic.

VII. The Gymnosperm tree (apart from Gnetales) is determined.

According to the tree given on page 9: Cycadales is first, Ginkgo second and at last Gnetales and Coniferales are sisters. Although some uncertainty about the position of Gnetales still may exist there other alternatives are unlikely with the present knowledge.

VIII. Coniferales family tree is determined. Pinaceae is the one half of Coniferales, all other conifer families (named: Coniferales II) are the other half.

The Coniferales family tree is stated on page 9 as a part of the Gymnosperm tree. The division into a Pinaceae part and a Coniferales II part is beyond any doubt, whereas until recently the relationship of all other conifers except Pinaceae has been very uncertain. Reviewing the latest results including the investigation done in connection with this report there is no doubt that the here stated Coniferales II tree is totally safe on family level.

IX. Pinaceae has most likely a Cedrus-like ancestor, which then is close to the ancestor of all Coniferales.

The "handcounting" shows that *Larix*, *Pseudotsuga*, *Cedrus* and partly *Picea sitchensis* have fewer mutations than the other Pinaceae. According to Szmidt et al. (2001) *Larix* (*Pseudotsuga* and *Cedrus* are not investigated) like *Ginkgo* in the *cox1* gene do not show the RNA-editing, which characterize the other Pinaceae (including *Picea abies*). This indicates that one of the genera *Cedrus*, *Larix* or *Pseudotsuga* may be the root of Pinaceae.

The most probable tree of the genera within Pinaceae is stated on page 9. The ancestor seems to be a *Cedrus*-like conifer according to Wang et al. (2000) using *matK*, *nad5* and *4CL*, and to both *rbcL* and the more unreliable 28S rRNA in this investigation, when Cycadales/Ginkgo is used as outgroup. As Chaw on two occasions has denied to release his 18S rRNA from Chaw et al. (1997B) it has not been possible to investigate this gene using *Ginkgo* as outgroup.

Wang et al. (2000) has chosen *Cycas* as outgroup: "*because sequence divergence of the rbcL gene is lower between Pinaceae and Cycas than between Pinaceae and Podocarpaceae or Araucariaceae (Wang et al. 1998a).*"

The "handcounting" in this investigation not only showed a close relationship between *Ginkgo* and Pinaceae in *rbcL*, which was the basis for constructing "standard Pinaceae", but *Cedrus* seemed also to be a genera which was rather close to "standard Pinaceae".

If Podocarpaceae/Araucariaceae/Sciadopitys, which at first may seem to be the nearest relatives, are chosen as outgroup, one of the genera from the very weak *Pinus/Cathaya/Picea*-clade will turn up as ancestor, which goes for *rbcL* in this investigation and 18S rRNA in Chaw et al. (1997B).

Morphologically there are at least three strong arguments for a *Cedrus*-like ancestor:

- (1) If the *Cedrus*-like ancestor had the same shoot-morphology as the recent *Cedrus* with both short and long shoots it gives a good explanation of the shoot-morphology of all other genera of Pinaceae.
- (2) Apart from having two seeds per scale in the *Cedrus* cone it is very similar to the Araucariaceae cone with one seed per scale, a fact which suggests a rather close relationship. The Araucariaceae family has like *Cedrus* preserved the upright and rather monstrous cones in all species, the cone position has changed into hanging in some of the other Pinaceae genera. Whereas it is rather easy to believe that a big, upright cone becomes hanging, it is rather impossible to imagine that a hanging cone changes by mutations into a big, upright cone, which not only demands a change of position of the cone axis but also requires a strong reinforcement of the conebearing branch at the same time.

(3) The mature cone dismember leaving the cone axis on the tree is a similarity between *Cedrus* and most *Araucariaceae*.

Pseudolarix (but not *Tsuga* and *Pseudotsuga*, which according to the cladogram are sisters to *Pseudolarix*), *Abies* and the close *Abies*-sister *Keteleeria* have the same upright cone as *Cedrus* (the *Keteleeria* cone do not dismember at maturity), i.e. that exclusively according to cone morphology they may be *Pinaceae* ancestors as well.

The abovementioned argumentation shows for certain that a rather big, upright cone, which dismember at maturity, originally has developed from an ancient ancestor to the conifers, and that it was still present at the time, when the conifers started the splitting up into *Pinaceae* and *Coniferales* II (including *Araucariaceae*).

Morphologically the *Pinus/Cathaya/Picea*- + *Larix/Pseudotsuga*-clades of *Pinaceae* are linked together due to two even more persistent characters which according to Farjon (1990) are

(1) wood anatomy: Resin canals (both vertical and horizontal) are present; and

(2) root anatomy: Two resin canals are present in vascular cylinder of young taproot.

This is true for both the *Larix/Pseudotsuga*-clade and the very weak *Pinus/Cathaya/Picea*-clade.

From the "handcounting" it is obvious due to the many unique mutations that *Cathaya* is not close related with any other *Pinaceae*. Using RFLP of *trnK* Wang et al. (1998B) have obtained the same result

A survey of the most important morphological characters within *Pinaceae* is found in Farjon (1990). *Pinaceae* is sharing two further characters with *Sciadopitys*, which make *Sciadopitys* very "Pinaceae-like" at distance. The one is the very quick lignification of the young shoots, the other is the often long, slim needles, which may be rather stiff.

Paleobotanically is the solution of the relationships within *Pinaceae* of the greatest importance. The earlier theory (among others based on cone fossils) that *Pinus* was the most likely ancestor has shown to be completely wrong. The great similarity between the early *Pinaceae* (*Cedrus*-like ancestor) and *Araucariaceae* cone is also very important knowledge to the paleobotanists.

X1 Within Pinaceae: *Abies* may be older than *Pinus*, and *Abies* may be more divided than *Pinus*.

According to the abovementioned *Pinus* is not the oldest genus within *Pinaceae* and neither is *Abies*. Although *Abies* is not the ancestor within *Pinaceae*, it may still be as old or even older than *Pinus*. Morphologically has *Abies* preserved the originally cone characters, but the suggestion is more based on molecular genetic basis. The splitting of *Abies* into "Japanese" and "Californian" sections seems according to the number of mutations involved in the "handcounting" to be almost as deep as the splitting of *Pinus* into haplo- and diploxyton. An earlier investigation using mtDNA polymorphism by Tsumura & Suyama (1998) of the 5 in Japan growing species of *Abies* has shown that *Abies mariesii* (which are mentioned "Californian" here) are entirely different from the 4 other species (mentioned "Japanese"). Another investigation using cp-DNA *trnL*-intergeneric spacer by Isoda et al. (2000) including 18 species of *Abies* (but unfortunately not *Abies magnifica*) indicates that a even deeper splitting may have taken place and not only into two but about five different sections.

X2. Within Pinaceae: *Keteleeria* is very close to *Abies*.

It appears from the "handcounting" and even more from the different cladograms that *Keteleeria* is very close to *Abies* (in some of the cladograms it is even inside *Abies*, which is an artifact).

X3. Within Pinaceae: *Picea*.

When the "handcounting" was done only the three species *Picea abies*, *P. pungens* and *P. sitchensis* were sequenced. *Picea sitchensis* was different from *P. abies* and *P. pungens* especially because the two latter had a row of 7 similar mutations, which were different from all other *Pinaceae*.

The sequence of the two species *Picea asperata* and *P. breweriana* done by Rydin et al. (2002) was unfortunately shorter, not showing the part of the sequence in which the 7 special mutations were present. Therefore further speculations regarding the relationship within the 5 *Picea* species will be omitted.

XI. Coniferales II is a single clade (monophyletic).

Almost all cladograms show Coniferales II to be monophyletic. Besides according to Gugerli et al. (2001) only the Conifers II have the loss of a nad1 intron 2, which is present in almost all other plants with the exception of a very few other losses scattered by chance.

XII. Araucariaceae/Podocarpaceae s.l. (= Phyllocladaceae + Podocarpaceae s.s.) is a single clade, which is the "first" half of Coniferales II.

The Araucariaceae investigation by Setoguchi et al. (1998) in rbcL including 30 species shows that *Wollemia* had derived prior to *Araucaria* (19 species) and *Agathis* (10 species) both of which are monophyletic. Unfortunately they used an inappropriate outgroup (*Podocarpus*, *Taxodium* and *Juniperus*) and their results are not in agreement with the cladograms in which *Ginkgo* is part of the outgroup. In all the latter cladograms using all algorithms *Araucaria* is sister to both *Wollemia* and *Agathis*, *Wollemia* and *Agathis* being sisters.

The Podocarpaceae-Phyllocladaceae investigation by Conran et al. (2000) in rbcL including 76+5 species shows that the 5 *Phyllocladus* species are situated inside the Podocarpaceae. Among others according to their fig. 1 strict consensus tree even their choice of outgroup may be inappropriate. In all my cladograms (except the NJ-trees) in which *Ginkgo* is part of the outgroup *Phyllocladus* is prior to the other two *Podocarpus* species, one of which is *Saxegothaea*, and thus giving a weak indication of *Phyllocladus* being first in Podocarpaceae s.l.

Conran et al. state that their investigation agree with the 4 Podocarpaceae species which were included in the investigations done in 18S rRNA by Chaw et al. (1997A, 1995), where *Saxegothaea* was not included, but contrary to the results in 18S rRNA done by Kelch (1998) according to whom *Phyllocladus* is prior to the rest of the Podocarpaceae. The 18S rRNA investigation stated here including 6 species confirm the results of Kelch (1998) in his fig. 2 in respect to the position of *Phyllocladus*, but disagree with Kelch according to *Saxegothaea*, which seems to be next and thus be prior to *Dacrycarpus*, where Kelch gets the opposite.

The development of the Araucariaceae and the Podocarpaceae is simultaneous and seems to have started at about the same time.

XIII. Phyllocladus seems to be a part of Podocarpaceae s. l.

Phyllocladus may be first in the Podocarpaceae clade as indicated by the 18S rRNA results and the rbcL-trees using *Ginkgo* in the outgroup, or it may be situated inside the clade as indicated by the abovementioned rbcL-results by Conran et al. (2000). But in both cases the cladograms show that *Phyllocladus* is a natural part of Podocarpaceae s. l. and not a family of its own.

XIV. Sciadopitys is a family of its own (= Sciadopityaceae) and is the "first" family of the "second" half of Coniferales II.

Sciadopitys verticillata has earlier often been classified as belonging to Taxodiaceae due to some morphological characters in the reproductive system although it by stature looks very similar to Pinaceae among others due to the early lignification of the young shoots and the long, stiff needles. Even the correct detailed morphology of shoots and needles, which will not be discussed here, may in some respects be homolog to the rather close Pinaceae family.

All molecular genetic investigations Chase et al. (1993), Price et al. (1993), Brunsfeld et al. (1994), Tsumura et al. (1995), Chaw et al. (1997) and Stefanovic et al. (1998) using rbcL, RFLP of 6 genes, 18S rRNA and 28S rRNA showed from the very beginning that *Sciadopitys* was a family of its own. Chemically there are many similarities and only small differences with other related Conifers according to Hegnauer (1962, 1986), where *Sciadopitys* is classified as belonging to Taxodiaceae.

The aroma of the needles by biting in them is very distinct like a mixture of *Picea*-taste and the taste of *Torreya* (near to *Pseudotsuga*-taste).

Paleobotanically fossils of Sciadopityaceae are very old and known as an independent group since the Upper Triassic according to Florin (1922), and for that reason already Seward (1919) presumed that *Sciadopitys* was the only recent member of an old family.

XV. Taxaceae s.l. (= Cephalotaxaceae + Taxaceae s.s.) is one family. Taxaceae is the "second" family of the "second" half of Con.II.

Moleculargenetically Taxaceae s.l. (which means Taxaceae s.s. plus Cephalotaxus) is one family or close connected according to Bowe (2000), Chase et al. (1993), Chaw et al. (1995, 1997A, 2000), Cheng et al. (2000), Källersjö et al. (1998), Stefanovic et al. (1998) and Tsumura et al. (1995). In the cladogrammes the three clades of the family: (1) Torreya/Amentotaxus, (2) Cephalotaxus and (3) Austrotaxus/Pseudotaxus/Taxus are rather weak related. According to rbcL in this investigation Torreya/Amentotaxus is sister to the two other clades with rather small statistical evidence, whereas Cheng et al. (2000) find that Cephalotaxus is prior to the two other clades, which allow them to state that Cephalotaxus is a family of its own.

By "handcounting" in rbcL the intergeneric relationship is very evident because Cephalotaxus besides the mutations of its own share almost the equal number of mutations with Torreya as it do with Taxus. Morphologically the Taxaceae s.l. seeds, which do not look like cones have caused great troubles. Stützel & Rövekamp (1999) and Mundry (2000) have shown that the female reproductive structures are totally homologous in Torreya, Cephalotaxus and Taxus, and therefore these structures define the Taxales s.l. family. Their results are in contrast to the results of the earlier botanists, who have not been able to reach the conclusions, which seem extremely obvious for non-specialists. Stützel & Rövekamp (1999) states: "*More recent descriptions of reproductive shoots in Taxus are often misleading, ambiguous, or even wrong in important points (e.g. Page 1990, p. 348-353)*".

According to Page (1990) the resin canals are still present in the needles of Torreya, Amentotaxus and Cephalotaxus, but absent in the rest of Taxaceae.

Chemically Taxus but not Torreya according to Gottlieb et al. (1990) is having a special oxidative biosynthetic power, which exclude the presence of the easy oxidable mono- and sesquiterpenes in Taxus.

Torreya needles have an aroma close to Pseudotsuga and with some relation to Sciadopitys. Cephalotaxus fortunei has a rather distinct aroma in the needles, whereas the needles of C. harringtonia, Amentotaxus and Taxus have no aromatic taste.

XVI. Cupressaceae s.l. (= Taxodiaceae + Cupressaceae s.s.) is a single family, the "third" and last family of Coniferales II.

From the very first molecular genetic cladograms it were obvious that Taxodiaceae would become paraphyletic, if Cupressaceae s.s. was kept as a separate family. Therefore the two families are now united in one big family named Cupressaceae s.l. (Using the name Taxocupressaceae instead would avoid many future mistakes, but practical and logical naming is forbidden by the Nomenclature Commission, just think of the contradictory name "Picea abies").

The former Taxodiaceae differs morphologically in many respects from Cupressaceae s.s. and is prior to them. Moleculargenetic Cunninghamia is the oldest in all cladograms. It is followed by first Taiwanian and then Arthrotaxis according to the matK-tree by Gadek et al. (2000), whereas these two genera may be sister due to the rbcL-cladograms. Afterwards follows the Sequoioid-clade including Metasequoia, Sequoia and Sequiadendron, where Metasequoia without certainty may be sister to the two others. At last in the former Taxodiaceae is the Taxodioid-clade including Cryptomeria, Glyptostrobus and Taxodium, where Cryptomeria without certainty may be sister to the two others.

The whole Taxocupressaceae = Cupressaceae s.l. ends with Cupressaceae s.s., consisting of the Callitroid-clade, which are sister to the Cupressoid-clade both of which contain many different genera. It is a complicated problem to state the intergeneric relationships within both of the two clades, and it will be considered outside the limits of this paper, although some of the relationships are rather certain. Yet one small detail is that the rather famous Chamaecyparis nootkatensis in the cladograms seem to belong to Cupressus rather than Chamaecyparis. According to Stephens (2002) it belong to the new genus Xanthocyparis together with a recently discovered, new species X. vietnamensis.

The Taxocupressaceae rbcL-tree is shown in appendix, but the matK-tree by Gadek et al (2000), which is rather similar to the rbcL-tree, offers a little better resolution.

Summary and Conclusion

It is an enormous and till now unsolved problem to find the family relations of the Gymnosperms. Why should this paper succeed in summarizing the several hundreds of earlier papers including cladograms combined with about two hundred more or less contradictive own cladograms preferably using the *rbcL*-gene and emphasizing common sense higher than all the usual cladistic parameters? The reader is the one to judge, and the future will show.

Gnetales is probably belonging to the Gymnosperms, where it is situated in Cladogram I (page 22), its position is not known for certain, it may be sister to both Angiosperms and Gymnosperms as well. 2005: Gnetales is not a sister to Pinaceae, it is a very old lineage, which is known back to 270 myr ago according to palaeobotanist Wang, Z.-Q. (2004) in *Annals of Botany* 94: 281-284.

The Coniferales family cladogram is correct beyond any reasonable doubt.

The very detailed Pinaceae cladogram is molecular genetically mainly based of the results of the genes *matK* and *nad5* , but it is not in conflict with the *rbcL* results using *Ginkgo* as outgroup. It is, however, the fabulous good agreement with the morphology which has seduced this author to put such a detailed cladogram forward as a qualified guess.

In Araucariaceae *Wollemia* may not be sister to both *Araucaria* and *Agathis*. Including *Ginkgo* in the outgroup indicates that *Araucaria* is sister to *Wollemia* and *Agathis*.

Podocarpaceae s.l. (including *Phyllocladus*) is molecular genetically a monophyletic family. *Ginkgo* in the outgroup gives weak evidence that *Phyllocladus* is prior to the rest of the family.

Taxaceae s.l. (including *Cephalotaxaceae*) is both molecular genetically and morphologically a monophyletic family.

Molecular geneticists must realize that it seems very important to include an ancestor in the outgroup if possible.

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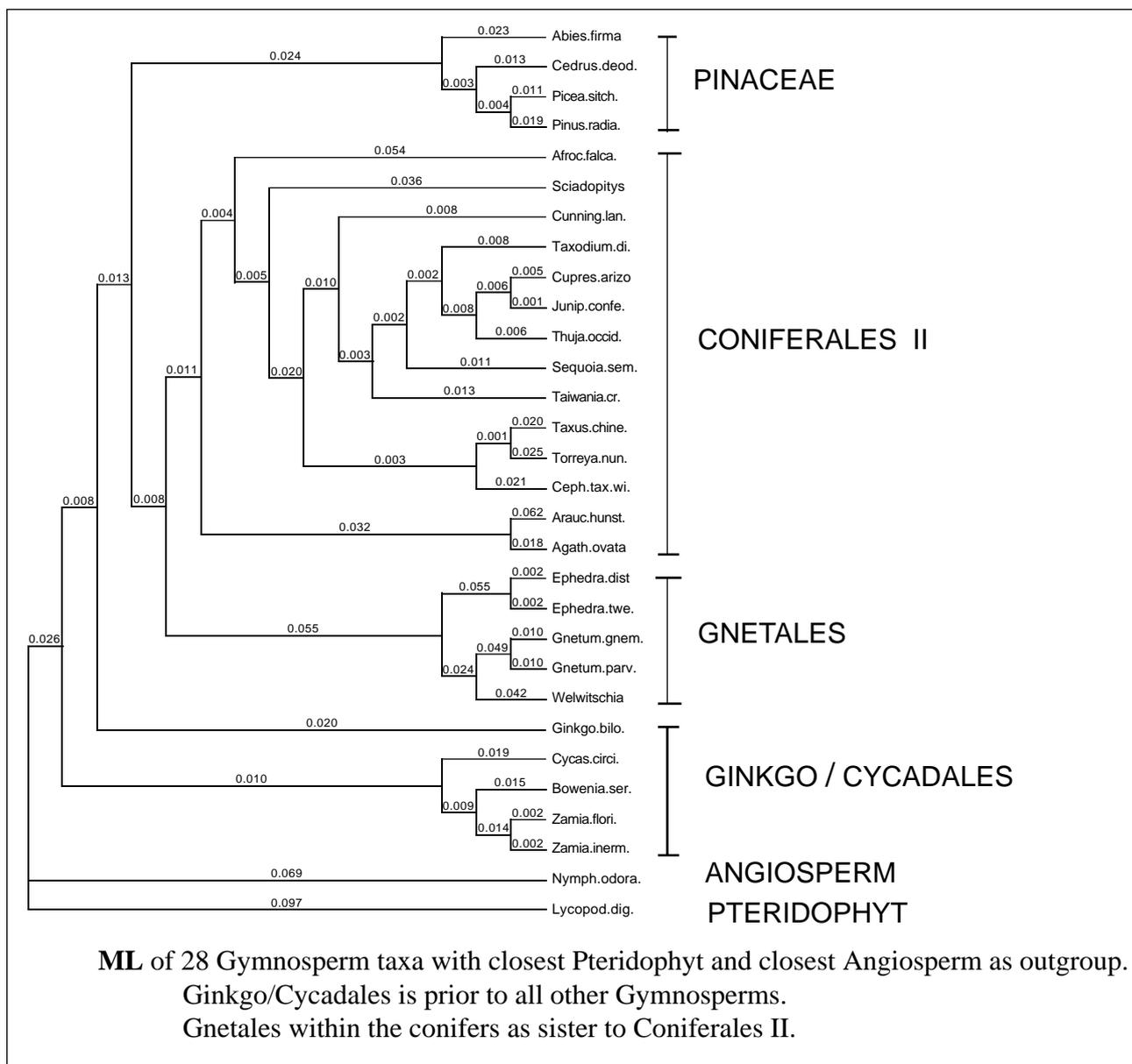
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Tables of taxa used for cladograms

rbcL: (about 1330-1550 bases)**18S rRNA:** (about 1740 bases)

Abies.firma	Abies firma	AB015647	Ab.la	Abies lasiocarpa	X79407
Abies.homol.	Abies homolepis	AB015648	Pn.wa	Pinus wallichiana	X75080
Abies.magni.	Abies magnifica	X58391	Picea	Picea mariana	L07059
Abies.marie.	Abies mariesii	AB015650	Larix	Larix leptolepis	D85294
Abies.sacha.	Abies sachalinensis	AB015651	Ar.cu	Araucaria cunninghamii	AF051794
Abies.veitc.	Abies veitchii	AB015649	Ag.bo	Agathis borneensis	D85302
Cathaya.arg.	Cathaya argyrophylla	AF015786	Pod.c	Podocarpus costalis	D38473
Cedrus.deod.	Cedrus deodora	X63662	Pod.t	Podocarpus totara	U87301
Keteleeria.d	Keteleeria davidiana	X63664	Dacry	Dacrycarpus imbricatus	D38247
Larix.occid.	Larix occidentalis	X63663	Nagei	Nageia nagi	D16447
Picea.abies	Picea abies	X75478	Saxeg	Saxegothaea conspicua	U87294
Picea.asper.	Picea asperata	AY056578	Phyll	Phyllocladus trichomanoides	D38244
Picea.brewe.	Picea breweriana	AY056579	Sciad	Sciadopitys verticillata	D85292
Picea.punge.	Picea pungens	X58136	C.tax	Cephalotaxus wilsoniana	D38241
Picea.sitch.	Picea sitchensis	X63660	Amtax	Amentotaxus formosana	D38248
Ps.larix..kae	Pseudolarix kaempferi	X58782	Torn	Torreya nuncifera	D38249
Ps.tsuga.me.	Pseudotsuga menziesii	X52937	Taxus	Taxus mairei	D16445
Tsuga.heter.	Tsuga heterophylla	X63659	Caloc	Calocedrus decurrens	D85293
Pinus.balfo.	Pinus balfouriana (hap.)	X63661	Cup.m	Cupressus macrocarpa	AF051797
Pinus.edulis	Pinus edulis (hap.)	X58137	Jun.c	Juniperus chinensis	D38243
Pinus.kremp.	Pinus krempfii (hap.)	X63665	Crypt	Cryptomeria japonica	D85304
Pinus.longa.	Pinus longaeva (hap.)	X58138	Msequ	Metasequoia stroboides	L00970
Pinus.walli.	Pinus wallichiana (hap.)	X58131	Taiwc	Taiwania cryptomerioides	D38250
Pinus.pinea	Pinus pinea (dip.)	X58133	Ep.ca	Ephedra californica	U42492
Pinus.radia.	Pinus radiata (dip.)	X58134	Gn.le	Gnetum leyboldii	L24045
Agath.borne.	Agathis borneensis	U96476	We.mi	Welwitschia mirabilis	D85299
Agath.ovata	Agathis ovata	U96483	Ginkg	Ginkgo biloba	D16448
Wollemia.no	Wollemia nobelis	AF030419	Cy.ta	Cycas taitungensis	D85297
Arauc.angus.	Araucaria angustifolia	U87750	Za.pu	Zamia pumilla	M20017
Arauc.hunst.	Araucaria hunsteinii	U87749	Nym.t	Nymphaea tuberosa	L24404
Afroc.falca.	Afrocarpus falcatus	X58135	Ly.in	Lycopodiella inundata	U18512
Saxegothaea	Saxegothaea conspicua	AF249662		28S rRNA: (about 640 bases)	
Phyllocl.hyp	Phyllocladus hypophyllis	AF027312	Ab.gra	Abies grandis	U90683
Sciadopitys	Sciadopitys verticillata	L25753	Pi.cem	Pinus cembra	U90681
Tax.bac.x.cu	Taxus baccata x T. cuspidata	AJ235811	Pi.nig	Pinus nigra	U90680
Taxus.chine.	Taxus chinensis	AB027316	Cederd	Cedrus deodora	U90684
Ceph.tax.wi.	Cephalotaxus wilsoniana	AB027312	Podo.m	Podocarpus macrophyllus	U90685
Torreya.nun.	Torreya nuncifera	AB027317	Po.Fal	Falcatifolium taxoides	U90687
Amentotax.ar	Amentotaxus argotaenia	L12580	Agatis	Agathis australis	U90692
Cunningham.lan.	Cunninghamia lanceolata	L25757	Ar.Wol	Wollemia nobilis	U90694
Taiwania.cr.	Taiwania cryptomeroides	L25756	Arau.a	Araucaria angustifolia	U90691
Athrotax.lax	Athrotaxis laxifolia	L25754	Sciado	Sciadopitys verticillata	U90698
Sequoia.sem.	Sequoia sempervirens	L25755	Torrgr	Torreya grandis	U90696
Sequ.den.gi.	Sequoiadendron giganteum	AY056580	Taxusb	Taxus baccata	U90695
Metasequoia	Metasequoia glyptostroboides	AJ235805	C.taxh	Cephalotaxus harringtonia	U90697
Taxodium.di.	Taxodium distichum	AF127427	Sequ.s	Sequoia sempervirens	U90701
Glyptostr.li	Glyptostrobus lineatus	L25750	Cunn.l	Cunninghamia lanceolata	U90699
Cryptomeria	Cryptomeria japonica	L25751	Taxodi	Taxodium distichum	U90702
Libocedrus.p	Libocedrus plumosa	L12574	Taiwcr	Taiwania cryptomerioides	U90700
Widdringtoni	Widdringtonia cedabergensis	L12538	Cryptj	Cryptomeria japonica	U90703
Diselma.arch	Diselma archeri	L12572	Cypres	Chamaecyparis lawsoniana	U90704
Callitris.rh	Callitris rhomboidea	L12537	Junipc	Juniperus communis	U90705
Neocall.arau	Neocallitropsis araucarioides	AF127426	Platyc	Platycladus orientalis	U90706
Chamec.noot.	Chamaecyperis nootkatensis	AF127431	C.cedd	Calocedrus decurrens	U90707
Chamec.obtu	Chamaecyparis obtuse	L12570	Ginkgo	Ginkgo biloba	U90672
Cupres.arizo	Cupressus arizonica	AF127430	Cyca.r	Cycas revoluta	U90673
Cupres.semp.	Cupressus sempervirens	L12571	Eph.ne	Ephedra nebrodensis	U90709
Junip.conf.	Juniperus conferta	L12573	Gne.gn	Gnetum gnemon	U90710
Junip.virgi.	Juniperus virginiana	AF119182	Welwit	Welwitschia mirabilis	U90708
Thuja.occid.	Thuja occidentalis	L12578	NymphN	Nymphaea nouchali	U90711
Thuja.stand.	Thuja standishii	AF127428	Fil.Po	Polypodium vulgare	U90712
Tjujopsis.do	Tjujopsis dolobrata	L12577			
Fokenia.hod	Fokenia hodginsii	AF127429			
Microbiota.d	Microbiota decussata	L12575			
Tetracl.arti	Tetraclinis articulata	L12576			
Platycl.o.fl	Platycladus orientalis flagelliflor.	L13172			
Calloedr.de	Calloedrus decurrens	L12569			
Ginkgo.bilo	Ginkgo biloba	D10733			
Cycas.circi.	Cycas circinalis	L12674			
Bowenia.ser.	Bowenia serrulata	L12671			
Zamia.flori	Zamia floridana	D10736			
Zamia.inerm.	Zamia inermis	L12683			
Ephedra.dist	Ephedra distachya	U72821			
Ephedra.twe.	Ephedra tweediana	U72822			
Welwitschia	Welwitschia mirabilis	AJ235814			
Gnetum.gnem.	Gnetum gnemon	L12680			
Gnetum.parv.	Gnetum parvifolium	D10735			
Nymph.odora.	Nymphaea odorata	M77034			
Lycopod.dig.	Lycopodium digitatum	L11055			

Position of Gnetales + Cycadales/Ginkgo in rbcL-cladograms I:



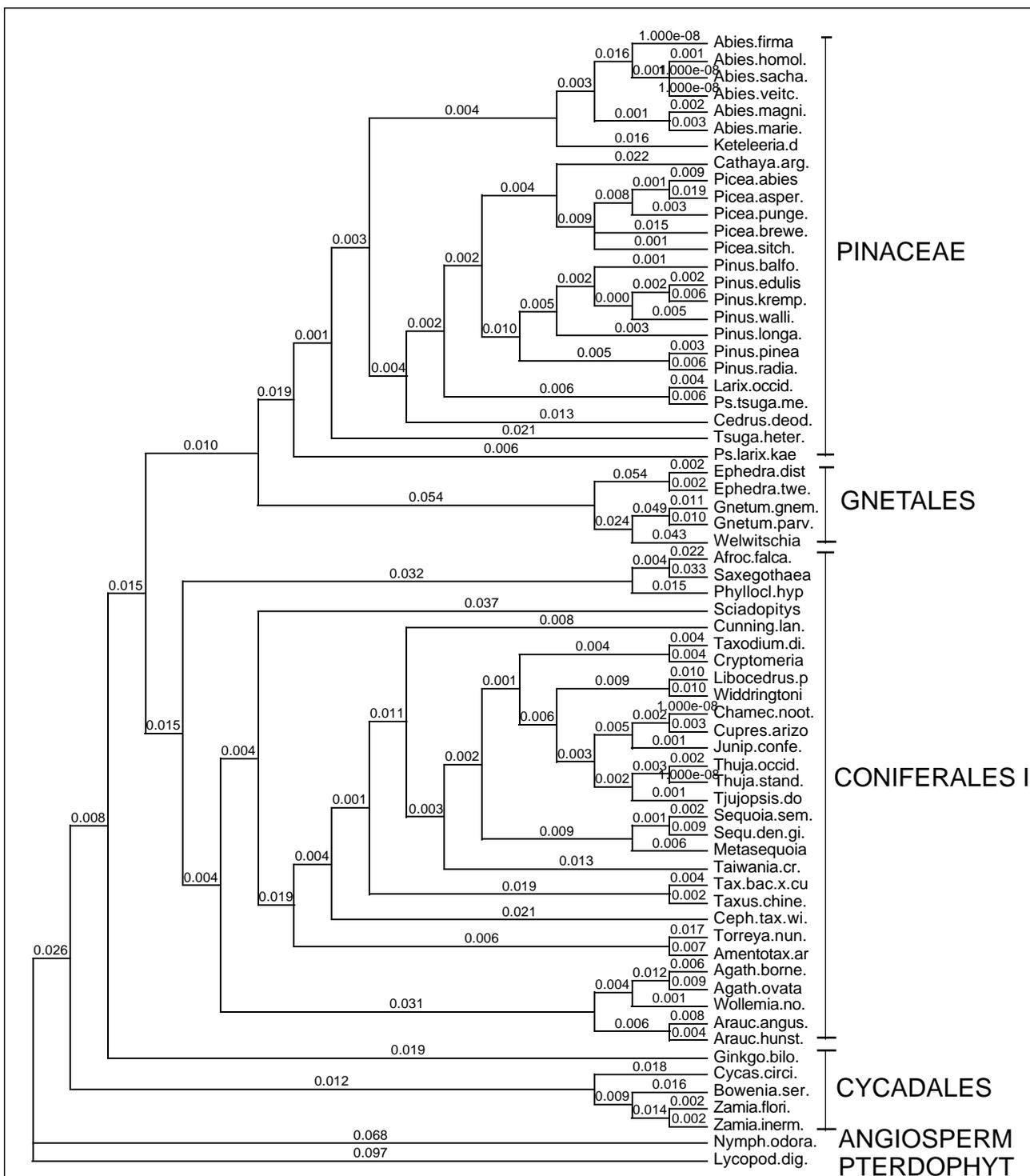
The same data-matrix yields other cladograms using other algorithms:

- NJ:** Ginkgo/Cycadales within the conifers as sister to Pinaceae.
Gnetales is in the outgroup as sister to the Pteridophyt, but not to the Angiosperm.
- MP:** Ginkgo/Cycadales is prior to the conifers.
Gnetales is in the outgroup as sister to the Pteridophyt, but not to the Angiosperm.

Bigger data-matrix of 64 Gymnosperm taxa using same outgroup and all algorithms:

- ML:** Ginkgo/Cycadales is prior to all other Gymnosperms.
Gnetales within the conifers as sister to Pinaceae. **See page 23.**
- NJ:** Ginkgo/Cycadales within the conifers as sister to Pinaceae.
Gnetales is prior to all other Gymnosperms (not in outgroup).
- MP:** Ginkgo/Cycadales is prior to the conifers.
Gnetales is in the outgroup as sister to the Pteridophyt, but not to the Angiosperm.

Position of Gnetales + Cycadales/Ginkgo in rbcL-cladogram II:



ML of 64 Gymnosperm taxa with closest Pteridophyt and closest Angiosperm as outgroup.
 Ginkgo/Cycadales is prior to all other Gymnosperms.
 Gnetales within the conifers as sister to Pinaceae. **See also page 22.**

The 28 taxa from the cladogram from the previous page is included in this cladogram.
Note that Gnetales has changed from sister to Coniferales II to sister to Pinaceae.
From this fact may be concluded that Gnetales may as well be prior to all conifers!!!

Note: The topology of other parts of the cladogram do also change with the number of taxa included. Pinaceae changes topology when the "outgroup" changes from Ginkgo to Gnetales.

