

An Undiscovered Country: *What Comparative Genomics Tells Us of Gymnosperm Genomes*

Ujwal R. Bagal, W. Walter Lorenz, Jeffrey F.D. Dean

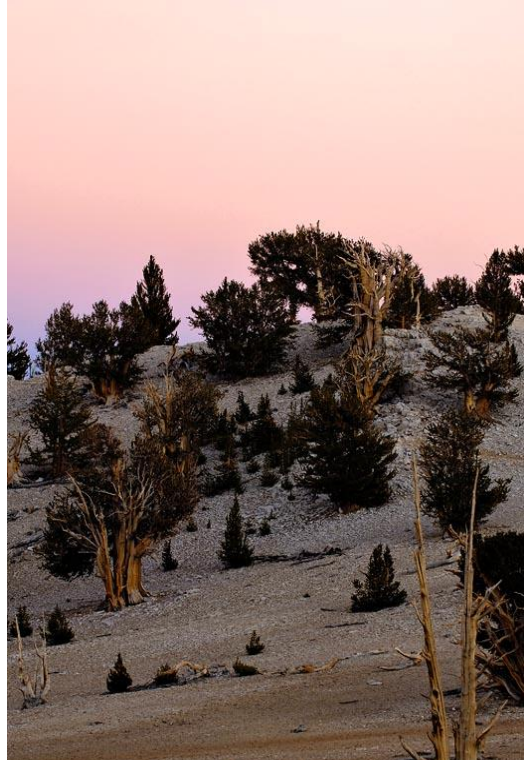
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University of Georgia



Diverse Form and Life History



SAGE Profiling and demonstration of differential gene expression along the axial developmental gradient of lignifying xylem in loblolly pine (*Pinus taeda*)

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Water stress-responsive genes in loblolly pine (*Pinus taeda*) roots identified by analyses of expressed sequence tag libraries

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Conifers represent an ancient and diverse branch in higher plant evolution. Some conifer species dominate modern-day ecosystems that are repositories for large amounts of terrestrial sequestered carbon, while others exist in populations numbering tens of individuals. Conifer forests are among the most productive in terms of annual lignocellulosic biomass generation, and coniferous trees are the preferred feedstock for much of the forest products industry, one of the most energy-intensive manufacturing sectors of the U.S. economy. Breeding programs to improve conifers have been in existence for more than 50 years, but progress has been slow because of the large size of the trees and their generally slow progress to sexual maturity.

Climate change and exotic forest pests are threatening certain conifer populations, but a general dearth of genomic resources and tools limits our capacity to address many of these issues and problems. One of the challenges of conifer genomics is that conifers do not have small genomes. Loblolly pine, for example, has a genome roughly seven times the size of the human genome. Thus, complete sequencing of the pine genome is unlikely until such time as sequencing and assembly costs are greatly reduced. Expressed sequence tags (ESTs), which represent the actively expressed portion of conifer genomes, are the most cost-effective route to identify the genes underpinning conifer growth, development, and response to the environment.

The proposed work will more than triple the number of publicly available ESTs for loblolly pine, the species currently serving as the primary reference species for conifer genomics. ESTs will also be generated for 22 other species, some of which have breeding programs and are of economic importance, but many of which have no existing genomic resources. New EST resources will be of immediate use in development of single-nucleotide polymorphism markers for ongoing association genetics studies and breeding for biomass accumulation, as well as for the development of improved oligonucleotide microarrays for functional genomics work. Comparative genomics studies performed using new sequences from previously unstudied conifers will facilitate phylogenetic analyses and greatly improve our understanding of higher plant evolution.

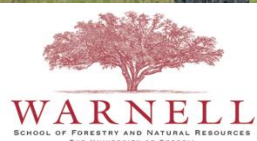
Principal Investigators: Jeffrey Dean (Univ. of Georgia), Glenn T. Howe (Oregon State Univ.), Kathleen D. Jermstad (U.S. Forest Service), David B. Neale and Deborah L. Rogers (Univ. of California, Davis)

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JGI CSP - Conifer EST Project

Transcriptome Assemblies Statistics

Pinaceae	Reads	Contigs*
• <i>Pinus taeda</i>	4,074,360	164,506
• <i>Pinus palustris</i>	542,503	44,975
• <i>Pinus lambertiana</i>	1,096,017	85,348
• <i>Picea abies</i>	623,144	36,867
• <i>Cedrus atlantica</i>	408,743	30,197
• <i>Pseudotsuga menziesii</i>	1,216,156	60,504
Other Conifer Families		
• <i>Wollemia nobilis</i>	471,719	35,814
• <i>Cephalotaxus harringtonia</i>	689,984	40,884
• <i>Sequoia sempervirens</i>	472,601	42,892
• <i>Podocarpus macrophylla</i>	584,579	36,624
• <i>Sciadopitys verticillata</i>	479,239	29,149
• <i>Taxus baccata</i>	398,037	33,142

*Assembled using MIRA

Conifer DBMagic: a database housing multiple de novo transcriptome assemblies for 12 diverse conifer species

W. Walter Lorenz • Savavanaraj Ayyampalayam •
John M. Bordeaux • Glenn T. Howe •
Kathleen D. Jermstad • David B. Neale •
Deborah L. Rogers • Jeffrey F. D. Dean

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Abstract Conifers comprise an ancient and widespread plant lineage of enormous commercial and ecological value. However, compared to model woody angiosperms, such as *Populus* and *Eucalyptus*, our understanding of conifers remains

using three different assembly algorithms: Newbler, MiraEST, and NGen. In addition, libraries from 11 other conifer species, as well as one member of the Gnetales (*Gnetum gnemon*), produced 0.4 to 1.2 million sequence reads each. Among the

http://ancangio.uga.edu/ng-genediscovery/conifer_dbMagic.jnlp

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Phenylalanine Ammonia-Lyase from Loblolly Pine¹

Purification of the Enzyme and Isolation of Complementary DNA Clones

Ross W. Whetten* and Ronald R. Sederoff

Department of Forestry, North Carolina State University, Raleigh, North Carolina 27695–8008

Plant Molecular Biology
May 1998, Volume 37, Issue 1, pp 15-24

A diverse family of phenylalanine ammonia-lyase genes expressed in pine trees and cell cultures

Stefanie L. Butland, Monica L. Chow, Brian E. Ellis



Loblolly pine PAL amino acid sequence alignment

PAL1	MVA AAE.....ITQANEVQ.....VKSTGLC.....TDFSSG...SDPLNWVRAAKAMEGSHFEEVAMVDSYFGAKESISEKSLT	70
PAL2	MVAGAD.....LGAVQANGNQNGGFHHVHSDVLC.....IQNG....PDPLNWGQAAKALQGSHFEEVAMVESYFGSEESISEKSLT	76
PAL4	MAPQEF.....TGEVKFC.....AGNGGTASLNDPLNWA...AAESMKGSHFEEVAMWEEF...RSYVRIQQSGSLT	83
PAL5	M.....NLCA.....AG.....NDPLNHWASVAESMKGSHFEEVAMWEEF...RAPVRIQQSGSLT	47
PAL3	NSSSVGEESSRMEIMSSSVGEESSRMENINGSMRSMELCRPPTLPLPGPLDGNKPDHVTFTTHWKKAAEAEMQCSHYEEVAMIKQNTTRKIVIRGTTLLT	100
PAL1	ISDVAAVAERS-QVWVWLDAAAKRSRVEESSHWVLTQMTEGTDITVGVITGFGATSHRRTNQGAELQKELIRFLNAGVVGKCPENVISEITTAAMLVRT	168
PAL2	LADVTAVARPEAKWELDAVSAKARVDESSHWVLTQNMLKGTDTVGVITGFGATSHRRTNQGAELQKELIRFLNSGVLT---DGNVLPQETTRAAMLVRT	172
PAL4	IAQVAAVARRMGSVWVWELTQCARARVDESSHWVMDSMANGTDSYGVITGFGATSHRRTRQGEALQKELIRFLNAGIFGGCGDSNSLPRDTRAAMLVRA	162
PAL5	IAQVAAVARRLGSVWVWELDTCARARVDESSHWVMDSIANGKAVITGVITGFGATSHRRTRSHGEALQKEMARFLNAGIFGGCGDSNTLPRDTRAAMLVRT	146
PAL3	VAEVTAVTERV-EVWVWLDDEASAEERVRSYQWYAKNVARGTDTYGVITGFGATSHRRTRDKAADLQKELIRFLNAGVVGK--ERLCLPAEYTRAAMLVRT	197
PAL1	NLLLQGYSGVRWDILETVEKLLNWLTPRLPLRGTITASGDLVPLSYIAGLITGRPNRSRVRSRDRGSIEMSGAEALKKVGLLEK-PFELQPKGLAVNNTSV	267
PAL2	NLLMQGYSGVRWEILETIQKLLNAGITPRLPLRGTITASGDLVPLSYIAGLITGRPNRSKARCRDGGKELGALEALQQIGVEK-PFELQPKGLAVNNTSV	271
PAL4	NLLLQGYSGIRWGIIEAMSGLLNAGITPRLPLRGTITASGDLVPLSYIAGLITGRSNARAVTADGKELGAALAAAGVENGPFELQPKGLAVNNTAV	262
PAL5	NLLLQGYSGIRWGIIEAMTOLLNAGITPRLPLRGTITASGDLVPLSYIAGLITGRPNARAVMADGTEVGAALAAAGVONGPFELQPKGLAVNNTAV	246
PAL3	NLLMQGYSGIRWEILEALRKLMDCNITPRLPLRGTITASGDLVPLSYIAGLITGRPNSEKALSPDGHLLDAMEALRKAGILE-PFKLQPKGLAVNNTAV	296
PAL1	GAALASIVCFDANVIALLESEVISAMFCEVMNPKPEFTDPLTHRLKHPHQAEMAAAIMEYVLDGSSYMEHAAKLH-EMNPLQEPQDRYALRTSPQWLGPQ	366
PAL2	GAALASIVCFDANVICIAAEVLSAMFCEVMKPKPEFTDPLTHRLKHPHQAEMAAAIMEYVLDGSSYMEKNAAKKH-EMNPLQEPQDRYALRTSPQWLGPQ	370
PAL4	GSALAAIVLFDANVVLLESEVLSALFCEVMQGNPEFTDHLTHRLKHPHQAIEAAAIMEHLLDGSYMEKAAAAKHQEADALSKEPQDRYALRTSPQWLGPQ	362
PAL5	GSALAAIVLFDANVVLLESEVLSALFCEVMQGNPEFTINHLIHRKHPHQAIEAAAIMEHLLDGSYMEKAAAANKKEADPLSKEPQDRYALRTSPQWLGPQ	346
PAL3	GSAAVAASVCFDANVGLLAELISALFCEVMKPKPEFVDPLTHRLKHPHQAIEAAAIMEHLLDGSYVREAAARLH-EKDPLEKPEQDRYALRTSPQWLGPQ	395
PAL1	VEVIRSAATHMIEEREINSVNDNPLVIDVARDKALHGGNFQGTPTGVSMDNLRSLASAIQKLMFAQFSELVNDYVYNGGLPSNLSGGPNPSIDYGFKGAETAMA	466
PAL2	VEVIRSAATHMIQREINSVNDNPLVIDVARDKALHGGNFQGTPTGVSMDNLRSLASAIQKLMFAQFSELVNDYVYNGGLPSNLSGGPNPSIDYGFKGAETAMA	470
PAL4	VEVIRSAATHMIQREINSVNDNPLVIDAARNKALHGGNFQGTPTGVSMDNLRSLAAAVGKLMFAQMSSELVNDYVYNGGLPSNLSGGPNPSIDYGFKGAETAMA	462
PAL5	VEVIRSAATHMIQREINSVNDNPLVIDAAGNKALHGGNFQGTPTGVSMDNLRSLAAAVGKLIFAQMSSELVNDYVYNGGLPSNLSGGPNPSIDYGFKGAETAMA	446
PAL3	VEVIRSAATHMIEREINSVNDNPLVIDVARDKALHGGNFQGTPTGVSMDNLRSLASAAVGLLFAQFSELVNDYVYNGGLPSNLSGGPNPSIDYGFKGAETAMA	495
PAL1	SYTSELQVLANPVTTHVQSAREQHNQDVNSLGLISARKSAEAIDIEKMLTSTYLVALCQAIDLRLHEENMLTAVRQVSVQVAEKTLSTGLNGELPGRFCE	566
PAL2	SYTSELQVLANPVTTHVQSAREQHNQDVNSLGLISARKSAEAIDIEKMLTSTYLVALCQAADLRLHEENMLTAVRQVSVSHVAEKMLSTHNGELTAORFCE	570
PAL4	SYTSELQVLANPVTTHVQSAREQHNQDVNSLGLISARMTAQAVIEIKMLTSTYLVALCQAIDLRLHEENLLAAVRQSVGQACEKTLVVGPRGELLPSRFCE	562
PAL5	SYTSELQVLANPVTTHVQSAREQHNQDVNSLGLISARMTAQAVIEIKMLTSTYLVALCQAIDLRLHEENLHAAVRQAVGQACEKTLVVGPRGELL-----	540
PAL3	SYTSELQVLANPVTTHVQSAREQHNQDVNSLGLISARKTAEAVIEIKMLTSTYLVALCQAIDLRLHEENMRSVVKHVVWLQAAEKTLCTAEDGSHDTGPFCE	595
PAL1	KDLLQVVDNEHVFYSYIDDPNCASYPLTQKLRNIIVHEHAF-KNAEGEKDPNTS.....IFNKIPVFEAEELKQALEPQVSLAES-YDK	646
PAL2	KDLLQAVENLHVFAYVDDPNCENYPLMQQLRQVVAHALTETAQIQTQTQSS.....IFNKIPAFEEELKQDMEAEVGRARQDYER	652
PAL4	KDLLKAVDREPVFYSYIDNPNCSATSVLTTKLRQVLFVHAIEKTA....NNDAS.....ILTRVPAFEELKARIVAEVQETEGA-FEK	639
PAL5	--LLKAVDREPVFYSYIDNPNCSATSVLTTVLRQVLFVHAIEKTT....DNDGS.....ILTRVPAFEELKARIVADVHETRAA-CEK	615
PAL3	KELLQVIDHQPVFYSYIDDPNTPSYALMLQLREVLVDEAL-KSSCPDGNAESDHNLPQAPAESAGAAIGILPNWVFSRIPVFEELKARLEEVTPKARER-FDN	693
PAL1	GTS-PLPDEIQECSRVPLEYFVFNQLGTKLLS GTRTISPGEVIVVVDIAISEDKVIVPLFKCLDGWEGTTLAHSSEINNLPRSPLYNDC.....YDLSPR	738
PAL2	GVAGSIPNRIQECRSVPLFYDFARSQLGTQLLSGDRVITSPGEGIKVITGRREGKIIISPLFKCLDGWSGTLPGFH.....GREGKIIISPLFKCLDGWSGTLPGFH	726
PAL4	GAA-LVFNRIKDCRSVPLYEFVRELVGASLLVGTNSRSPGEDFQKVFVAINEGKAVEPLFKCLERWNGAPIPI.....GREGKIIISPLFKCLDGWSGTLPGFH	711
PAL5	GTA-LVFNRIKDCRSVPLYEFVRAELGTSLLVGTDSRSPGEDFQKVFVAINEGKAVEPLFKCLERWNGAPIPI.....GREGKIIISPLFKCLDGWSGTLPGFH	687
PAL3	GDF-PLIANEINKCRVPLYEFVRES ELGTDLLTGPKWRSPGEDIKWEVGI CQGGIGDVLKCLDAGWGCAGPFTPRAYPASPAFNASYWAWFDS TKSPS	792
PAL1	NLLLMLLFSDPPEFDWS	754
PAL2S	727
PAL4	711
PAL5	687
PAL3	ATSGRGFWSAQQQQVL	808

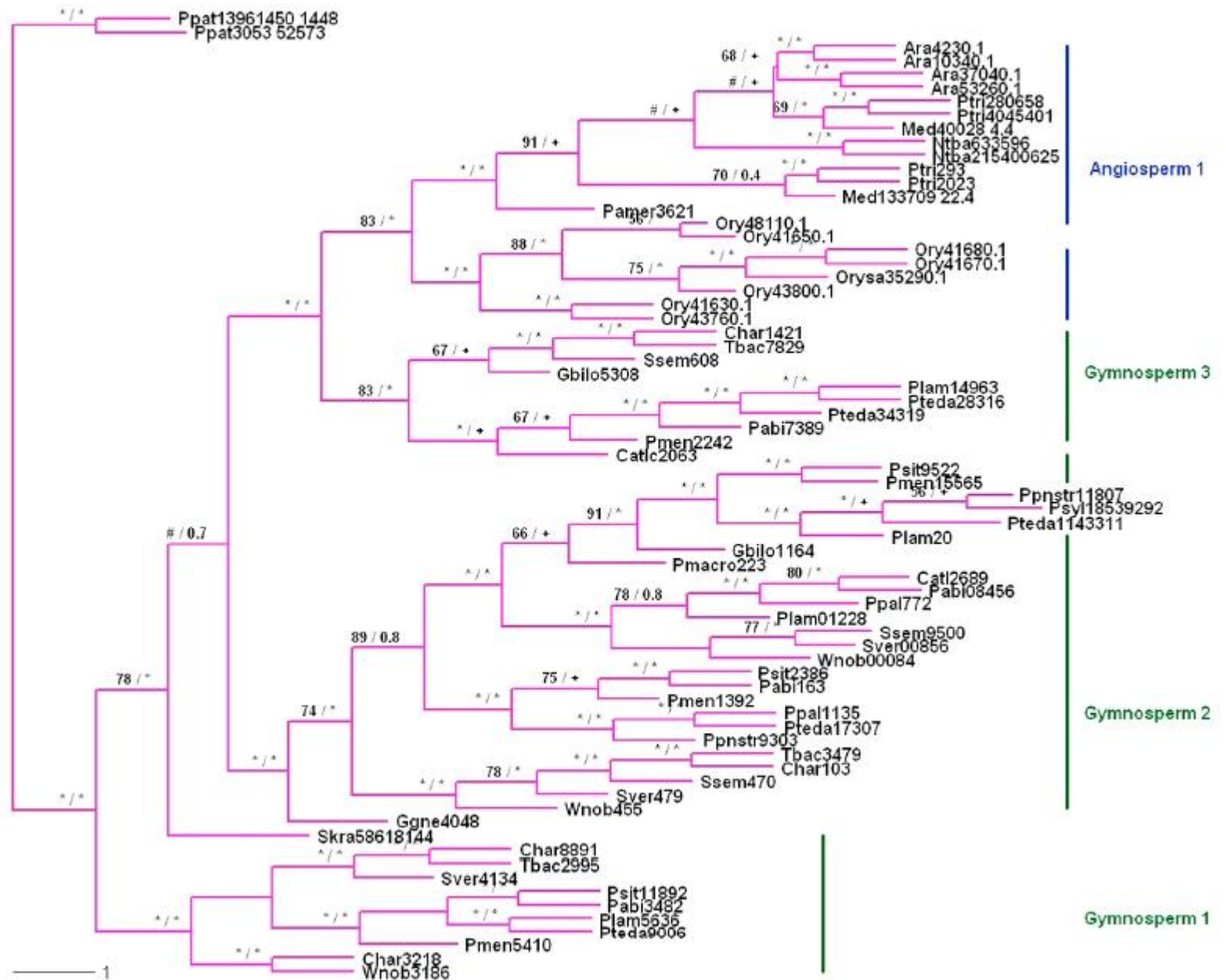


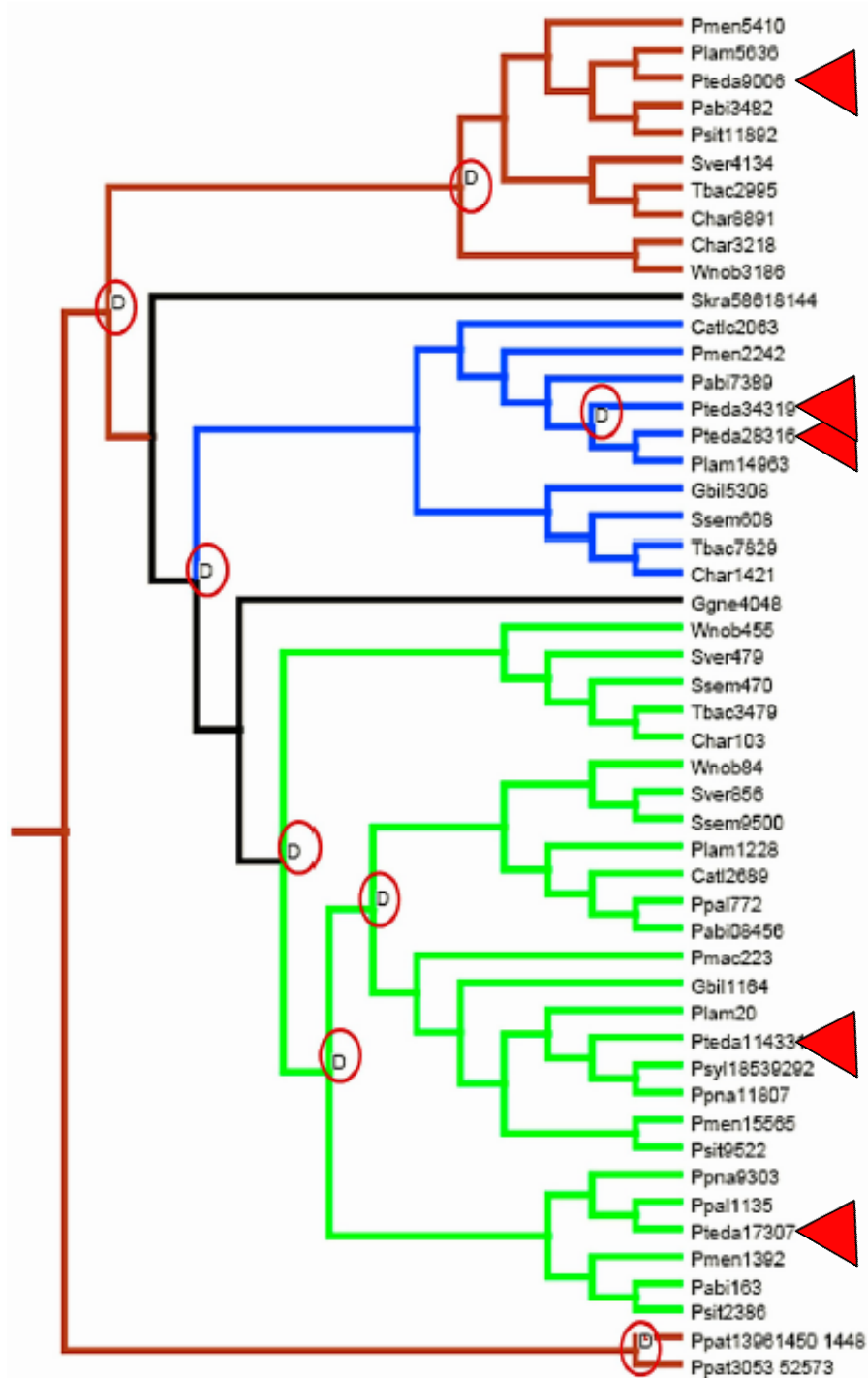


Analysis Method

- Sequence Collection
 - PlantTribe, PlantGDB, GenBank, Conifer DBMagic assemblies
 - 25 taxa comprising of 71 sequences
- Phylogenetic analysis
 - Maximum Likelihood: RAxML (Stamatakis *et al.*)
 - Bayesian Method: MrBayes (Huelsenbeck, *et al.*)
 - Tree reconciliation: NOTUNG 2.6 (Chen *et al.*)

Phylogenetic Tree of Vascular Plant PALs





Phylogenetic Analysis of Conifer PAL Gene Sequences

Conifer-specific branch shown in green

PROCEEDINGS

Open Access

The phenylalanine ammonia lyase (PAL) gene family shows a gymnosperm-specific lineage

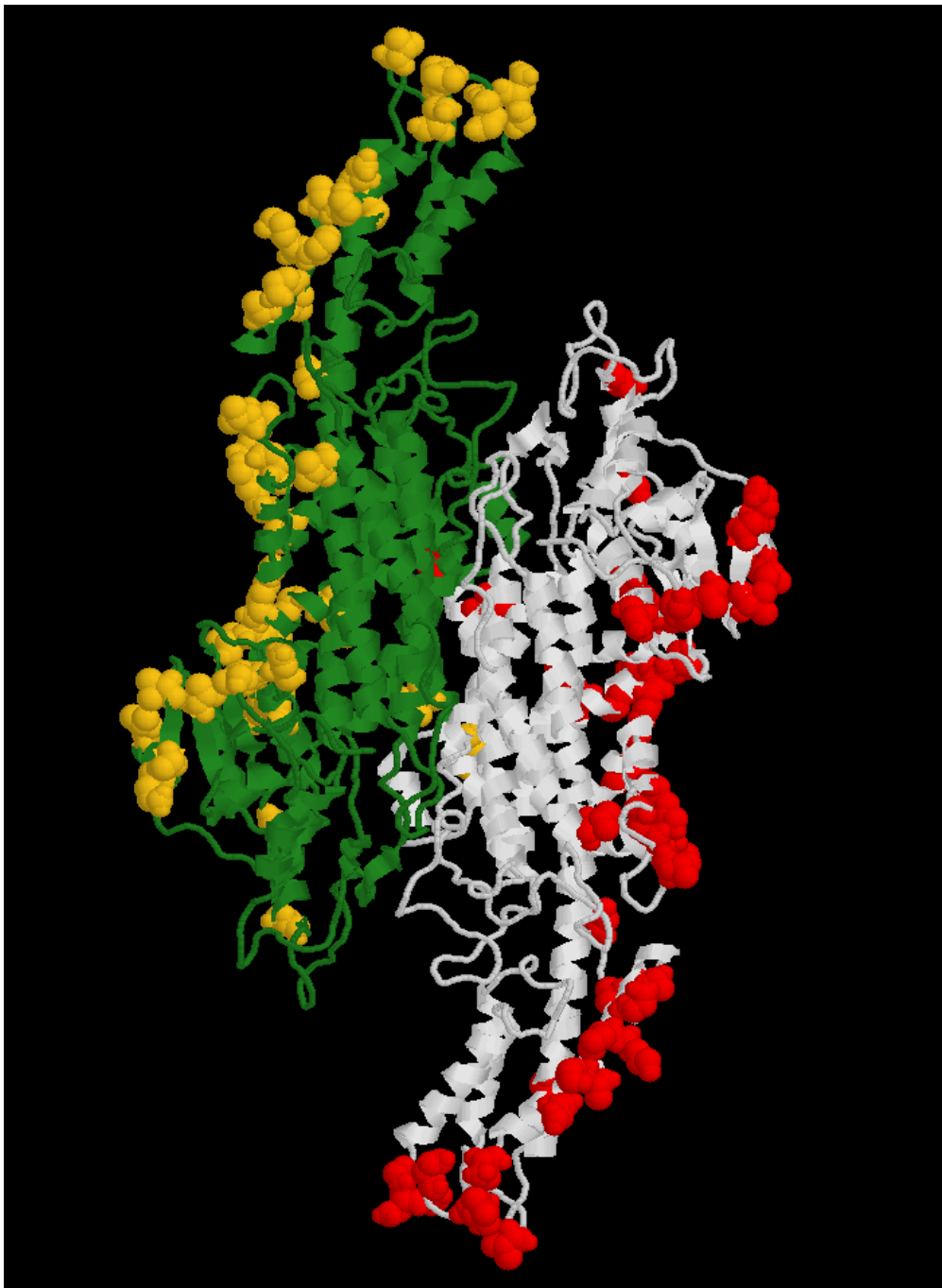
Ujwal R Bagal¹, James H Leebens-Mack^{1,2}, W Walter Lorenz³, Jeffrey FD Dean^{1,3,4*}

From IEEE International Conference on Bioinformatics and Biomedicine 2011
Atlanta, GA, USA. 12-15 November 2011

Amino Acids Under Relaxed Constraint

Maximum Likelihood analysis

- Nested codon substitution models
 - M0 : constant dN/dS ratio
 - M2a : rate ratio $\omega_1 < 1$, $\omega_2 = 1$ and $\omega_3 > 1$
 - M3 : ($\omega_1 < \omega_2 < \omega_3$)
 - (M0, M2a, M3, M2a+S1, M2a+S2, M3+S1, M3+S2)
- Fitmodel version 0.5.3 (Guindon et al. 2004)
 - S1 : equal switching rates (alpha = beta)
 - S2 : unequal switching rates (alpha \neq beta)



Variable
gymnosperm PAL
amino acid
residues mapped
onto a crystal
structure for
parsley PAL



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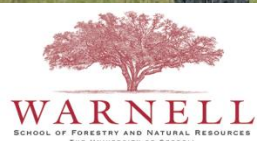
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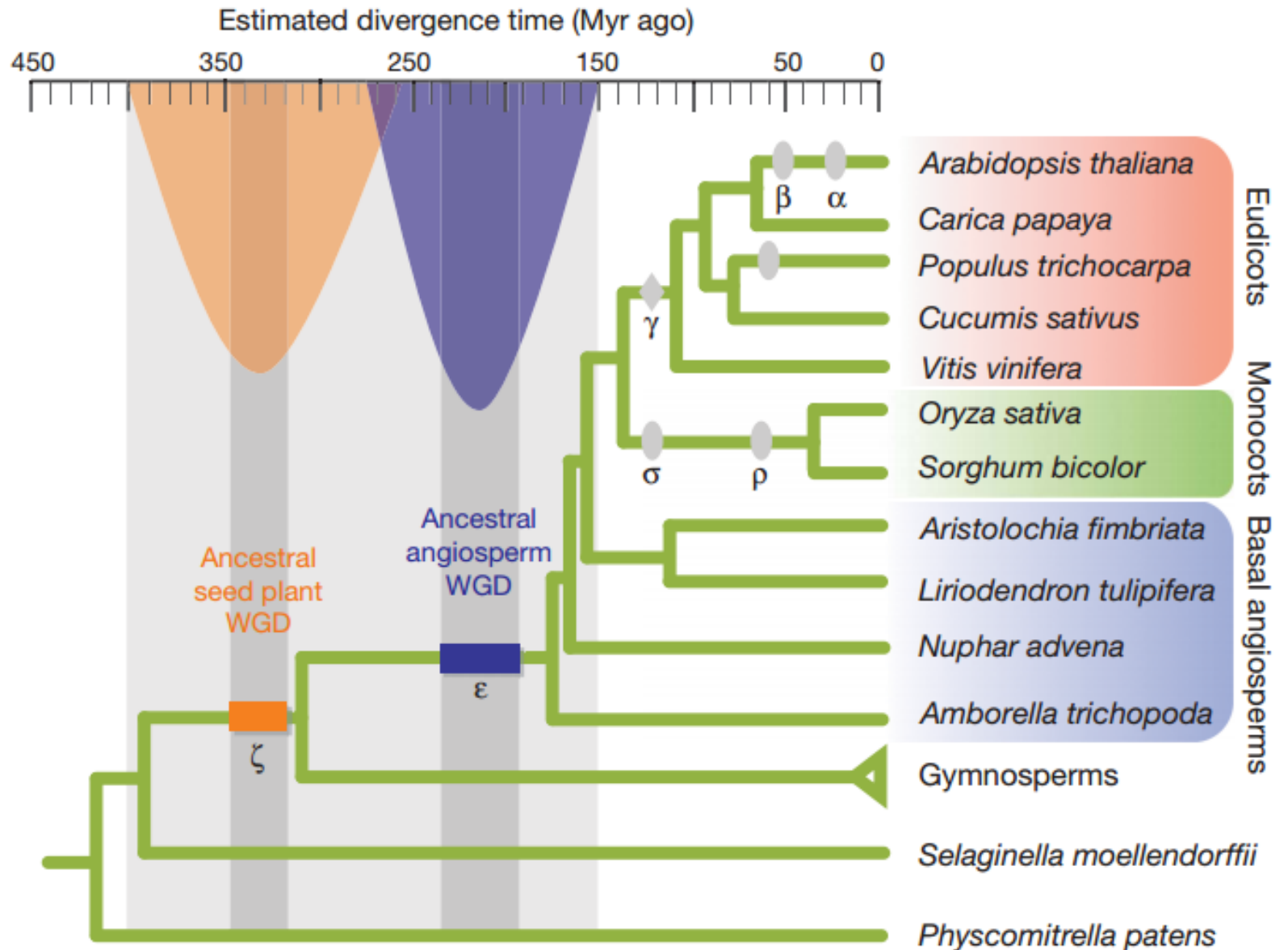
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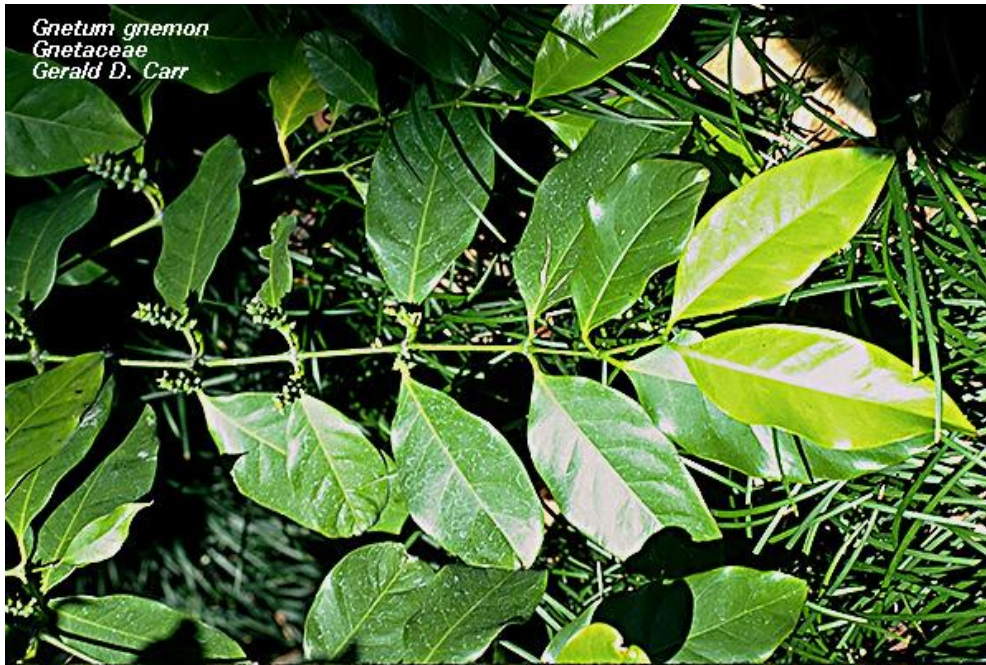
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Ancestral polyploidy in seed plants and angiosperms

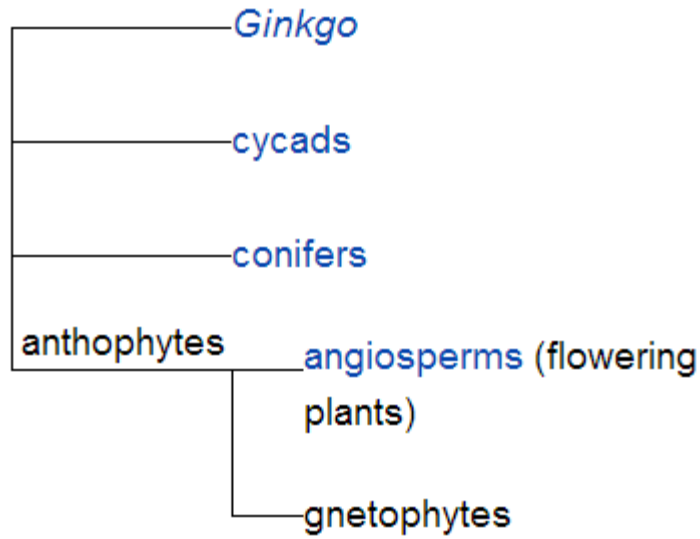




Some Confusing Gnetales Characteristics

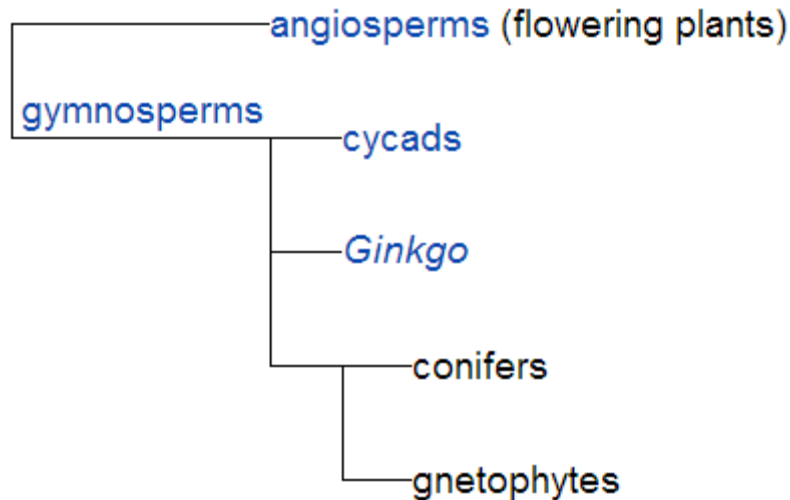
- S-lignin
- Vessel elements
- Cone-like structures
- Reduced bract-like leaves



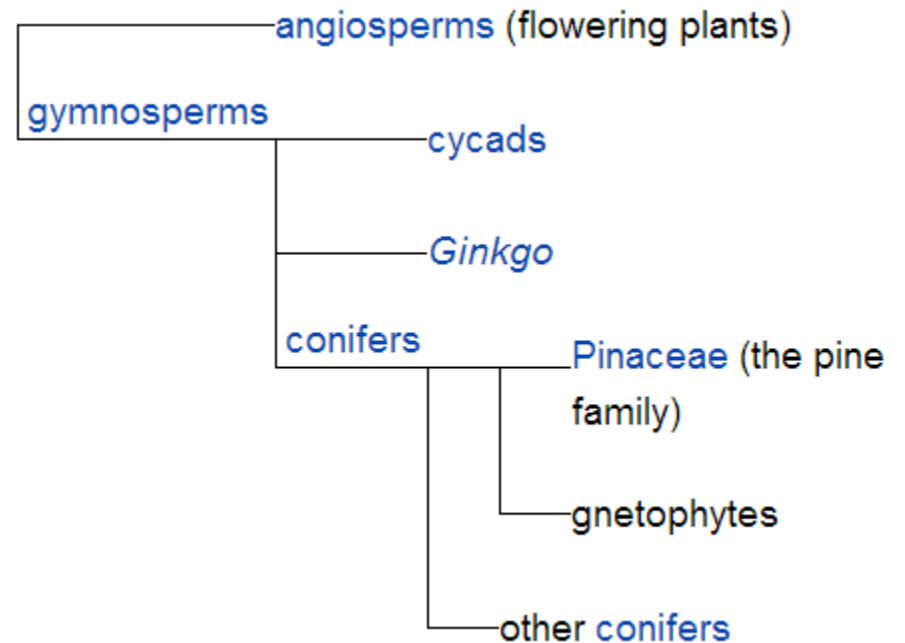


Are the Gnetales Derived Conifers?

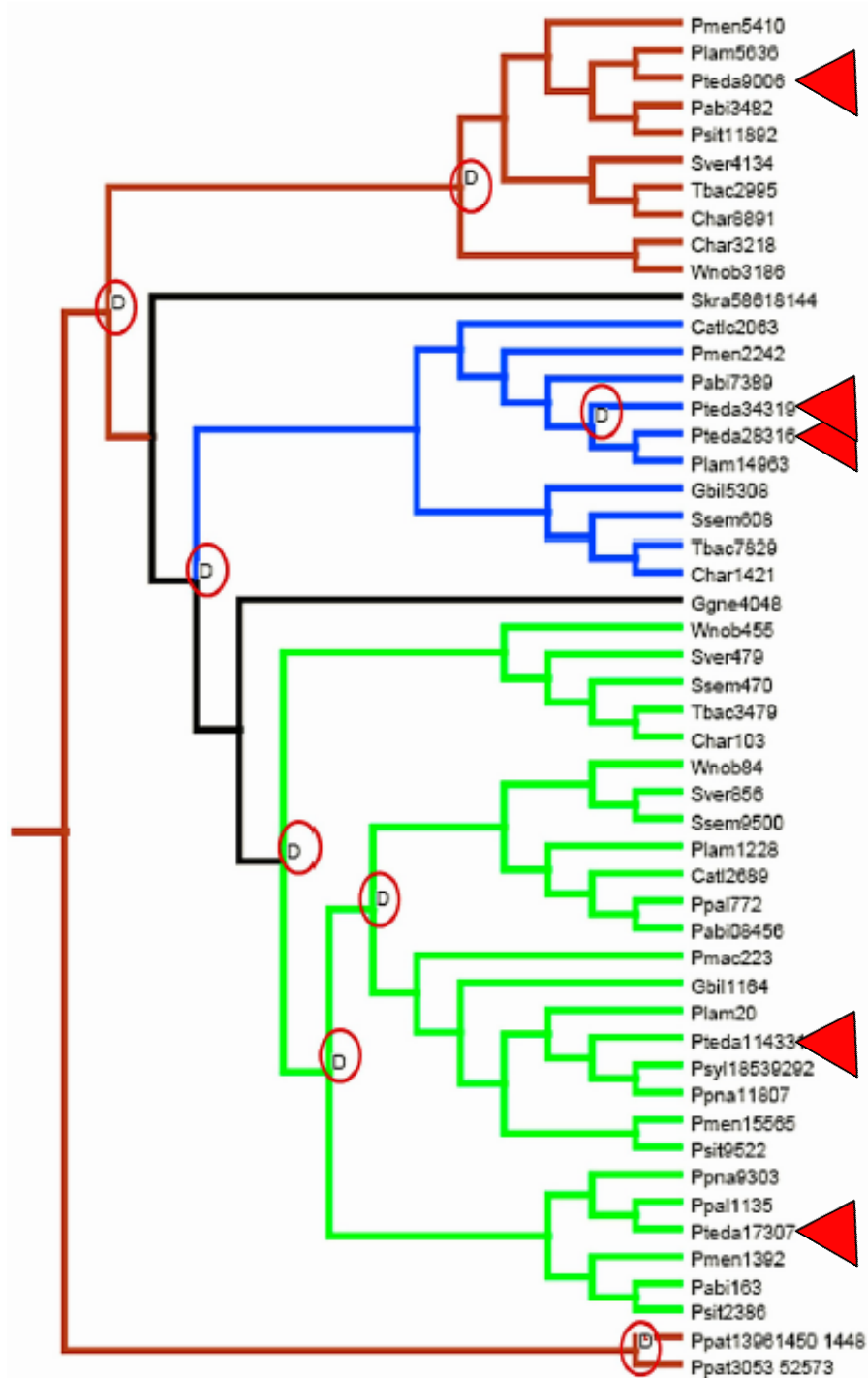
Anthophyte



'Gnetifer'



'Gnepines'



Phylogenetic Analysis of Conifer PAL Gene Sequences

Conifer-specific branch shown in green



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Three genome-based phylogeny of Cupressaceae *s.l.*: Further evidence for the evolution of gymnosperms and Southern Hemisphere biogeography

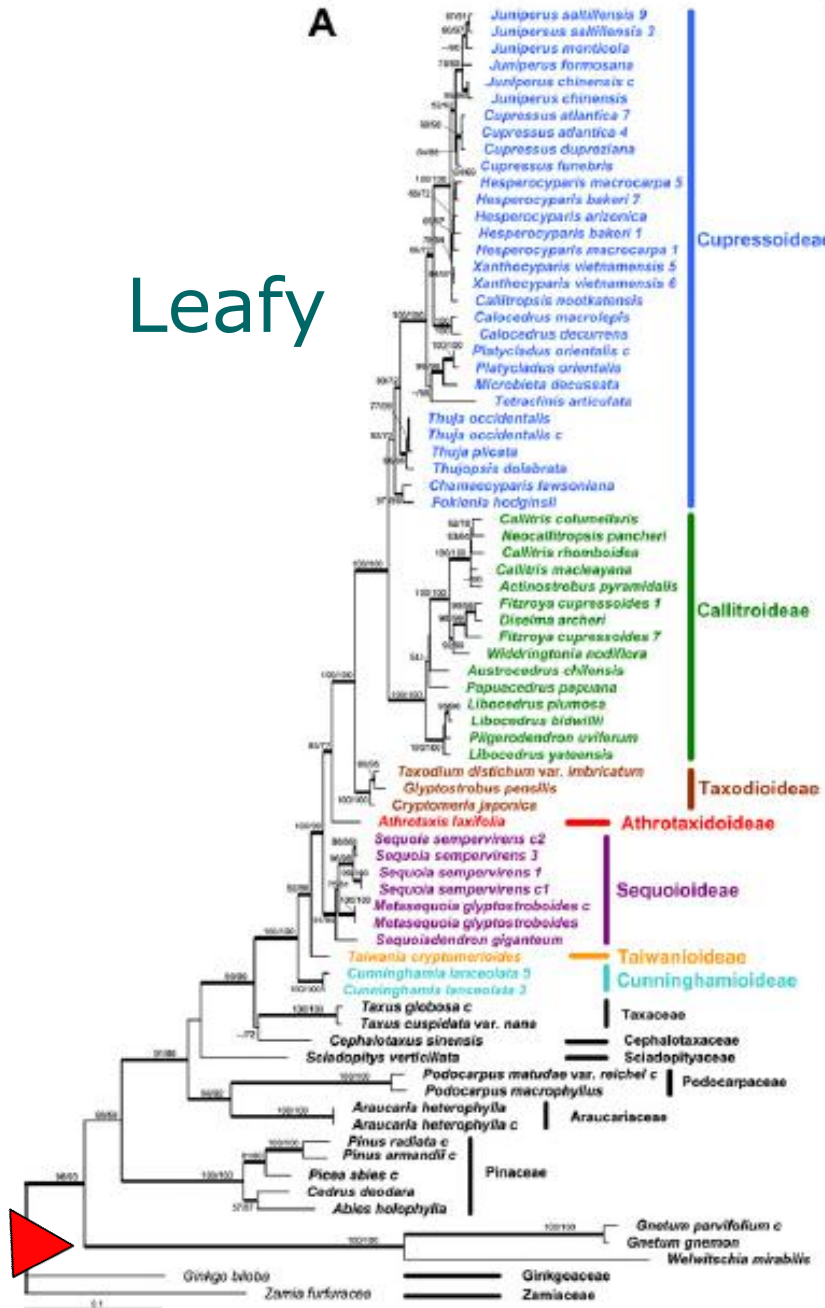
Zu-Yu Yang^{a,b}, Jin-Hua Ran^a, Xiao-Quan Wang^{a,*}

^a State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

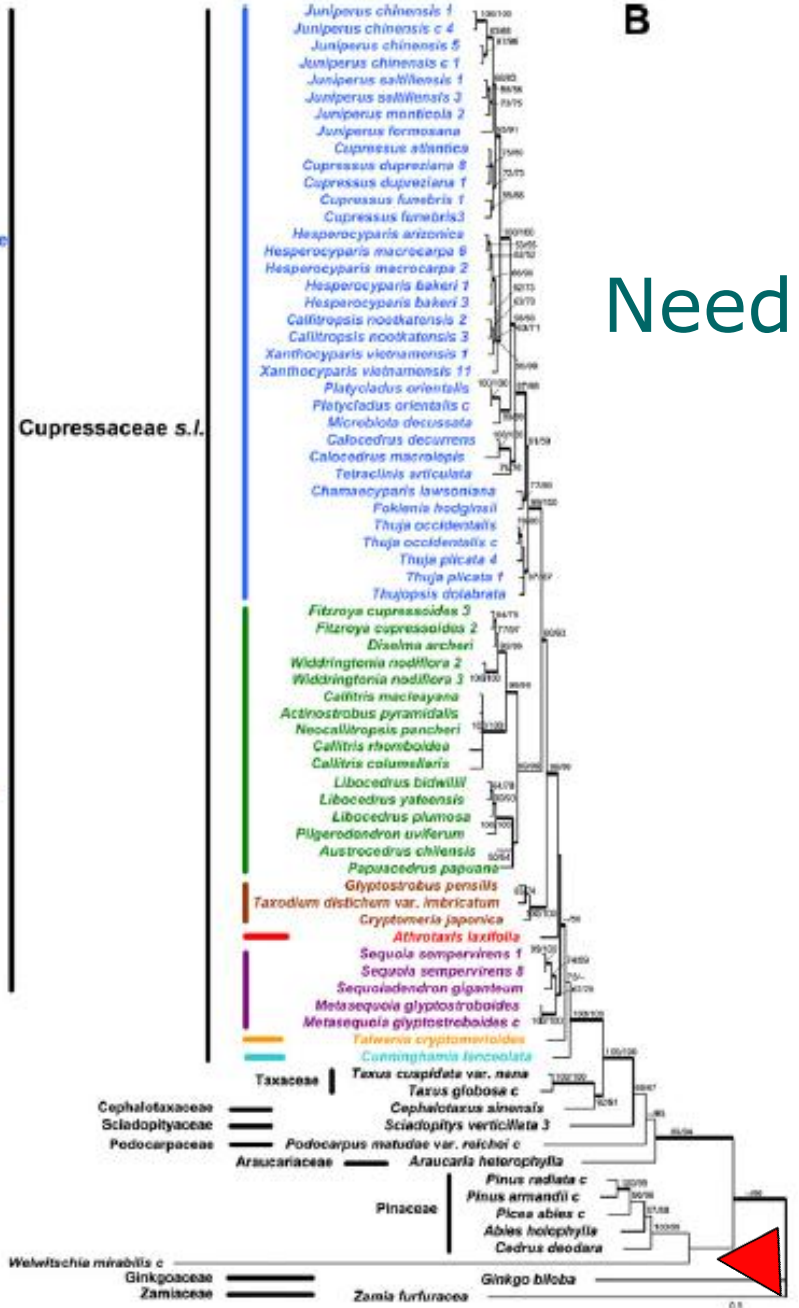
^b Graduate University of the Chinese Academy of Sciences, Beijing 100039, China



Leafy



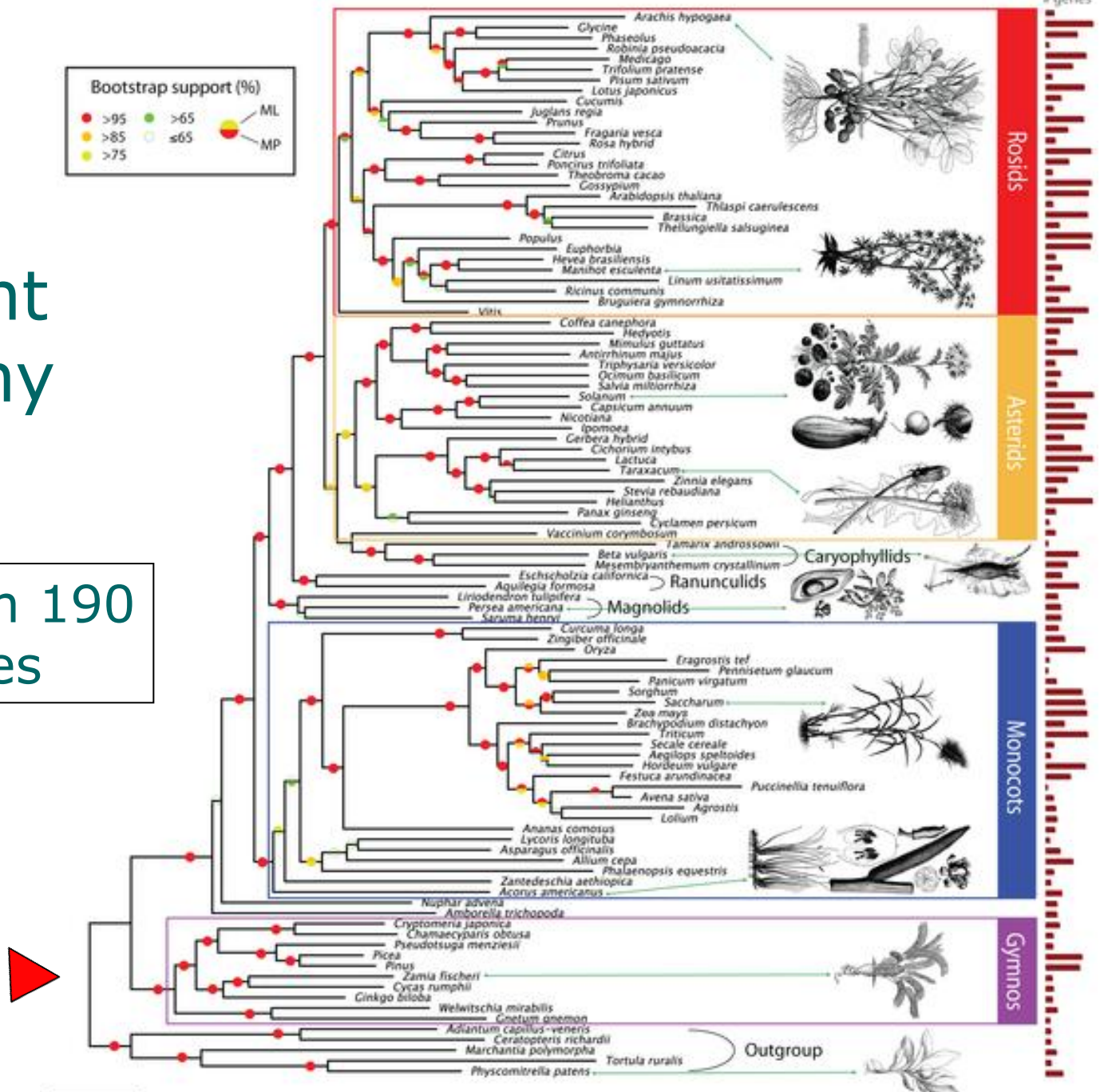
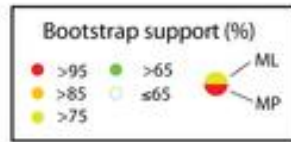
Needly





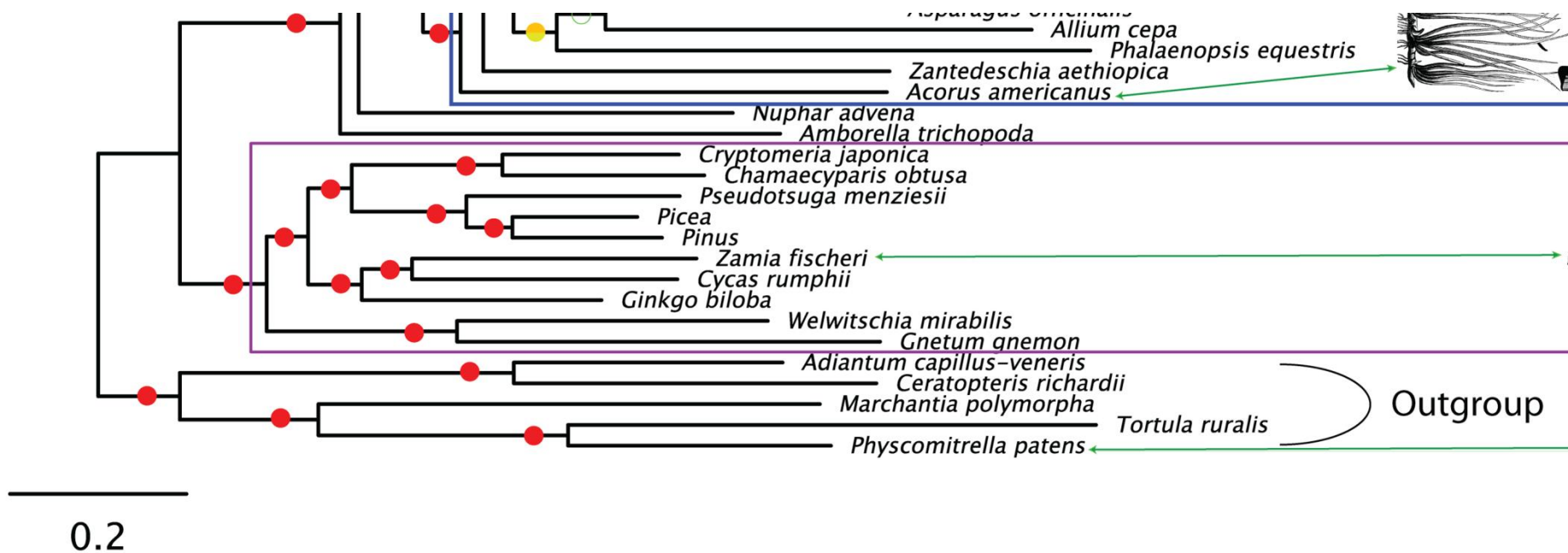
Land Plant Phylogeny

Tree based on 190 Core genes





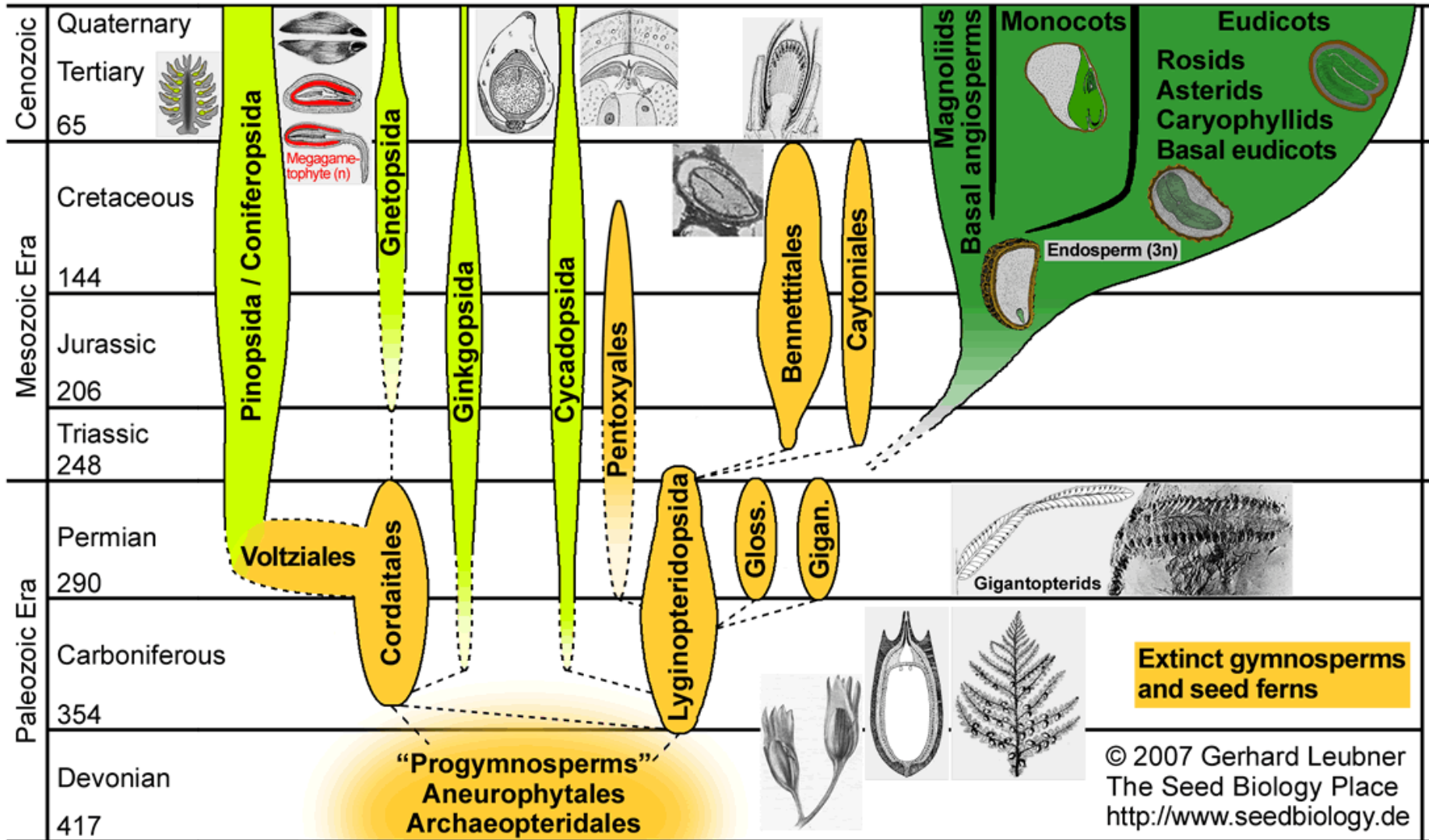
Land Plant Phylogeny





Gymnosperms

Angiosperms





The 1KP Project

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The 1000 plants (oneKP or 1KP) initiative is a public-private partnership generating large scale gene sequence information for 1000 different species of plants. Major supporters include Alberta's Department of Advanced Education and Technology (AET), Silicon Valley based Musea Ventures, Beijing Genomics Institute in Shenzhen, University of Alberta, and Alberta's Informatics Circle of Research Excellence (iCORE).

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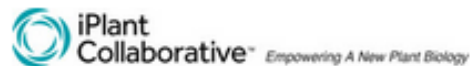


[View List of 1000 Plants](#)

[View Available Sequences](#)



musea ventures




www.onekp.com

51 Conifer Species in the 1KP Project


Araucariaceae	<i>Wollemia nobilis</i>	Pinaceae	<i>Abies lasiocarpa</i>
Cephalotaxaceae	<i>Cephalotaxus harringtonia</i>	Pinaceae	<i>Cedrus libani</i>
Cephalotaxaceae	<i>Amentotaxus argotaenia</i>	Pinaceae	<i>Keteleeria evelyniana</i>
Cupressaceae	<i>Athrotaxis cupressoides</i>	Pinaceae	<i>Larix speciosa</i>
Cupressaceae	<i>Austrocedrus chilensis</i>	Pinaceae	<i>Picea engelmannii</i>
Cupressaceae	<i>Calocedrus decurrens</i>	Pinaceae	<i>Pinus parviflora</i>
Cupressaceae	<i>Chamaecyparis lawsoniana</i>	Pinaceae	<i>Pinus radiata</i>
Cupressaceae	<i>Cryptomeria japonica</i>	Pinaceae	<i>Pinus jeffreyi</i>
Cupressaceae	<i>Cunninghamia lanceolata</i>	Pinaceae	<i>Pinus ponderosa</i>
Cupressaceae	<i>Diselma archeri</i>	Pinaceae	<i>Pseudotsuga wilsoniana</i>
Cupressaceae	<i>Fokienia hodginsii</i>	Pinaceae	<i>Tsuga heterophylla</i>
Cupressaceae	<i>Glyptostrobus pensilis</i>	Podocarpaceae	<i>Dacrycarpus compactus</i>
Cupressaceae	<i>Juniperus scopulorum</i>	Podocarpaceae	<i>Falcatifolium taxoides</i>
Cupressaceae	<i>Metasequoia glyptostroboides</i>	Podocarpaceae	<i>Halocarpus bidwillii</i>
Cupressaceae	<i>Microbiota decussata</i>	Podocarpaceae	<i>Manoao colensoi</i>
Cupressaceae	<i>Papuacedrus papuana</i>	Podocarpaceae	<i>Microcachrys tetragona</i>
Cupressaceae	<i>Pilgerodendron uviferum</i>	Podocarpaceae	<i>Nageia nagi</i>
Cupressaceae	<i>Platyclusus orientalis</i>	Podocarpaceae	<i>Phyllocladus hypophyllus</i>
Cupressaceae	<i>Sequoia sempervirens</i>	Podocarpaceae	<i>Podocarpus coriaceus</i>
Cupressaceae	<i>Taiwania cryptomerioides</i>	Podocarpaceae	<i>Podocarpus rubens</i>
Cupressaceae	<i>Taxodium distichum</i>	Podocarpaceae	<i>Prumnopitys andina</i>
Cupressaceae	<i>Thuja plicata</i>	Podocarpaceae	<i>Sundacarpus amarus</i>
Cupressaceae	<i>Thujopsis dolabrata</i>	Sciadopityaceae	<i>Sciadopitys verticillata</i>
Cupressaceae	<i>Widdringtonia cedarbergensis</i>	Taxaceae	<i>Taxus baccata</i>
		Taxaceae	<i>Taxus cuspidata</i>
		Taxaceae	<i>Torreya taxifolia</i>
		Taxaceae	<i>Torreya nucifera</i>





“Evolution is, so to speak, an experimentalist that has been running experiments for three and a half billion years, since the origin of life on Earth. And, wonderfully, the genomes of today's organisms retain the lab notes of these experiments, so that we can go back and reconstruct the events that took place.”

-Eric Lander ("Genetics" in Biology, 7th Ed., 2005)



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Our challenge will be to reconstruct the notebooks lost to retirements and laboratory disasters

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Tree Biotechnology 2013

Hosted by



May 26th - June 1st, 2013 | Asheville, NC, USA

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IUFRO Tree Biotechnology Conference

Started in 1985 and now meeting for the 16th time, the International Union of Forest Research Organization (IUFRO) Tree Biotechnology 2013 Conference has established a solid tradition for over 20 years as the official meeting of the IUFRO working group 2.04.06 - Molecular biology of forest trees. The Conference is the premier international venue for presenting and discussing new developments and ideas for current and future research in tree genetics, genomics and biotechnology. The IUFRO Tree Biotechnology 2013 Conference will be held in Asheville (NC, USA), and is being co-chaired by M. Kirst (University of Florida) and J. Dean (University of Georgia).

Conference Theme

The theme of the Conference will be “Forest Biotechnology: Meeting the Needs of a Changing World” to reflect the changing role of forest biotechnology in addressing the growing need for sustainable, renewable sources of biomass, in the face of climate change.

Conference Dates

May 26 (Sunday) to June 1 (Saturday), 2013

Conference Venue

The conference will take place at the Convention Center of the Marriott Renaissance Hotel (<http://www.wcghotels.com/asheville-hotel/>) in Asheville, NC. Asheville, located in the Appalachian Mountains, is one of the most charming cities in the US, with a great arts community, diverse outdoor adventures and numerous historic landmarks. For more information visit www.exploreasheville.com. Asheville is conveniently served by the

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