Toward the ultimate phylogeny of Magnoliaceae: phylogenomic approach

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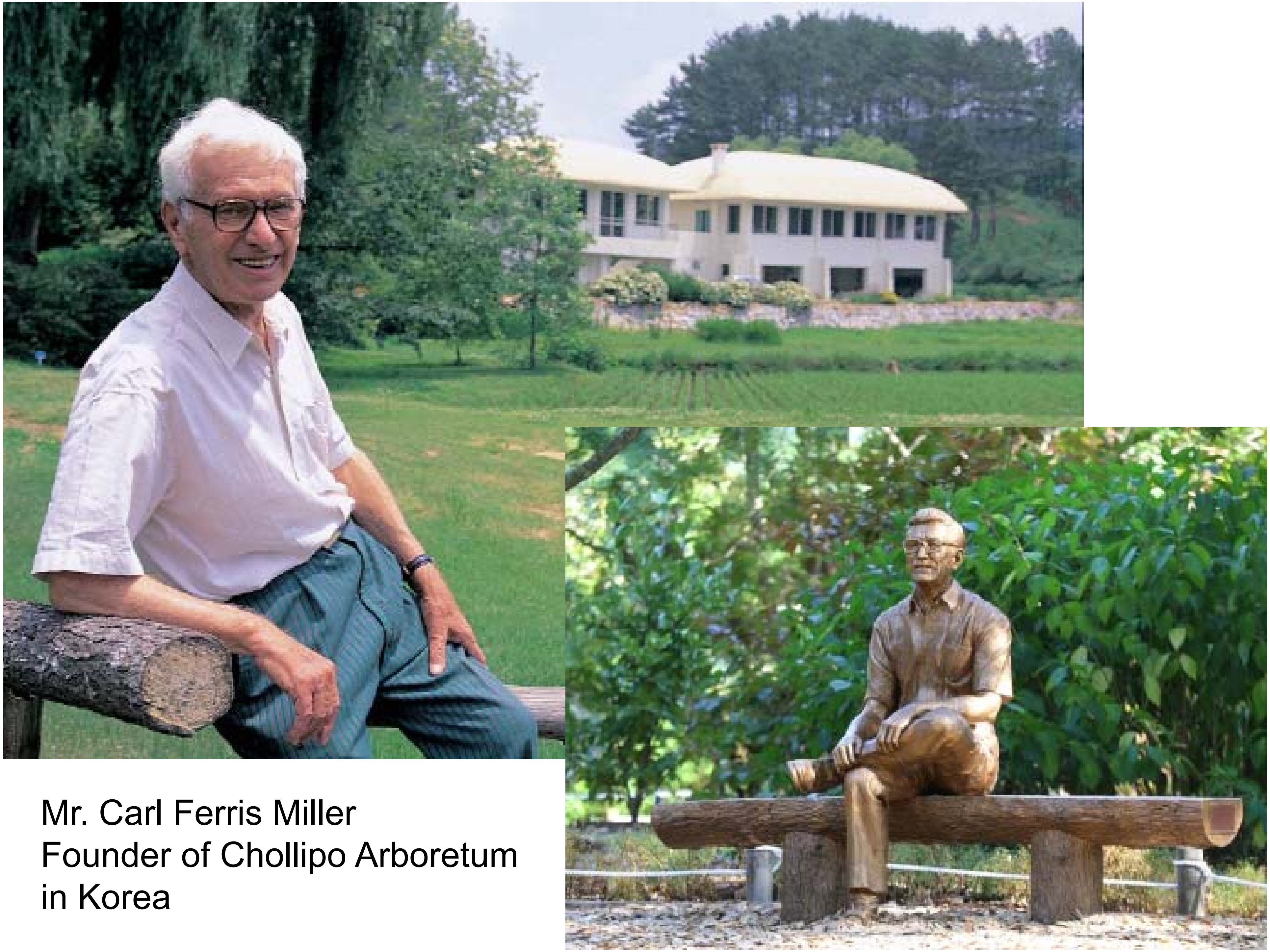
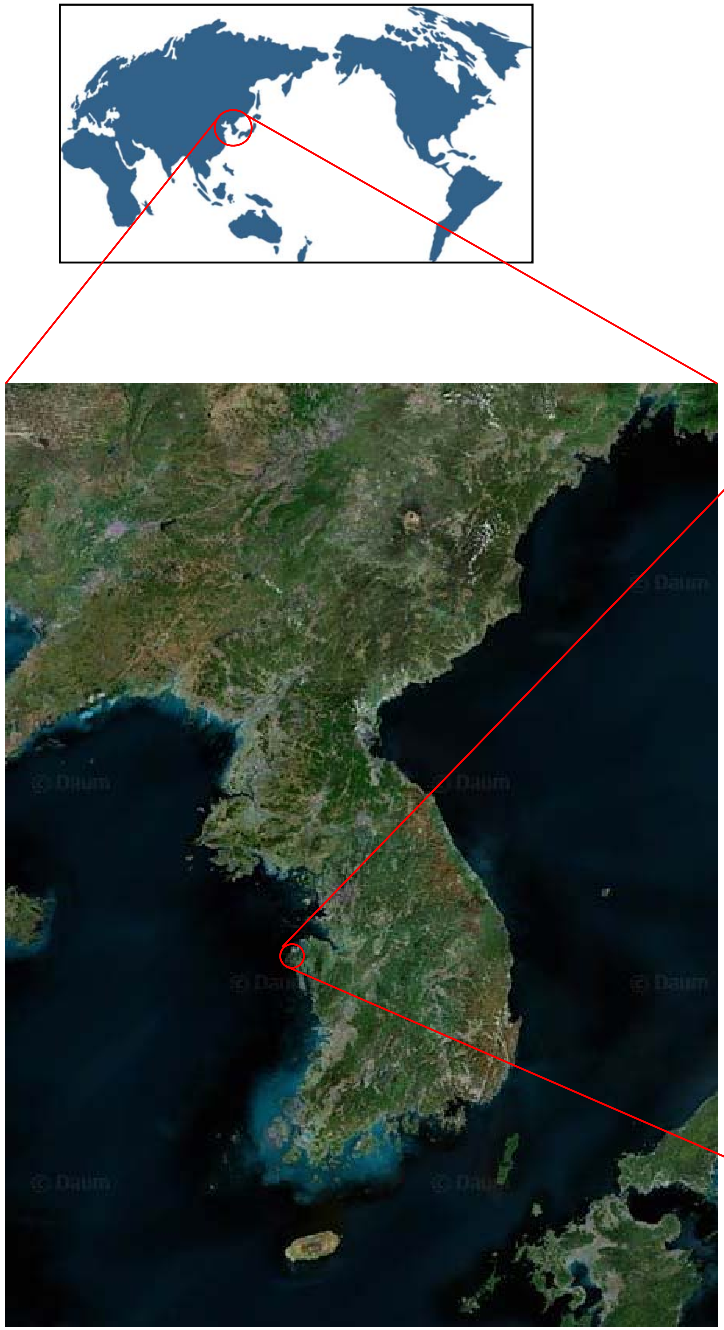


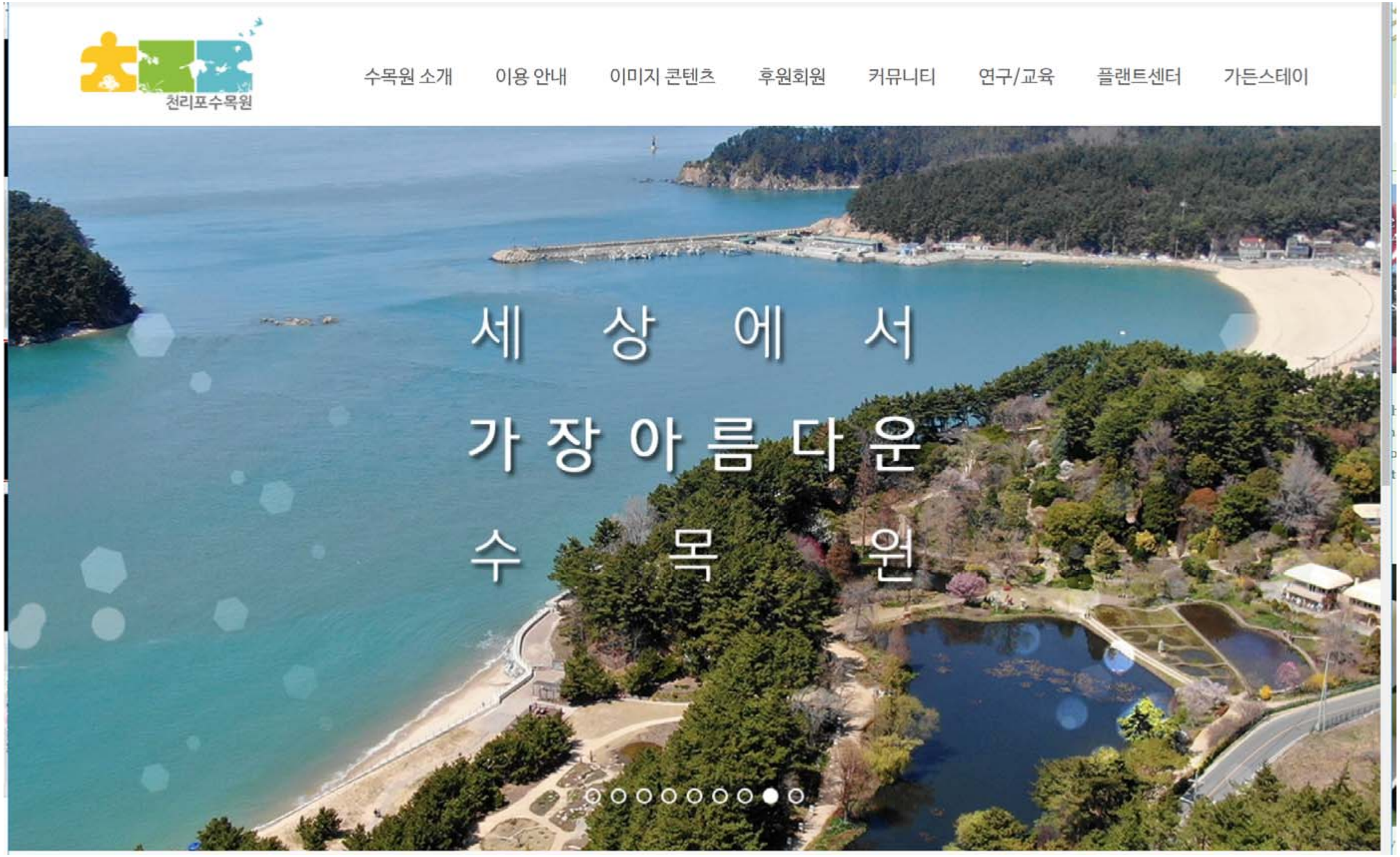
Mr. Carl Ferris Miller
Founder of Chollipo Arboretum
in Korea

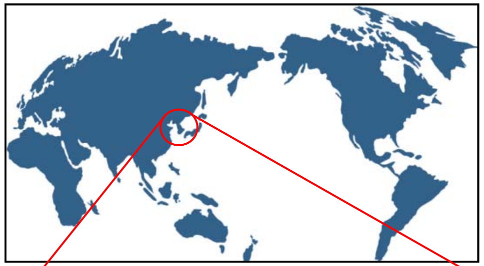
Chollipo Arboretum
Famous for its magnolia collection

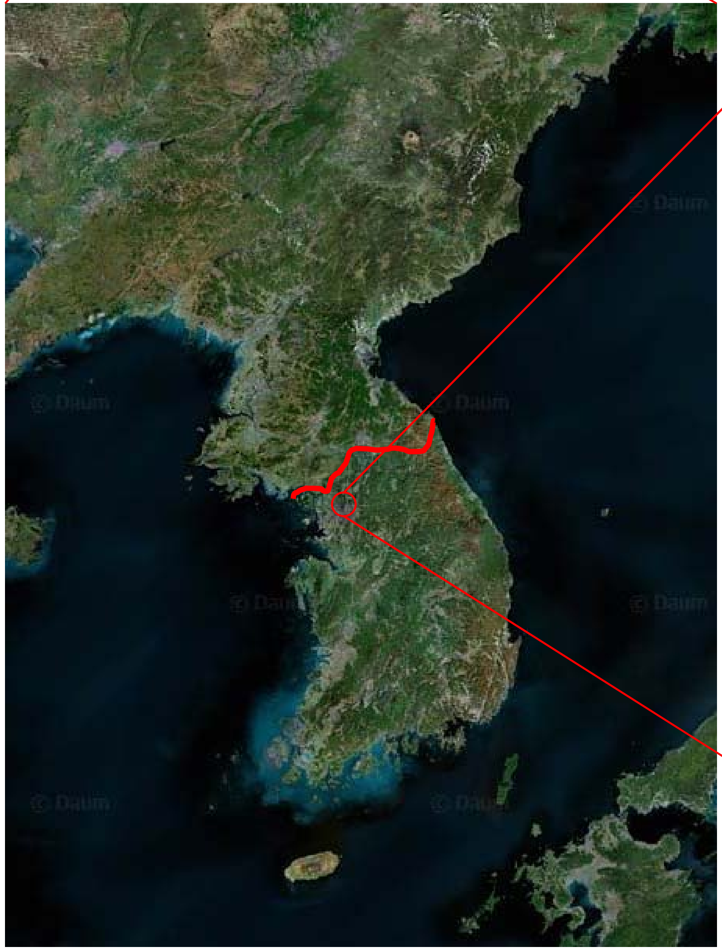


2020. Annual Meeting of Magnolia Society International Cholliop Arboretum in Korea. April 13th~22th, 2020

http://WWW.Chollipo.org







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Dr. Hans Nooteboom **Dr. Liu Yu-Hu**

Twenty-one years ago... in 1998
The 1st International Symposium on the Family Magnoliaceae, Gwangzhow


Dr. Hiroshi Azuma


Dr. Weibang Sun


Mr. Richard Figlar


Handsome young boy


Dr. Hans Nooteboom


Dr. Yong-kang Sima


Dr. Qing-wen Zeng


Dr. Yu-wu Law

Presented ITS study on Magnoliaceae - never published

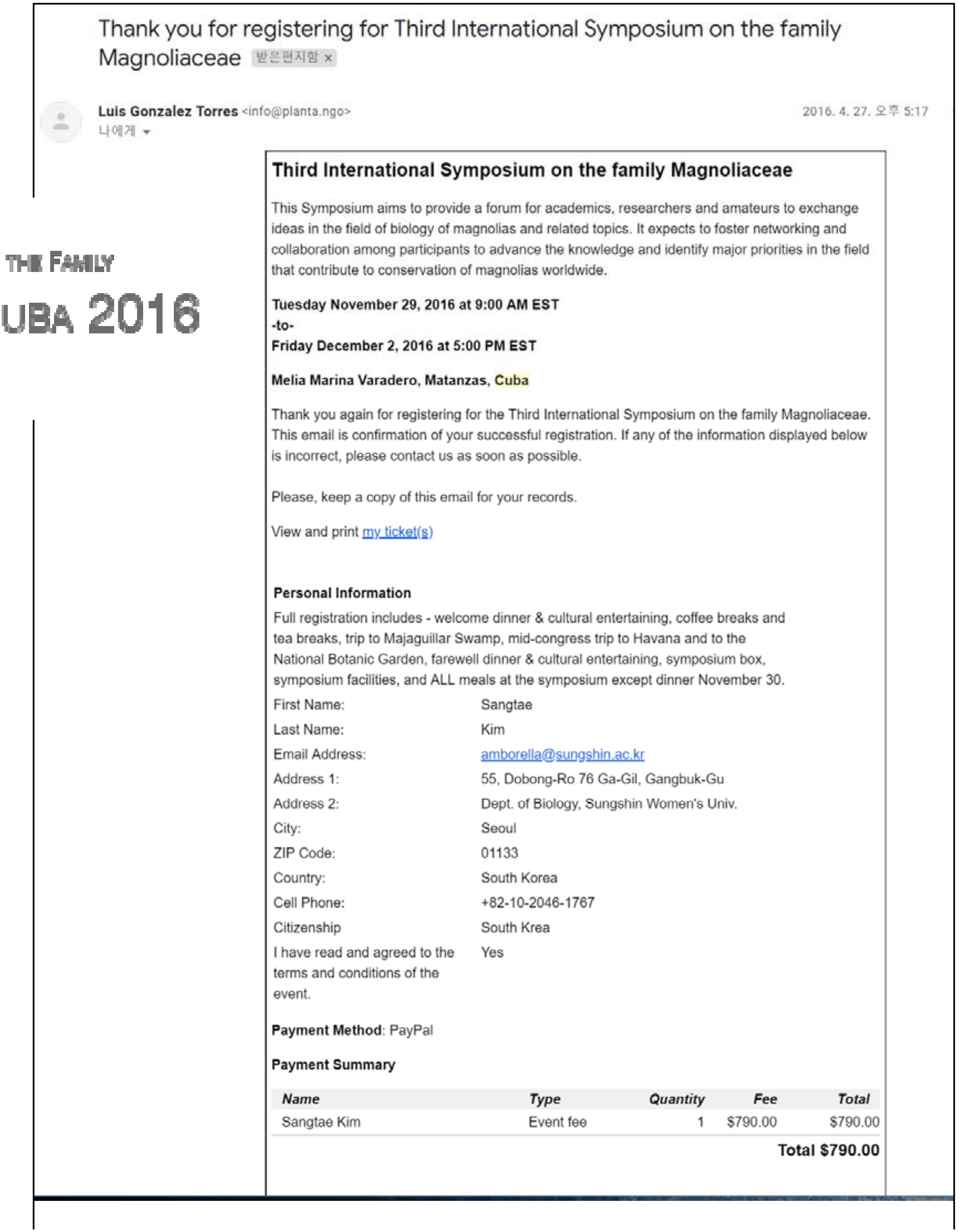
Ten years ago… **in 2009**

Presented nine cp genome region study (9.2 kbp) on Magnoliaceae – published in 2013

2015

1st International Sympodium on Neotropical Magnoliaceae

THIRD INTERNATIONAL SYMPOSIUM ON THE FAMILY
MAGNOLIACEAE, CUBA 2016



Thank you for registering for Third International Symposium on the family Magnoliaceae 받은편지함 ×

Luis Gonzalez Torres <info@planta.ngo>
나에게

2016. 4. 27. 오후 5:17

Third International Symposium on the family Magnoliaceae

This Symposium aims to provide a forum for academics, researchers and amateurs to exchange ideas in the field of biology of magnolias and related topics. It expects to foster networking and collaboration among participants to advance the knowledge and identify major priorities in the field that contribute to conservation of magnolias worldwide.

Tuesday November 29, 2016 at 9:00 AM EST
-to-
Friday December 2, 2016 at 5:00 PM EST

Melia Marina Varadero, Matanzas, Cuba

Thank you again for registering for the Third International Symposium on the family Magnoliaceae. This email is confirmation of your successful registration. If any of the information displayed below is incorrect, please contact us as soon as possible.

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Personal Information

Full registration includes - welcome dinner & cultural entertaining, coffee breaks and tea breaks, trip to Majaguillar Swamp, mid-congress trip to Havana and to the National Botanic Garden, farewell dinner & cultural entertaining, symposium box, symposium facilities, and ALL meals at the symposium except dinner November 30.

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Last Name:	Kim
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Country:	South Korea
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Name	*Type*	*Quantity*	*Fee*	*Total*
Sangtae Kim	Event fee	1	$790.00	$790.00

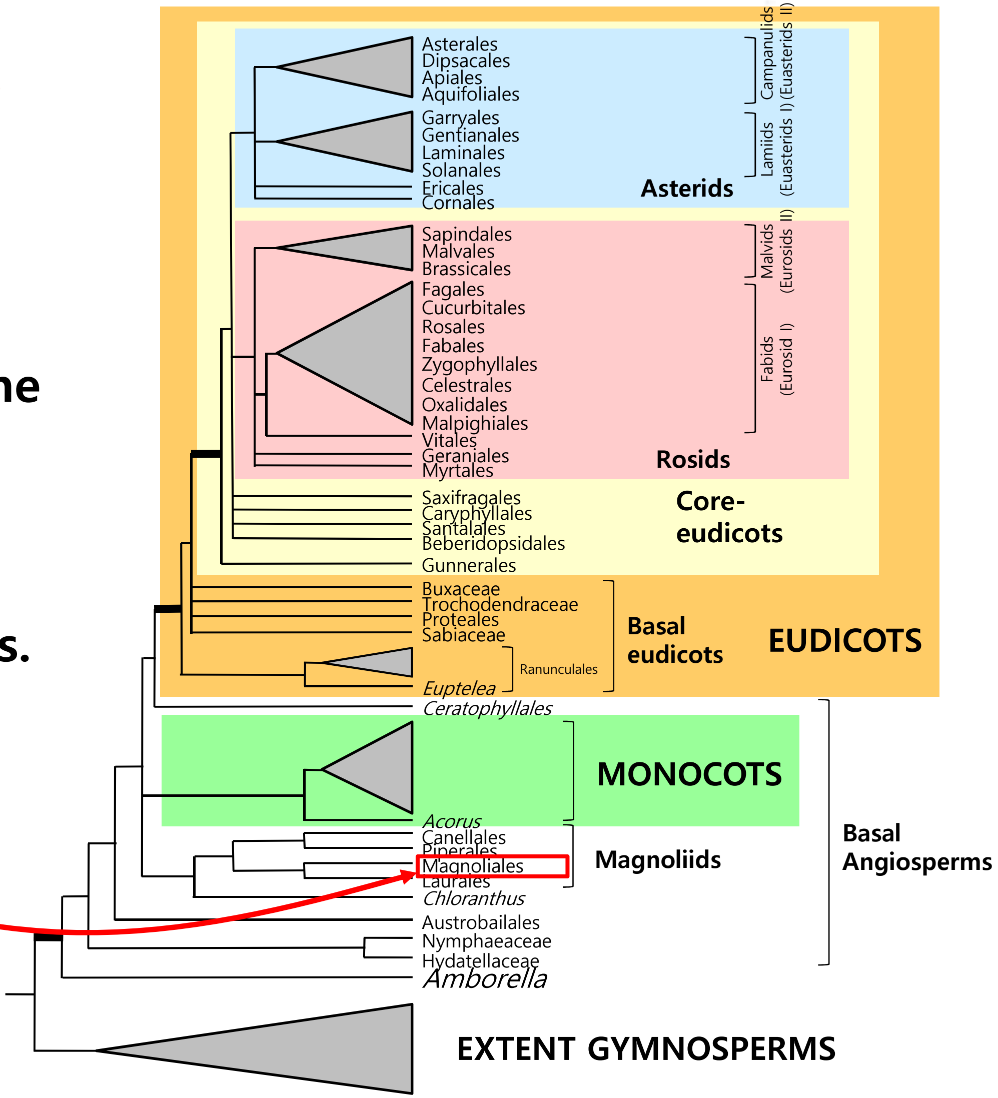
Total $790.00

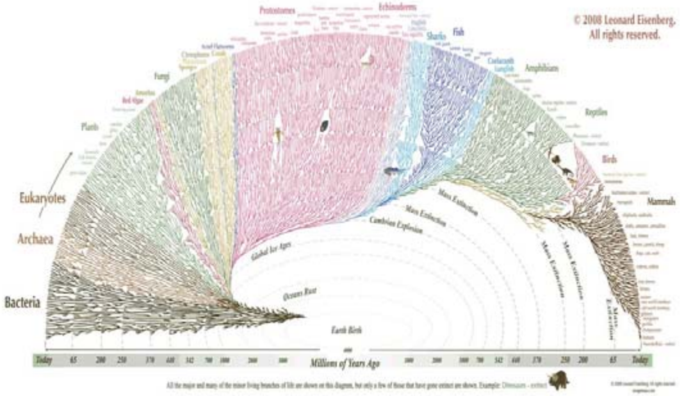
Gadalajara, 2019

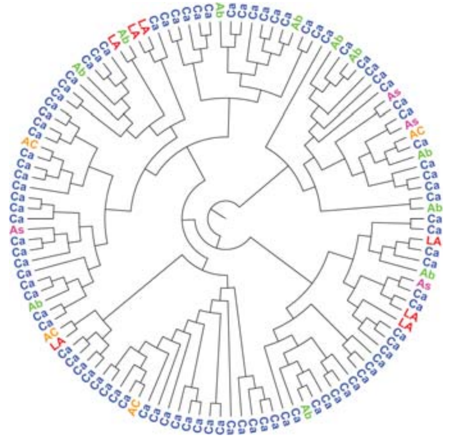
3rd International Sympodium and Workshop on Neotropical Magnoliaceae

Why magnolia study is important in botany?

- **As a member of early-diverging angiosperms, reconstruction of the phylogeny of Magnoliaceae will play a key role in understanding the diversification of angiosperms.**

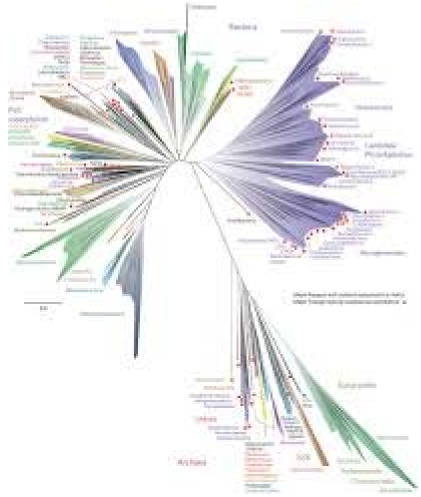


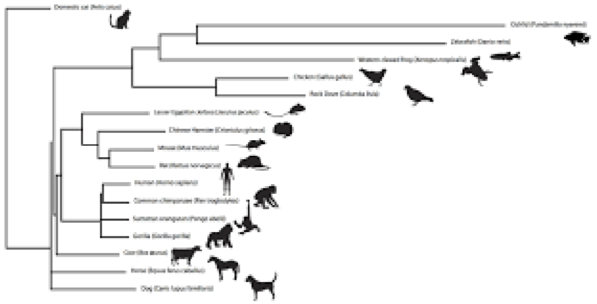




Why Phylogeny?

→ Reconstruction of phylogenetic tree is the first step for understanding **EVOLUTION** and **CLASSIFICATION** of a group of taxa





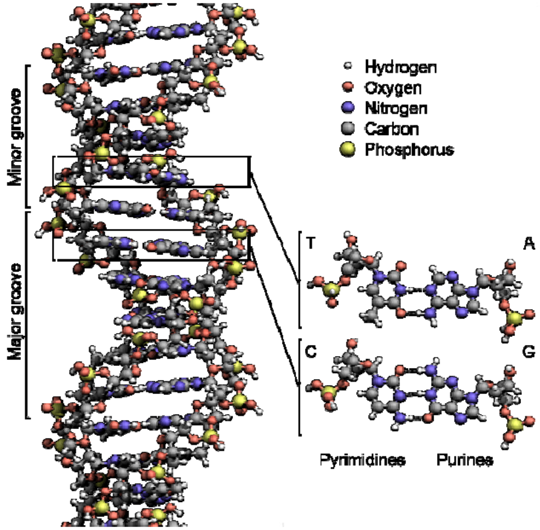
How can we make phylogenetic tree?

- (Traditionally,)

  Morphological character analysis

- (After the recognition that DNA is basic genetic material of life,)

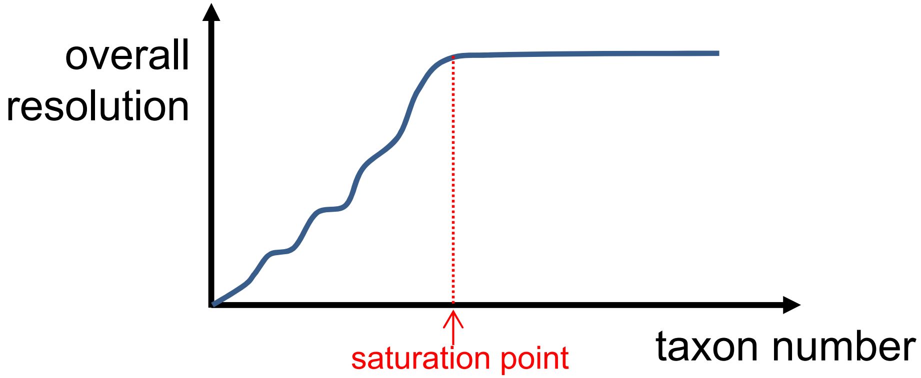
  DNA analysis



Toward the ultimate phylogeny…

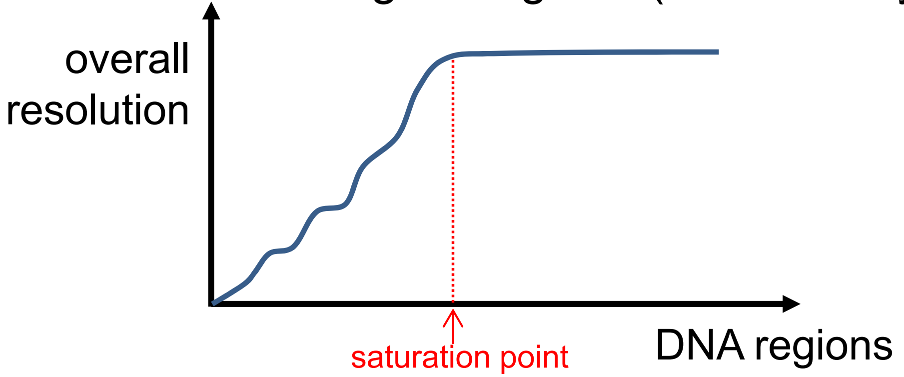
How can we make GOOD phylogenetic tree?

1) **Complete sampling** representing ALL subgroups of a target plant group

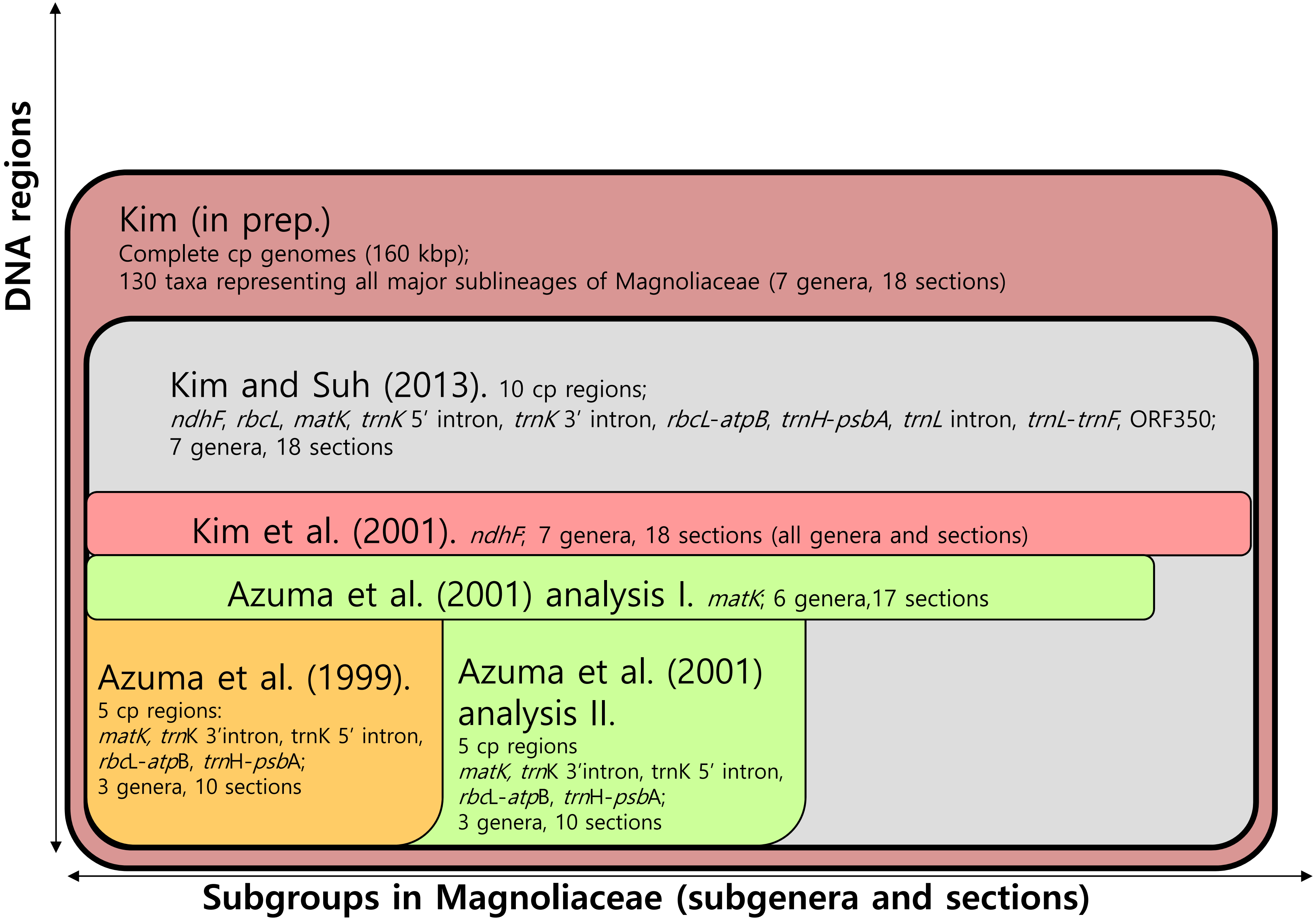


2) **Complete data collection: include DNA regions as many as possible (at lease over the saturation point)**

- have to compare orthologous genes: **exclude multiple copy genes**
- exclude too divergent regions (theoretically, > 25%)



History of Molecular Phylogenetic Studies on Magnoliaceae



DNA regions

Kim (in prep.)
Complete cp genomes (160 kbp);
130 taxa representing all major sublineages of Magnoliaceae (7 genera, 18 sections)

Kim and Suh (2013). 10 cp regions;
ndhF, *rbcL*, *matK*, *trnK* 5′ intron, *trnK* 3′ intron, *rbcL-atpB*, *trnH-psbA*, *trnL* intron, *trnL-trnF*, ORF350;
7 genera, 18 sections

Kim et al. (2001). *ndhF*; 7 genera, 18 sections (all genera and sections)

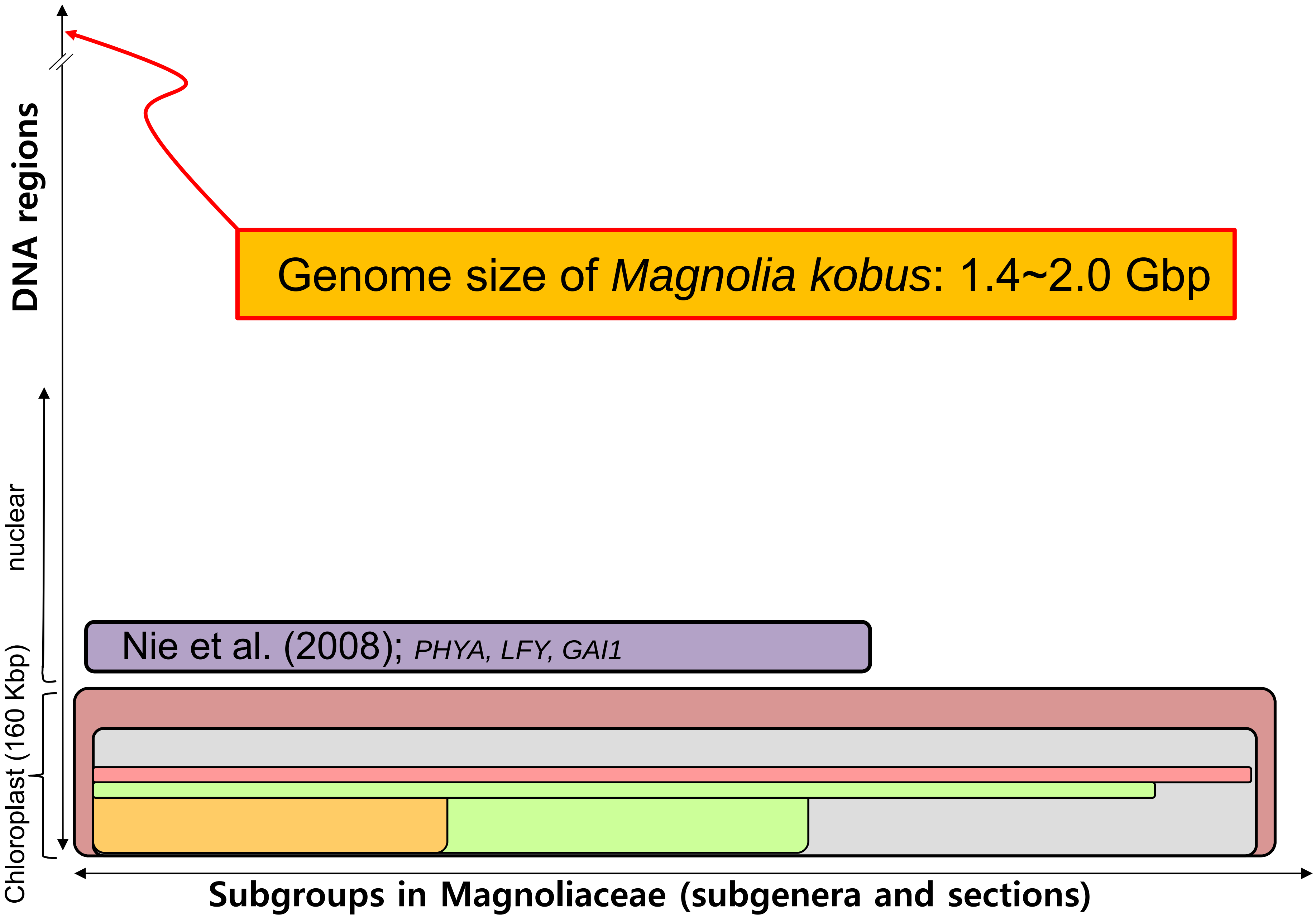
Azuma et al. (2001) analysis I. *matK*; 6 genera,17 sections

Azuma et al. (1999).
5 cp regions:
*matK, trn*K 3′intron, trnK 5′ intron,
*rbc*L-*atp*B, *trn*H-*psb*A;
3 genera, 10 sections

Azuma et al. (2001) analysis II.
5 cp regions
*matK, trn*K 3′intron, trnK 5′ intron,
*rbc*L-*atp*B, *trn*H-*psb*A;
3 genera, 10 sections

Subgroups in Magnoliaceae (subgenera and sections)

History of Molecular Phylogenetic Studies on Magnoliaceae

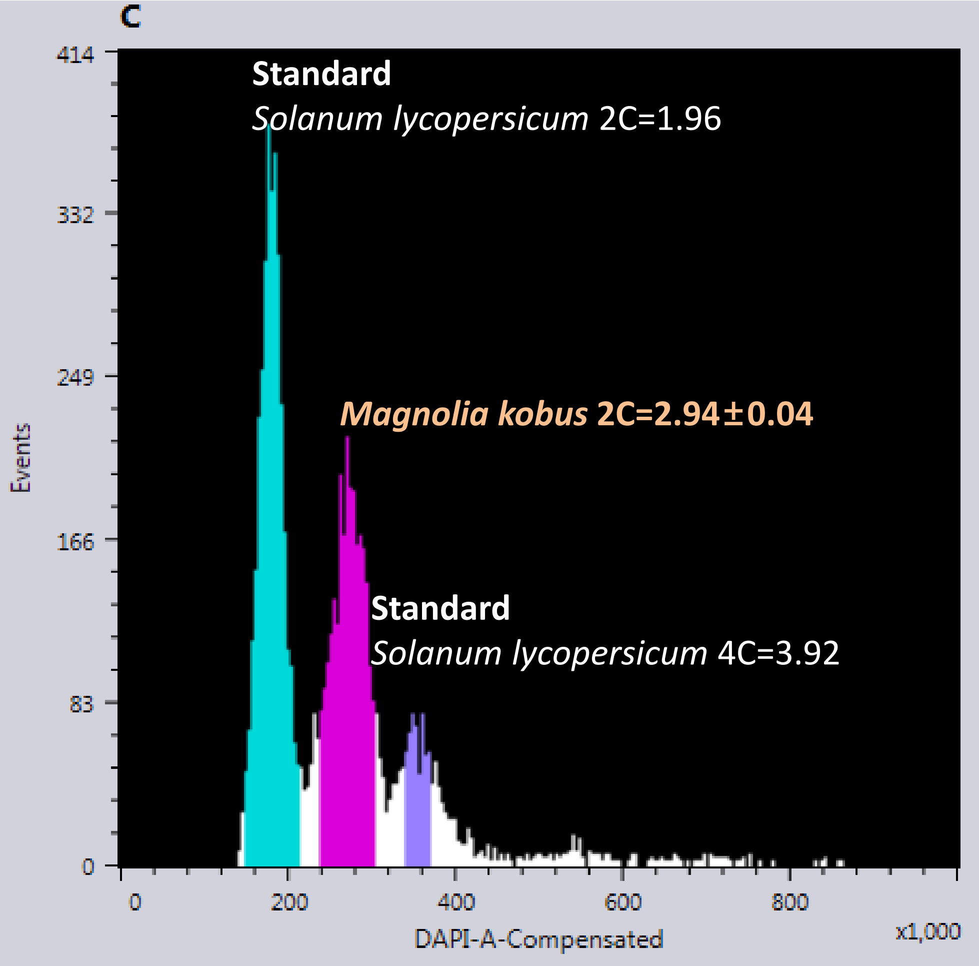


Genome size of *Magnolia kobus*: 1.4~2.0 Gbp

Nie et al. (2008); *PHYA, LFY, GAI1*

Estimation of genome size of *M. kobus* based on flow cytometry

1C = 1.42pg (~1.39Gbp)



Estimation of genome size of *M. kobus* by k-mer analyses

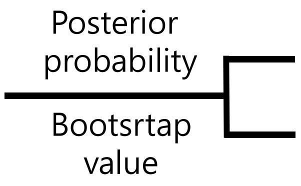
Estimated size is ca. 2 Gbp k=73.

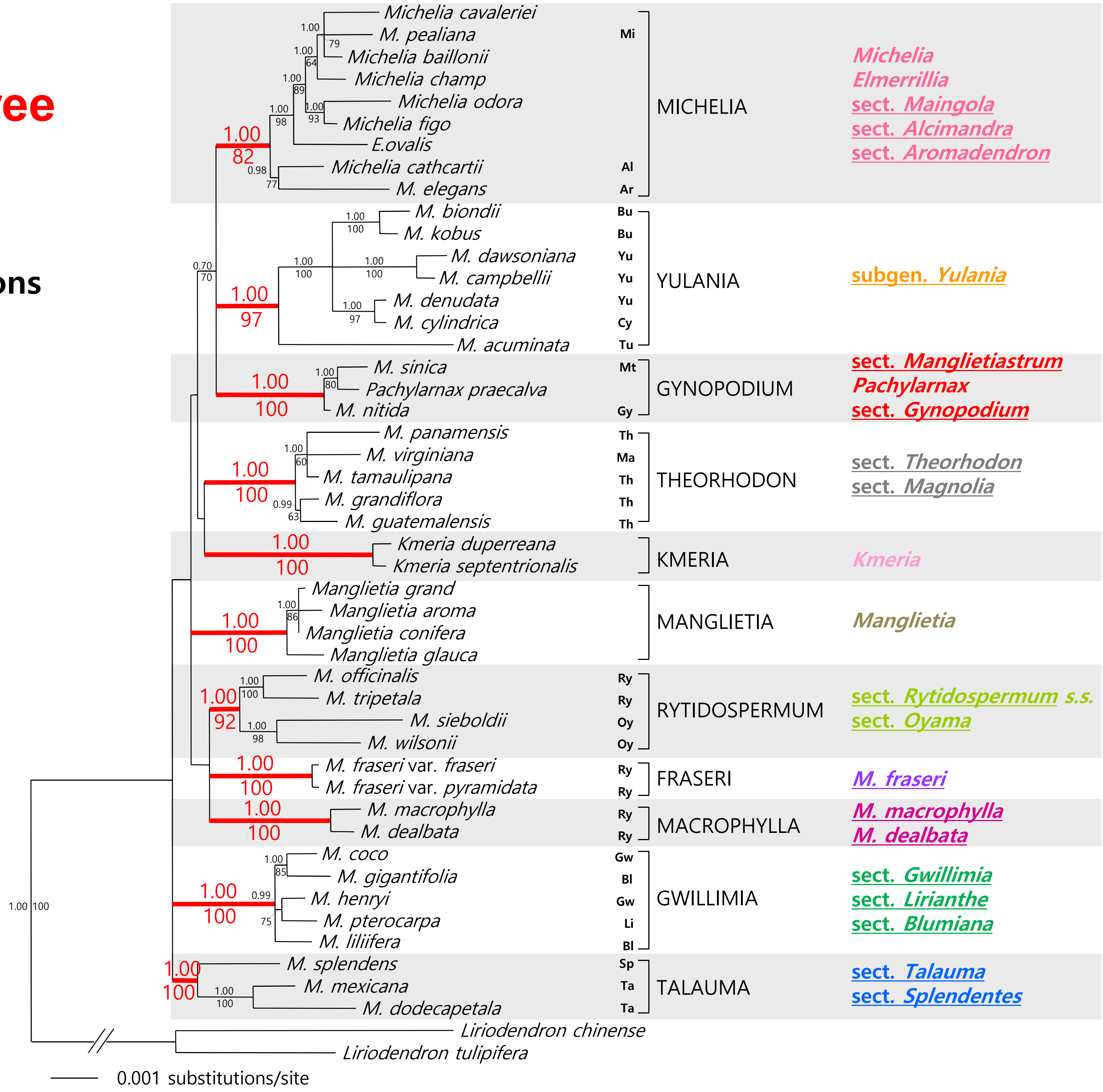
k=73, 2,079,028,389bp

k=33, 4,599,062,800bp

Previous Chloroplast tree

Kim et al. (2013)
10 chloroplast regions
= 9.2 Kbp





Previous
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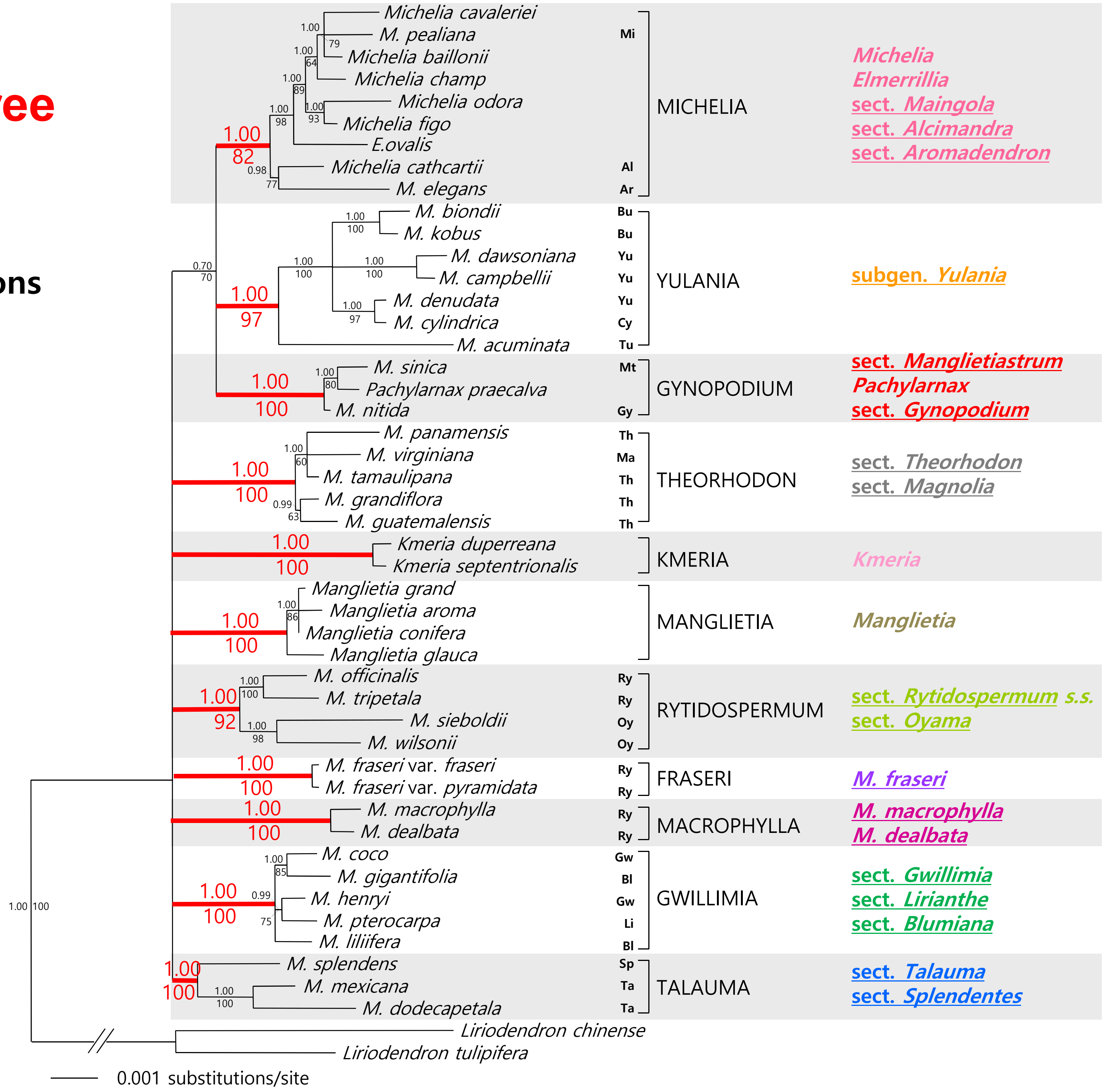
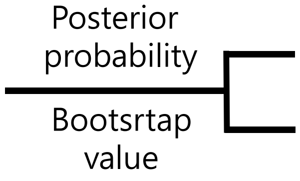
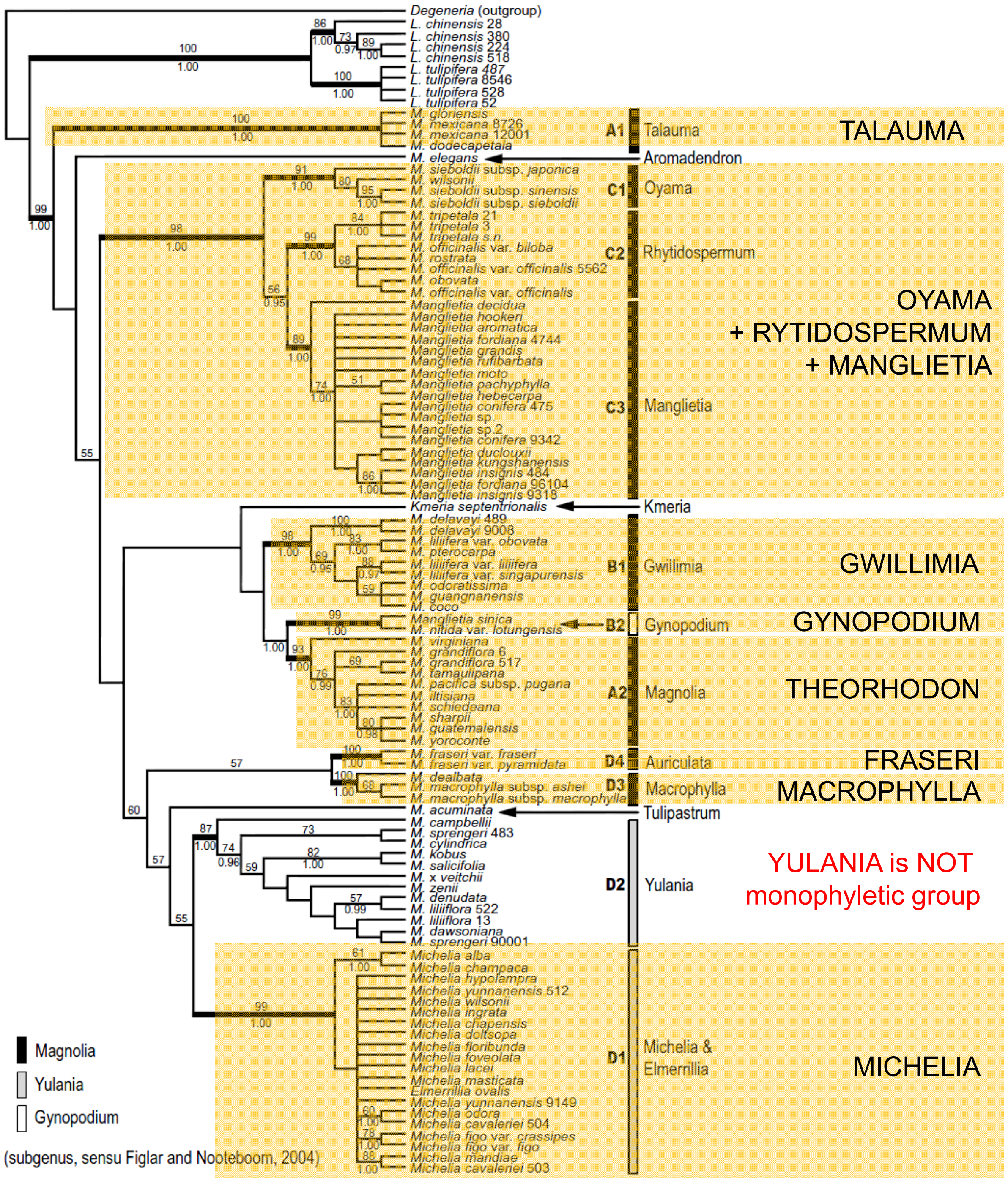




Previous Nuclear tree

Nie et al. (2008)
Nuclear *PHYA, LFY, GAI1*
= 2.3 Kbp

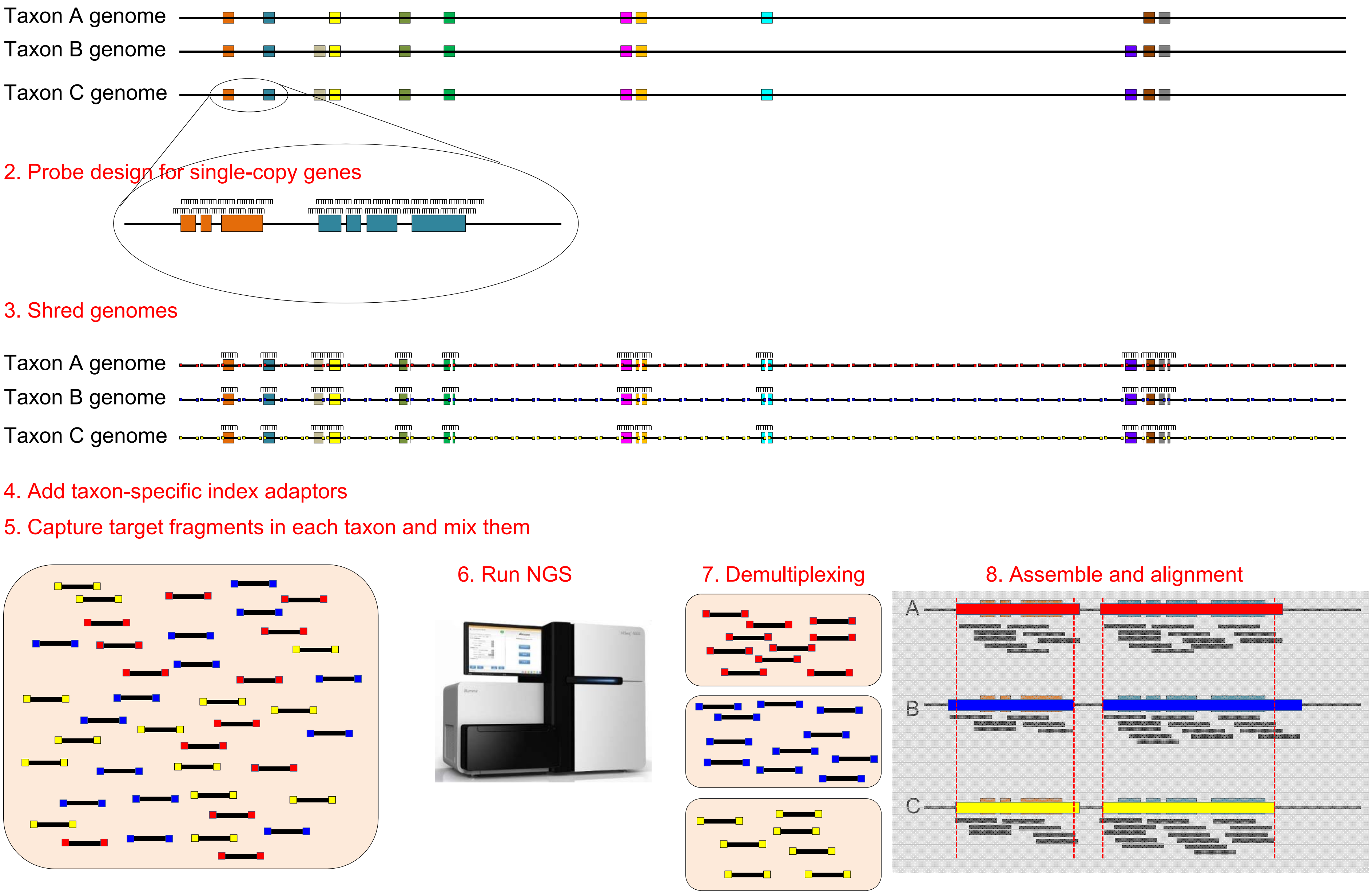


YULANIA is NOT monophyletic group

In this study,

- We tried a genome-wide approach on the phylogeny of Magnoliaceae with high-throughput **target-enrichment sequencing (Hyb-Seq)** using NGS.

Overview of NGS based target-enrichment sequencing (Hyb-Seq)



1. Detection of single-copy genes

2. Probe design for single-copy genes

3. Shred genomes

4. Add taxon-specific index adaptors

5. Capture target fragments in each taxon and mix them

6. Run NGS

7. Demultiplexing

8. Assemble and alignment

Target-enrichment sequencing = Hyb-Seq = Targeted sequencing

- Already have been applied in human exome sequencing.

- Tools for Hyb-Seq
  - MarkerMiner (Chamala et al., 2015): detecting single copy-genes
  - HybPiper (Johnson et al., 2016): mapping and alignment

- **Recent publications**

Animals

Prum et al. (2015). **Birds**. Nature
Breinholt et al. (2017). **Butterflies**. Systematic Biology
…

Plants

Mandel et al. (2016). **Asteraceae**. Applications in Plant Sciences
Schmickl et al. (2016). African ***Oxalis*** (Oxalidaceae). Molecular Ecology Resources
Stefan et al. (2017). **Aristolochiaceae** Molecular Phylogenetics and Evolution
Tamara et al. (2018). ***Euphobia*** (Euphobiaceae). New Phytologist
Global *Carex* Group (submitted). ***Carex*** (Cyperaceae).
…

Materials and Method

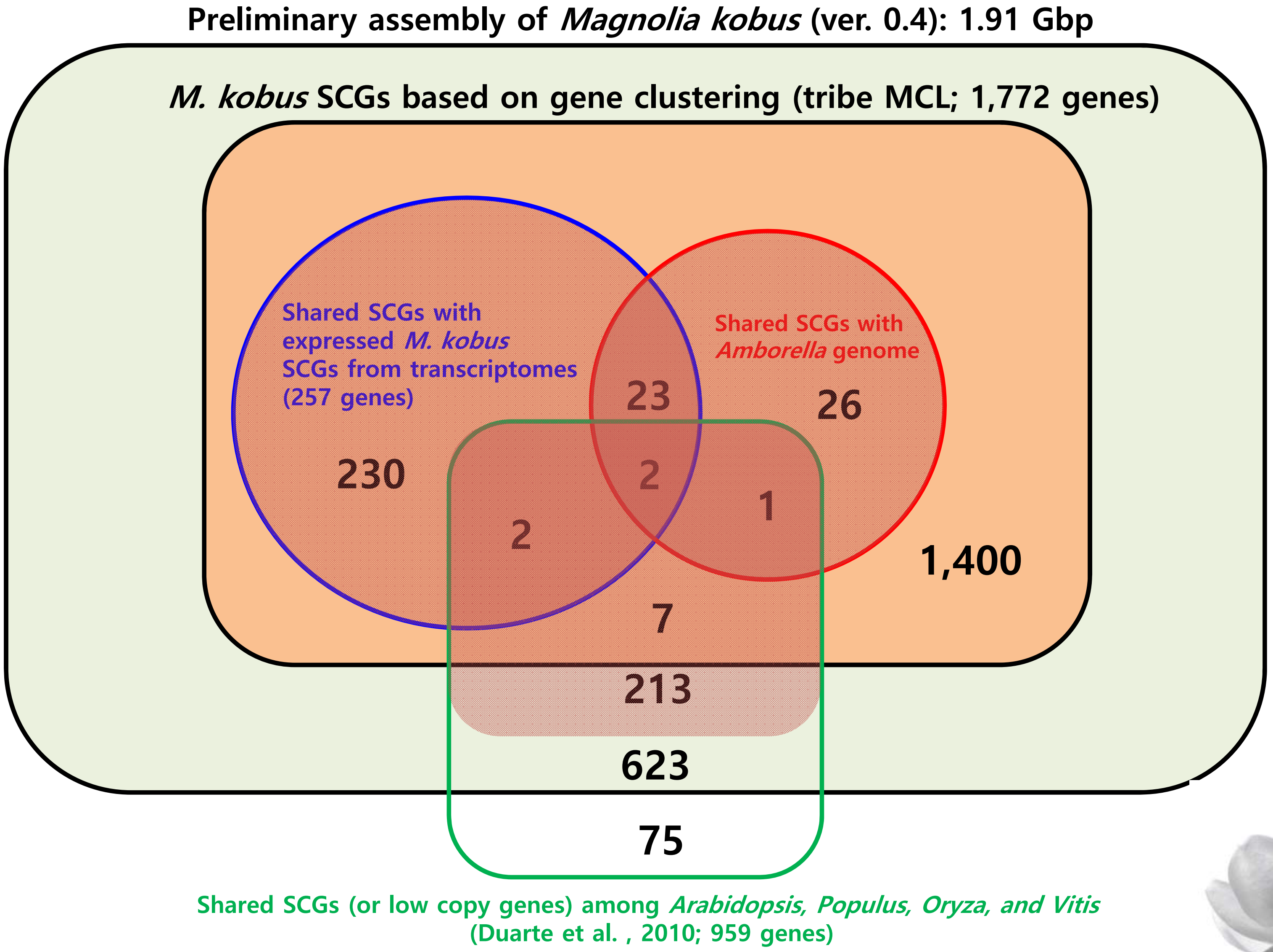
Materials

- **130 taxa** of Magnoliaceae
  - Representatives of previously reported **ALL** subgroups of Magnoliaceae (subgenera, sections, major clades)

Method

- **Target regions:**
  chloroplast whole genome (160 Kbp) + **504 nuclear single-copy gene regions** (443 Kbp)
- Capturing and NGS running: RapidGenomics Co.
  - 32 Gbp of 100 bp paired-end sequencing using HiSeq3000
- Extraction of othologos gene regions: HybPiper (Johnson et al., 2016)
- Matrix combining and sequence statistics: MEGA (ver. 7.0)
- Phylogenetic analyses: ML with 500 bootstrap using RAxML (GUI 1.5 beta)

Nuclear Target Regions for Hyb-Seq: 504 single-copy genes (443 Kbp)



SCG: single copy gene

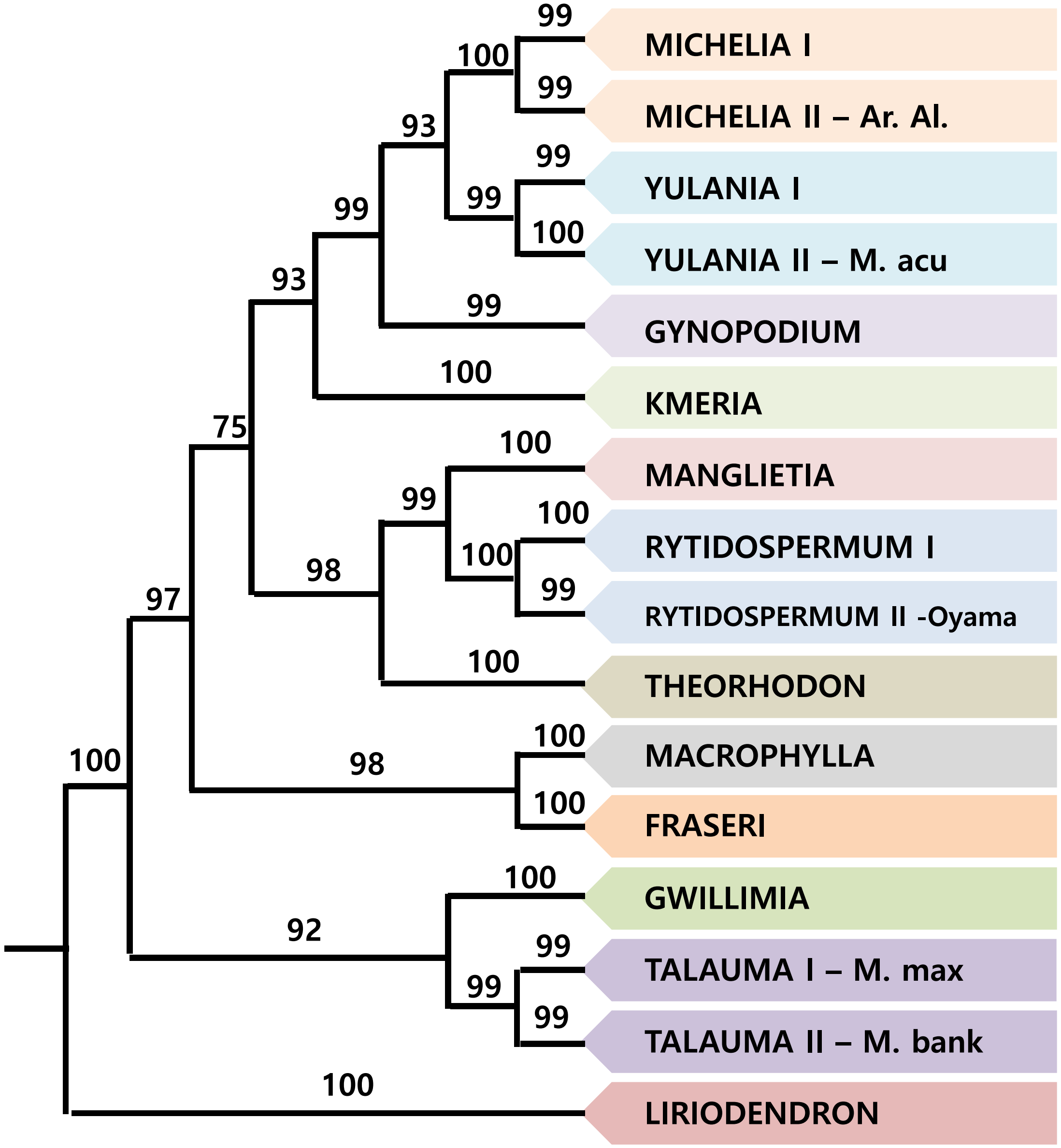
Results

Chloroplast genome

- **130 magnolia cp genomes were COMPLETED (more than 1,000X)**
  - uncovered gaps: only 0.06 % (three regions)
    - → later, completed by PCR-based Sanger sequencing
- Size of cp genome
  - subfamily Magnolioideae: 159.1 Kbp (mean of 127 taxa)
  - subfamily Liriodendroideae: 159.7 Kbp (mean of two taxa)
- Structural variations
  - ***trnV-GAC* deleted in *M.liliiflora***
  - 299 bp deletion in *M. acuminate*
  - 658 bp deletion in *M. kobus*

- Aligned matrix: 136.4 Kbp
  - Phylogenetically informative sites in Magnoliaceae: 6,014 (4.8%)
  - Phylogenetically informative sites in subfamily Magnolioideae: 4,984 (3.7%)

Summary of Chloroplast phylogeny (BS>50%)



Results

Nuclear regions

- 504 nuclear single-copy genes were determined.

  (+ introns and adjacent intergenic spacer regions)

  → Only 20 gene regions were analyzed in this talk


A243

Results

Nuclear regions

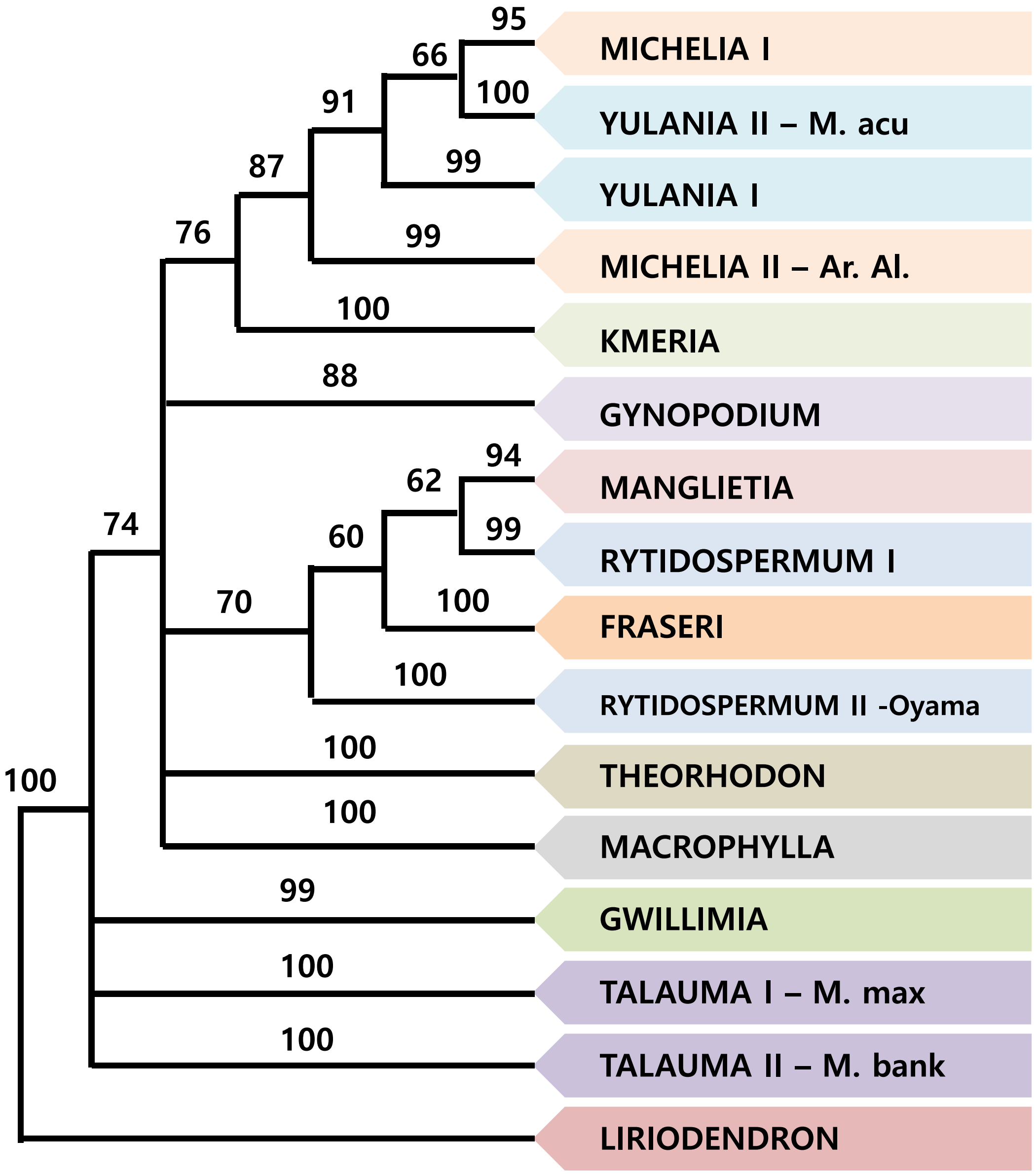
- 504 nuclear single copy genes were determined.

  (+ introns and adjacent intergenic spacer regions)

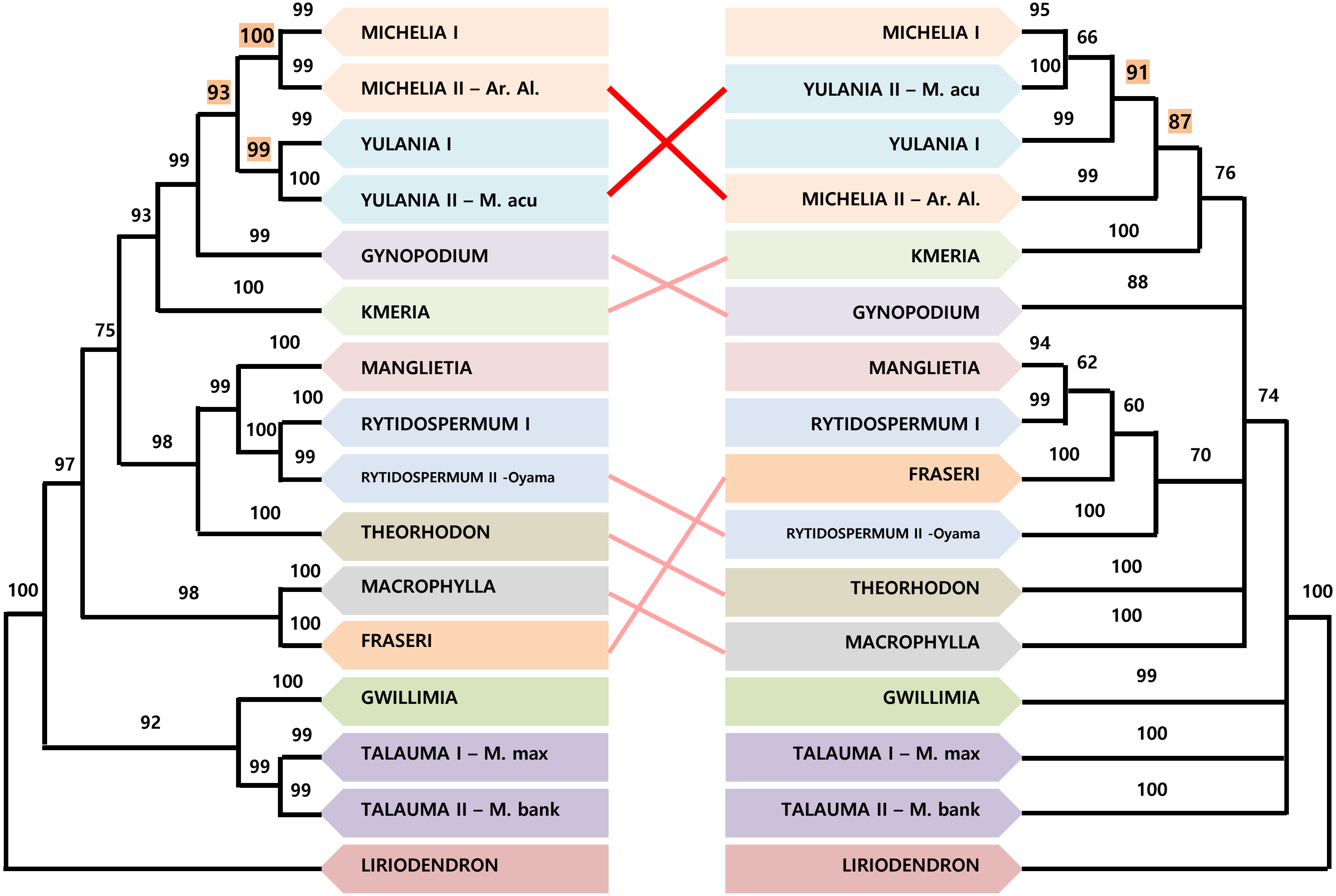
  → Only 20 gene regions were analyzed in this talk

	nuclear 20 gene regons		cp genome	
aligned length (Kbp)	**24**		**136**	
	Magnoliaceae	Magnolioideae	Magnoliaceae	Magnolioideae
# of informative sites (bp)	4,255	**3,514**	6,014	**4,984**
% of informative sites (%)	17.8	**14.7**	4.8	**3.7**

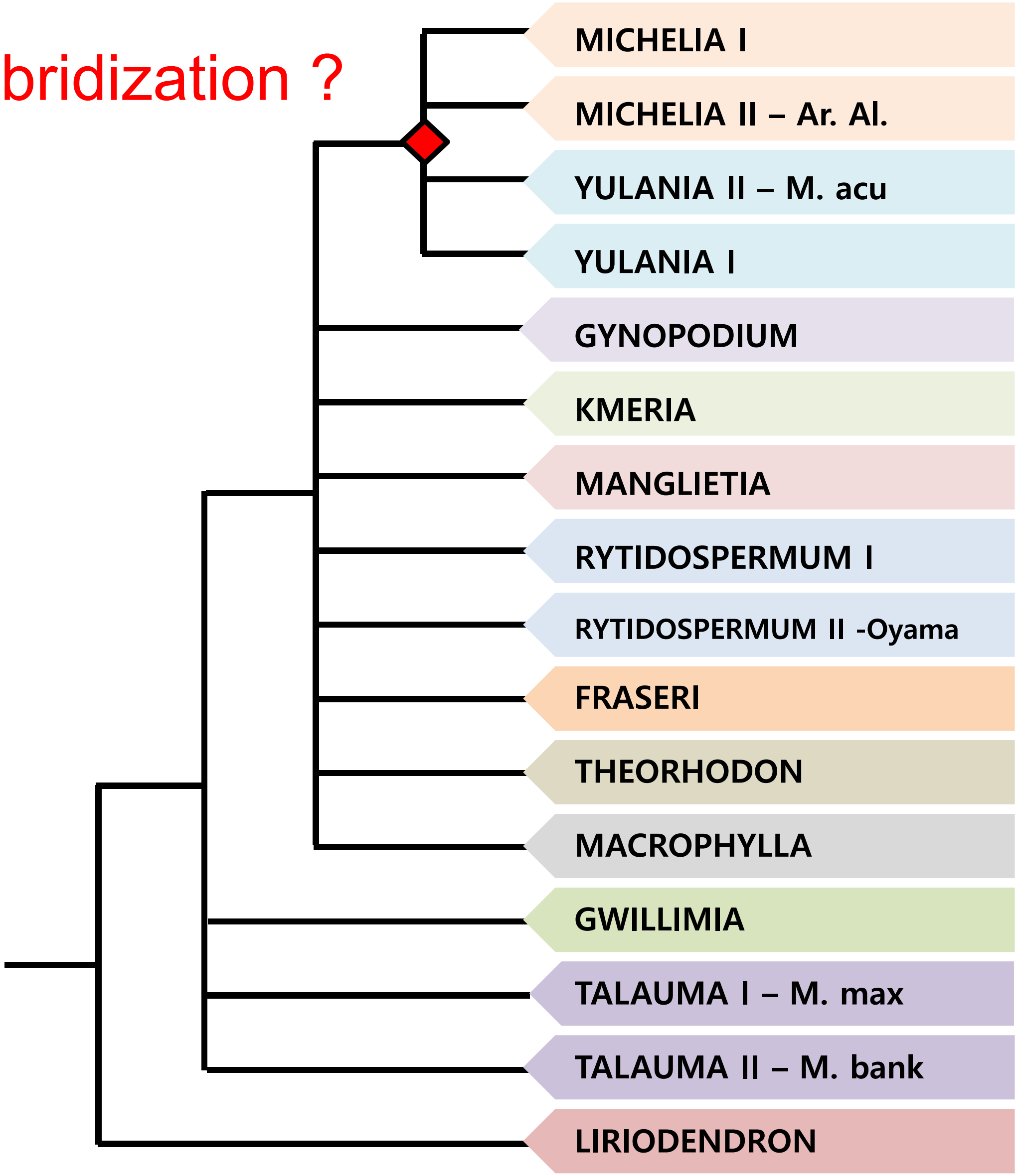
Summary of nuclear phylogeny (BS>50%)



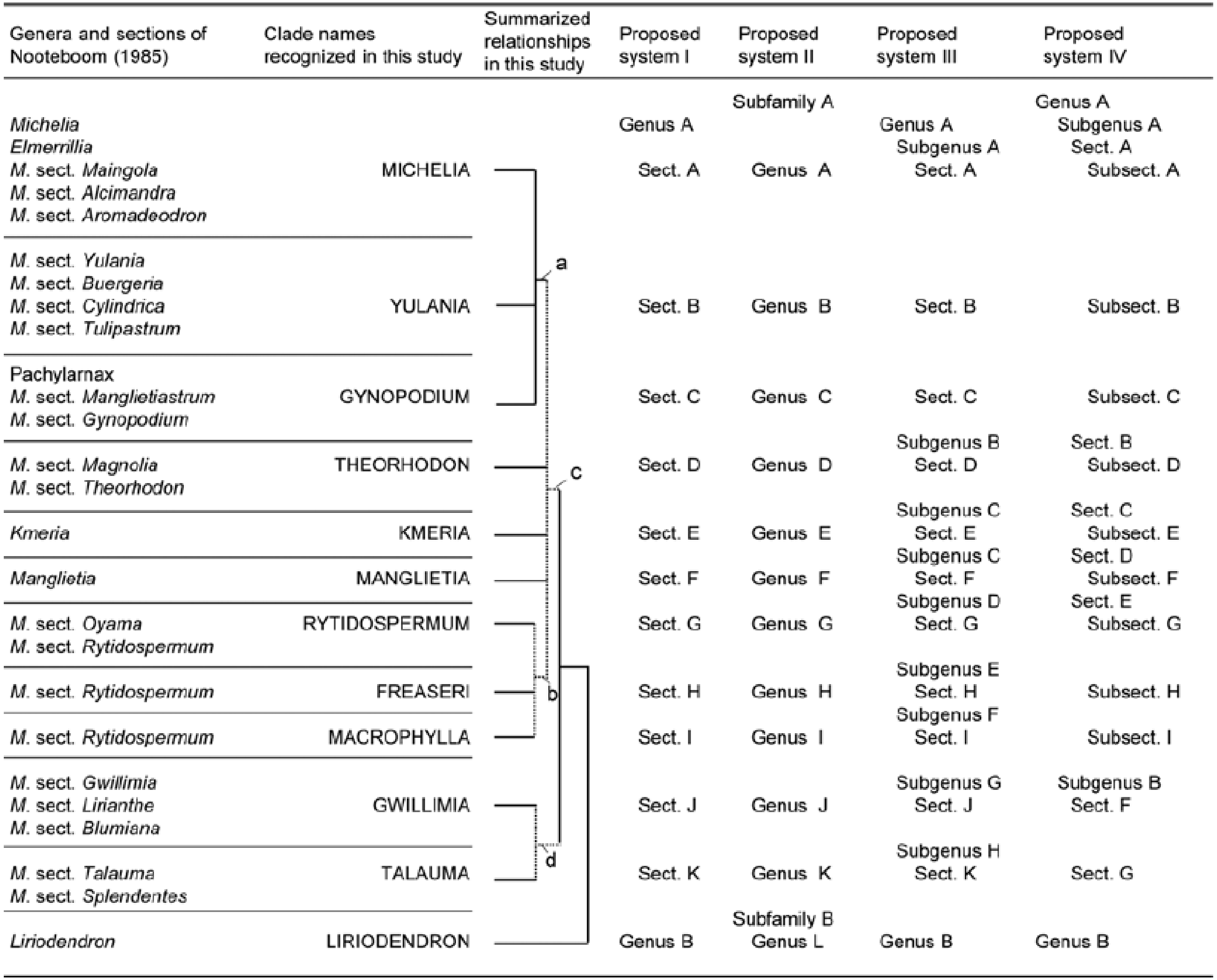
Chloroplast vs. Nuclear (BS>50%)



Strict consensus tree between cp tree and nuclear tree



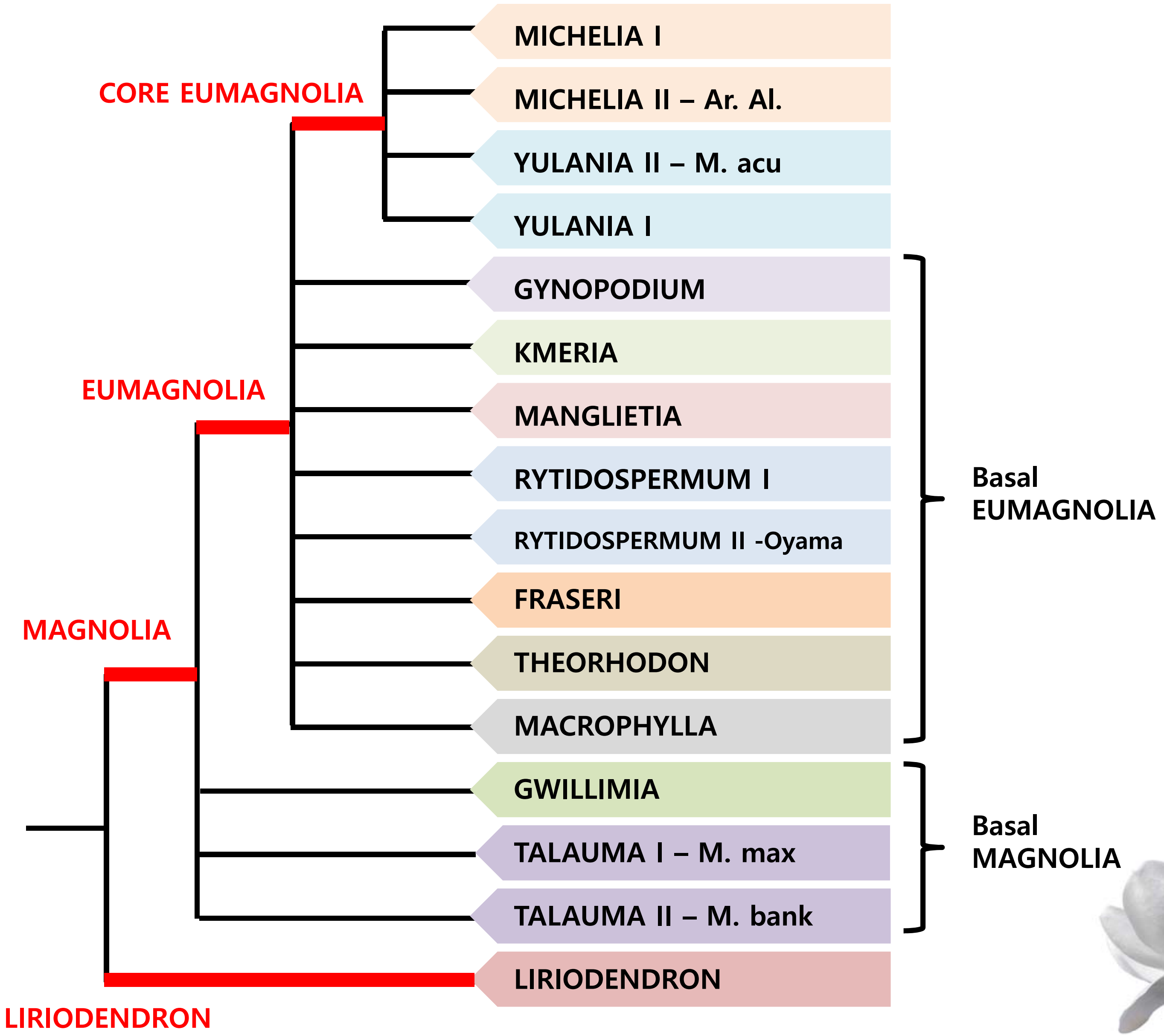
Taxonomic structures of possible classification systems proposed in Kim and Suh (2013) based on the analyses of chloroplast 10 regions



Genera and sections of Nooteboom (1985)	Clade names recognized in this study	Summarized relationships in this study	Proposed system I	Proposed system II	Proposed system III	Proposed system IV
Michelia *Elmerrillia* *M.* sect. *Maingola* *M.* sect. *Alcimandra* *M.* sect. *Aromadeodron*	MICHELIA		Genus A Sect. A	Subfamily A Genus A	Genus A Subgenus A Sect. A	Genus A Subgenus A Sect. A Subsect. A
M. sect. *Yulania* *M.* sect. *Buergeria* *M.* sect. *Cylindrica* *M.* sect. *Tulipastrum*	YULANIA	a	Sect. B	Genus B	Sect. B	Subsect. B
Pachylarnax *M.* sect. *Manglietiastrum* *M.* sect. *Gynopodium*	GYNOPODIUM		Sect. C	Genus C	Sect. C	Subsect. C
M. sect. *Magnolia* *M.* sect. *Theorhodon*	THEORHODON	c	Sect. D	Genus D	Subgenus B Sect. D	Sect. B Subsect. D
Kmeria	KMERIA		Sect. E	Genus E	Subgenus C Sect. E	Sect. C Subsect. E
Manglietia	MANGLIETIA		Sect. F	Genus F	Subgenus C Sect. F	Sect. D Subsect. F
M. sect. *Oyama* *M.* sect. *Rytidospermum*	RYTIDOSPERMUM		Sect. G	Genus G	Subgenus D Sect. G	Sect. E Subsect. G
M. sect. *Rytidospermum*	FREASERI	b	Sect. H	Genus H	Subgenus E Sect. H	Subsect. H
M. sect. *Rytidospermum*	MACROPHYLLA		Sect. I	Genus I	Subgenus F Sect. I	Subsect. I
M. sect. *Gwillimia* *M.* sect. *Lirianthe* *M.* sect. *Blumiana*	GWILLIMIA		Sect. J	Genus J	Subgenus G Sect. J	Subgenus B Sect. F
M. sect. *Talauma* *M.* sect. *Splendentes*	TALAUMA	d	Sect. K	Genus K	Subgenus H Sect. K	Sect. G
Liriodendron	LIRIODENDRON		Genus B	Subfamily B Genus L	Genus B	Genus B

Strict consensus tree between cp tree and nuclear tree

PhyloCode (Cantino and de Queiroz, 2010)



SUMMARY

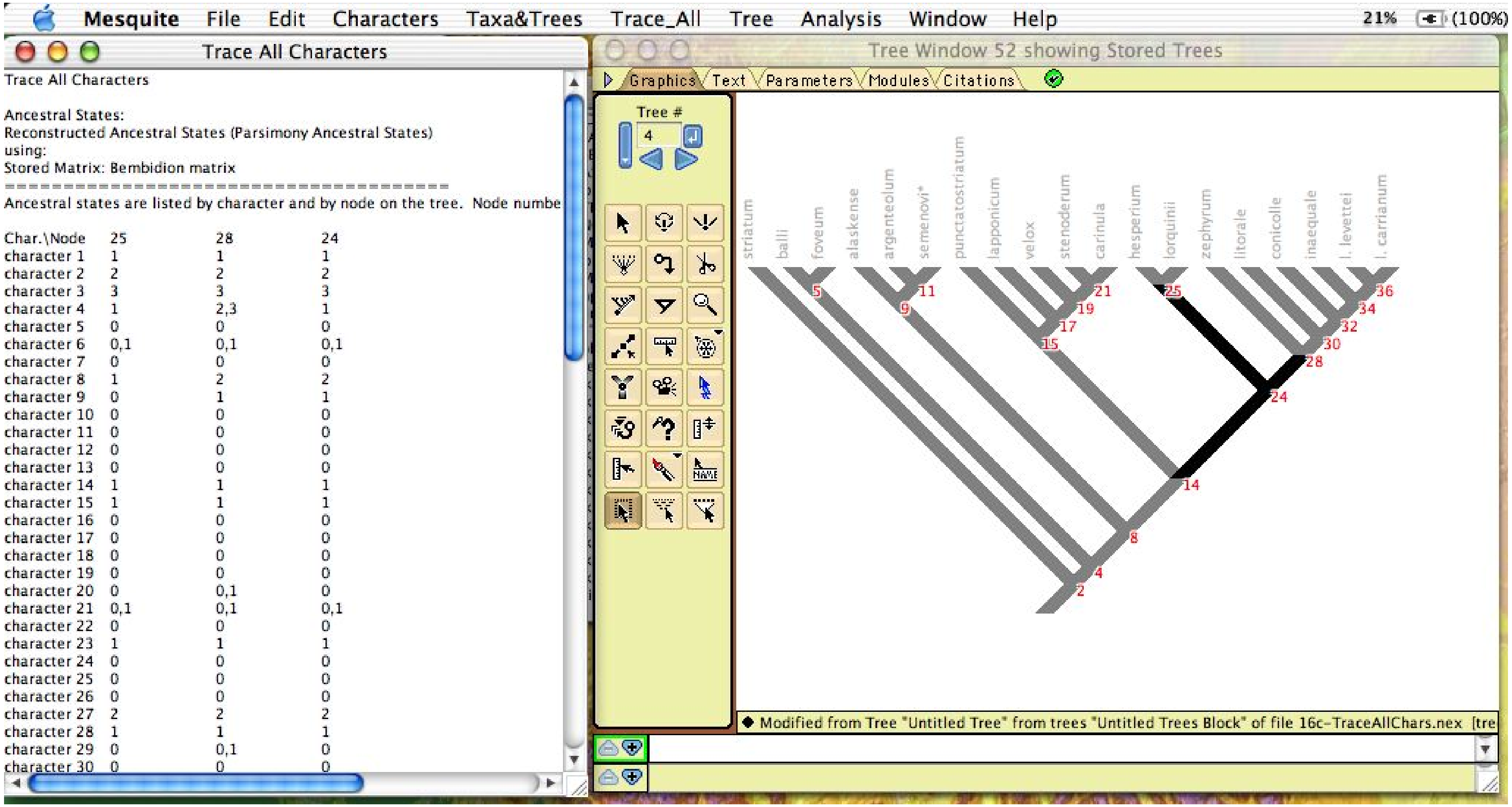
- For **130 taxa** representing all subgroups in Magnoliaceae, **complete chloroplast genomes** and **20 nuclear DNA regions** were determined.
- Some conflict relationships were recognized between chloroplast tree and nuclear tree suggesting possible **ancient hybridization**, especially between ancestors of MICHELIA and YULANIA clades.
- Highly supported clades from both chloroplast tree and nuclear tree were named as **EUMAGNOLIA** and **CORE EUMAGNOLIA** based on the concept of PhyloCode.
- Target-enrichment NGS (Hyb-Seq) will be the **standard method** of future phylogenetic studies.

→ **Ultimate Phylogeny**

Further studies

1) **Evolutionary character reconstruction**
2) Re-investigation of genome size
3) Suggestion of a minimum set of genes for phylogenetic or barcoding studies

Evolutionary character reconstruction



```
Trace All Characters

Ancestral States:
Reconstructed Ancestral States (Parsimony Ancestral States)
using:
Stored Matrix: Bembidion matrix
========================================
Ancestral states are listed by character and by node on the tree.  Node numbe

Char.\Node    25          28          24
character 1   1           1           1
character 2   2           2           2
character 3   3           3           3
character 4   1           2,3         1
character 5   0           0           0
character 6   0,1         0,1         0,1
character 7   0           0           0
character 8   1           2           2
character 9   0           1           1
character 10  0           0           0
character 11  0           0           0
character 12  0           0           0
character 13  0           0           0
character 14  1           1           1
character 15  1           1           1
character 16  0           0           0
character 17  0           0           0
character 18  0           0           0
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character 30  0           0           0
```

◆ Modified from Tree "Untitled Tree" from trees "Untitled Trees Block" of file 16c-TraceAllChars.nex [tre

Key characters in the classification system of Nooteboom (1985)

- Family Magnoliaceae
  - Subfamily Magnolioideae
    - Tribe Magnolieae
      - Genus *Magnolia*
        - Subgenus *Magnolia*
          - Section *Magnolia*
          - Section *Gwillimia*
          - Section *Lirianthe*
          - Section *Rytidospermum*
          - Section *Oyama*
          - Section *Theorhodon*
          - Section *Gynopodium*
          - Section *Maingola*
          - Section *Alcimandra*
        - Subgenus *Yulania*
          - Section *Yulania*
          - Section *Buergeria*
          - Section *Tulipastrum*
        - Subgenus *Talauma*
          - Section *Talauma*
          - Section *Blumiana*
          - Section *Aromadendron*
          - Section *Manglietiastrum*
      - Genus *Kmeria*
      - Genus *Manglietia*
      - Genus *Pachylarnax*
    - Tribe Michelieae
      - Genus *Elmerrillia*
      - Genus *Michelia*
  - Subfamily Liriodendroideae
    - Genus *Liriodendron*

Terminal flower

Axillary flower

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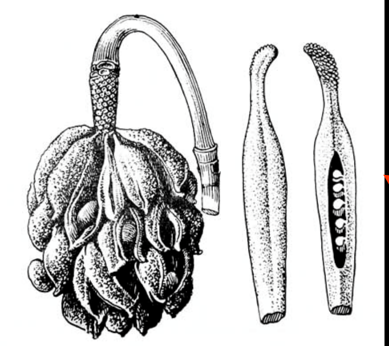
      - Genus *Elmerrillia*
      - Genus *Michelia*
  - Subfamily Liriodendroideae
    - Genus *Liriodendron*

Unisexual flower

Ovules 4 or more



Capsule like fruit

- Family Magnoliaceae
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      - Genus *Kmeria*
      - Genus *Manglietia*
      - Genus *Pachylarnax*
    - Tribe Michelieae
      - Genus *Elmerrillia*
      - Genus *Michelia*
  - Subfamily Liriodendroideae
    - Genus *Liriodendron*


Anthers introrse


Anthers latrorse

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          - Section *Lirianthe*
          - Section *Rytidospermum*
          - Section *Oyama*
          - Section *Theorhodon*
          - Section *Gynopodium*
          - Section *Maingola*
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      - Genus *Kmeria*
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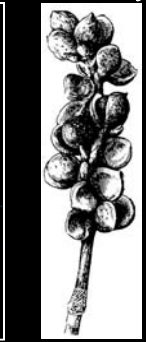
      - Genus *Pachylarnax*
    - Tribe Michelieae
      - Genus *Elmerrillia*
      - Genus *Michelia*
  - Subfamily Liriodendroideae
    - Genus *Liriodendron*

Anthers	Carpels; fruits
introrse 	free; ellipsoidal 
latrorse 	free; cylindrical 
introrse 	connate; ovoidal 

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Diverse morphology of magnolia seeds

Well described in Tiffney (1977)

CHALAZAL TYPES

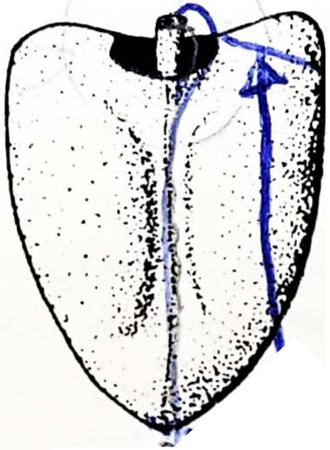
Moat and Stalk

Terminal Pore

Lateral Pore

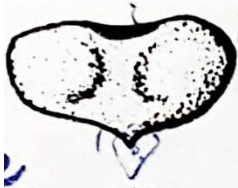
V-SHAPED MOAT

VENTRAL FACE

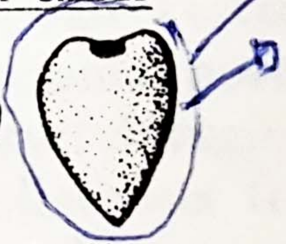


COLLAR

EXTREMES OF SHAPES

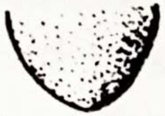


Bean-Like



Cordiforme

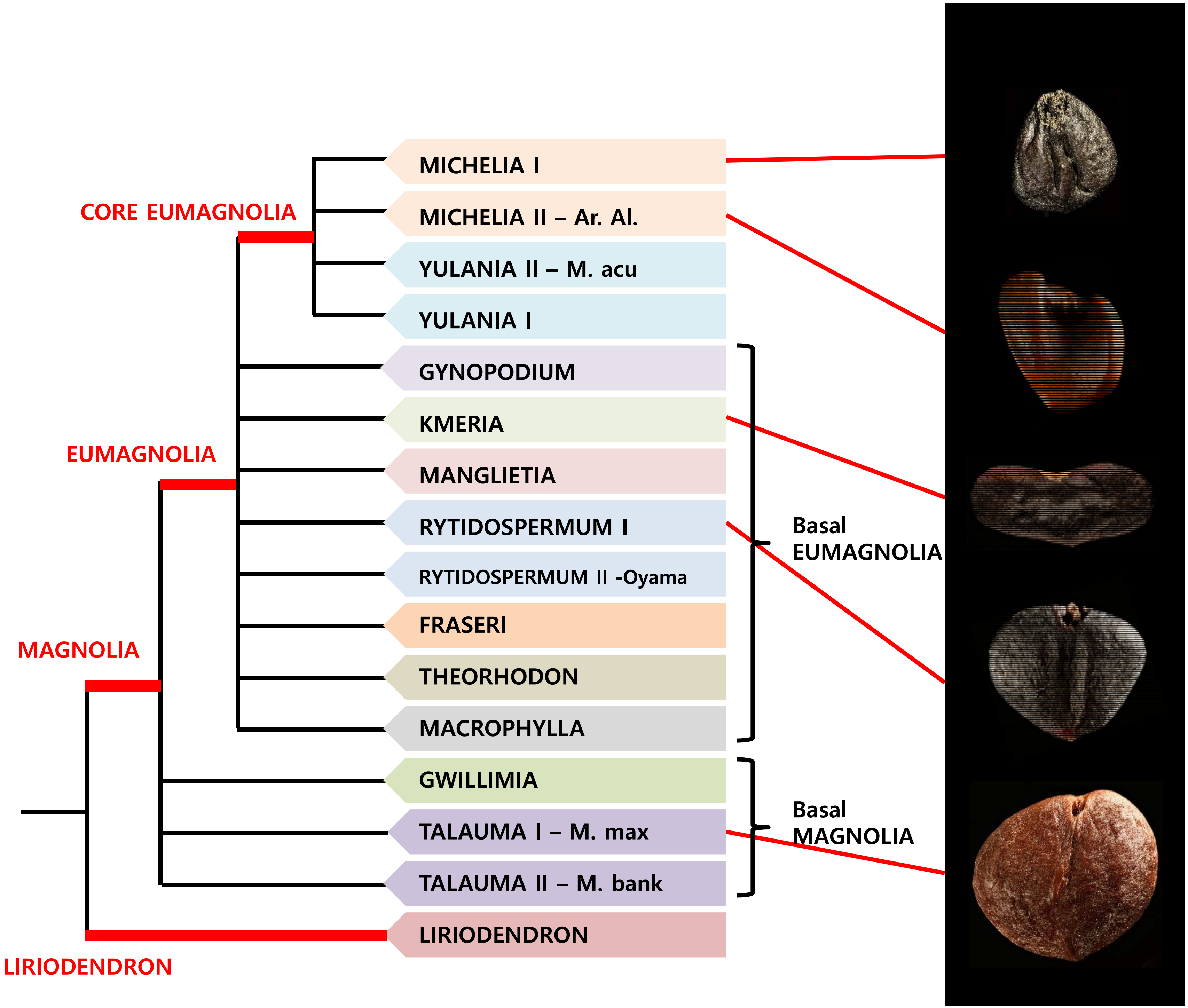
MICROPYLAR FORMS



Rounded

Beaked

Tiffney (1977)



CORE EUMAGNOLIA

EUMAGNOLIA

MAGNOLIA

LIRIODENDRON

MICHELIA I

MICHELIA II – Ar. Al.

YULANIA II – M. acu

YULANIA I

GYNOPODIUM

KMERIA

MANGLIETIA

RYTIDOSPERMUM I

RYTIDOSPERMUM II -Oyama

FRASERI

THEORHODON

MACROPHYLLA

GWILLIMIA

TALAUMA I – M. max

TALAUMA II – M. bank

LIRIODENDRON

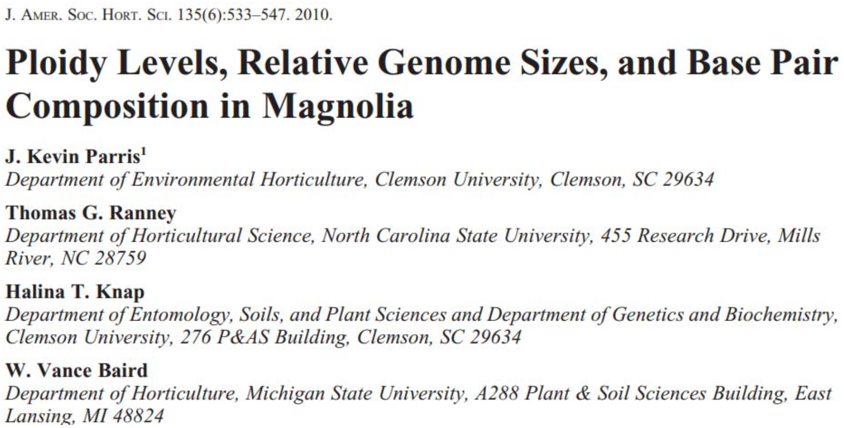
Basal EUMAGNOLIA

Basal MAGNOLIA

Further studies

1) Evolutionary character reconstruction
2) **Re-investigation** of genome size
3) Suggestion of a minimum set of genes for phylogenetic or barcoding studies

Genome size estimation



J. Amer. Soc. Hort. Sci. 135(6):533–547. 2010.

Ploidy Levels, Relative Genome Sizes, and Base Pair Composition in Magnolia

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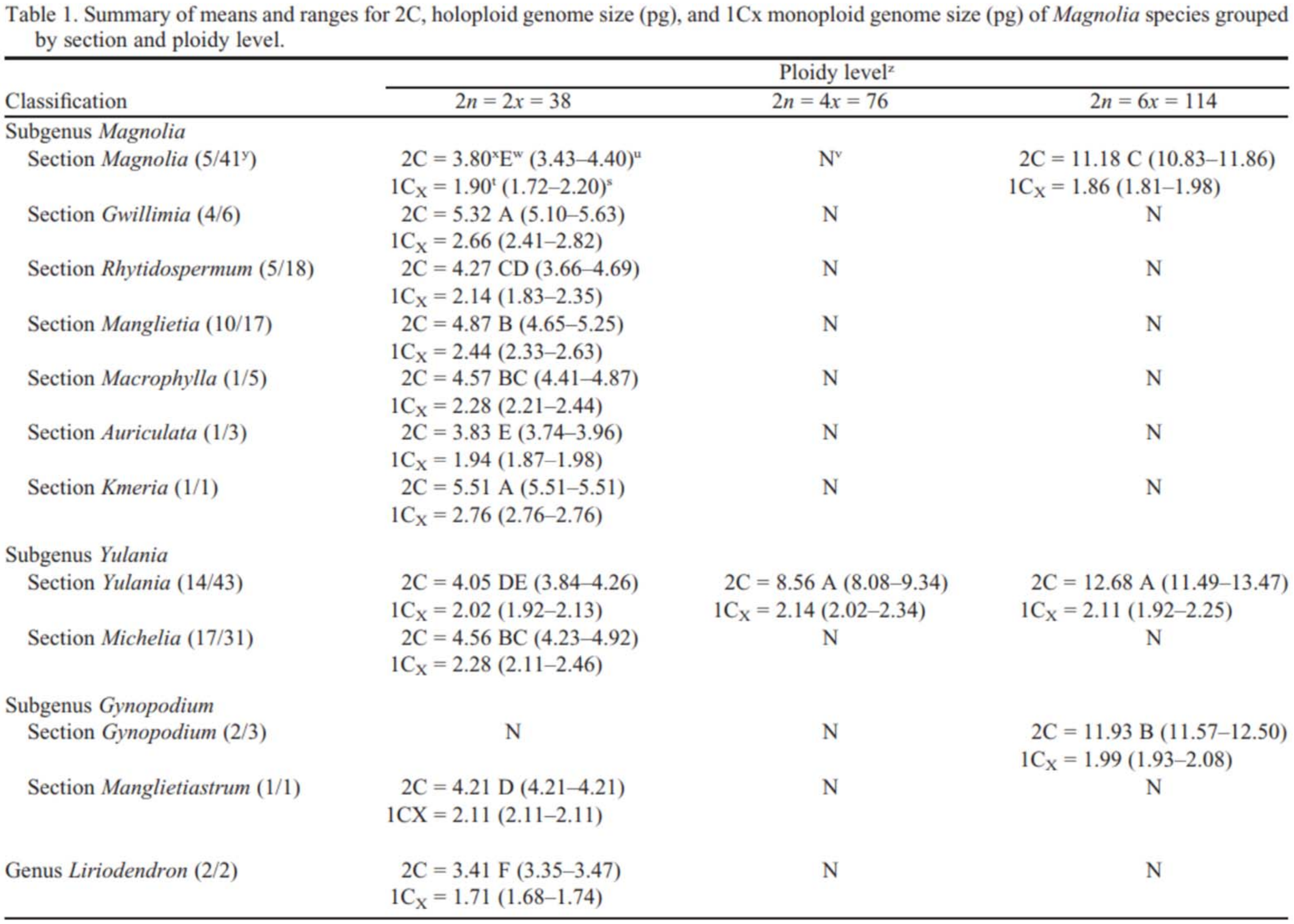
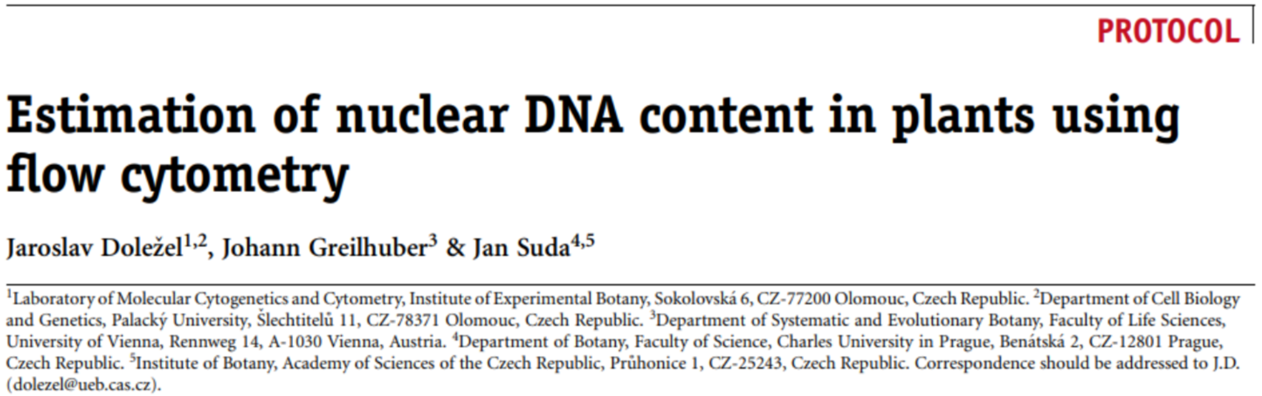


Table 1. Summary of means and ranges for 2C, holoploid genome size (pg), and 1Cx monoploid genome size (pg) of *Magnolia* species grouped by section and ploidy level.

	Ploidy level[z]		
Classification	$2n = 2x = 38$	$2n = 4x = 76$	$2n = 6x = 114$
Subgenus *Magnolia*			
Section *Magnolia* (5/41[y])	2C = 3.80[x]E[w] (3.43–4.40)[u] $1C_X$ = 1.90[t] (1.72–2.20)[s]	N[v]	2C = 11.18 C (10.83–11.86) $1C_X$ = 1.86 (1.81–1.98)
Section *Gwillimia* (4/6)	2C = 5.32 A (5.10–5.63) $1C_X$ = 2.66 (2.41–2.82)	N	N
Section *Rhytidospermum* (5/18)	2C = 4.27 CD (3.66–4.69) $1C_X$ = 2.14 (1.83–2.35)	N	N
Section *Manglietia* (10/17)	2C = 4.87 B (4.65–5.25) $1C_X$ = 2.44 (2.33–2.63)	N	N
Section *Macrophylla* (1/5)	2C = 4.57 BC (4.41–4.87) $1C_X$ = 2.28 (2.21–2.44)	N	N
Section *Auriculata* (1/3)	2C = 3.83 E (3.74–3.96) $1C_X$ = 1.94 (1.87–1.98)	N	N
Section *Kmeria* (1/1)	2C = 5.51 A (5.51–5.51) $1C_X$ = 2.76 (2.76–2.76)	N	N
Subgenus *Yulania*			
Section *Yulania* (14/43)	2C = 4.05 DE (3.84–4.26) $1C_X$ = 2.02 (1.92–2.13)	2C = 8.56 A (8.08–9.34) $1C_X$ = 2.14 (2.02–2.34)	2C = 12.68 A (11.49–13.47) $1C_X$ = 2.11 (1.92–2.25)
Section *Michelia* (17/31)	2C = 4.56 BC (4.23–4.92) $1C_X$ = 2.28 (2.11–2.46)	N	N
Subgenus *Gynopodium*			
Section *Gynopodium* (2/3)	N	N	2C = 11.93 B (11.57–12.50) $1C_X$ = 1.99 (1.93–2.08)
Section *Manglietiastrum* (1/1)	2C = 4.21 D (4.21–4.21) 1CX = 2.11 (2.11–2.11)	N	N
Genus *Liriodendron* (2/2)	2C = 3.41 F (3.35–3.47) $1C_X$ = 1.71 (1.68–1.74)	N	N


PROTOCOL

Estimation of nuclear DNA content in plants using flow cytometry

Jaroslav Doležel[1,2], Johann Greilhuber[3] & Jan Suda[4,5]

[1]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic. [2]Department of Cell Biology and Genetics, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic. [3]Department of Systematic and Evolutionary Botany, Faculty of Life Sciences, University of Vienna, Rennweg 14, A-1030 Vienna, Austria. [4]Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-12801 Prague, Czech Republic. [5]Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice 1, CZ-25243, Czech Republic. Correspondence should be addressed to J.D. (dolezel@ueb.cas.cz).

- Using a proper standard plant is a key to estimate precise genome size in flow cytometry
- Parris et al. (2010) used just one internal standard (*Psium sativum;* 2C=0.09)
- Genome size of *M. kobus:*
  1.4 Gbp in this study
  2.0 Gbp in Parris et al. (2010)
- Recognition of genome size is very important for the selection of taxon for future intensive genome sequencing

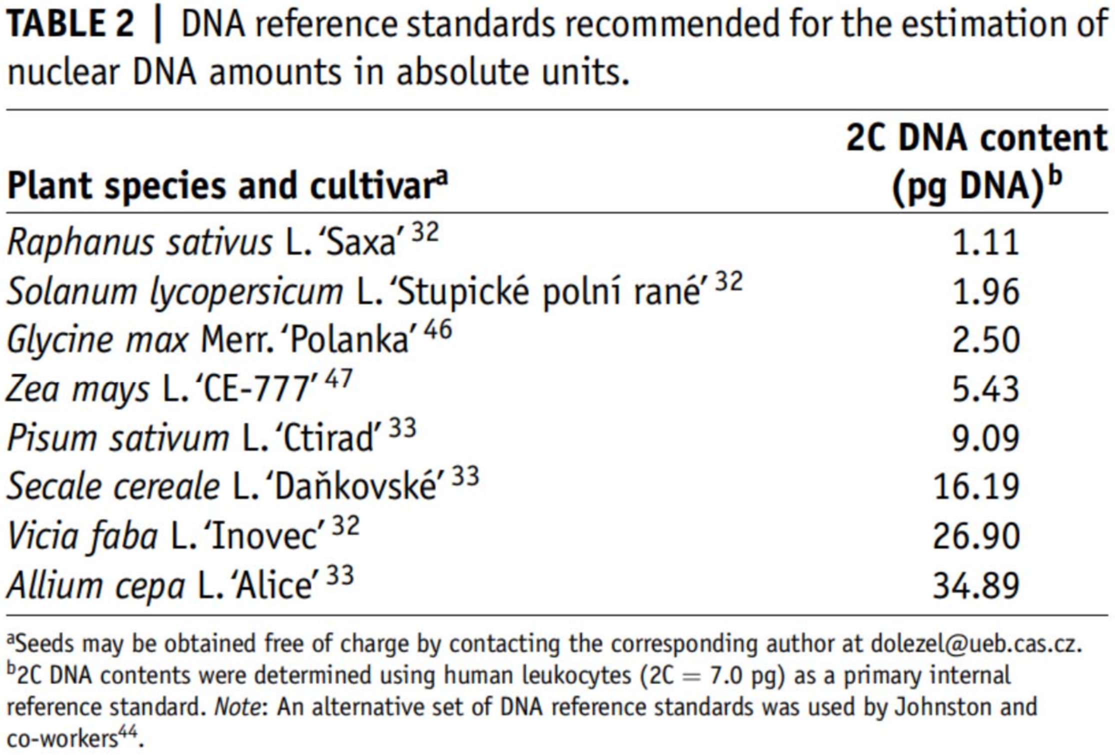


TABLE 2 | DNA reference standards recommended for the estimation of nuclear DNA amounts in absolute units.

Plant species and cultivar[a]	2C DNA content (pg DNA)[b]
Raphanus sativus L. 'Saxa'[32]	1.11
Solanum lycopersicum L. 'Stupické polní rané'[32]	1.96
Glycine max Merr. 'Polanka'[46]	2.50
Zea mays L. 'CE-777'[47]	5.43
Pisum sativum L. 'Ctirad'[33]	9.09
Secale cereale L. 'Daňkovské'[33]	16.19
Vicia faba L. 'Inovec'[32]	26.90
Allium cepa L. 'Alice'[33]	34.89

[a]Seeds may be obtained free of charge by contacting the corresponding author at dolezel@ueb.cas.cz.
[b]2C DNA contents were determined using human leukocytes (2C = 7.0 pg) as a primary internal reference standard. *Note*: An alternative set of DNA reference standards was used by Johnston and co-workers[44].

Further studies

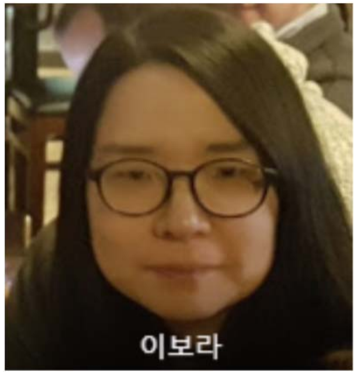
1) Evolutionary character reconstruction

2) Re-investigation of genome size

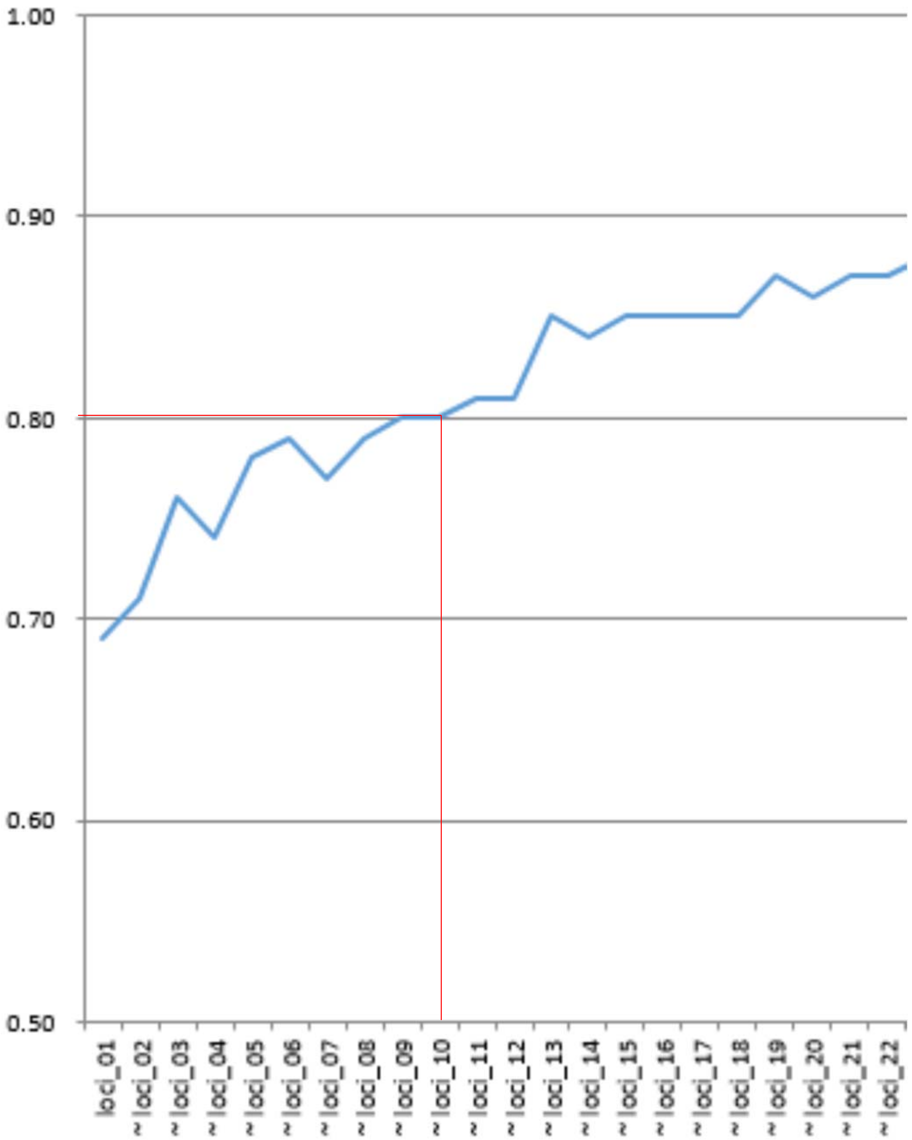
3) **Suggestion of a minimum set of genes for phylogenetic or barcoding studies**

Suggestion of a minimum set of genes for phylogenetic or barcoding studies

- In case of *Carex* Hyb-Seq study, we successfully extracted 10 best nuclear regions showing **80% of clade-resolution** compare to total gene (340 genes) analysis.
- We may suggest a set of the best regions for the studies of additional taxa.



Bora Lee, Ph.D. student, Sungshin Univ.



Thanks to Suhyun Park, Ph.D. student
Dr. Jongsun Park, CEO, InfoBoss Co.

Many thanks to

Dr. Antonia Vazquez

Dr. Weerakit Harnpariphan

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