


 *Jenny Helander* 

M.Sc: Biology + Chemistry

Poul Møllers Vej 7, DK - 2000 Frbg.

FAX +  (45) 38 34 34 07

Email: info@conifers.dk

Phylogeny of Gymnosperms: Fact and Fiction.

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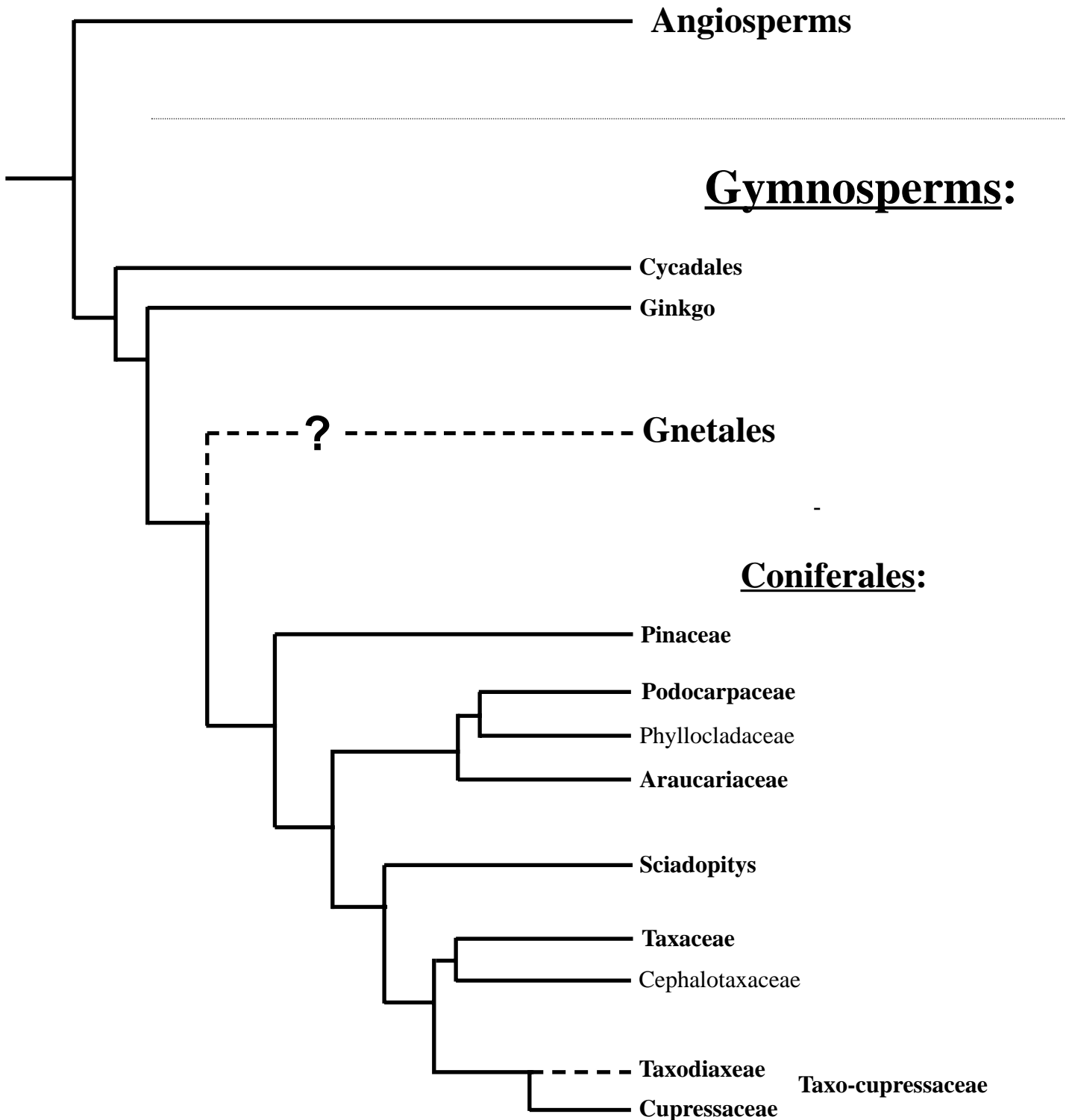
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Pinus jeffreyi. Fra Aljos Farjon (1984)

Phylogeny of Coniferales

Fig. 1



Families of Coniferales:

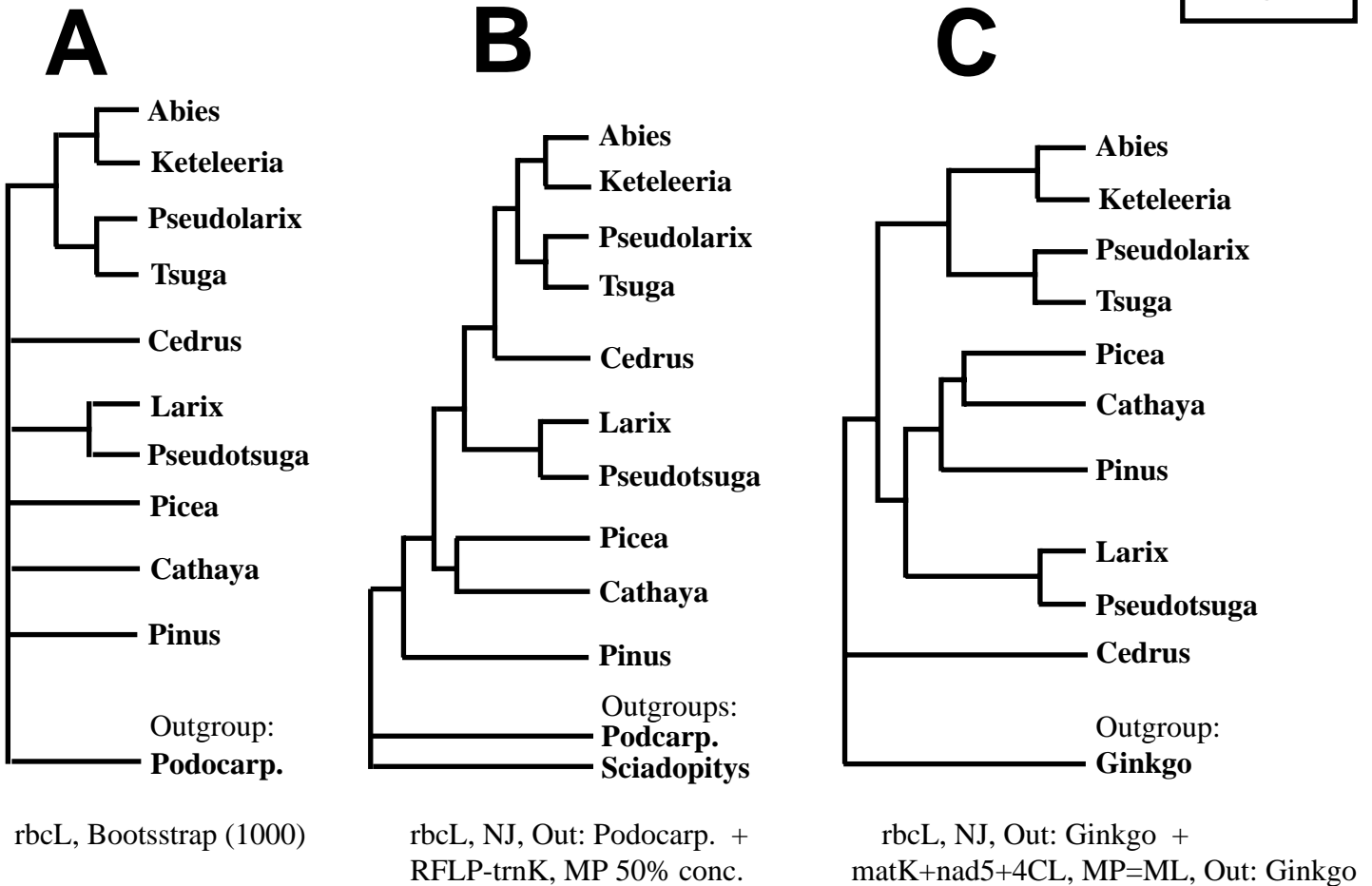
Some investigations indicate that Gnetales is a part of the Coniferales, but it is rather unlikely.

Sciadopitys forms a family of its own.

Cypressaceae is a part of Taxodiaceae, but even the Taxo-cypressaceae family may be seen as a development of the Taxaceae-Amenotaxus-Cephalotaxaceae families to which the nearest Taxodiaceae end is rather close.

Phylogeny of Pinaceae

Fig. 2



Families of Pinaceae:

NOTE 1: The relationship between Pinus, Cathaya and Picea is unknown, but they seem distant related. Cathaya does not form a sure clade with Picea. Cathaya is a very special genus with many particular mutations.

NOTE 2: It is still unknown, whether the root of Pinaceae is near to Pinus, Cathaya or Picea or it is nearer to Cedrus and/or the Larix-Pseudotsuga clade.

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Phylogeny of Gymnosperms

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Phylogeny of Gymnosperms: Fact and Fiction.

Gymnosperms, Gnetales, Coniferales, Pinaceae, Pinus, Abies.

Jenny Helander, MSc: Biology and Chemistry. Poul Mollers Vej 7, Copenhagen, 2000 Frbg., Denmark.

Abstract

A review of the phylogeny of the Gymnosperms, in particular the families of Coniferales; e.g. Pinaceae including the genera Pinus and Abies. In order not to mislead readers, who are not trained cladists, only a very few cladograms are shown. Originally the author reconstructed some molecular genetic cladograms (not published) in 1999 based on the rbcL, 18S rRNA and 28S rRNA genesequences from the GenBank. This paper from 2001 is based on the cladograms from 1999 together with almost all other investigations done by other scientists concerning the subject. As a result of this new material, the root of Gnetales in the 2001 edition is placed near Coniferales (but probably not inside it), whereas in the 1999 edition Gnetales was placed as a sistergroup to the Angiosperms (and not together with the other Gymnosperms), due to the cladograms of rbcL and 28S rRNA. Ordinary chemical evidence has not helped to solve any problems, while the flavour of the essential oils in the conifer-needles (estimated by biting in the needles) supports the found family relations rather good.

Key words. Phylogeny, Gymnosperms, Gnetales, Coniferales, Pinaceae, Pinus, Abies. Taste of essential oils in conifer-needles.

Introduction

The molecular genetics has during the last ten years showed to be superior to morphology in cladistics, and it has an almost unlimited future, because the genetic message contains an immense amount of information, which is still waiting to be revealed.

Unfortunately most of the phylogenetic trees are published in a way, which mislead not only outsiders and those biologists, who are not familiar with cladistics, but it appears that even trained cladists neither understand the published evidence nor are able to express their results in a way, which make them easily understandable and useful for other scientists.

The main reason for the abovementioned problem is that molecular genetics is based on the statistic mutations, which by chance have been so succesful that they have been preserved during the evolution. At present it is not yet possible to deal with those mutations in a way, which leads to an unambiguous phylogenetic tree. On the contrary, there are not only several different programs for constructing trees such as Maximum Parsimony (MP), Neighbour Joining (NJ), Maximum Likelihood (ML), UPGMA, etc., but also the aligment of the basesequences may be done in different ways, the different mutations may be given different weight according to both their location in the codon and if they are transversion/transition mutations, and finally the choise of outgroup may be important too. Thus the same basesequences may lead to very different trees, when treated by different geneticists.

This investigation, which originally included plants from green algae to flowering plants, are mainly based on the three genes rbcL, 18S rRNA and 28S rRNA, and it was carried out during the summer 1999 due to personal curiosity. In order to state the recent facts and ask questions within the Gymnosperm-phylogeny it has been supplied with newer information, which is referred without reconstruction.

This survey is published to the benefit for morphologists, paleobotanics and even other geneticists, as much confusion still seemes to exist in this area even by the so called experts.

Material and methods

The molecular genetic investigations are exclusively based on the basesequences, which were listed in GenBank 1999. Nearly all the reconstructions have been mentioned earlier in the referred papers. Therefore further specifications are not repeated in this paper. The alignment is done manually and the trees are constructed using PAUP v. 4.0b2. The MP and NJ-trees were chosen as representative, because they showed the best agreement with each other, although similarities and differences is thought to be the essential in molecular genetics. Within the *rbcL*-gene much "handcounting" has been done, i.e. comparing the mutations without the use of computer. Due to human intelligence the "handcounting" method is more safe than computing, when the material are limited.

rbcL-alignment:

The *rbcL* basesequences seemed very reliable. There were no conspicuous errors or problems (except for *Selaginella*, which is not dealt with in this paper). On the contrary one felt very confident with the alignment, and it was possible to create both a "standard Pinaceae" and a "standard Coniferales" basesequence, where only very few bases were depending on the choice of outgroup. In fact with only few species involved, one felt more safe by using "handcounting", when you compared the mutations, instead of letting PAUP compute the trees, but both methods gave the same conclusions.

The planned statistics was cancelled as the irregularities did not seem to follow the laws of statistics. Some species (e.g. *Ginkgo*) had almost none mutations, while others (e.g. *Cathaya*) had many mutations, and although most species had a majority of transitions, majority of transversions was found too. See also Kjällersjö et al. (1998). The main problem by using *rbcL* to class the Gymnosperms is that only one Podocarpaceae ("*Nageia falcatus*") is sequenced, and *N. falcatus* is not one of the basic Podocarpus-species.

18S rRNA-alignment:

The 18S rRNA basesequences, which by chance was aligned at first, seemed very unreliable. There were errors in the sequences as well as tremendous problems with the alignment, and some sequences seemed to tell more about the scientist than the plant. The alignment of those "unreliable" species was carried out similar to the description of the alignment of 16S rDNA done by Manhart (1995), but only few of those species concern the part of the investigation, which is mentioned in this paper. Contrary to the just mentioned, the Coniferales-part sequenced by Chaw et al. (1993, 1995, 1997A), which by chance was aligned at last, was apparently without errors and the alignment was easy (and was consistent with the final alignment of the "unreliable" species).

The alignment problems caused so much suspicion to the 18S rRNA-sequences that they were only treated by PAUP and not by "handcounting".

Pinaceae is of special interest for this investigation, but in spite of two requests Chaw has refused to release his Pinaceae-sequences from Chaw et al. (1997 B).

28S rRNA-alignment:

The 28S rRNA basesequences was almost like 18S rRNA.

Chemical investigations, literature:

Flavonoides and terpenes and other essential oils may be used to investigate the relationship between the different Gymnosperms, but not only the presence (and absence!) of the different compounds from each group must be known, but especially for the essential oils also the actual amount of each component is important.

Flavonoides: It seems from Harborne & Baxter (1999) that some *Zamia* and some Podocarpaceae contain the same flavonoides, whereas flavonoides, which are listed for many other Coniferales, are not mentioned for Pinaceae.

Essential oils: The information about the essential oils given in Hegnauer (1962, 1986) do not enable any conclusions.

Chemistry of Gnetales: Whereas Hegnauer (1962) concludes that Gnetales is far more related with Angiosperms than Gymnosperms. The later investigation by Gottlieb & Kubitzki (1984) concludes quite the opposite!

Chemical investigations, flavour (taste):

Most botanists and chemists as well may laugh by the thought of determining the relationship of plants by use of the flavour (taste). Never the less, the human sense of taste is far better in analysing the relation between the essential oils in the needles of Coniferales than any other analysis yet done. (Glycosinolates are characteristic of Caprales and the phylogeny of some Brassicaceae species has been rearranged due to the chemical structure of the mustard oils, which they contain). The comparison of flavours has in most cases only been determined by biting in a needle and comparing the taste to the taste of *Picea abies* as memorized.

Results

rbcL:

The resulting trees are similar to the trees stated in Brunfield et al. (1994), Chase et al. (1993), Price et al. (1993), Kjällersjö et al. (1998), Wang et al. (1997) and for the few Araucariaceae species here also Setoguchi et al. (1998).

18S rRNA:

The resulting trees are similar to the trees stated in Chaw (1993, 1995, 1997 A) [the sequences from Chaw (1997 B) are not available], Doyle et al. (1994), Hamsby & Zimmer (1992), Qui et al. (1999), Troitsky et al. (1991) and for the few Podocarpaceae species here also Kelch (1998). Not like Kranz & Huss (1996).

28S rRNA:

The resulting trees are similar to the trees stated in Stefanovic et al. (1998). While all the mutations were used by the author to obtain the strict consensus tree, Stefanovic et al. (fig. 4) in their alignment have to "manipulate" their data by only using transversions in order to get the same tree.

Flavour (taste) of essential oils from the needles:

Definition: The taste of *Picea abies* needles (by biting in them) is mentioned as Picea-taste.

Pinaceae with a distinct Picea-taste: *Picea*, *Abies*, *Tsuga*, *Cedrus*.

Pinaceae without prominent taste: *Larix*.

Pinaceae with a distinct taste different from Picea-taste: *Pinus* (the taste is familiar with the Picea-taste), *Pseudotsuga* (with an orange-like aroma).

Araucaria araucana and *A. heterophylla* has no prominent taste.

Podocarpaceae with a distinct Picea-taste: *Saxogothea conspicua*, *Podocarpus nivalis*, *P. alpinus*, *P. cunninghamii*.

Podocarpaceae with no prominent taste: *Podocarpus henkelii*, *P. latifolius*, *P. macrophyllus*, *Decussocarpus mannii*.

Podocarpaceae with a distinct taste different from Picea-taste: *Podocarpus gracilior*, *Decussocarpus falcatus*.

Sciadopitys has a taste, which clearly contain Picea-taste, but it is more *Torreya*-like.

Torreya nucifera, *T. californica*, and *T. taxifolium* have a special aromatic taste.

Taxus, *Cephalotaxus* and *Cunninghamii* has no prominent taste.

Summarizing result. The phylogeny of the Gymnosperms is outlined in Fig.1.

The conifer family-tree is mainly based on the rbcL, 18S rRNA and 28S rRNA data.

The supposed position of Gnetales in Fig. 1 is due to conclusions, which include all the information given in all the papers mentioned below (see: III. Gnetales are probably close to Pinaceae).

Cycadales and Ginkgo are close to each other, but it is uncertain whether Cycadales is prior to Ginkgo or if they have developed simultaneously. (Some of the trees show Ginkgo/Cycadales within the Coniferales).

Chemical evidence of essential oils: The Coniferales part of the tree in Fig. 1 is in good agreement with the relations of the flavour (taste) from the needles, which is caused by chemical similarities in the essential oils. Pinaceae is closest related to Podocarpaceae (and Araucariaceae), which is closest related to *Sciadopitys*, which again is close related to *Torreya*.

I. Angiosperms are monophyletic.

All molecular genetic investigations done so far show that the extant Angiosperms form a monophyletic group. The latest investigations in Qui et al. (1999) and partly in Kjällersjö et al. (1998), Bowe et al. (2000) and Chaw et al. (2000) show that the oldest angiosperms seem to be *Amborella*, *Nymphaeles* and *Illiciales*. The family relationships within the Angiosperms are not dealt with in this paper.

II. Gymnosperms are monophyletic.

All molecular genetic investigations show that the extant Gymnosperms are monophyletic except for the rbcL- and 28S rRNA-trees, but these are artifacts (see below). Donohue & Doyle (2000) reach the same conclusion, and they state that more morphological work must be done in order to strengthen the possibilities for the paleobotanists to find new evidence. [Note that Doyle now has changed his mind totally about the position of Gnetales compared to his opinion in Doyle (1998)]. You can no longer assert that a primitive nymphaeae-like plant has developed from a rather specialized Conifer, which is hard to believe.

III. Gnetales are probably close to Pinaceae.

The phylogeny of the odd group Gnetales is very interesting. Unfortunately the differences in opinion on just that point tend to slow down the whole process of clearing up the phylogeny of all the other seed plants too, although all the other seed plants are completely independent of Gnetales. The purpose by this paper is firstly to state that Angiosperms have not derived from Coniferales, and secondly to state the relations within the Coniferales. Therefore it may be rather stupid to deal with Gnetales too, as it may blur the two main purposes, but never the less it will be done. *Int. Jour. Pl. Sci.* 157(6): S1-S125 (1996) is review on all aspects on Gnetales by different authors.

The anthophyte theory, which suggests a near connexion between Gnetales and Angiosperms, is based on morphological similarities between the two groups. However, the small but essential differences in the apparent analogies (the vessels for instance) might perhaps better be interpreted as parallel development than as a near relationship.

The chemistry of Gnetales is described as very close to that of the Angiosperms by Hegnauer (1962), who then believed in the anthophyt theory, whereas Gottlieb & Kubizki (1982) showed that the chemistry of Gnetales are much closer related to the Coniferales than to the Angiosperms.

Unfortunately two of the first molecular genetic investigations, which was carried out, used *rbcL* and 28S rRNA genes. From both of which the resulting trees strongly indicate that Gnetales are closer related to Angiosperms than to Coniferales. But the below-mentioned 15 investigations involving more than 25 different genes are all indicating, that Gnetales are much closer related to Coniferales than they are to Angiosperms.

List of the 15 investigations:

cox1: In Bowe et al. (2000): Sistergroup of Gnetales is Pinaceae (ML), Conifer II (MP), and all Conifers (NJ).

m-atpA: In Bowe et al. (2000) both ML, MP, and NJ show Pinaceae as the closest sistergroup to Gnetales.

18S rRNA: In Chaw et al. (1997) both NJ and MP show Gnetales as sistergroup to Coniferales.

mtSSU rDNA: In Chaw et al. (2000) ML: Gnetales is as a sistergroup to Pinaceae (Ginkgo is inside Conifers too!).

nuSSU rDNA: In Chaw et al. (2000) ML shows Gnetales as sistergroup to all the Conifers.

LEAFY-genes: In Frohlich & Meyerowitz (1997) are stated that the LEAFY-genes support the Gnetales - Coniferales relationship.

cpITS: In Goremykin et al. (1996) NJ shows Gnetales as sistergroup to Pinaceae (inside the Conifers!).

9149 sites of different genes: In Hansen et al. (1999) Mp, ML, and NJ show Gnetales as a sistergroup to Pinus.

cox3: In Malek et al. (1996) MP and ML show Gnetales as a sistergroup to Conifers (Picea).

16S rRNA: In Manhart (1995) MP show Gnetales as a sistergroup to Conifers (Juniperus).

m-atp1+m-matR+pl-atpB+pl-rbcL+n-18S rDNA (total 8733 sites): In Qui et al. (1999) MP show Gnetales as a sistergroup to Pinus in Pinaceae (Pinaceae are inside the Conifers!).

rpoC1: In Samigullin et al. (1999) NJ, ML and MP show Gnetales as a sistergroup to Pinus (MP: Ginkgo/Cycas inside Pinaceae).

Legumin: In Shutov et al. (1998) TREE show Gnetales as a sistergroup to Pinaceae (Ginkgo inside Pinaceae).

cyt-18S rRNA, *chl-4,5S rRNA*, *cyt-5,8S rRNA*, *cyt-5S rRNA*: In Troitsky et al. (1991) show that in the 4 trees (from each of the 4 genes) Gnetales are closer related to Pinaceae/Ginkgo/Cycas than to Angiosperms.

MADS-box genes: In Winter et al. (1999) NJ-trees support strongly the Gnetales - Coniferales relationship.

Isolated only a few of the abovementioned 15 investigations are convincing evidence of the Gnetales-Coniferales relation, but in all they are very persuasive. Many of them are discussed further in Donohue & Doyle (2000).

IV. Ginkgo and Cycadales are closely related.

Ginkgo and Cycadales are closely related. They are either sistergroups as shown in Fig. 1, or they are a close clade, many different cladograms show one of the two configurations.

V. Coniferales are a monophyletic group, and the tree is structured as shown in Fig. 1.

A. Monophylety. For those, who have dealt with Conifers, it is hard to understand that it is necessary to prove that Coniferales are a monophyletic group (maybe except for Ginkgo).

Angiosperms are not a part of Coniferales, as the Gymnosperms are a monophyletic group.

According to the headlines in Chaw et al. (2000) the origin of Gnetales is the Conifers. It is a very surprising assertion, and it is not supported in the paper to an extent, which makes one wish to control the cladograms. As a matter of fact there is more support for Gnetales being Conifers in Bowe et al. (2000), although the support is weak there too. By the way, in Chaw et al. (2000) there is a very good example of manipulating data in molecular genetics: The *rbcL*-data, which originally confirm the anthophyte hypothesis, are changed in a way, which makes them unambiguous showing that Gnetales are a sistergroup to Pinaceae (inside the Coniferales!).

Fortunately Raubeson & Jansen (1992) have, due to a very rare chloroplast DNA mutation, proved with almost certainty that the Conifers are monophyletic. The work of Raubeson & Jansen (1992) includes not only all three families of Gnetales, as well as Ginkgo and Cycadales, but also 9 different Pteridophytes.

As a matter of fact Ginkgo and Cycas are mixed into the Conifers in the cladograms more frequently than Gnetales, but without the assertion that Ginkgo and/or Cycas are belonging to the conifers.

B. Coniferales 1: Pinaceae. Pinaceae are a sistergroup to all other Coniferales. The problems of the family relations within Pinaceae are not solved yet. There are three main questions, which are illustrated in Fig. 2.

1. Which is the oldest genus? It is unknown, whether Pinus/Picea/Cathaya are the first among the Pinaceae, or it may be Ceder and/or the Larix-Pseudotsuga clade.

2. The relationship between *Pinus*, *Picea* and *Cathaya* (none of those are near related!) are unknown.

3. The choice of Outgroup seems to influence the result.

As demonstrated in Fig. 2A, which shows the most reliable relations, *Keteleeria* forms a very close clade with *Abies*; *Pseudolarix* forms a clade with *Tsuga*; and all four genera together form a clade. (*Nothotsuga* is situated just outside *Tsuga*). *Larix* and *Pseudotsuga* form another clade. None other relations are certain!

Cathaya has a lot of mutations, and it is not closely related to any other genera within Pinaceae.

There are two very uncertain relations: *Pinus*, *Picea* and *Cathaya* usually seem far related in the cladograms, but their relationship are very uncertain, and *Picea* and *Cathaya* do not form a distinct clade, as Fig. 2B and Fig 2C seem to suggest. Furthermore *Cedrus* and the *Larix*-*Pseudotsuga* clade are usually situated in some far mutual relation.

Choice of outgroup: The mothergroup *Ginkgo* is the outgroup, which is closest to Pinaceae, if you are counting mutations, but the sistergroup Podocarpaceae-Araucariaceae may be of equal quality as outgroup.

α. Outgroup: Podocarpaceae, Araucariaceae, Sciadopitys.

Podocarpaceae as outgroup gives a *rbcL*-tree with *Pinus* as root (Fig. 2B), which tree is identical with the result of the unrooted RFLP-trnK investigation by Wang et al. (1998 A). – Chaw et al. (1997 B) have done an investigation with the 18S rRNA gene using the same outgroup, but the trees are not published, and Chaw denies to release the base sequences. The very short review of the work states: "The *Larix*-*Pseudotsuga* clade are root and sister to the remaining Pinaceous genera, where *Abies* *Keteleeria*, *Pseudolarix* and *Tsuga* form a clade, while the positions of the clades *Cedrus*, *Picea* and *Pinus*-*Cathaya* are uncertain." (If it means that *Cedrus* and *Picea* are one clade and *Pinus* and *Cathaya* are another clade, then these results are not in agreement with the other abovementioned investigations).

Tsumura et al. (1995) have done a Coniferales investigation using RFLP (6 chloroplast genes), in which *Sciadopitys* becomes outgroup for Pinaceae. *Pinus* is the root, and then the tree is splitting up to a clade composed of the subclades *Picea*-*Cedrus* and *Larix*-*Pseudotsuga* and another part starting with *Pseudolarix*, which is sistergroup to *Keteleeria*, which again is sistergroup to a *Tsuga*-*Abies* clade. The Pinaceae part of this cladogram is slightly different from Fig. 2B +2C (among other are *Cedrus* and *Picea* one clade, and *Keteleeria* is a little misplaced).

β. Outgroup: *Ginkgo*, Cycadales.

Ginkgo-outgroup gives a *rbcL*-tree with *Cedrus* as root (Fig. 2C), which is identical with the cladogram (named fig.2) in the investigation by Wang et al. (2000) including the *matK*, *nad5* and *4CL* genes. Unfortunately Wang et al. (2000) have not tried to construct another cladogram using Podocarpaceae as outgroup. In an earlier *rbcL* investigation Wang et al. (1998 B) state a MP-tree (rather similar to Fig.2B) and a NJ-tree (Fig. 2C) both with *Cycas* (+ *Podocarpus*, *Araucaria*) as outgroup.

– Summarizing the abovementioned it must be concluded that the molecular genetics still is far from answering the very interesting question of the phylogenetic interrelationship within the Pinaceae-family.

Traditionally Pinaceae are classified morphological according to their shoots and foliage "the Christmas tree theory", which is obvious phylogenetic wrong. Many other suggestions have been forwarded. Farjon (1990) has made a phylogenetic investigation, where he has weighted the morphology of the reproductive organs higher than the morphology of the foliage. Farjons cladogram is rather close to the Fig. 2B tree, but in his tree *Cedrus* is sistergroup directly to *Abies*.

Pinaceae: *Pinus* (haplo- and diploxylon).

The authors investigation has been done with the bases from the *rbcL*-gene by "handcounting" from a total amount of about 1440 bases. The 4 haploxylon species: *Pinus balfouriana*, *P. edulis*, *P. krempfii* and *P. longaeva* had the same 21 changes in bases (besides some mutations, which are different for each species) in relation to "standard" Pinaceae. The two diploxylon species: *Pinus pinea* and *P. radiata* had also 21 changes in bases different from "standard" Pinaceae. Of the 21 changes for haploxylon and the other 21 changes for diploxylon 15 of the changes were identical, which means that the difference between all haploxylon and all diploxylon involved are $(21-15) + (21-15) = 12$ specific mutations. (The Bootstrap value on the cladogram was near 100 for both the haploxylon clade and the diploxylon clade).

The abovementioned *Pinus* investigation is not very interesting, and it is only stated here in order to allow a comparison with the deep splitting of the genus *Abies*, which is mentioned below.

A much better *Pinus* investigation including 32 species, which has been done later than the abovementioned, is stated in Wang et al. (1999).

Pinaceae: Abies ("Japanese" Abies contra "Californian" Abies).

The under Pinus abovementioned investigation was repeated with the Abies species with a total amount of about 1330 bases. The 4 "Japanese" Abies species: *Abies firma*, *A. homolepsis*, *A. sachalinensis* and *A. veichii* had the same 19 changes in bases (besides some mutations, which are different for each species) in relation to "standard" Pinaceae. **Abies mariesii is from Japan, but genetic it is a "Californian" Abies!** The 2 "Californian" Abies species: *Abies magnifica* and *A. mariesii* had 18 changes in bases different from "standard" Pinaceae. Of the 19 changes for the "Japanese" and the 18 changes for the "Californian" Abies 14 changes were identical, which means that the difference between all "Japanese" and all "Californian" Abies involved are $(19-14) + (18-14) = 9$ specific mutations. (The Bootstrap value on the cladogram was near 100 for both the "Japanese" clade and over 50 for the "Californian" clade).

Pinaceae: Picea.

Picea sitchensis was also genetic very different from *P. abies* and *P. pungens*, the difference was not due to scattered mutations, but *P. abies* and *P. pungens* had a row of similar mutations (different from the other Pinaceae).

C. Coniferales 2.

1. Araucariaceae, Phyllocladaceae-Podocarpaceae clade.

The "Southern Conifers" = the Araucariaceae, Phyllocladaceae-Podocarpaceae clade is the basis of Coniferales 2. Some unreliability occurs because Araucariaceae is mainly investigated using *rbcL* by Setoguchi et al. (1998), which investigation the author of this paper has not repeated due to lack of time, whereas the Phyllocladaceae-Podocarpaceae are mainly investigated using 18S rRNA by Kelch (1998) and by Chaw et al. (1995 and 1997A). Araucariaceae may be prior to the rest (but it is not certain), and Phyllocladaceae seem to be prior to the rest of the Podocarpaceae.

2. Sciadopitys.

Sciadopitys has earlier been classified in Taxodiaceae (which is completely incomprehensible by the author of this paper), but already the first molecular genetic investigation and the following by Chase et al. (1993), Price et al. (1993), Brunsfeld et al. (1994), Tsumura et al. (1995), Chaw et al. (1997) & Stefanovic et al. (1998), which include *rbcL*, RFLP, 18S rRNA and 28S rRNA, showed that *Sciadopitys* was a family of its own situated between Araucariaceae-Podocarpaceae and Taxaceae. A "handcounting" by the author in *rbcL* showed that *Sciadopitys* was far from Pinaceae, Podocarpaceae and Taxodiaceae, but closest to Podocarpaceae. Besides the chemical similarities, which are stated in Hegnauer (1962, 1986), it must be mentioned that the *Picea*-taste, which is present in many Pinaceae, also is present in some Podocarpaceae and partly in *Sciadopitys*, where it is mixed with the *Torreya* flavour.

3. Taxaceae-Cephalotaxaceae, Taxodiaceae, Cupressaceae clade.

Phylogenetic there is a rather even and gradual development down through the clade, so it is a matter of personal opinion, whether the old classifying in four families shall be kept, or some of the families should be united. Taxaceae-Cephalotaxaceae is definite a clade, so it is convenient to keep it as one (or even two) separate family (families). For some years there has been an ongoing knowledge of the fact that the Taxodiaceae and Cupressaceae must be seen as one family. According to the international rules this family should be named Cupressaceae s.l. Instead of using this confusing, for most lay (unlearned) people misleading name the author propose that the international board of nomenclature depart from their rules in this particular case and name the family Taxo-cupressaceae instead. As names are supposed to inform and not to mislead, the author suggest anyone else to use the name Taxo-cupressaceae (in spite of the rules).

3a. Taxaceae-Cephalotaxaceae.

From the published data in Bowe (2000), Chase et al. (1993), Chaw (1993, 1995, 1997A, 2000), Källersjö et al. (1998), Stefanovic et al. (1998), Tsumura et al. (1995), Cheng et al. (2000) and the authors unpublished trees (*rbcL*, 18S rRNA and 28S rRNA from GenBank) it is impossible to draw conclusions. There is no evidence that Taxaceae and Cephalotaxaceae are two separate families. The trees from 18S and 28S show *Taxus* prior to Cephalotaxus, but *mtSSU*, *nuSSU*, *matK* and *ITS* show the opposite. Only the 28S-tree has *Sciadopitys* closest to *Torreya*, although they two alone have a similar taste, but *Torreya* is situated quite opposite in Cheng et al. (2000), which is the newest and seems to be the most reliable paper.

Likewise it is a question whether Taxaceae-Cephalotaxaceae and the Taxo-cupressaceae are separate families or they shall be united to one big family. The distance from Taxaceae s.l. to *Cunninghamia*, which is among the oldest members of the Taxo-cupressaceae family, is not remarkable long!

3b. Taxo-cupressaceae.

From the very first cladograms dealing with both Cupressaceae s.s. and Taxodiaceae. it was revealed that Taxodiaceae was not monophyletic and that Cupressaceae s.s. was developed inside Taxodiaceae as a branch of this family. The two former families is now generally accepted to be one monophyletic family, but as mentioned above it is a question, whether the whole Taxaceae s.l. – Taxo-cupressaceae clade shall be regarded as one or more families. As earlier mentioned *Cunninghamia* is the oldest genus in Taxo-cupressaceae, and it is followed by *Taiwania*, but then there a little disagreement of the order of the genera.

The first four investigations were done using *rbcL* by Gadek & Quinn (1993) [Cupressaceae s.s.], Chase et al. (1993), Price et al. (1993) and Brunselft et al. (1994). Tsumura et al. (1994) used RFLP of six genes, while Chaw et al. (1995, 1997) used 18S rRNA, Stefanovic et al. (1998) used 28S rRNA, Källersjö et al. (1998) used *rbcL*, and Gadek et al. (2000) used *matK* + *rbcL*. Of course there are not total agreement between the different investigations, but the Taxodiaceae genus *Cunninghamia* seemes to be the oldest, and in according to Gadek et al. (2000) and others it seemes that the whole Cupressaceae clade is the youngest of the Taxo-cupressaceae.

Discussion

It must now be considered beyond any doubt that the Gymnosperms are monophyletic and a sistergroup to the monophyletic Angiosperms. This fact is supported by the many new, abovementioned investigations, which proves with certainty that the Gnetales are a part of the Gymnosperms. Most investigations seem to prove that Gnetales are a sistergroup to Pinaceae, but Gnetales are an odd order with a development which is very different from Coniferales, Ginkgo and Cycadales, and therefore Gnetales may be placed almost anywhere within the Gymnosperms (even inside Pinaceae, although almost none evidence support this teori so far).

The phylogeny of the main families within the Coniferales are now stated with certainty, but there are still many questions left regarding the exact position of the genera within each family.

Phylogenetic cladistics is revolutionized due to the immense number of molecular genetic characters, but beginner problems are not yet overwon, which goes both for the right determination of the basesequences as for the correct use of cladistics as well for the final conclusions.

In many respects even chemistry may contribute with better characters for phylogeny than morphology does, but for most chemical compounds you have to know both presence and absence of both the component and similar compounds, and in other cases it is the chemical pathways which matters. Hegnauer (1962, 1986) is very informative (but still there is a problem with the absence), whereas "The Handbook of Natural Flavonoids" by Harborne & Baxter (1999) only is a list of positive findings.

Needle flavour of essential oils in Conifers seem to verify the cladistic found relationships (except may be for the *Torreya* taste).

Acknowledgements

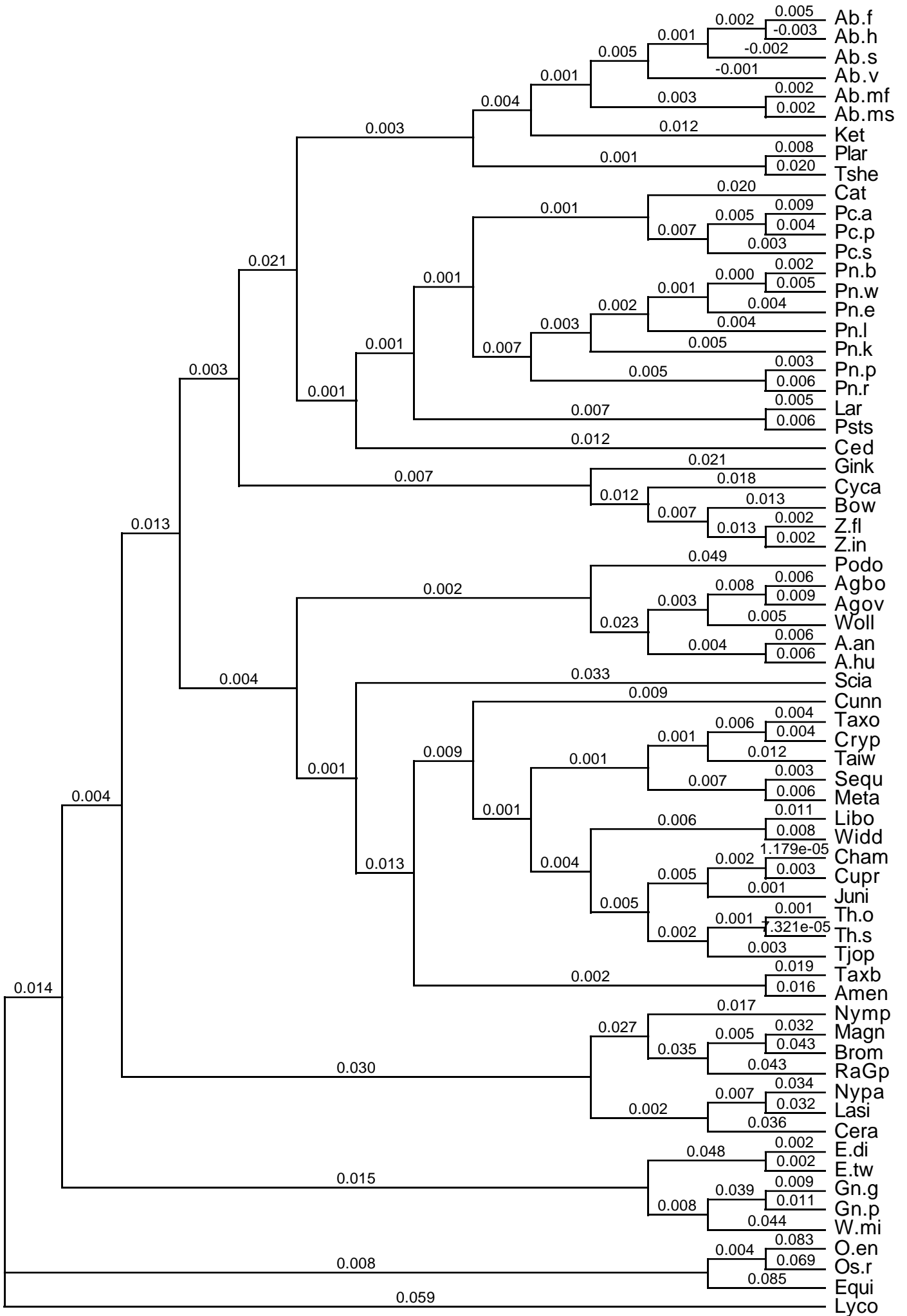
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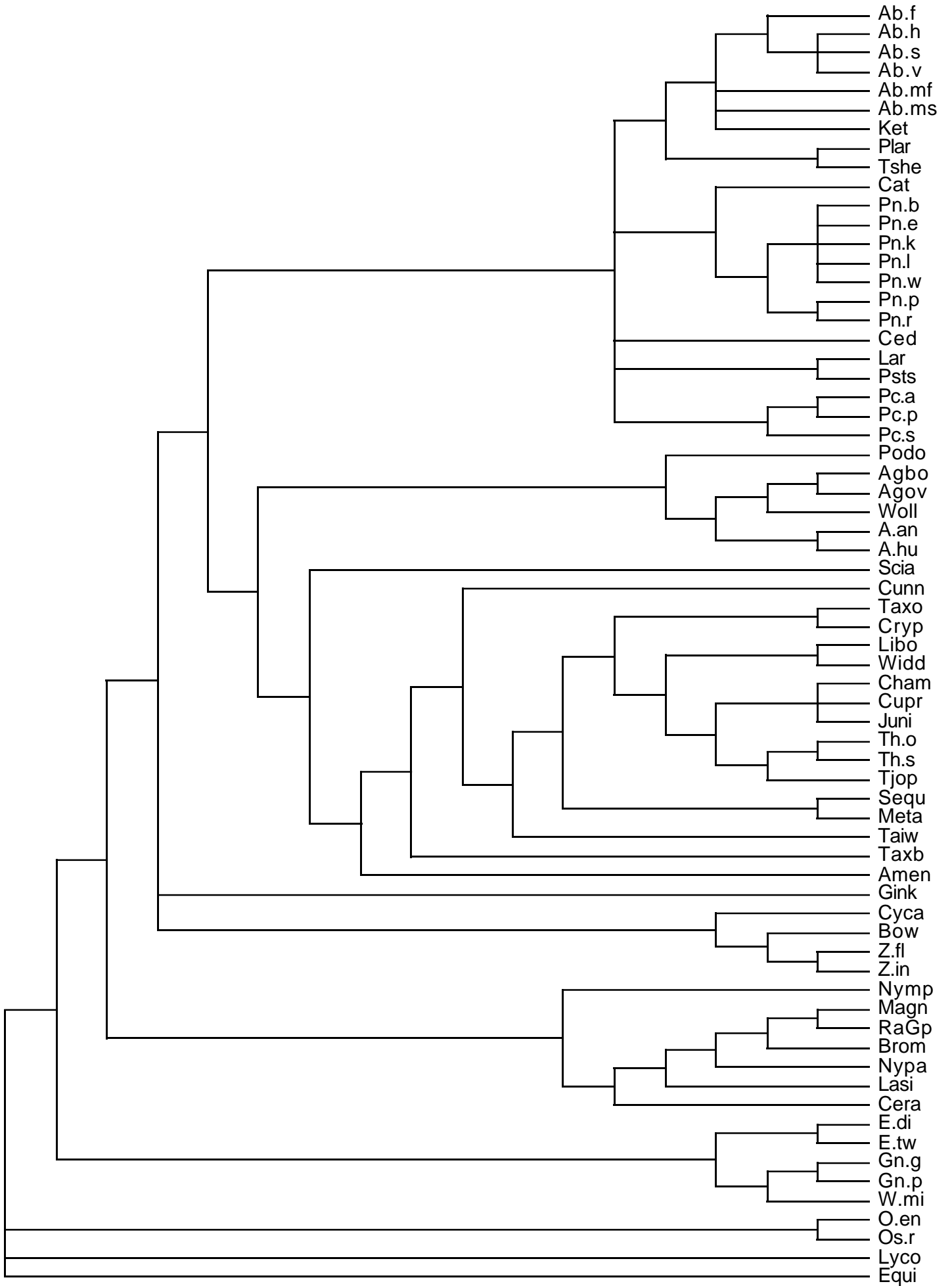
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Strict



Bootstrap

