Supplementary Material

Continued Adaptation of C_4 Photosynthesis After an Initial Burst of Changes in the Andropogoneae Grasses

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This Supplementary Material contains seven figures and six tables. Note that tables S1, S2, S3 and S4 are in separate .xlsx files. An additional 31 data files, including scripts and alignments, can be found in the compressed folder "Bianconi_et_al_Syst_Biol_2019.zip" (see the accompanying README file for details).

Figure S1. Effect of different filtering stringencies and reference species on the set of nuclear genes retained for phylogenetic analyses. (a,f) Number of genes retained; (b,g) taxon occupancy (i.e. proportion of species represented); (c,h) proportion of the alignment that is complete (completeness); (d,i) number of parsimony informative sites; (e,j) proportion of the branches with bootstrap support values ≥ 50%. Analyses were performed on the reduced subset of 37 species (a-e) and the complete set of 66 species (f-j), and using either *Setaria italica* or *Sorghum bicolor* as the reference. Each analysis was repeated with a different level of trimming (no trim = no trimming; 50% trim = sites covered by less than 50% of sequences were trimmed; 70% trim = sites covered by less than 70% of sequences were trimmed). Finally, analyses considered either all gene trees ('all') or only gene trees with ≥ 50% of branches with bootstrap support ≥ 50%. Dashed rectangles highlight the datasets used to generate the multigene coalescent species tree in Fig. 1 (in black), and Fig. S3 (in grey).

Figure S2. Bayesian phylogram inferred from non-coding sequences of plastomes. Black circles on nodes indicate Bayesian posterior probability ≥ 0.95.

Figure S3. Multigene coalescent species trees inferred from 3,127 nuclear genes using *Sorghum bicolor* as a reference. Pie charts on nodes indicate the proportion of quartet trees that support the main topology (in blue), the first alternative (in red), and the second alternative (in orange). Local

posterior probabilities are indicated near nodes. Branch lengths are given in coalescent units. The major groups of Panicoideae are indicated with shades, as in Fig. 1.

Figure S4. Bayesian phylograms inferred from individual nuclear markers. (a) *apo1*, (b) *arodeh*, (c) *dwarf8*, (d) *floricaula*, (e) *kn1*, (f) *phyB*, (g) *rep1* and (h) *waxy*. Bayesian posterior probability values ≥ 70 are indicated near nodes. The main groups of Panicoideae are delimited with shades.

Figure S5. Time-calibrated phylogenetic tree based on coding sequences of plastomes. Bars on nodes indicate the 95% HPD intervals for the ages. The main groups of Panicoideae are delimited with shades.

Figure S6. Bayesian phylograms inferred for C_4-related genes. These phylograms were inferred based on 3rd positions of codons of several core C_4 genes: (a) *nadpmdh-1P1*, (b) *nadpme-1P4*, (c) *pck-1P1*, (d) *ppc-1P3*, (e) *ppdk-1P2*; paralogs of core C_4 genes: (f) *nadpme-1P1*, (g) *nadpme-1P2*, (h) *nadpme-1P3*, (i) *ppc-1P4*, (j) *ppc-1P5*, (k) *ppc-1P7*, (l) *ppdk-1P1*; and other nuclear phylogenetic markers: (m) *arodeh*, (n) *dwarf8*, (o) *kn1*, (p) *phyB* and (q) *waxy*. Branches in red lead to C_4 species. Bayesian posterior probability values ≥ 70 are indicated near nodes. The main groups of Panicoideae are delimited with shades.

Figure S7. Phylograms with branch lengths based on amino acid sequences. The tree topologies were fixed to those obtained using 3rd positions of codons used for branch model tests. Results are shown for core C_4 genes: (a) *nadpmdh-1P1*, (b) *nadpme-1P4*, (c) *pck-1P1*, (d) *ppc-1P3*, (e) *ppdk-1P2*; paralogs of core C_4 genes: (f) *nadpme-1P1*, (g) *nadpme-1P2*, (h) *nadpme-1P3*, (i) *ppc-1P4*, (j) *ppc-1P5*, (k) *ppc-1P7*, (l) *ppdk-1P1*; and other nuclear genes: (m) *arodeh*, (n) *dwarf8*, (o) *kn1*, (p) *phyB* and (q) *waxy*. Branches in red lead to C_4 species. Scale bars = 0.01 expected amino acid substitutions per site.

Table S1. Genomic data information.

Table S2. Read mapping success and nuclear genome coverage in datasets assembled using *Setaria italica* or *Sorghum bicolor* sequences as references.

Table S3. NCBI accession numbers of sequences of nuclear markers assembled in this study.

Table S4. Photosynthetic types and leaf anatomy data of the 66 grass species used in this study.

Table S5. Summary of multigene coalescent species trees inferred from nuclear data under different filtering stringencies.

Table S6. Summary of branch model comparisons performed using the species tree topology.

Fig. S1

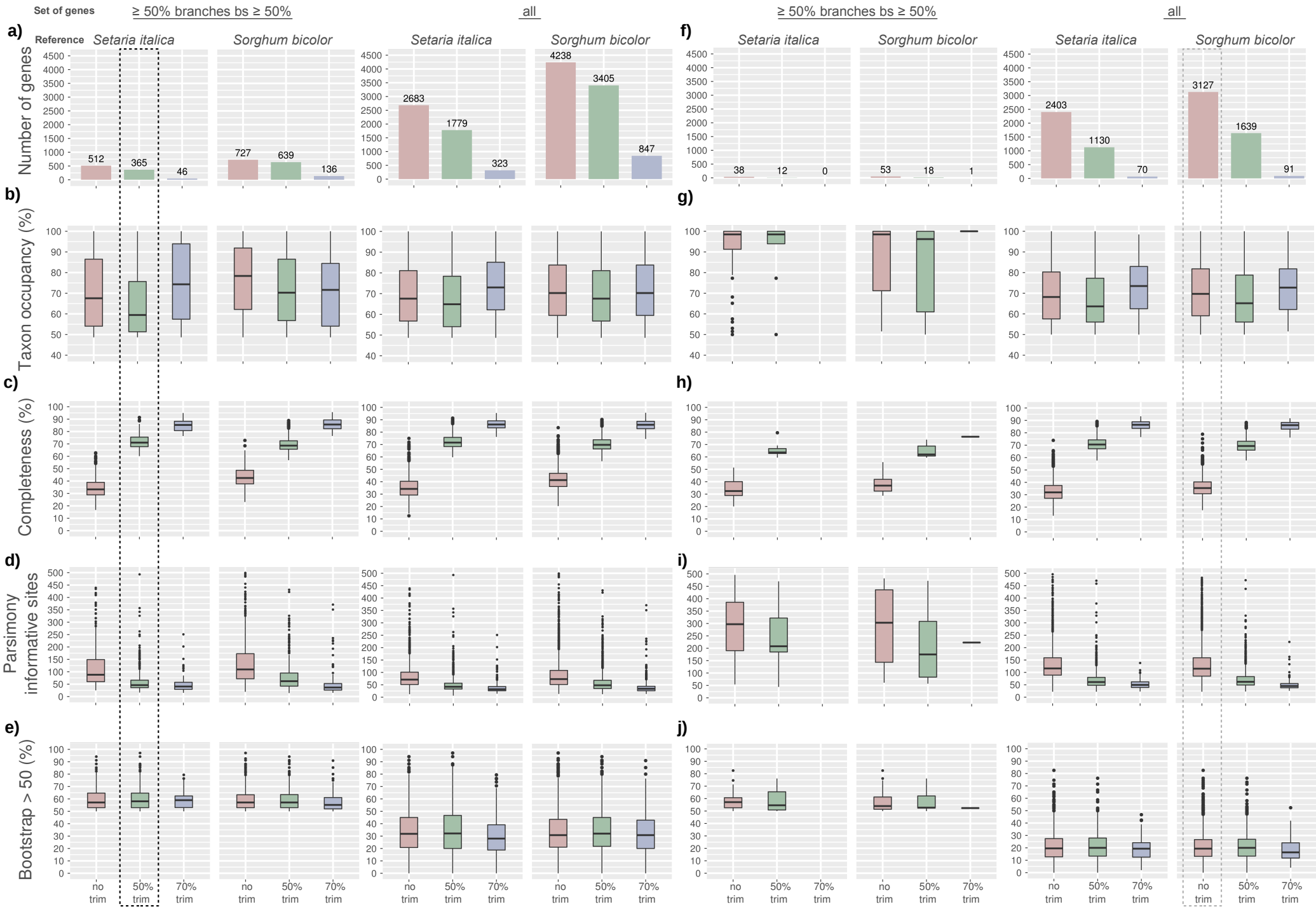

Set of genes
≥ 50% branches bs ≥ 50%
all
≥ 50% branches bs ≥ 50%
all
Reference
Setaria italica
Sorghum bicolor
Setaria italica
Sorghum bicolor
Setaria italica
Sorghum bicolor
Setaria italica
Sorghum bicolor
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
Number of genes
Taxon occupancy (%)
Completeness (%)
Parsimony informative sites
Bootstrap > 50 (%)
512
365
46
727
639
136
2683
1779
323
4238
3405
847
38
12
0
53
18
1
2403
1130
70
3127
1639
91
no trim
50% trim
70% trim

Fig. S2

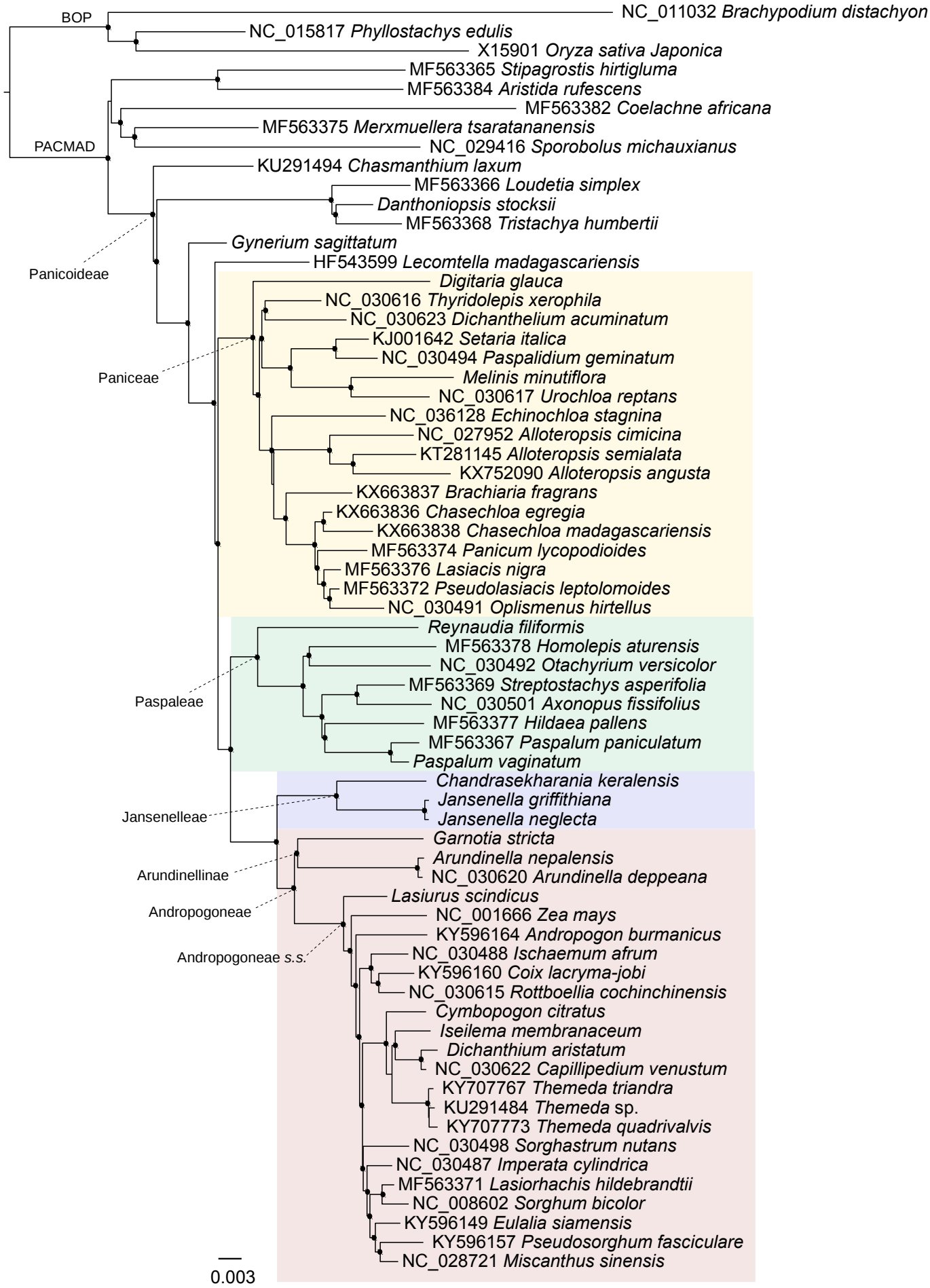

BOP
PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
NC_011032 Brachypodium distachyon
NC_015817 Phyllostachys edulis
X15901 Oryza sativa Japonica
MF563365 Stipagrostis hirtigluma
MF563384 Aristida rufescens
MF563382 Coelachne africana
MF563375 Merxmuellera tsaratananensis
NC_029416 Sporobolus michauxianus
KU291494 Chasmanthium laxum
MF563366 Loudetia simplex
Danthoniopsis stocksii
MF563368 Tristachya humbertii
Gynerium sagittatum
HF543599 Lecomtella madagascariensis
Digitaria glauca
NC_030616 Thyridolepis xerophila
NC_030623 Dichanthelium acuminatum
KJ001642 Setaria italica
NC_030494 Paspalidium geminatum
Melinis minutiflora
NC_030617 Urochloa reptans
NC_036128 Echinochloa stagnina
NC_027952 Alloteropsis cimicina
KT281145 Alloteropsis semialata
KX752090 Alloteropsis angusta
KX663837 Brachiaria fragrans
KX663836 Chasechloa egregia
KX663838 Chasechloa madagascariensis
MF563374 Panicum lycopodioides
MF563376 Lasiacis nigra
MF563372 Pseudolasiacis leptolomoides
NC_030491 Oplismenus hirtellus
Reynaudia filiformis
MF563378 Homolepis aturensis
NC_030492 Otachyrium versicolor
MF563369 Streptostachys asperifolia
NC_030501 Axonopus fissifolius
MF563377 Hildaea pallens
MF563367 Paspalum paniculatum
Paspalum vaginatum
Chandrasekharania keralensis
Jansenella griffithiana
Jansenella neglecta
Garnotia stricta
Arundinella nepalensis
NC_030620 Arundinella deppeana
Lasiurus scindicus
NC_001666 Zea mays
KY596164 Andropogon burmanicus
NC_030488 Ischaemum afrum
KY596160 Coix lacryma-jobi
NC_030615 Rottboellia cochinchinensis
Cymbopogon citratus
Iseilema membranaceum
Dichanthium aristatum
NC_030622 Capillipedium venustum
KY707767 Themeda triandra
KU291484 Themeda sp.
KY707773 Themeda quadrivalvis
NC_030498 Sorghastrum nutans
NC_030487 Imperata cylindrica
MF563371 Lasiorhachis hildebrandtii
NC_008602 Sorghum bicolor
KY596149 Eulalia siamensis
KY596157 Pseudosorghum fasciculare
NC_028721 Miscanthus sinensis
0.003

Fig. S3

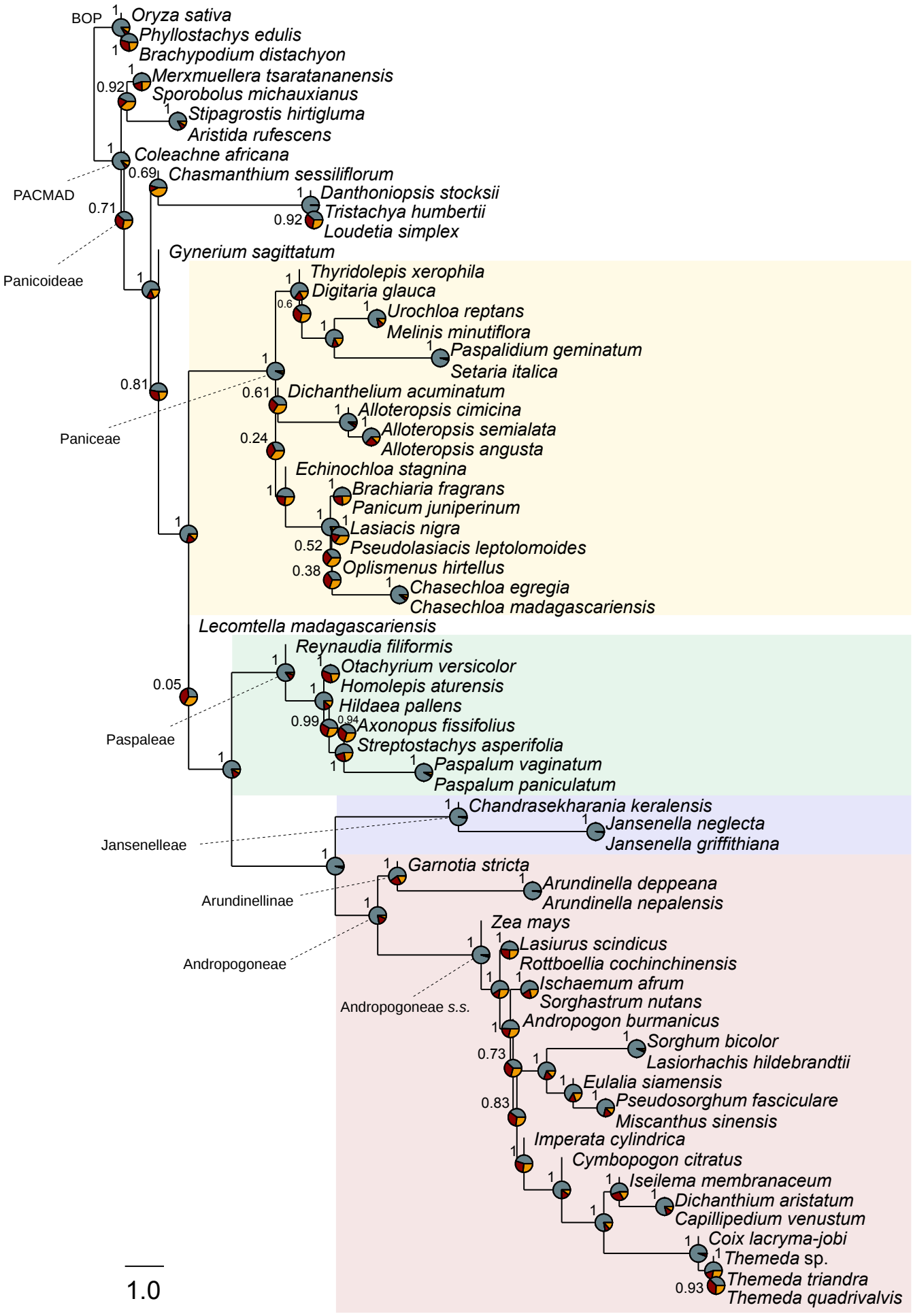
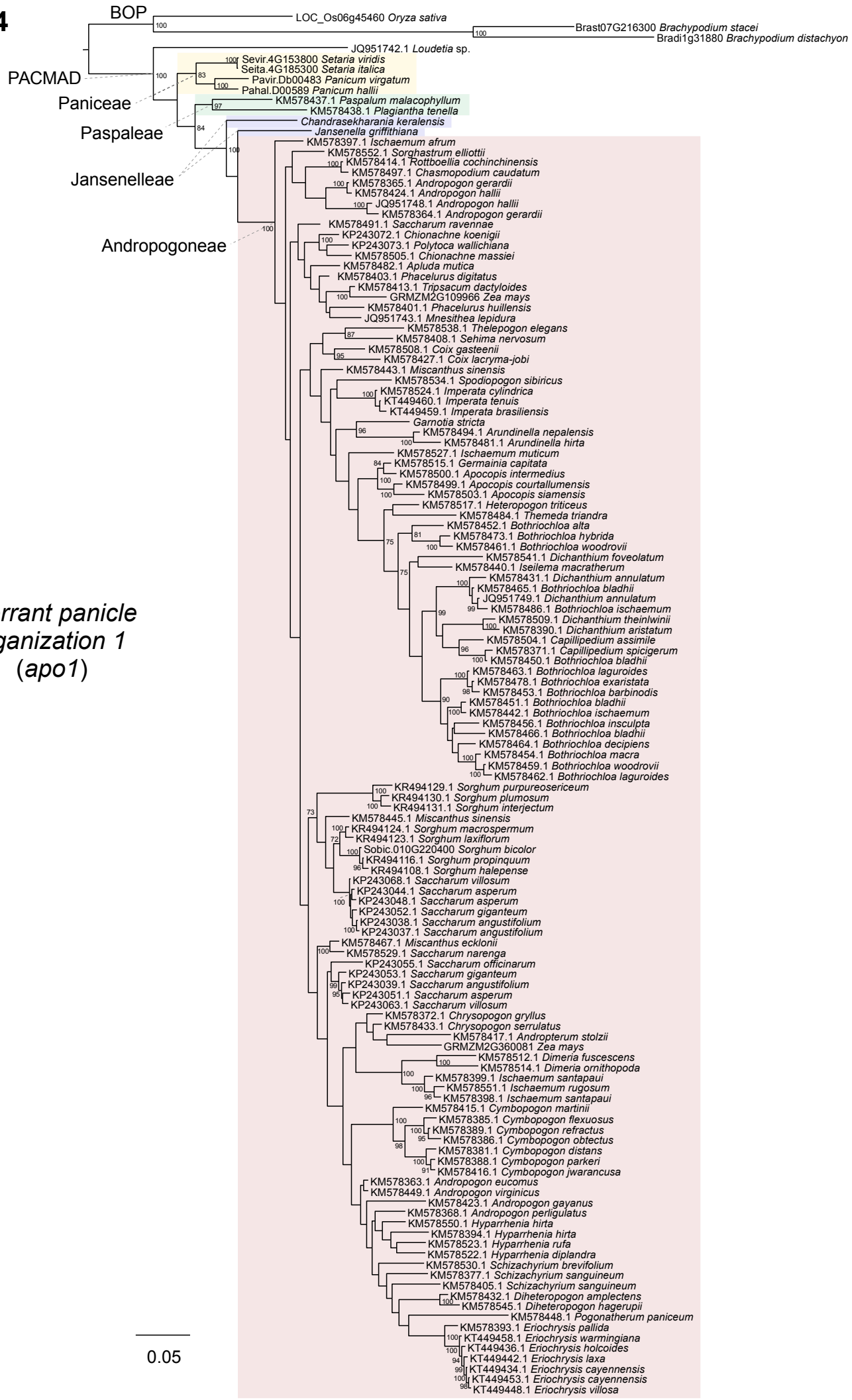

BOP
PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
Oryza sativa
Phyllostachys edulis
Brachypodium distachyon
Merxmuellera tsaratananensis
Sporobolus michauxianus
Stipagrostis hirtigluma
Aristida rufescens
Coleachne africana
Chasmanthium sessiliflorum
Danthoniopsis stocksii
Tristachya humbertii
Loudetia simplex
Gynerium sagittatum
Thyridolepis xerophila
Digitaria glauca
Urochloa reptans
Melinis minutiflora
Paspalidium geminatum
Setaria italica
Dichanthelium acuminatum
Alloteropsis cimicina
Alloteropsis semialata
Alloteropsis angusta
Echinochloa stagnina
Brachiaria fragrans
Panicum juniperinum
Lasiacis nigra
Pseudolasiacis leptolomoides
Oplismenus hirtellus
Chasechloa egregia
Chasechloa madagascariensis
Lecomtella madagascariensis
Reynaudia filiformis
Otachyrium versicolor
Homolepis aturensis
Hildaea pallens
Axonopus fissifolius
Streptostachys asperifolia
Paspalum vaginatum
Paspalum paniculatum
Chandrasekharania keralensis
Jansenella neglecta
Jansenella griffithiana
Garnotia stricta
Arundinella deppeana
Arundinella nepalensis
Zea mays
Lasiurus scindicus
Rottboellia cochinchinensis
Ischaemum afrum
Sorghastrum nutans
Andropogon burmanicus
Sorghum bicolor
Lasiorhachis hildebrandtii
Eulalia siamensis
Pseudosorghum fasciculare
Miscanthus sinensis
Imperata cylindrica
Cymbopogon citratus
Iseilema membranaceum
Dichanthium aristatum
Capillipedium venustum
Coix lacryma-jobi
Themeda sp.
Themeda triandra
Themeda quadrivalvis
1.0

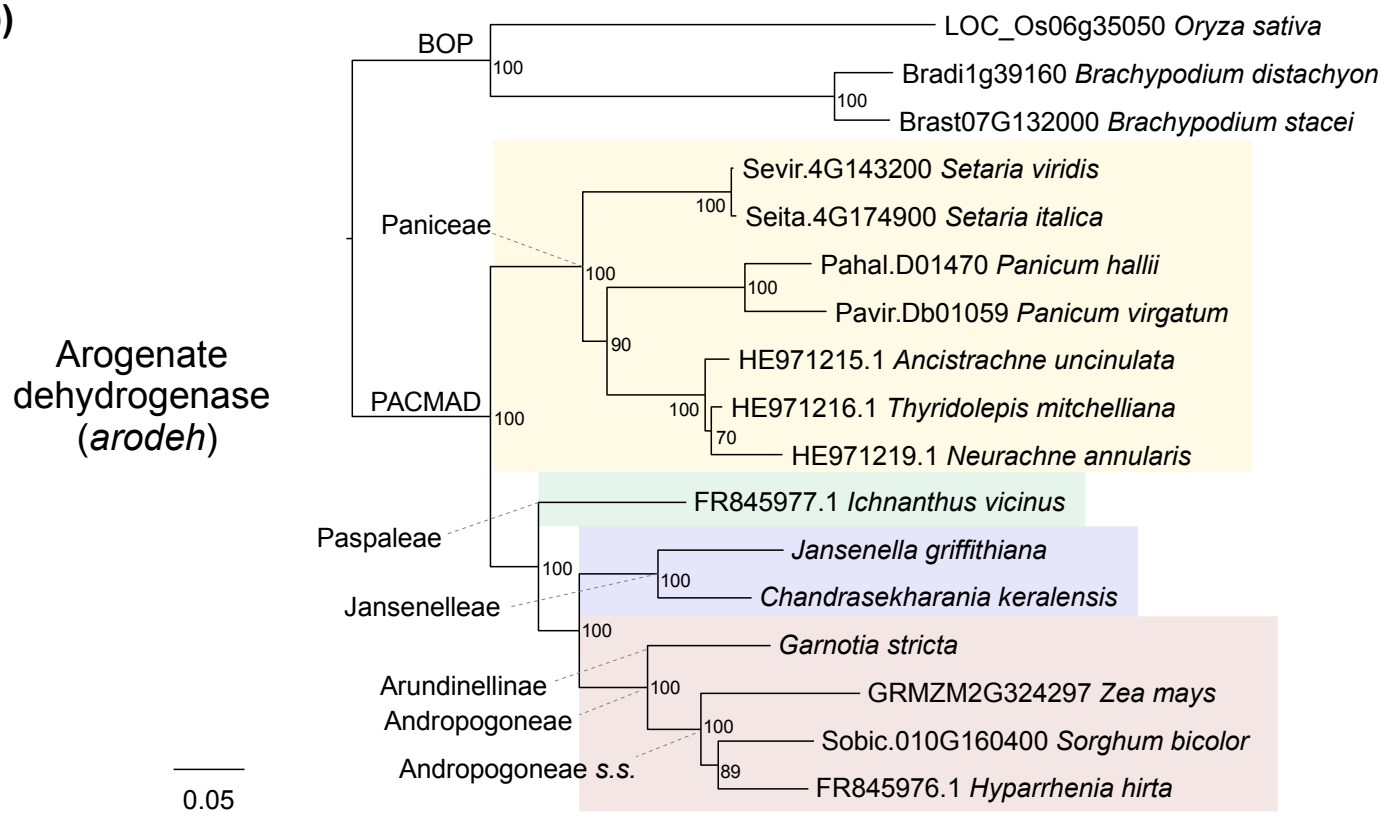
Fig. S4

a)

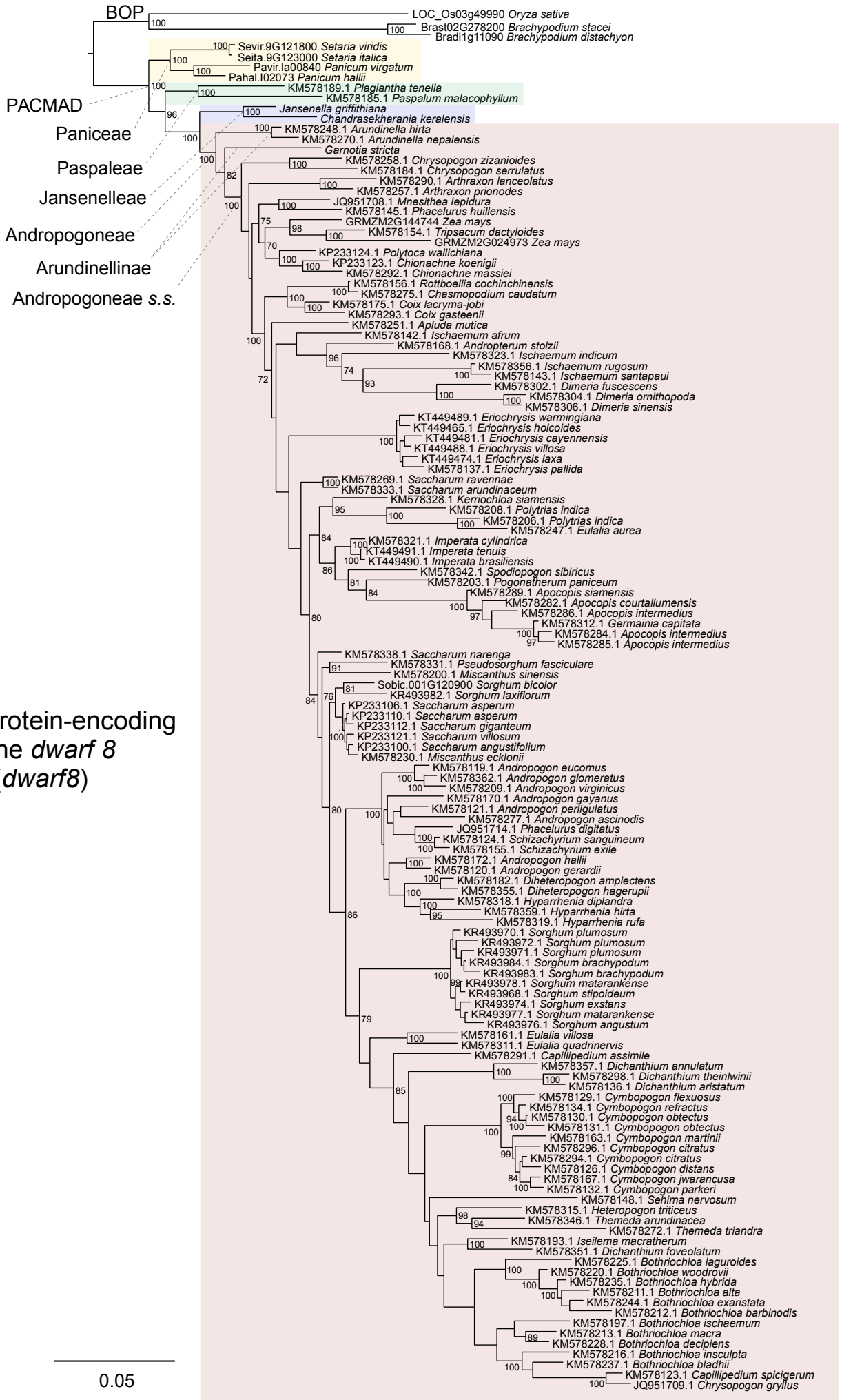


aberrant panicle organization 1 (*apo1*)

b)

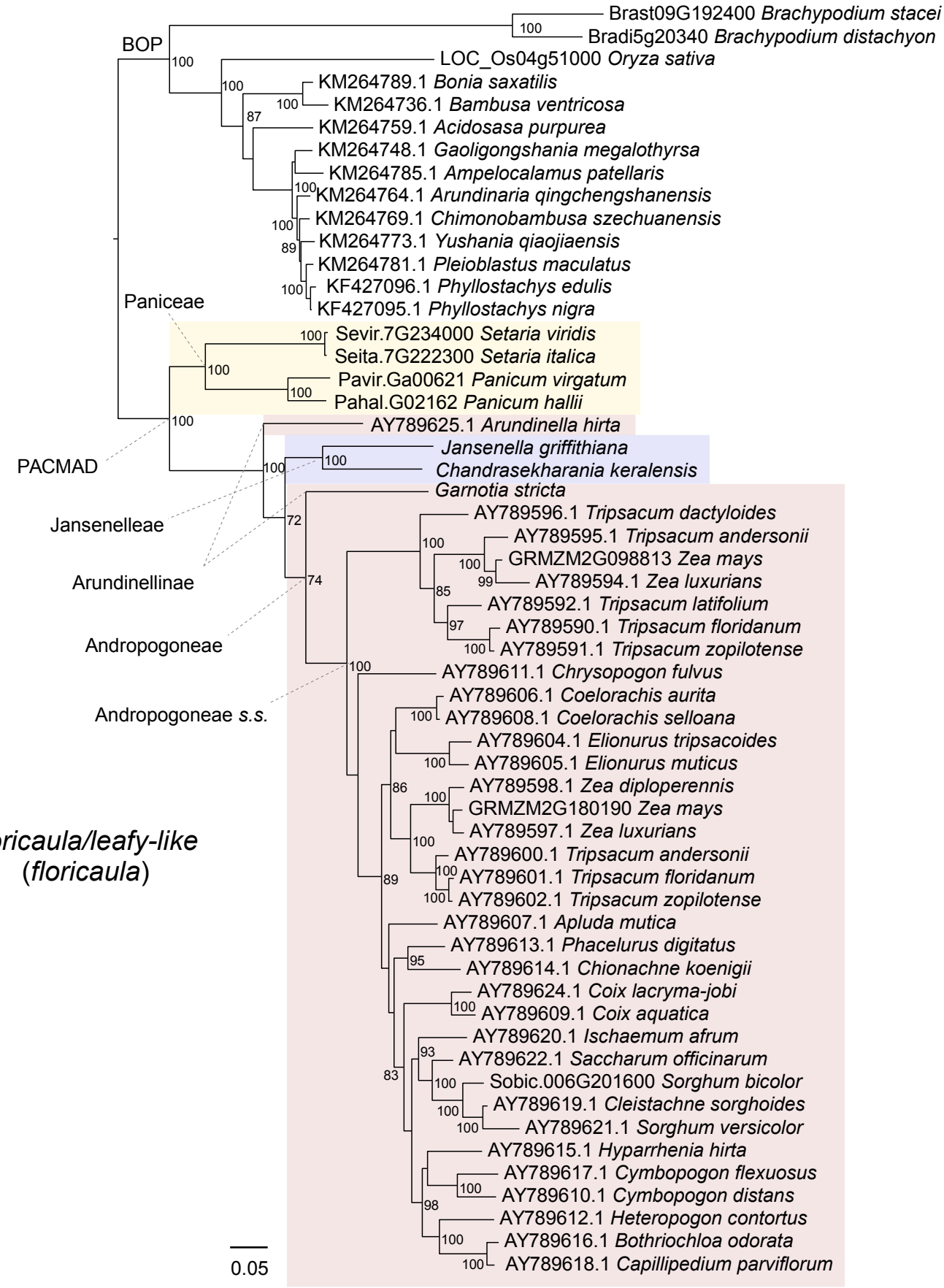

Arogenate dehydrogenase (*arodeh*)
BOP
PACMAD
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae *s.s.*
LOC_Os06g35050 *Oryza sativa*
Bradi1g39160 *Brachypodium distachyon*
Brast07G132000 *Brachypodium stacei*
Sevir.4G143200 *Setaria viridis*
Seita.4G174900 *Setaria italica*
Pahal.D01470 *Panicum hallii*
Pavir.Db01059 *Panicum virgatum*
HE971215.1 *Ancistrachne uncinulata*
HE971216.1 *Thyridolepis mitchelliana*
HE971219.1 *Neurachne annularis*
FR845977.1 *Ichnanthus vicinus*
Jansenella griffithiana
Chandrasekharania keralensis
Garnotia stricta
GRMZM2G324297 *Zea mays*
Sobic.010G160400 *Sorghum bicolor*
FR845976.1 *Hyparrhenia hirta*
100
100
100
100
100
90
100
100
70
100
100
100
100
89
0.05

c)



DELLA protein-encoding gene *dwarf 8* (*dwarf8*)

d)


Brast09G192400 Brachypodium stacei
Bradi5g20340 Brachypodium distachyon
BOP
LOC_Os04g51000 Oryza sativa
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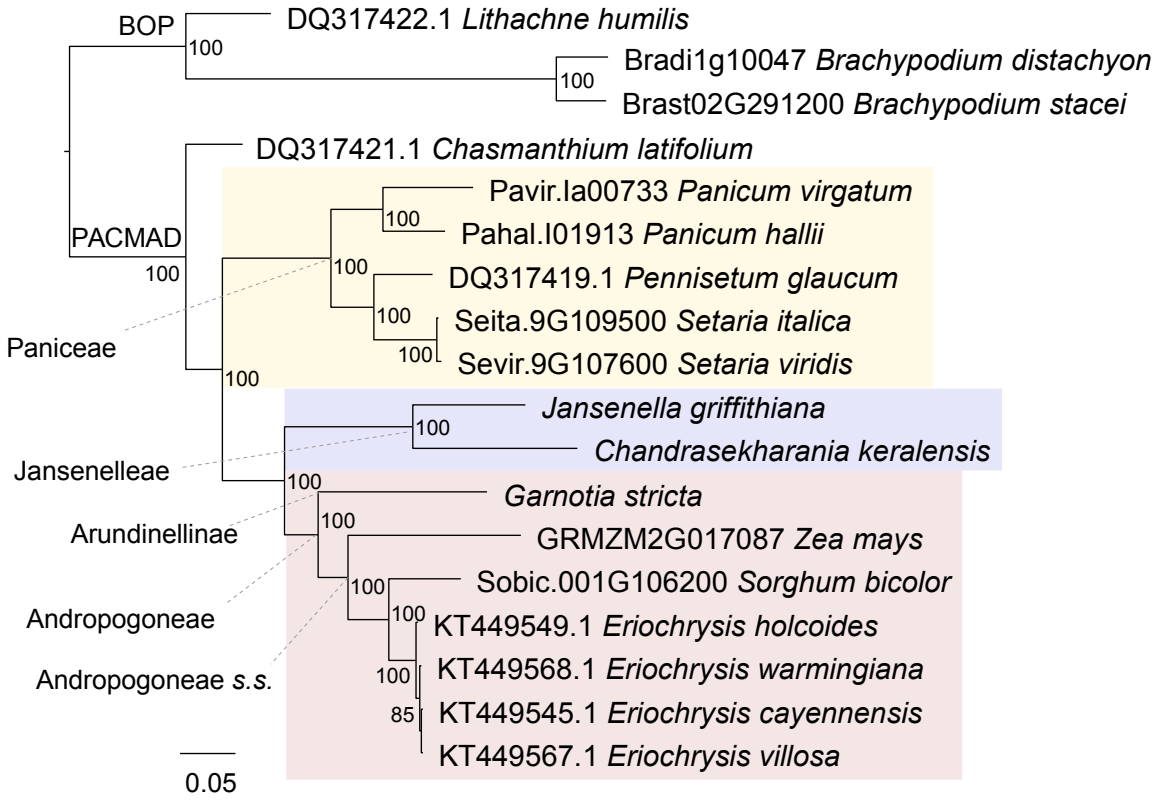
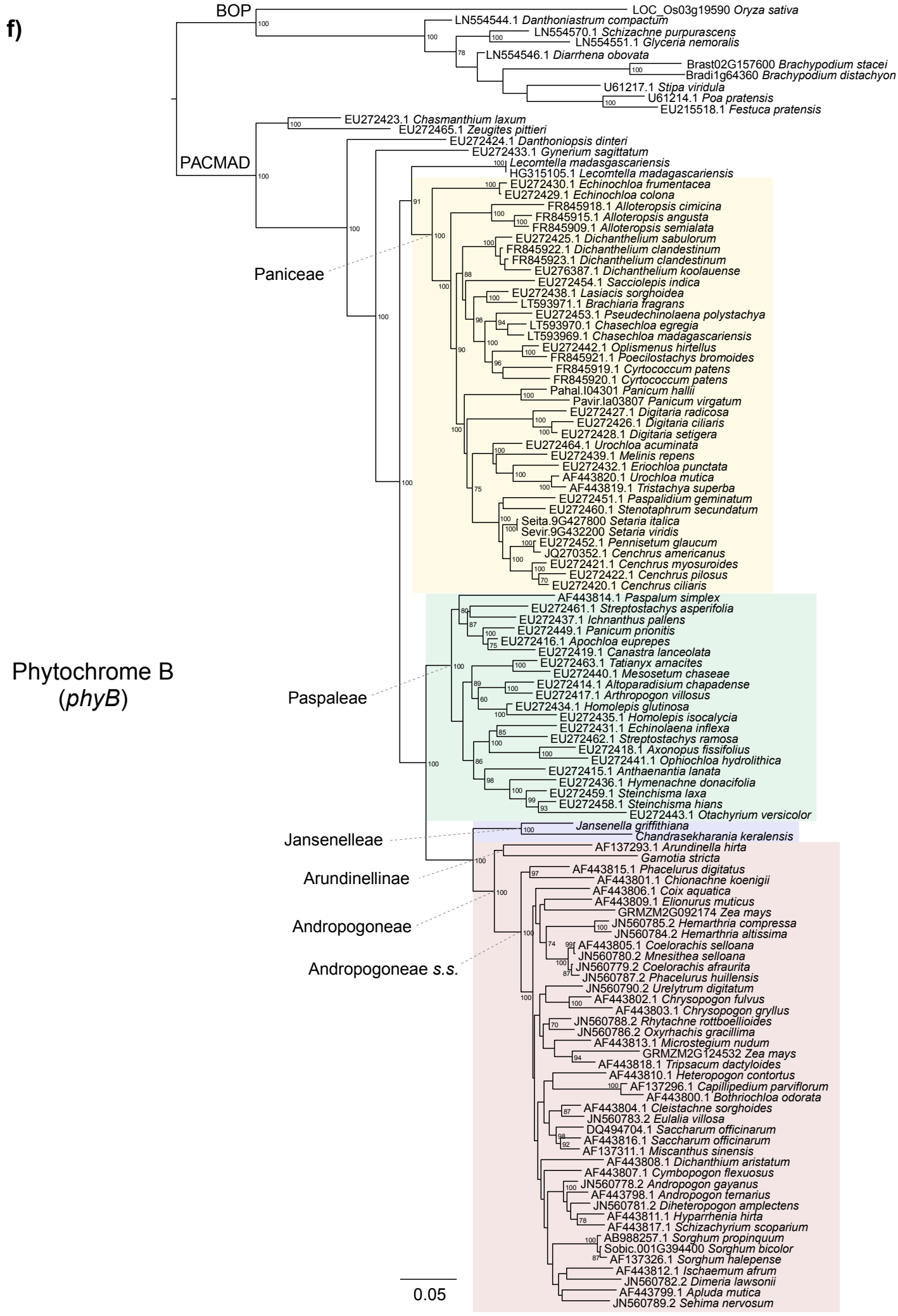
KM264736.1 Bambusa ventricosa
KM264759.1 Acidosasa purpurea
KM264748.1 Gaoligongshania megalothyrsa
KM264785.1 Ampelocalamus patellaris
KM264764.1 Arundinaria qingchengshanensis
KM264769.1 Chimonobambusa szechuanensis
KM264773.1 Yushania qiaojiaensis
KM264781.1 Pleioblastus maculatus
KF427096.1 Phyllostachys edulis
KF427095.1 Phyllostachys nigra
Paniceae
Sevir.7G234000 Setaria viridis
Seita.7G222300 Setaria italica
Pavir.Ga00621 Panicum virgatum
Pahal.G02162 Panicum hallii
AY789625.1 Arundinella hirta
PACMAD
Jansenella griffithiana
Chandrasekharania keralensis
Garnotia stricta
Jansenelleae
AY789596.1 Tripsacum dactyloides
AY789595.1 Tripsacum andersonii
GRMZM2G098813 Zea mays
AY789594.1 Zea luxurians
AY789592.1 Tripsacum latifolium
AY789590.1 Tripsacum floridanum
AY789591.1 Tripsacum zopilotense
Arundinellinae
Andropogoneae
AY789611.1 Chrysopogon fulvus
AY789606.1 Coelorachis aurita
AY789608.1 Coelorachis selloana
AY789604.1 Elionurus tripsacoides
AY789605.1 Elionurus muticus
AY789598.1 Zea diploperennis
GRMZM2G180190 Zea mays
AY789597.1 Zea luxurians
AY789600.1 Tripsacum andersonii
AY789601.1 Tripsacum floridanum
AY789602.1 Tripsacum zopilotense
Andropogoneae s.s.
AY789607.1 Apluda mutica
AY789613.1 Phacelurus digitatus
AY789614.1 Chionachne koenigii
AY789624.1 Coix lacryma-jobi
AY789609.1 Coix aquatica
AY789620.1 Ischaemum afrum
AY789622.1 Saccharum officinarum
Sobic.006G201600 Sorghum bicolor
AY789619.1 Cleistachne sorghoides
AY789621.1 Sorghum versicolor
AY789615.1 Hyparrhenia hirta
AY789617.1 Cymbopogon flexuosus
AY789610.1 Cymbopogon distans
AY789612.1 Heteropogon contortus
AY789616.1 Bothriochloa odorata
AY789618.1 Capillipedium parviflorum
floricaula/leafy-like
(floricaula)
0.05

e)

knotted 1
(*kn1*)




f)
Phytochrome B
(phyB)
BOP
PACMAD
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
LOC_Os03g19590 Oryza sativa
LN554544.1 Danthoniastrum compactum
LN554570.1 Schizachne purpurascens
LN554551.1 Glyceria nemoralis
LN554546.1 Diarrhena obovata
Brast02G157600 Brachypodium stacei
Bradi1g64360 Brachypodium distachyon
U61217.1 Stipa viridula
U61214.1 Poa pratensis
EU215518.1 Festuca pratensis
EU272423.1 Chasmanthium laxum
EU272465.1 Zeugites pittieri
EU272424.1 Danthoniopsis dinteri
EU272433.1 Gynerium sagittatum
Lecomtella madasgascariensis
HG315105.1 Lecomtella madagascariensis
EU272430.1 Echinochloa frumentacea
EU272429.1 Echinochloa colona
FR845918.1 Alloteropsis cimicina
FR845915.1 Alloteropsis angusta
FR845909.1 Alloteropsis semialata
EU272425.1 Dichanthelium sabulorum
FR845922.1 Dichanthelium clandestinum
FR845923.1 Dichanthelium clandestinum
EU276387.1 Dichanthelium koolauense
EU272454.1 Sacciolepis indica
EU272438.1 Lasiacis sorghoidea
LT593971.1 Brachiaria fragrans
EU272453.1 Pseudechinolaena polystachya
LT593970.1 Chasechloa egregia
LT593969.1 Chasechloa madagascariensis
EU272442.1 Oplismenus hirtellus
FR845921.1 Poecilostachys bromoides
FR845919.1 Cyrtococcum patens
FR845920.1 Cyrtococcum patens
Pahal.I04301 Panicum hallii
Pavir.Ia03807 Panicum virgatum
EU272427.1 Digitaria radicosa
EU272426.1 Digitaria ciliaris
EU272428.1 Digitaria setigera
EU272464.1 Urochloa acuminata
EU272439.1 Melinis repens
EU272432.1 Eriochloa punctata
AF443820.1 Urochloa mutica
AF443819.1 Tristachya superba
EU272451.1 Paspalidium geminatum
EU272460.1 Stenotaphrum secundatum
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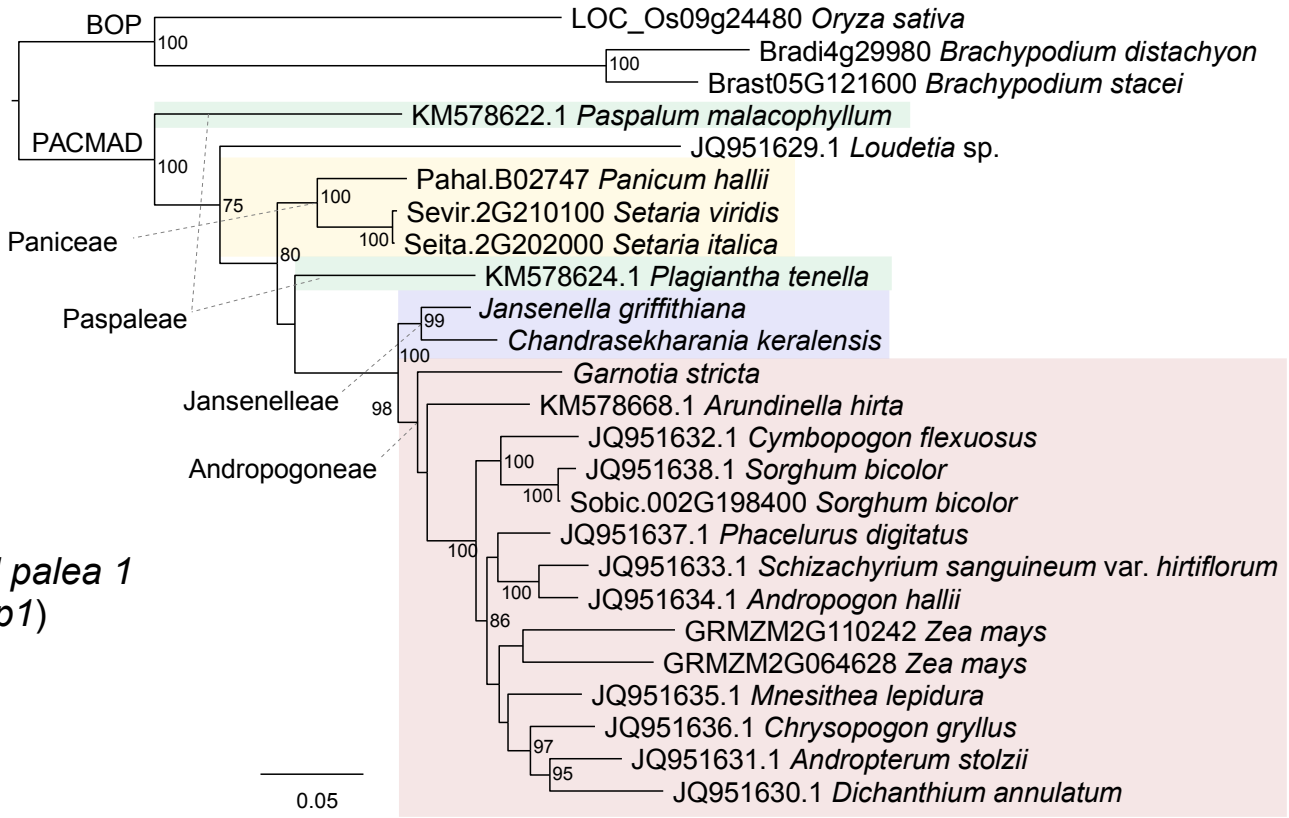
EU272452.1 Pennisetum glaucum
JQ270352.1 Cenchrus americanus
EU272421.1 Cenchrus myosuroides
EU272422.1 Cenchrus pilosus
EU272420.1 Cenchrus ciliaris
AF443814.1 Paspalum simplex
EU272461.1 Streptostachys asperifolia
EU272437.1 Ichnanthus pallens
EU272449.1 Panicum prionitis
EU272416.1 Apochloa euprepes
EU272419.1 Canastra lanceolata
EU272463.1 Tatianyx arnacites
EU272440.1 Mesosetum chaseae
EU272414.1 Altoparadisium chapadense
EU272417.1 Arthropogon villosus
EU272434.1 Homolepis glutinosa
EU272435.1 Homolepis isocalycia
EU272431.1 Echinolaena inflexa
EU272462.1 Streptostachys ramosa
EU272418.1 Axonopus fissifolius
EU272441.1 Ophiochloa hydrolithica
EU272415.1 Anthaenantia lanata
EU272436.1 Hymenachne donacifolia
EU272459.1 Steinchisma laxa
EU272458.1 Steinchisma hians
EU272443.1 Otachyrium versicolor
Jansenella griffithiana
Chandrasekharania keralensis
AF137293.1 Arundinella hirta
Garnotia stricta
AF443815.1 Phacelurus digitatus
AF443801.1 Chionachne koenigii
AF443806.1 Coix aquatica
AF443809.1 Elionurus muticus
GRMZM2G092174 Zea mays
JN560785.2 Hemarthria compressa
JN560784.2 Hemarthria altissima
AF443805.1 Coelorachis selloana
JN560780.2 Mnesithea selloana
JN560779.2 Coelorachis afraurita
JN560787.2 Phacelurus huillensis
JN560790.2 Urelytrum digitatum
AF443802.1 Chrysopogon fulvus
AF443803.1 Chrysopogon gryllus
JN560788.2 Rhytachne rottboellioides
JN560786.2 Oxyrhachis gracillima
AF443813.1 Microstegium nudum
GRMZM2G124532 Zea mays
AF443818.1 Tripsacum dactyloides
AF443810.1 Heteropogon contortus
AF137296.1 Capillipedium parviflorum
AF443800.1 Bothriochloa odorata
AF443804.1 Cleistachne sorghoides
JN560783.2 Eulalia villosa
DQ494704.1 Saccharum officinarum
AF443816.1 Saccharum officinarum
AF137311.1 Miscanthus sinensis
AF443808.1 Dichanthium aristatum
AF443807.1 Cymbopogon flexuosus
JN560778.2 Andropogon gayanus
AF443798.1 Andropogon ternarius
JN560781.2 Diheteropogon amplectens
AF443811.1 Hyparrhenia hirta
AF443817.1 Schizachyrium scoparium
AB988257.1 Sorghum propinquum
Sobic.001G394400 Sorghum bicolor
AF137326.1 Sorghum halepense
AF443812.1 Ischaemum afrum
JN560782.2 Dimeria lawsonii
AF443799.1 Apluda mutica
JN560789.2 Sehima nervosum
0.05

g)



retarded palea 1
(*rep1*)

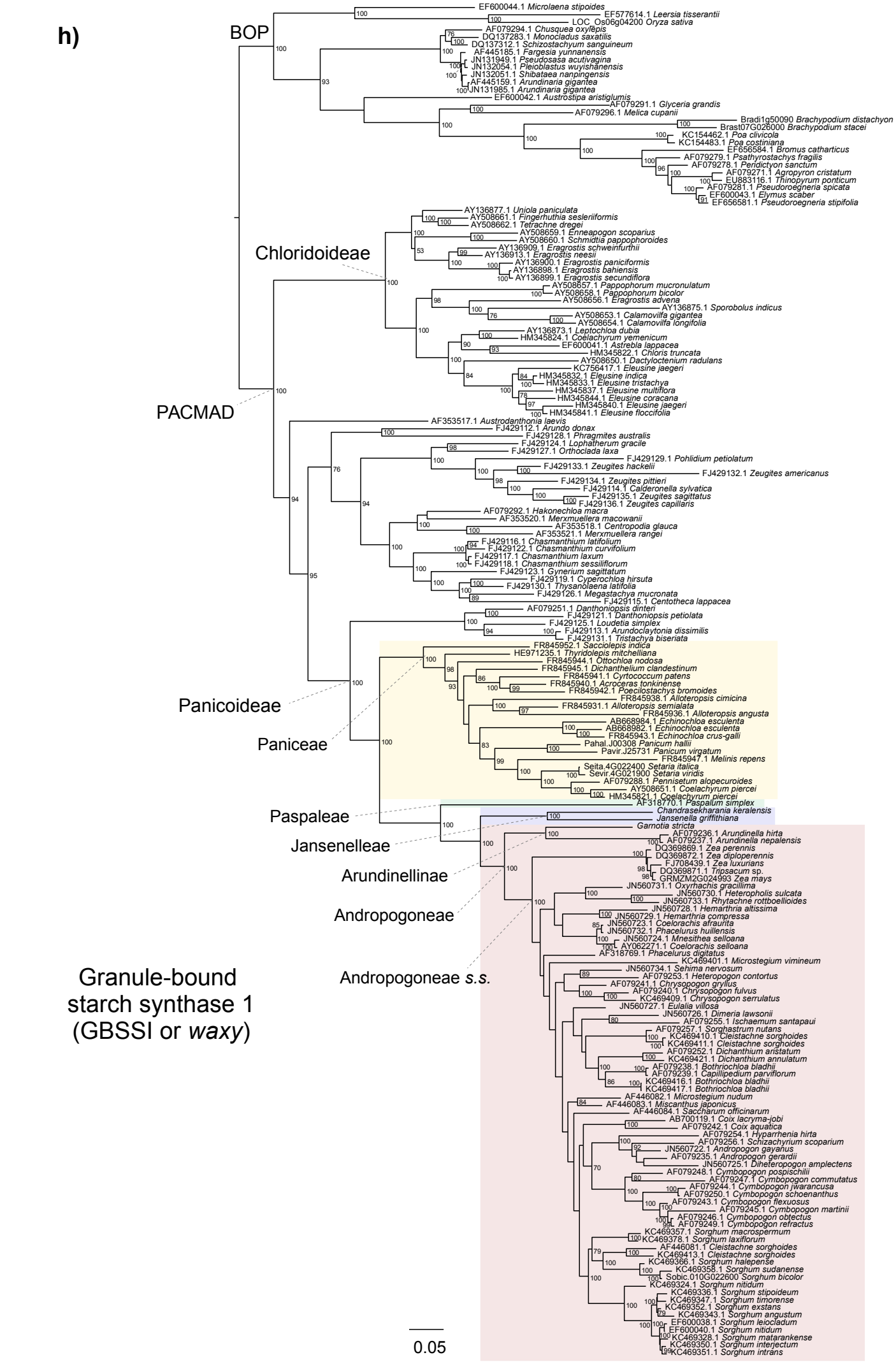
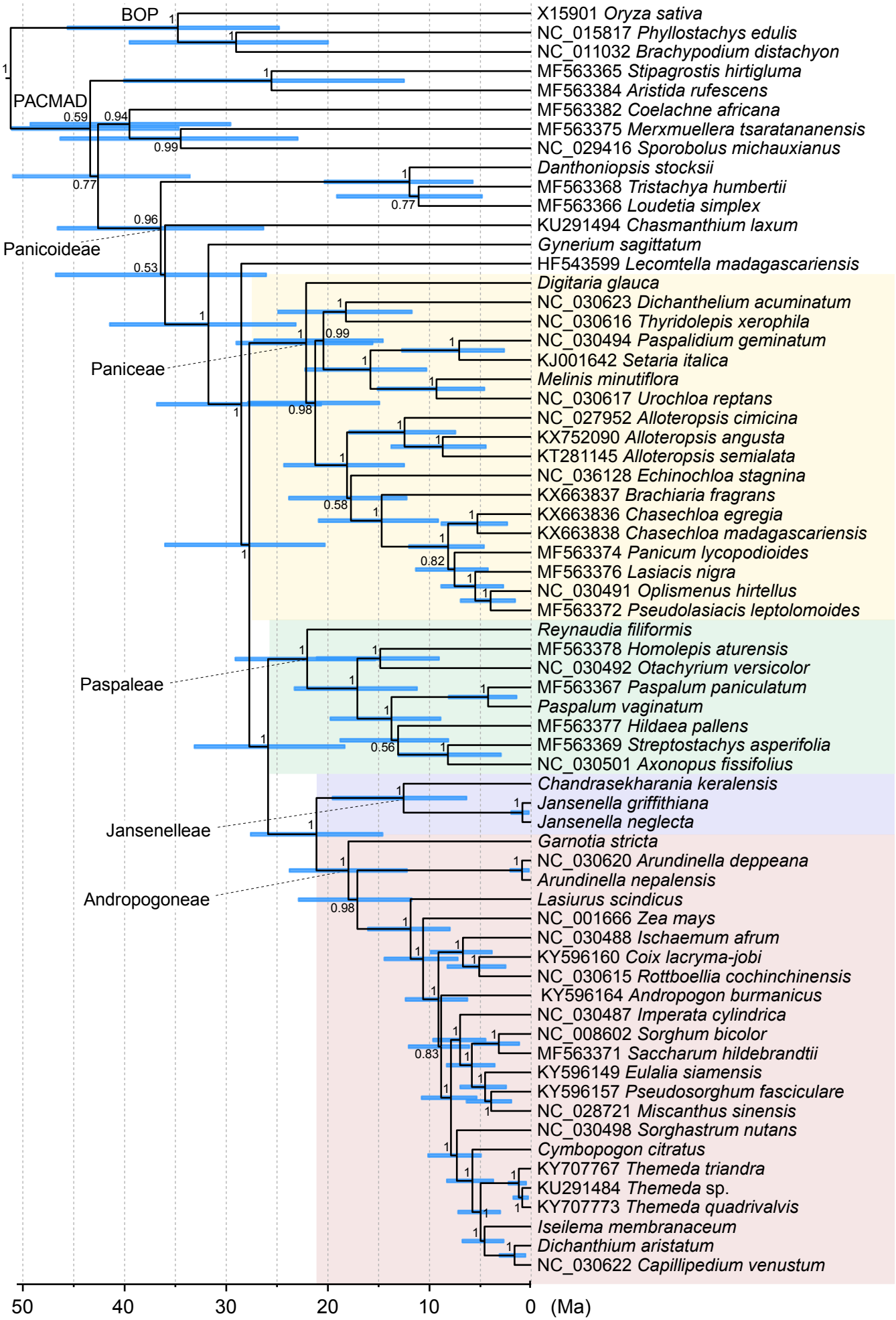

h)
BOP
Chloridoideae
PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
Granule-bound starch synthase 1 (GBSSI or waxy)
0.05

Fig. S5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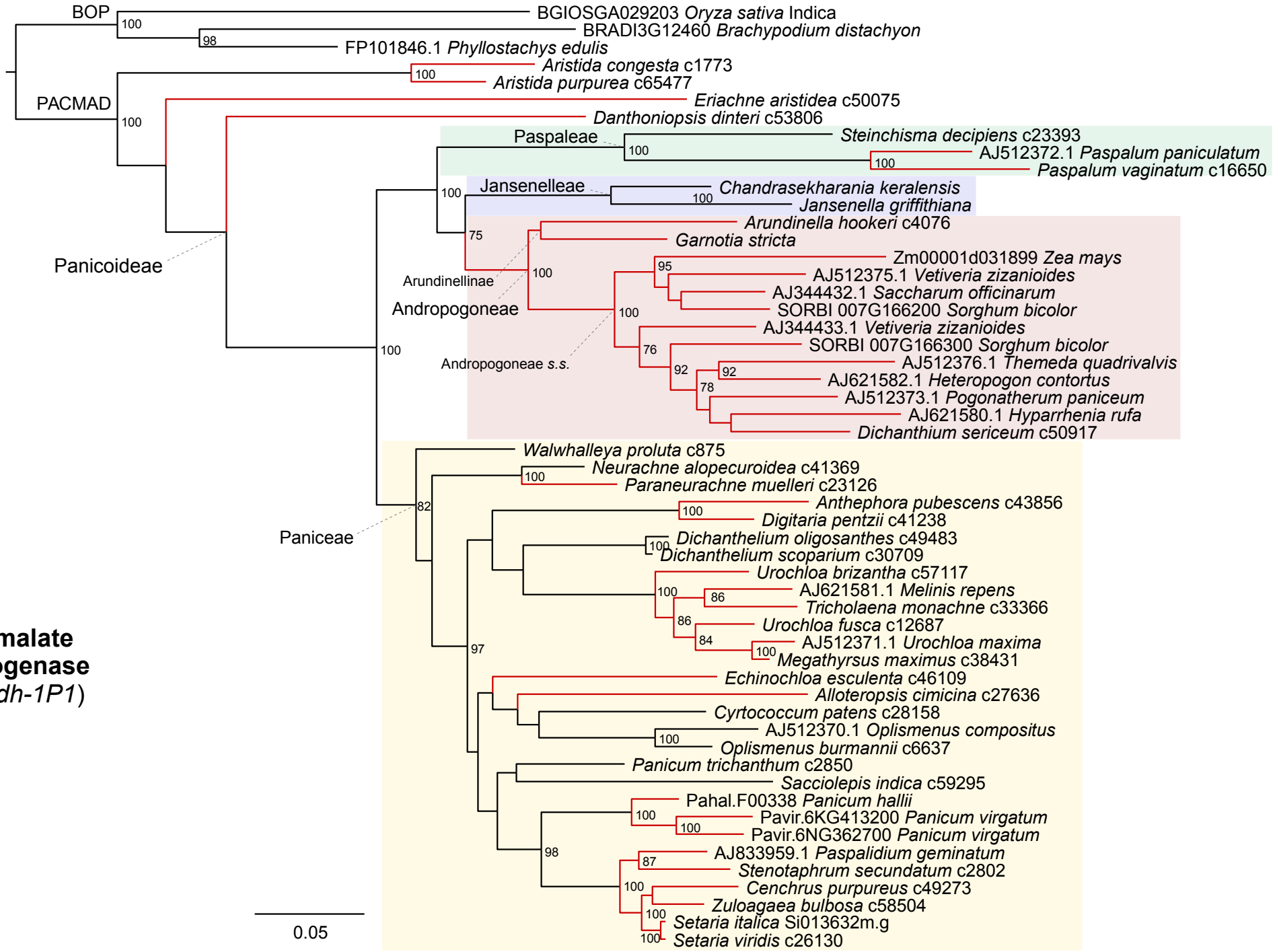
PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Andropogoneae
X15901 Oryza sativa
NC_015817 Phyllostachys edulis
NC_011032 Brachypodium distachyon
MF563365 Stipagrostis hirtigluma
MF563384 Aristida rufescens
MF563382 Coelachne africana
MF563375 Merxmuellera tsaratananensis
NC_029416 Sporobolus michauxianus
Danthoniopsis stocksii
MF563368 Tristachya humbertii
MF563366 Loudetia simplex
KU291494 Chasmanthium laxum
Gynerium sagittatum
HF543599 Lecomtella madagascariensis
Digitaria glauca
NC_030623 Dichanthelium acuminatum
NC_030616 Thyridolepis xerophila
NC_030494 Paspalidium geminatum
KJ001642 Setaria italica
Melinis minutiflora
NC_030617 Urochloa reptans
NC_027952 Alloteropsis cimicina
KX752090 Alloteropsis angusta
KT281145 Alloteropsis semialata
NC_036128 Echinochloa stagnina
KX663837 Brachiaria fragrans
KX663836 Chasechloa egregia
KX663838 Chasechloa madagascariensis
MF563374 Panicum lycopodioides
MF563376 Lasiacis nigra
NC_030491 Oplismenus hirtellus
MF563372 Pseudolasiacis leptolomoides
Reynaudia filiformis
MF563378 Homolepis aturensis
NC_030492 Otachyrium versicolor
MF563367 Paspalum paniculatum
Paspalum vaginatum
MF563377 Hildaea pallens
MF563369 Streptostachys asperifolia
NC_030501 Axonopus fissifolius
Chandrasekharania keralensis
Jansenella griffithiana
Jansenella neglecta
Garnotia stricta
NC_030620 Arundinella deppeana
Arundinella nepalensis
Lasiurus scindicus
NC_001666 Zea mays
NC_030488 Ischaemum afrum
KY596160 Coix lacryma-jobi
NC_030615 Rottboellia cochinchinensis
KY596164 Andropogon burmanicus
NC_030487 Imperata cylindrica
NC_008602 Sorghum bicolor
MF563371 Saccharum hildebrandtii
KY596149 Eulalia siamensis
KY596157 Pseudosorghum fasciculare
NC_028721 Miscanthus sinensis
NC_030498 Sorghastrum nutans
Cymbopogon citratus
KY707767 Themeda triandra
KU291484 Themeda sp.
KY707773 Themeda quadrivalvis
Iseilema membranaceum
Dichanthium aristatum
NC_030622 Capillipedium venustum
50
40
30
20
10
0
(Ma)

Fig. S6

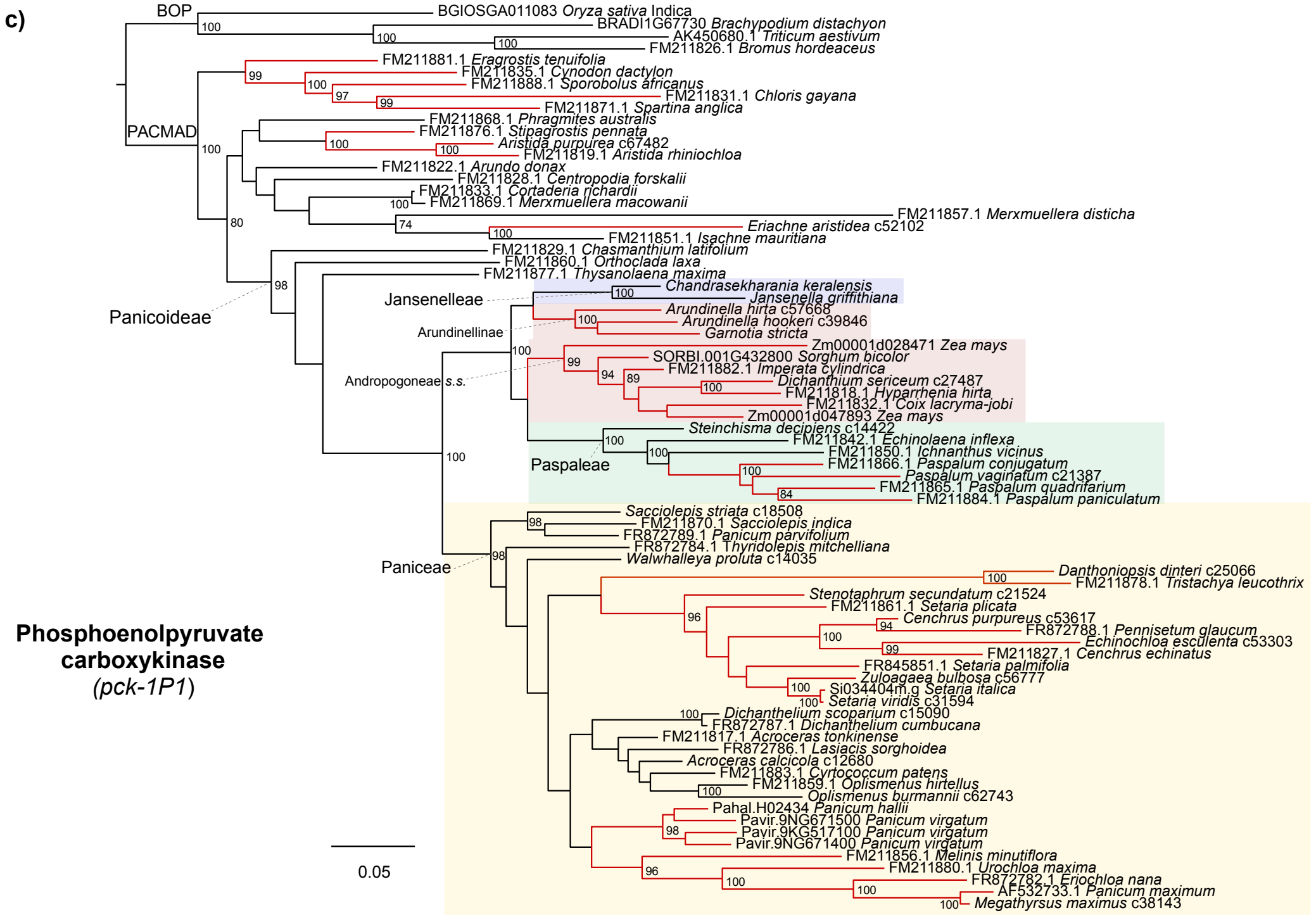
a)



NADP-malate dehydrogenase (*nadpmdh-1P1*)

b)

NADP-malic enzyme *(nadpme-1P4)*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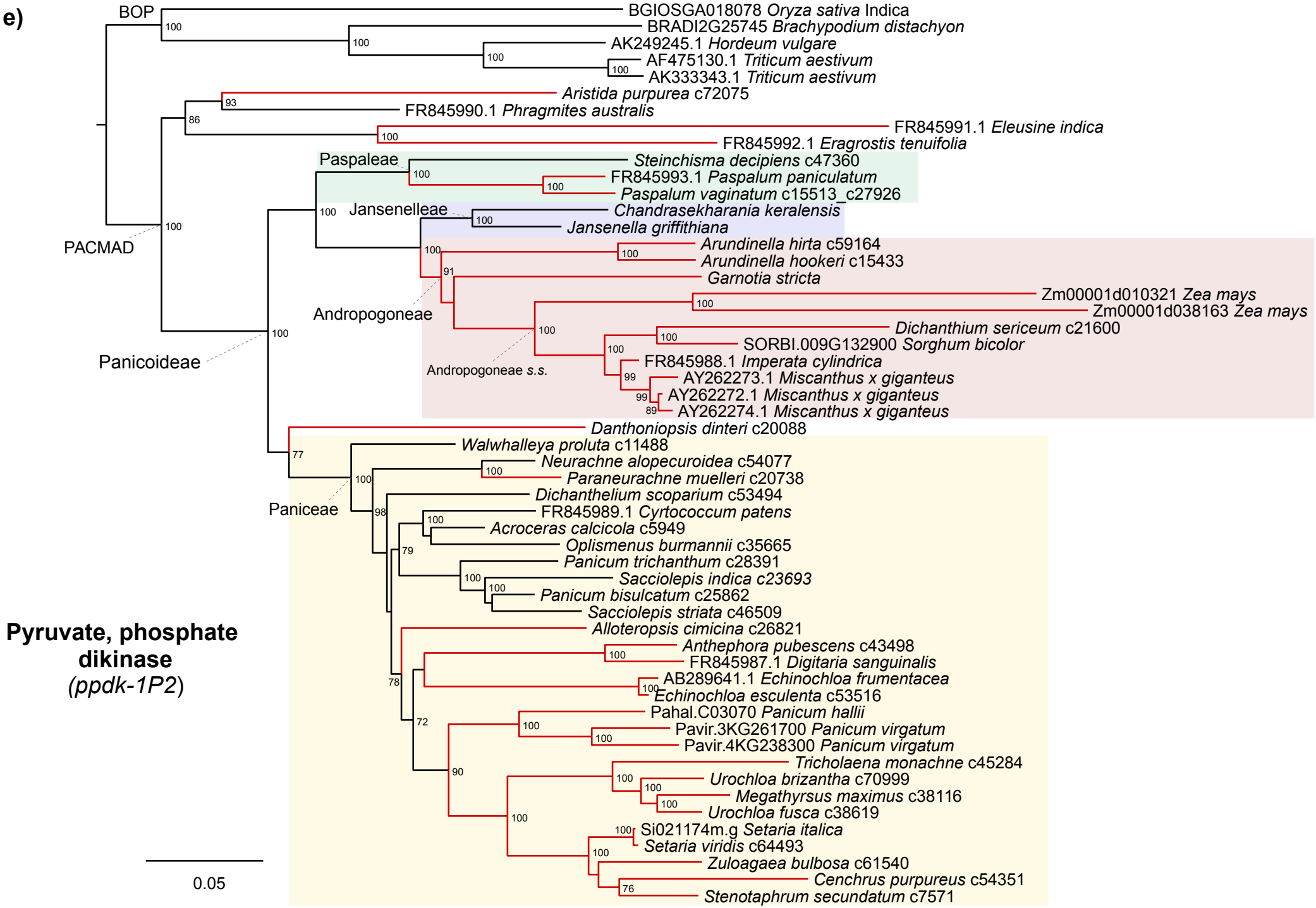
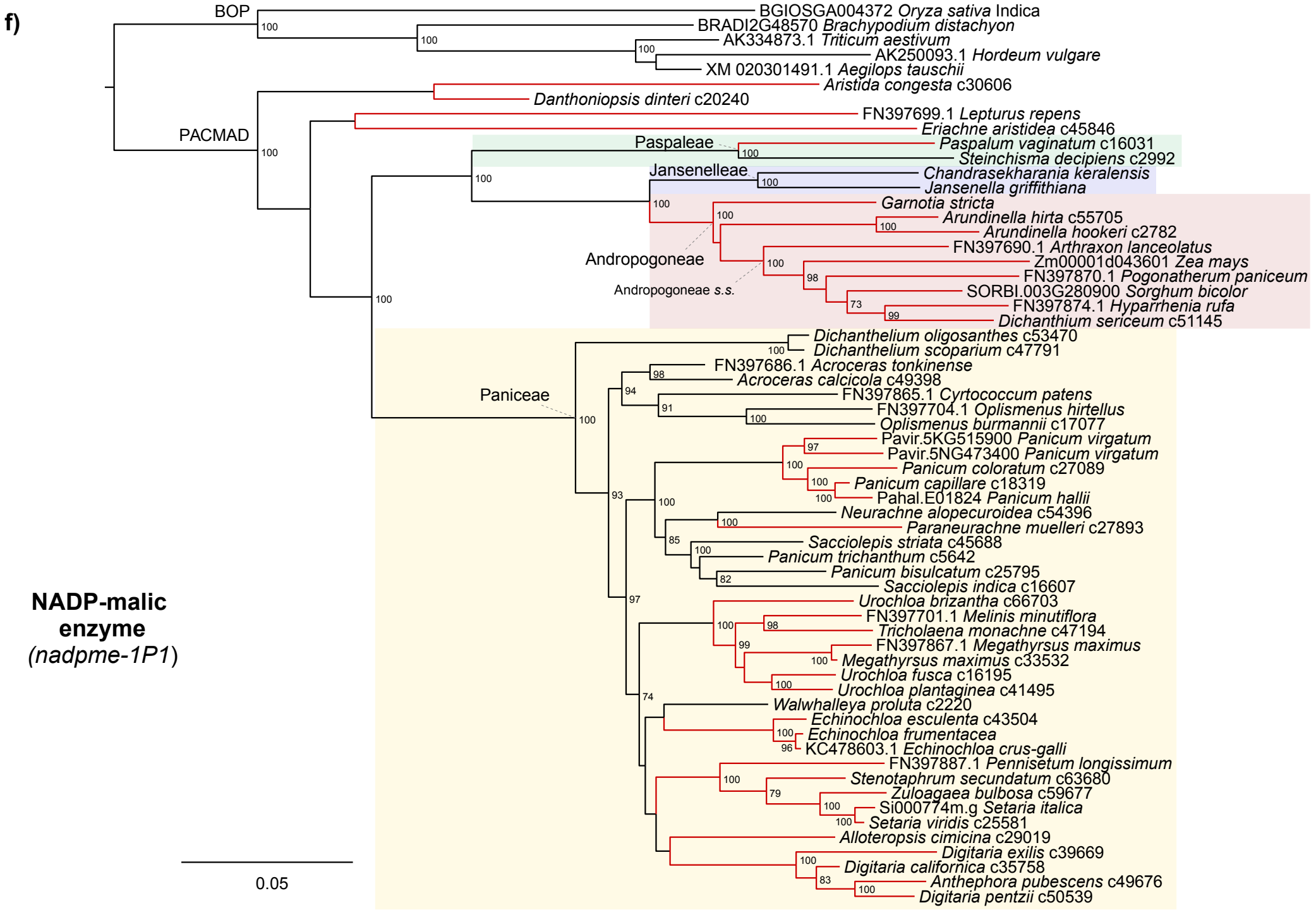
FM211881.1 Eragrostis tenuifolia
FM211835.1 Cynodon dactylon
FM211888.1 Sporobolus africanus
FM211831.1 Chloris gayana
FM211871.1 Spartina anglica
PACMAD
FM211868.1 Phragmites australis
FM211876.1 Stipagrostis pennata
Aristida purpurea c67482
FM211819.1 Aristida rhiniochloa
FM211822.1 Arundo donax
FM211828.1 Centropodia forskalii
FM211833.1 Cortaderia richardii
FM211869.1 Merxmuellera macowanii
FM211857.1 Merxmuellera disticha
Eriachne aristidea c52102
FM211851.1 Isachne mauritiana
FM211829.1 Chasmanthium latifolium
FM211860.1 Orthoclada laxa
FM211877.1 Thysanolaena maxima
Panicoideae
Chandrasekharania keralensis
Jansenelleae
Jansenella griffithiana
Arundinella hirta c57668
Arundinellinae
Arundinella hookeri c39846
Garnotia stricta
Zm00001d028471 Zea mays
SORBI.001G432800 Sorghum bicolor
FM211882.1 Imperata cylindrica
Andropogoneae s.s.
Dichanthium sericeum c27487
FM211818.1 Hyparrhenia hirta
FM211832.1 Coix lacryma-jobi
Zm00001d047893 Zea mays
Steinchisma decipiens c14422
FM211842.1 Echinolaena inflexa
FM211850.1 Ichnanthus vicinus
FM211866.1 Paspalum conjugatum
Paspaleae
Paspalum vaginatum c21387
FM211865.1 Paspalum quadrifarium
FM211884.1 Paspalum paniculatum
Sacciolepis striata c18508
FM211870.1 Sacciolepis indica
FR872789.1 Panicum parvifolium
FR872784.1 Thyridolepis mitchelliana
Walwhalleya proluta c14035
Paniceae
Danthoniopsis dinteri c25066
FM211878.1 Tristachya leucothrix
Stenotaphrum secundatum c21524
FM211861.1 Setaria plicata
Cenchrus purpureus c53617
FR872788.1 Pennisetum glaucum
Echinochloa esculenta c53303
FM211827.1 Cenchrus echinatus
FR845851.1 Setaria palmifolia
Zuloagaea bulbosa c56777
Si034404m.g Setaria italica
Setaria viridis c31594
Dichanthelium scoparium c15090
FR872787.1 Dichanthelium cumbucana
FM211817.1 Acroceras tonkinense
FR872786.1 Lasiacis sorghoidea
Acroceras calcicola c12680
FM211883.1 Cyrtococcum patens
FM211859.1 Oplismenus hirtellus
Oplismenus burmannii c62743
Pahal.H02434 Panicum hallii
Pavir.9NG671500 Panicum virgatum
Pavir.9KG517100 Panicum virgatum
Pavir.9NG671400 Panicum virgatum
FM211856.1 Melinis minutiflora
FM211880.1 Urochloa maxima
FR872782.1 Eriochloa nana
AF532733.1 Panicum maximum
Megathyrsus maximus c38143
0.05
Phosphoenolpyruvate carboxykinase
(pck-1P1)

d)

Phosphoenolpyruvate carboxylate
(ppc-1P3)

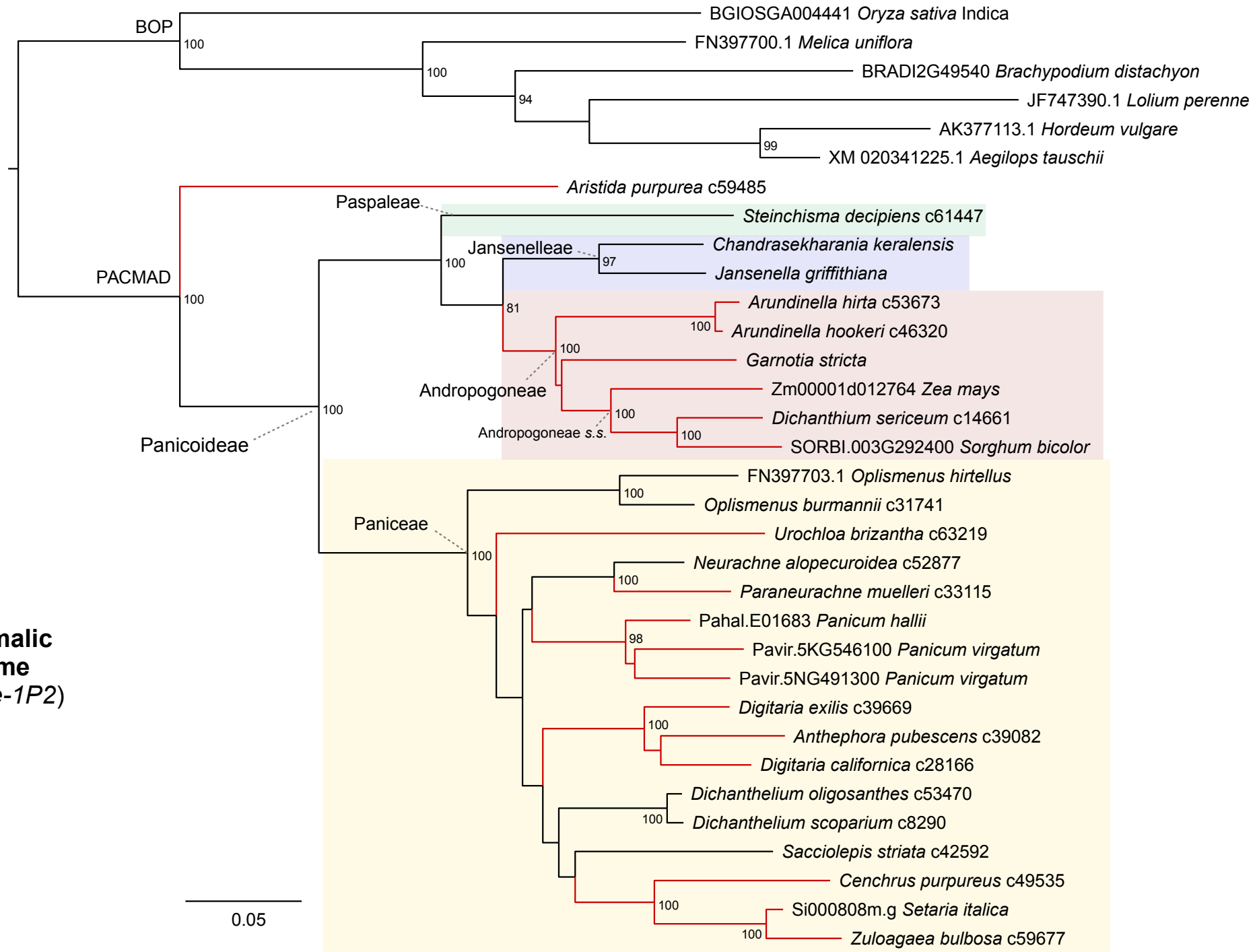


Pyruvate, phosphate dikinase
(ppdk-1P2)

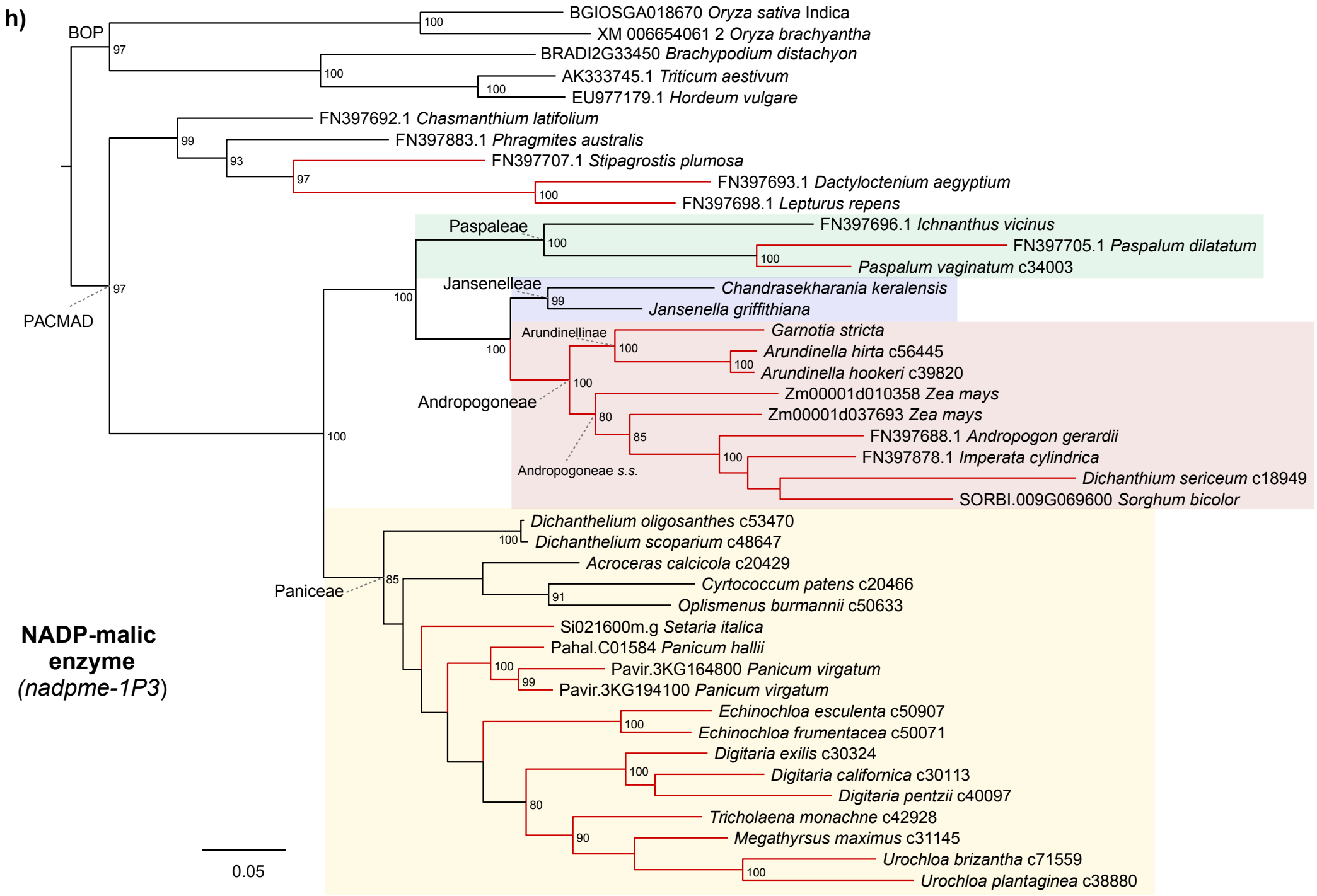


NADP-malic enzyme *(nadpme-1P1)*

g)



NADP-malic enzyme
(nadpme-1P2)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Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
Paniceae
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XM 006654061 2 Oryza brachyantha
BRADI2G33450 Brachypodium distachyon
AK333745.1 Triticum aestivum
EU977179.1 Hordeum vulgare
FN397692.1 Chasmanthium latifolium
FN397883.1 Phragmites australis
FN397707.1 Stipagrostis plumosa
FN397693.1 Dactyloctenium aegyptium
FN397698.1 Lepturus repens
FN397696.1 Ichnanthus vicinus
FN397705.1 Paspalum dilatatum
Paspalum vaginatum c34003
Chandrasekharania keralensis
Jansenella griffithiana
Garnotia stricta
Arundinella hirta c56445
Arundinella hookeri c39820
Zm00001d010358 Zea mays
Zm00001d037693 Zea mays
FN397688.1 Andropogon gerardii
FN397878.1 Imperata cylindrica
Dichanthium sericeum c18949
SORBI.009G069600 Sorghum bicolor
Dichanthelium oligosanthes c53470
Dichanthelium scoparium c48647
Acroceras calcicola c20429
Cyrtococcum patens c20466
Oplismenus burmannii c50633
Si021600m.g Setaria italica
Pahal.C01584 Panicum hallii
Pavir.3KG164800 Panicum virgatum
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Echinochloa esculenta c50907
Echinochloa frumentacea c50071
Digitaria exilis c30324
Digitaria californica c30113
Digitaria pentzii c40097
Tricholaena monachne c42928
Megathyrsus maximus c31145
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Urochloa plantaginea c38880
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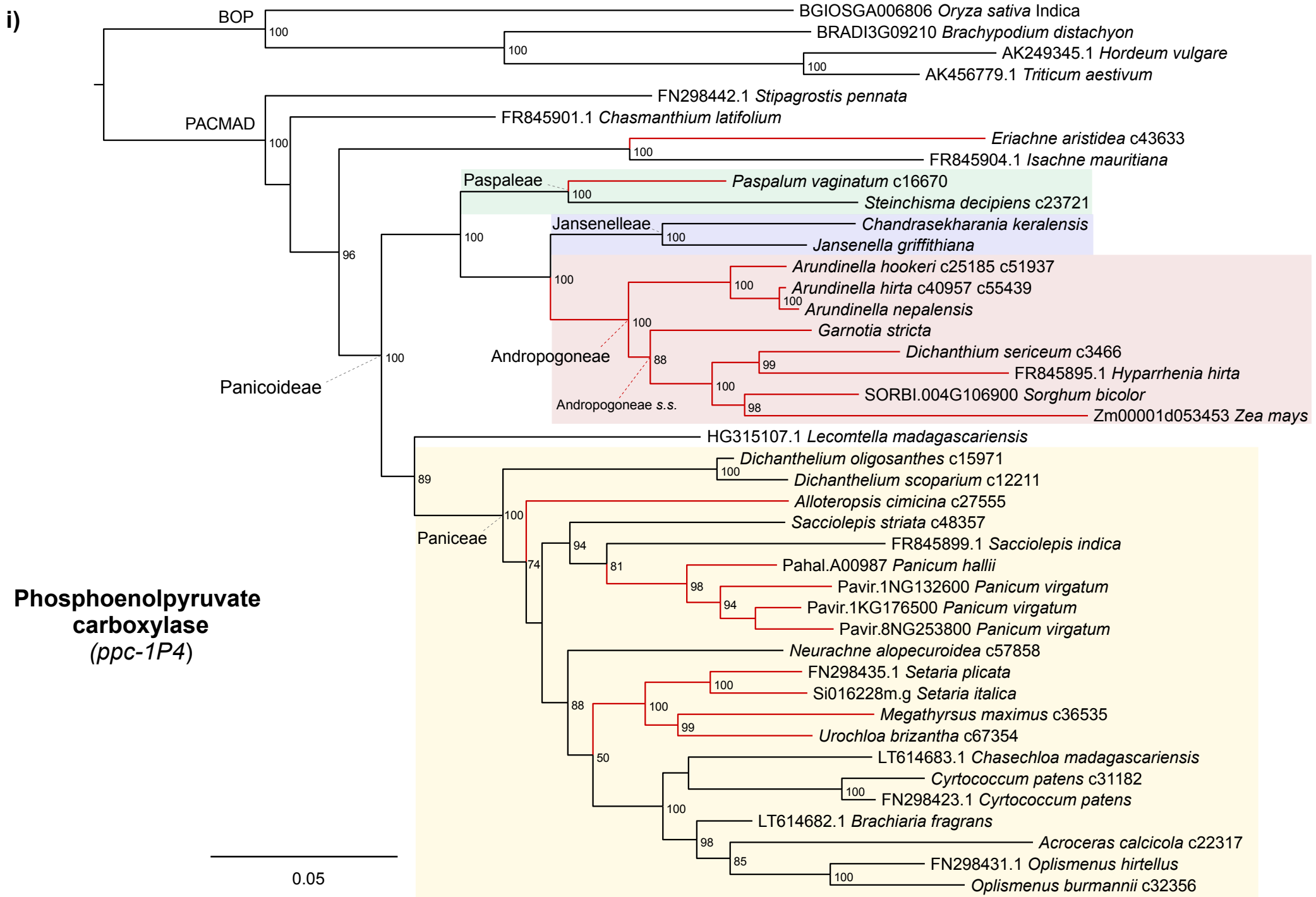
NADP-malic enzyme (nadpme-1P3)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Panicoideae
Paspaleae
Jansenelleae
Andropogoneae
Andropogoneae s.s.
Paniceae
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BRADI3G09210 Brachypodium distachyon
AK249345.1 Hordeum vulgare
AK456779.1 Triticum aestivum
FN298442.1 Stipagrostis pennata
FR845901.1 Chasmanthium latifolium
Eriachne aristidea c43633
FR845904.1 Isachne mauritiana
Paspalum vaginatum c16670
Steinchisma decipiens c23721
Chandrasekharania keralensis
Jansenella griffithiana
Arundinella hookeri c25185 c51937
Arundinella hirta c40957 c55439
Arundinella nepalensis
Garnotia stricta
Dichanthium sericeum c3466
FR845895.1 Hyparrhenia hirta
SORBI.004G106900 Sorghum bicolor
Zm00001d053453 Zea mays
HG315107.1 Lecomtella madagascariensis
Dichanthelium oligosanthes c15971
Dichanthelium scoparium c12211
Alloteropsis cimicina c27555
Sacciolepis striata c48357
FR845899.1 Sacciolepis indica
Pahal.A00987 Panicum hallii
Pavir.1NG132600 Panicum virgatum
Pavir.1KG176500 Panicum virgatum
Pavir.8NG253800 Panicum virgatum
Neurachne alopecuroidea c57858
FN298435.1 Setaria plicata
Si016228m.g Setaria italica
Megathyrsus maximus c36535
Urochloa brizantha c67354
LT614683.1 Chasechloa madagascariensis
Cyrtococcum patens c31182
FN298423.1 Cyrtococcum patens
LT614682.1 Brachiaria fragrans
Acroceras calcicola c22317
FN298431.1 Oplismenus hirtellus
Oplismenus burmannii c32356
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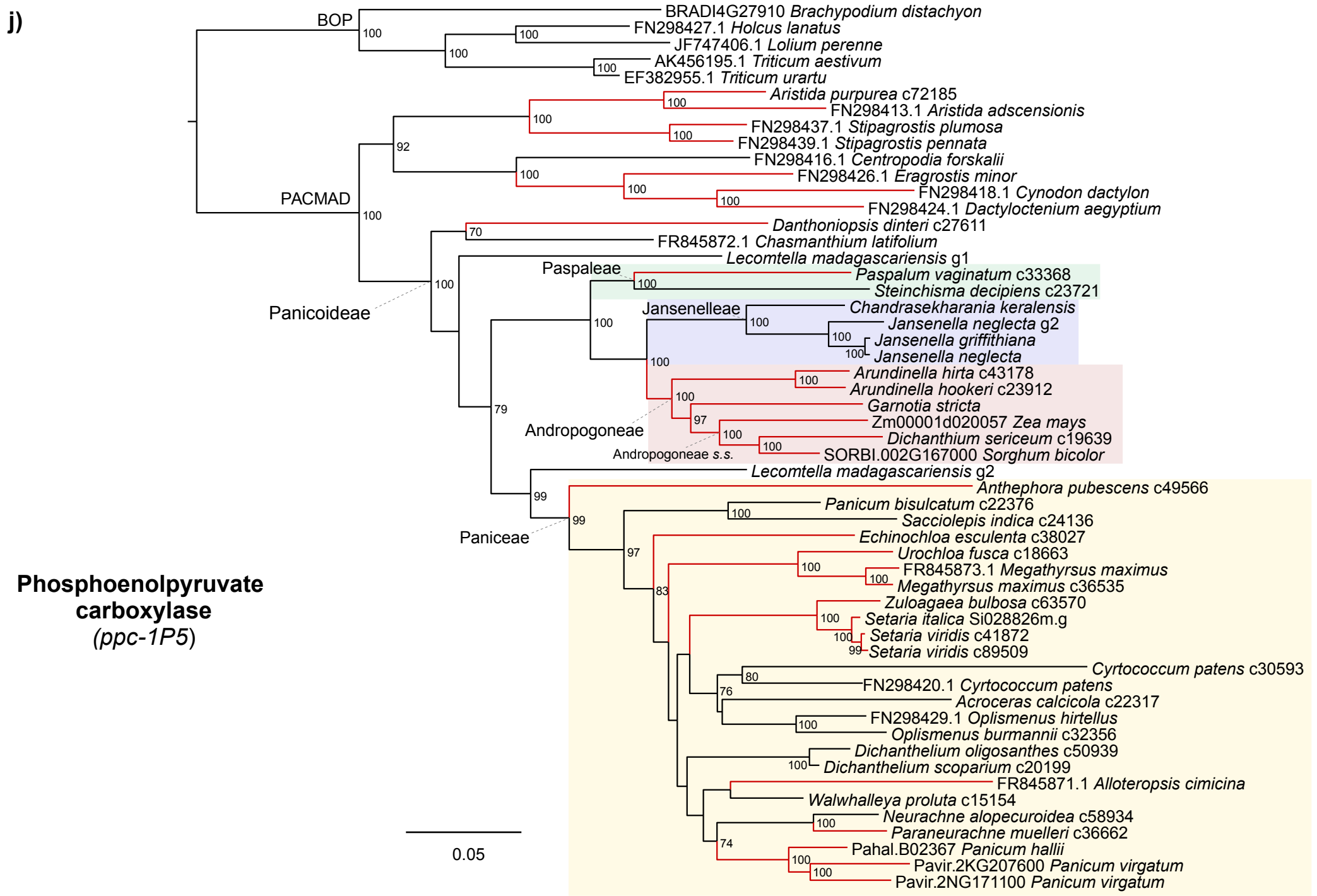
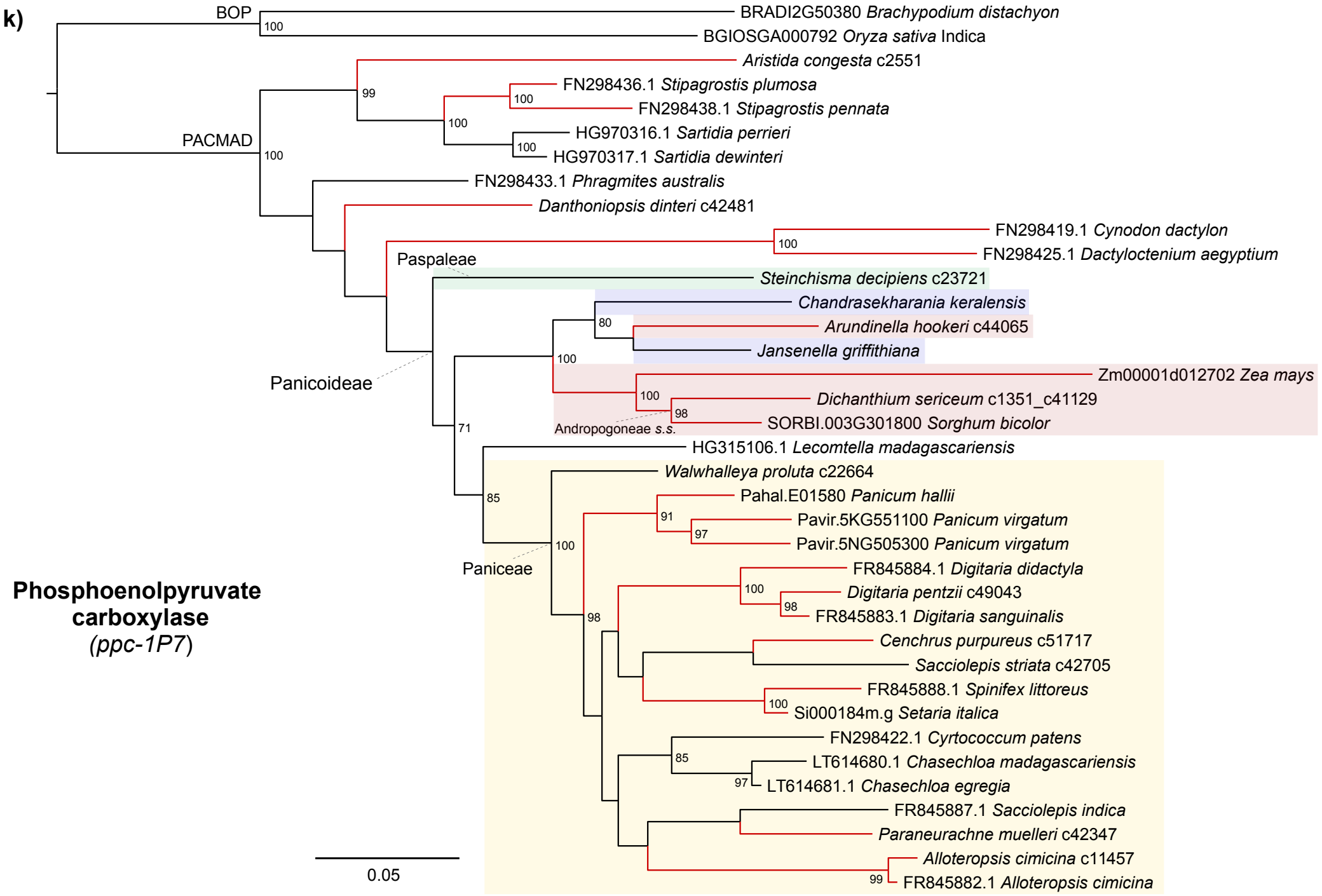
Phosphoenolpyruvate carboxylase (ppc-1P4)



Phosphoenolpyruvate carboxylase
(ppc-1P5)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FN298438.1 Stipagrostis pennata
HG970316.1 Sartidia perrieri
HG970317.1 Sartidia dewinteri
FN298433.1 Phragmites australis
Danthoniopsis dinteri c42481
FN298419.1 Cynodon dactylon
FN298425.1 Dactyloctenium aegyptium
Paspaleae
Steinchisma decipiens c23721
Chandrasekharania keralensis
Arundinella hookeri c44065
Jansenella griffithiana
Panicoideae
Zm00001d012702 Zea mays
Dichanthium sericeum c1351_c41129
SORBI.003G301800 Sorghum bicolor
Andropogoneae s.s.
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Walwhalleya proluta c22664
Pahal.E01580 Panicum hallii
Pavir.5KG551100 Panicum virgatum
Pavir.5NG505300 Panicum virgatum
Paniceae
FR845884.1 Digitaria didactyla
Digitaria pentzii c49043
FR845883.1 Digitaria sanguinalis
Cenchrus purpureus c51717
Sacciolepis striata c42705
FR845888.1 Spinifex littoreus
Si000184m.g Setaria italica
FN298422.1 Cyrtococcum patens
LT614680.1 Chasechloa madagascariensis
LT614681.1 Chasechloa egregia
FR845887.1 Sacciolepis indica
Paraneurachne muelleri c42347
Alloteropsis cimicina c11457
FR845882.1 Alloteropsis cimicina
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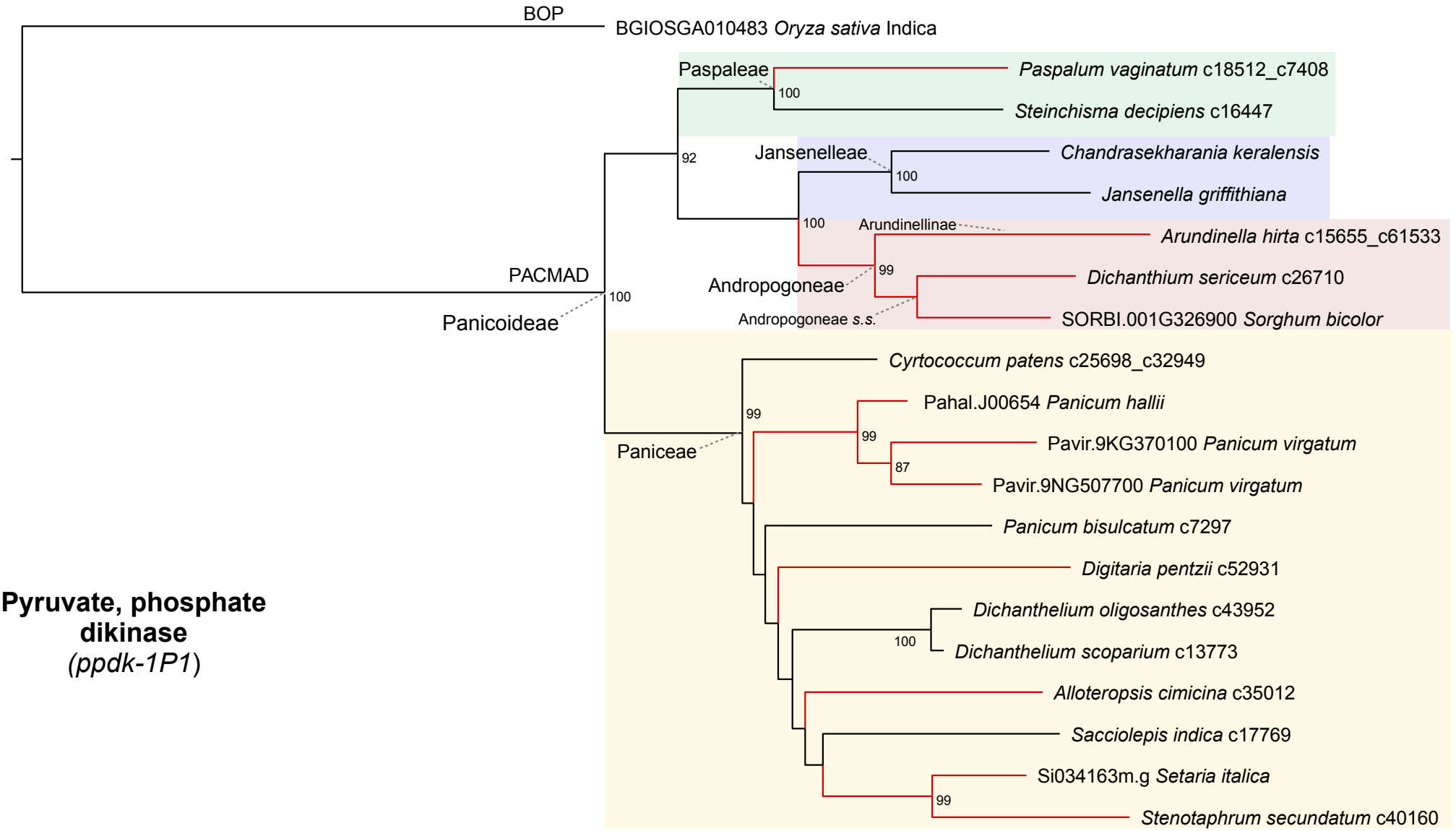
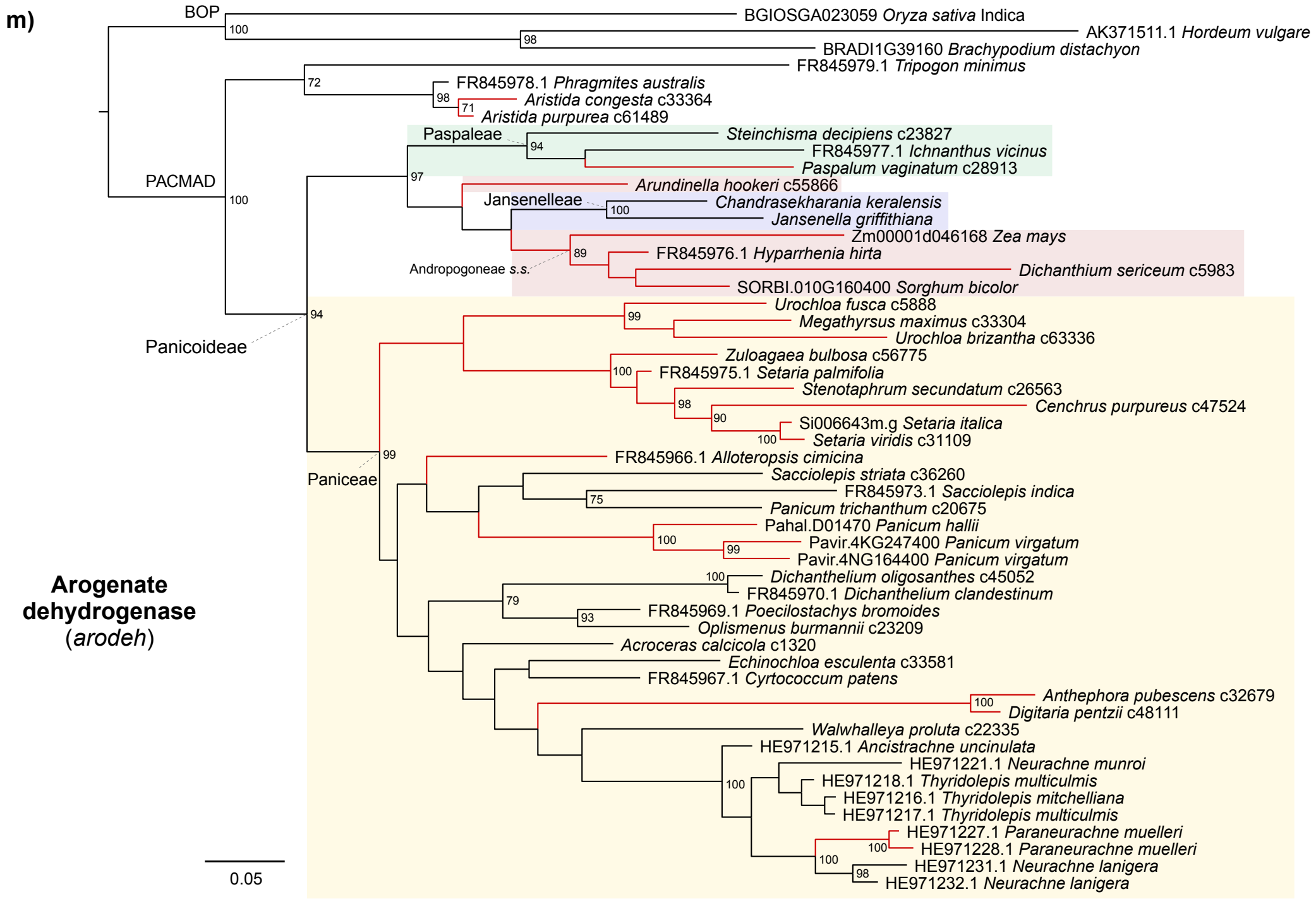
Phosphoenolpyruvate carboxylase
(ppc-1P7)

I)



Pyruvate, phosphate dikinase
(ppdk-1P1)

0.05


m)
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PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Andropogoneae s.s.
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AK371511.1 Hordeum vulgare
BRADI1G39160 Brachypodium distachyon
FR845979.1 Tripogon minimus
FR845978.1 Phragmites australis
Aristida congesta c33364
Aristida purpurea c61489
Steinchisma decipiens c23827
FR845977.1 Ichnanthus vicinus
Paspalum vaginatum c28913
Arundinella hookeri c55866
Chandrasekharania keralensis
Jansenella griffithiana
Zm00001d046168 Zea mays
FR845976.1 Hyparrhenia hirta
Dichanthium sericeum c5983
SORBI.010G160400 Sorghum bicolor
Urochloa fusca c5888
Megathyrsus maximus c33304
Urochloa brizantha c63336
Zuloagaea bulbosa c56775
FR845975.1 Setaria palmifolia
Stenotaphrum secundatum c26563
Cenchrus purpureus c47524
Si006643m.g Setaria italica
Setaria viridis c31109
FR845966.1 Alloteropsis cimicina
Sacciolepis striata c36260
FR845973.1 Sacciolepis indica
Panicum trichanthum c20675
Pahal.D01470 Panicum hallii
Pavir.4KG247400 Panicum virgatum
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Dichanthelium oligosanthes c45052
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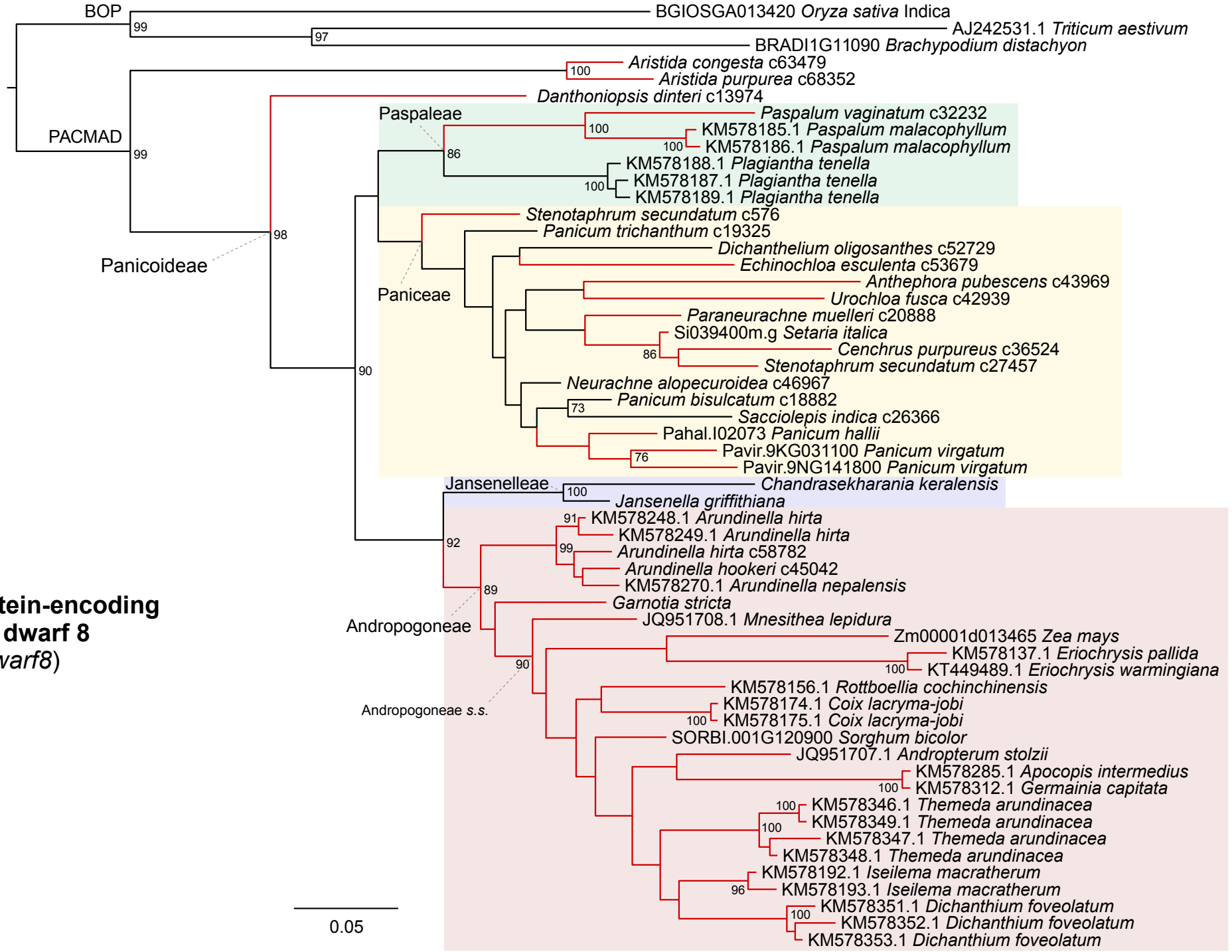
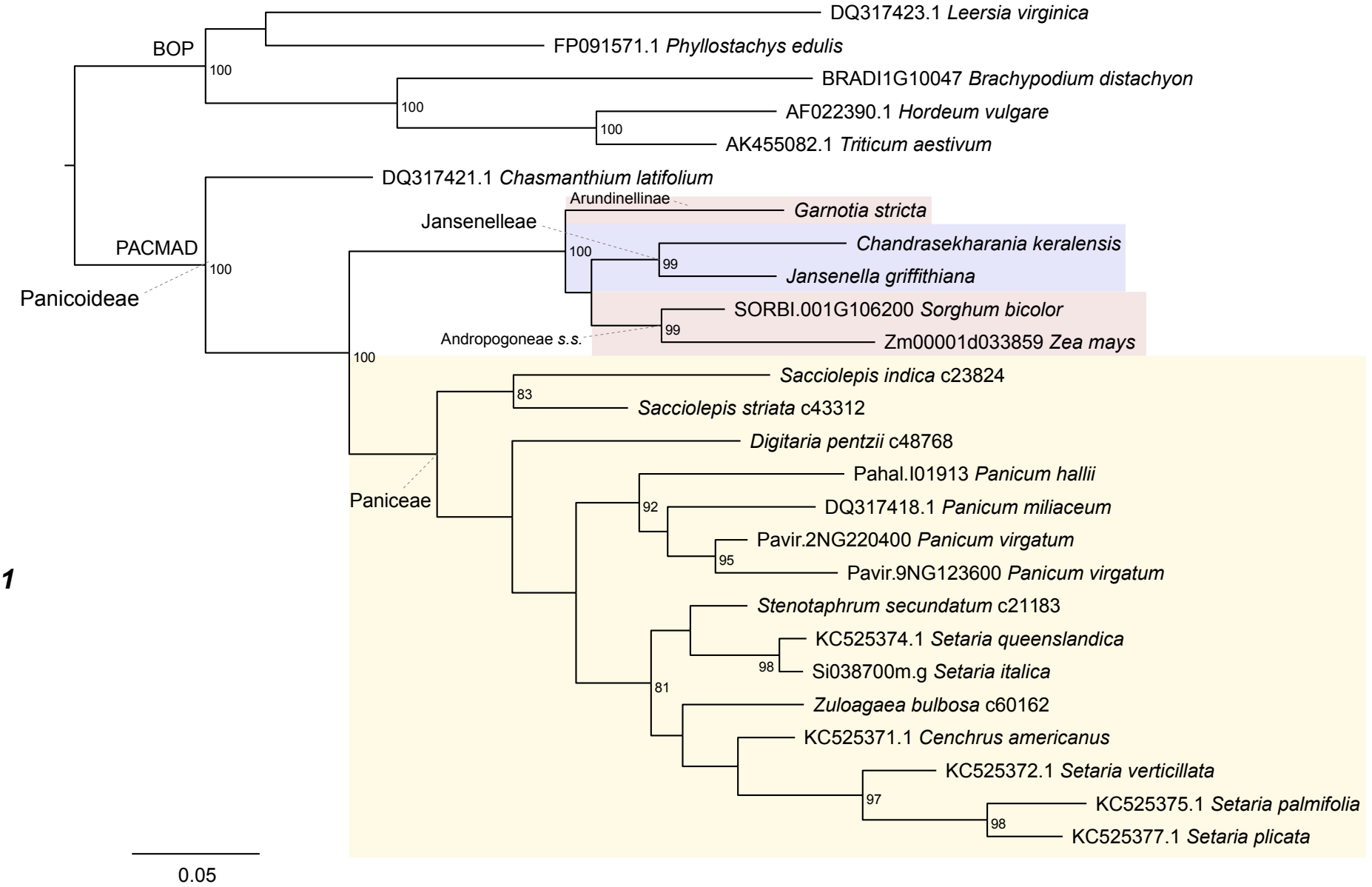
FR845969.1 Poecilostachys bromoides
Oplismenus burmannii c23209
Acroceras calcicola c1320
Echinochloa esculenta c33581
FR845967.1 Cyrtococcum patens
Anthephora pubescens c32679
Digitaria pentzii c48111
Walwhalleya proluta c22335
HE971215.1 Ancistrachne uncinulata
HE971221.1 Neurachne munroi
HE971218.1 Thyridolepis multiculmis
HE971216.1 Thyridolepis mitchelliana
HE971217.1 Thyridolepis multiculmis
HE971227.1 Paraneurachne muelleri
HE971228.1 Paraneurachne muelleri
HE971231.1 Neurachne lanigera
HE971232.1 Neurachne lanigera
Arogenate dehydrogenase (arodeh)
0.05

n)



DELLA protein-encoding gene dwarf 8 (*dwarf8*)

o)



Knotted 1
(kn1)

p)

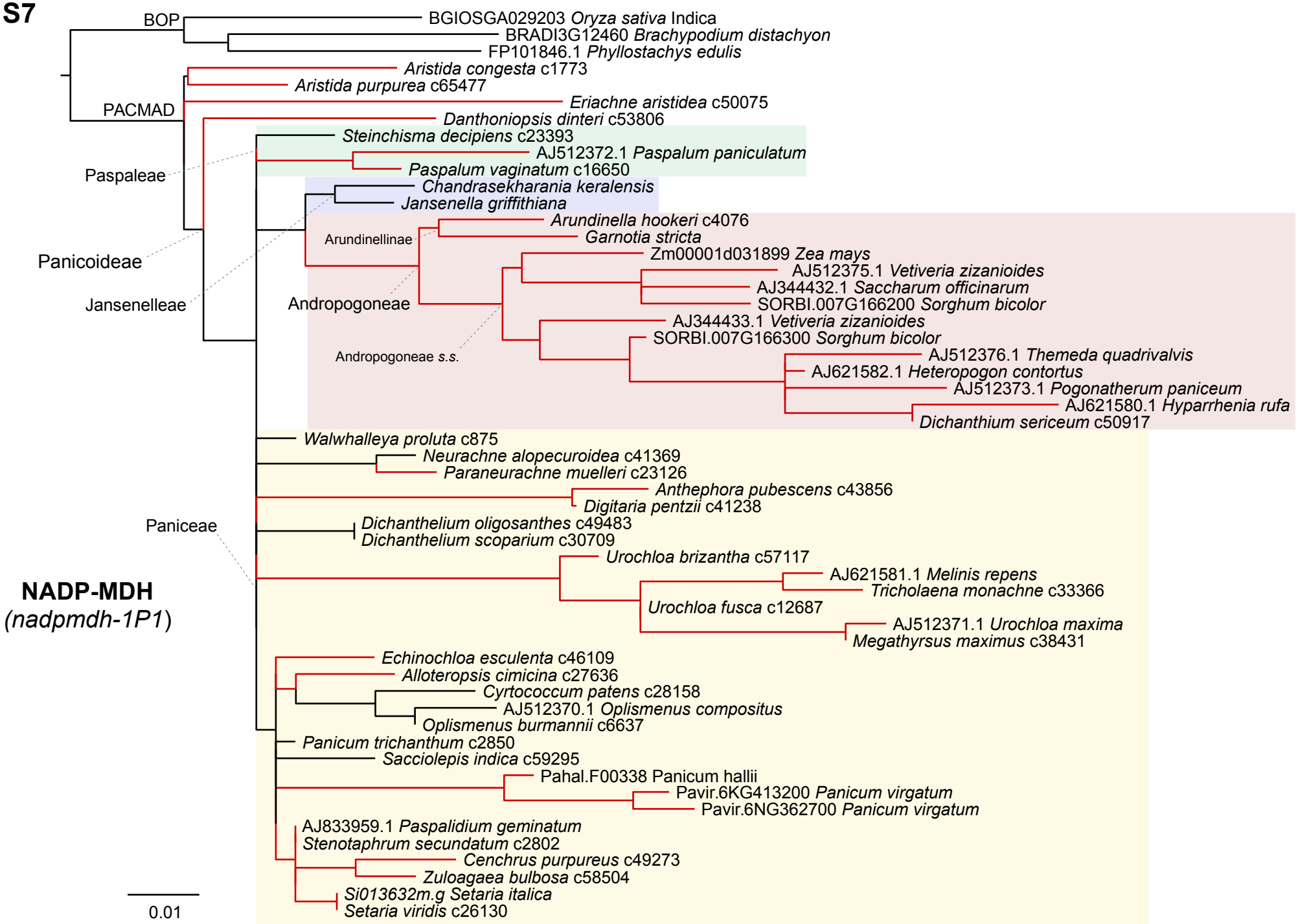
phytochrome B
(phyB)

q)

Granule-bound starch synthase 1
(waxy)

Fig. S7

a)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PACMAD
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Aristida purpurea c65477
Eriachne aristidea c50075
Danthoniopsis dinteri c53806
Paspaleae
Steinchisma decipiens c23393
AJ512372.1 Paspalum paniculatum
Paspalum vaginatum c16650
Chandrasekharania keralensis
Jansenella griffithiana
Panicoideae
Arundinellinae
Arundinella hookeri c4076
Garnotia stricta
Zm00001d031899 Zea mays
AJ512375.1 Vetiveria zizanioides
AJ344432.1 Saccharum officinarum
SORBI.007G166200 Sorghum bicolor
Jansenelleae
Andropogoneae
AJ344433.1 Vetiveria zizanioides
SORBI.007G166300 Sorghum bicolor
Andropogoneae s.s.
AJ512376.1 Themeda quadrivalvis
AJ621582.1 Heteropogon contortus
AJ512373.1 Pogonatherum paniceum
AJ621580.1 Hyparrhenia rufa
Dichanthium sericeum c50917
Walwhalleya proluta c875
Neurachne alopecuroidea c41369
Paraneurachne muelleri c23126
Anthephora pubescens c43856
Digitaria pentzii c41238
Paniceae
Dichanthelium oligosanthes c49483
Dichanthelium scoparium c30709
Urochloa brizantha c57117
AJ621581.1 Melinis repens
Tricholaena monachne c33366
Urochloa fusca c12687
AJ512371.1 Urochloa maxima
Megathyrsus maximus c38431
Echinochloa esculenta c46109
Alloteropsis cimicina c27636
Cyrtococcum patens c28158
AJ512370.1 Oplismenus compositus
Oplismenus burmannii c6637
Panicum trichanthum c2850
Sacciolepis indica c59295
Pahal.F00338 Panicum hallii
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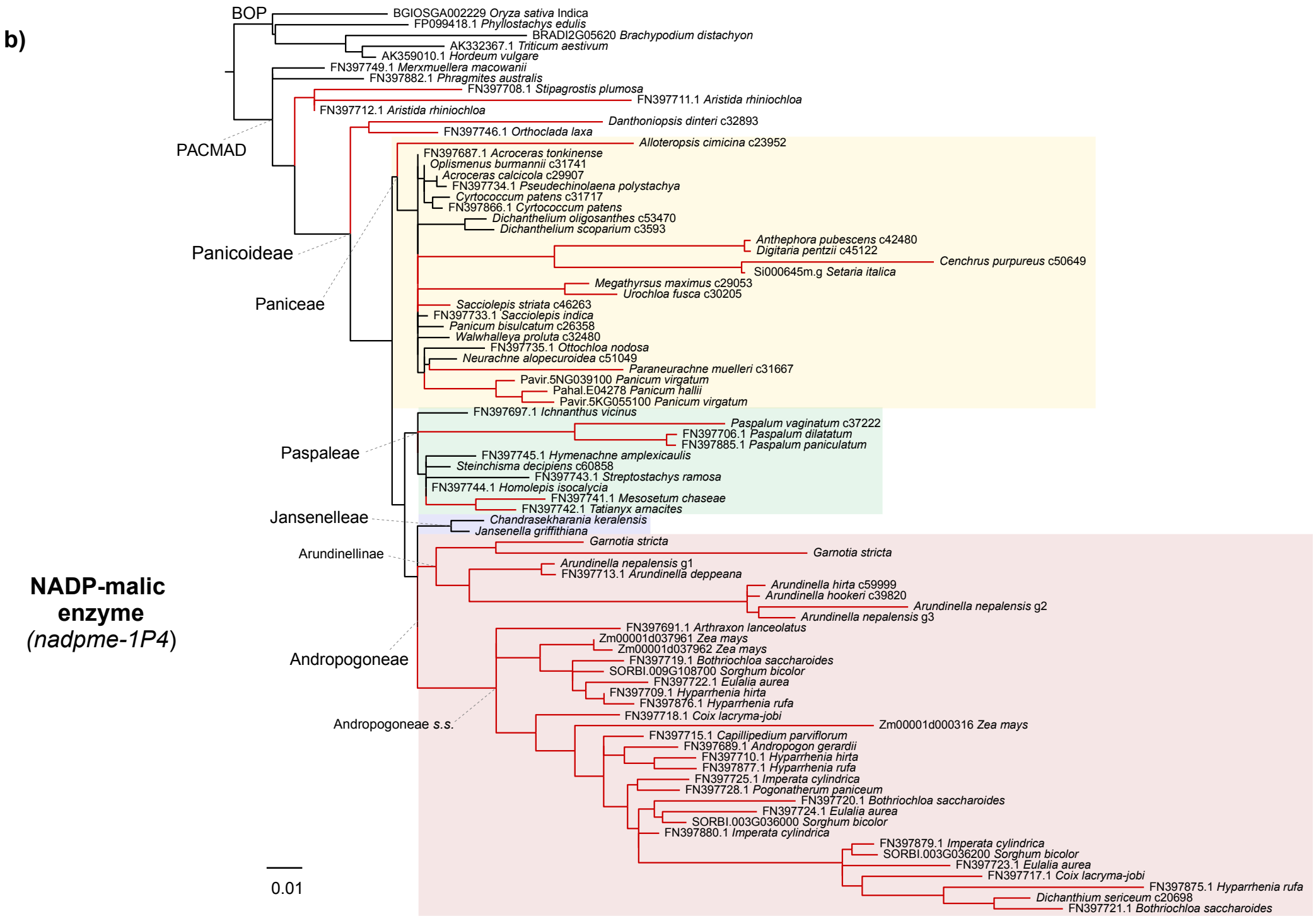
Pavir.6NG362700 Panicum virgatum
AJ833959.1 Paspalidium geminatum
Stenotaphrum secundatum c2802
Cenchrus purpureus c49273
Zuloagaea bulbosa c58504
Si013632m.g Setaria italica
Setaria viridis c26130
NADP-MDH
(nadpmdh-1P1)
0.01


b)
NADP-malic enzyme (nadpme-1P4)
BOP
PACMAD
Panicoideae
Paniceae
Paspaleae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
BGIOSGA002229 Oryza sativa Indica
FP099418.1 Phyllostachys edulis
BRADI2G05620 Brachypodium distachyon
AK332367.1 Triticum aestivum
AK359010.1 Hordeum vulgare
FN397749.1 Merxmuellera macowanii
FN397882.1 Phragmites australis
FN397708.1 Stipagrostis plumosa
FN397711.1 Aristida rhiniochloa
FN397712.1 Aristida rhiniochloa
Danthoniopsis dinteri c32893
FN397746.1 Orthoclada laxa
Alloteropsis cimicina c23952
FN397687.1 Acroceras tonkinense
Oplismenus burmannii c31741
Acroceras calcicola c29907
FN397734.1 Pseudechinolaena polystachya
Cyrtococcum patens c31717
FN397866.1 Cyrtococcum patens
Dichanthelium oligosanthes c53470
Dichanthelium scoparium c3593
Anthephora pubescens c42480
Digitaria pentzii c45122
Cenchrus purpureus c50649
Si000645m.g Setaria italica
Megathyrsus maximus c29053
Urochloa fusca c30205
Sacciolepis striata c46263
FN397733.1 Sacciolepis indica
Panicum bisulcatum c26358
Walwhalleya proluta c32480
FN397735.1 Ottochloa nodosa
Neurachne alopecuroidea c51049
Paraneurachne muelleri c31667
Pavir.5NG039100 Panicum virgatum
Pahal.E04278 Panicum hallii
Pavir.5KG055100 Panicum virgatum
FN397697.1 Ichnanthus vicinus
Paspalum vaginatum c37222
FN397706.1 Paspalum dilatatum
FN397885.1 Paspalum paniculatum
FN397745.1 Hymenachne amplexicaulis
Steinchisma decipiens c60858
FN397743.1 Streptostachys ramosa
FN397744.1 Homolepis isocalycia
FN397741.1 Mesosetum chaseae
FN397742.1 Tatianyx arnacites
Chandrasekharania keralensis
Jansenella griffithiana
Garnotia stricta
Garnotia stricta
Arundinella nepalensis g1
FN397713.1 Arundinella deppeana
Arundinella hirta c59999
Arundinella hookeri c39820
Arundinella nepalensis g2
Arundinella nepalensis g3
FN397691.1 Arthraxon lanceolatus
Zm00001d037961 Zea mays
Zm00001d037962 Zea mays
FN397719.1 Bothriochloa saccharoides
SORBI.009G108700 Sorghum bicolor
FN397722.1 Eulalia aurea
FN397709.1 Hyparrhenia hirta
FN397876.1 Hyparrhenia rufa
FN397718.1 Coix lacryma-jobi
Zm00001d000316 Zea mays
FN397715.1 Capillipedium parviflorum
FN397689.1 Andropogon gerardii
FN397710.1 Hyparrhenia hirta
FN397877.1 Hyparrhenia rufa
FN397725.1 Imperata cylindrica
FN397728.1 Pogonatherum paniceum
FN397720.1 Bothriochloa saccharoides
FN397724.1 Eulalia aurea
SORBI.003G036000 Sorghum bicolor
FN397880.1 Imperata cylindrica
FN397879.1 Imperata cylindrica
SORBI.003G036200 Sorghum bicolor
FN397723.1 Eulalia aurea
FN397717.1 Coix lacryma-jobi
FN397875.1 Hyparrhenia rufa
Dichanthium sericeum c20698
FN397721.1 Bothriochloa saccharoides
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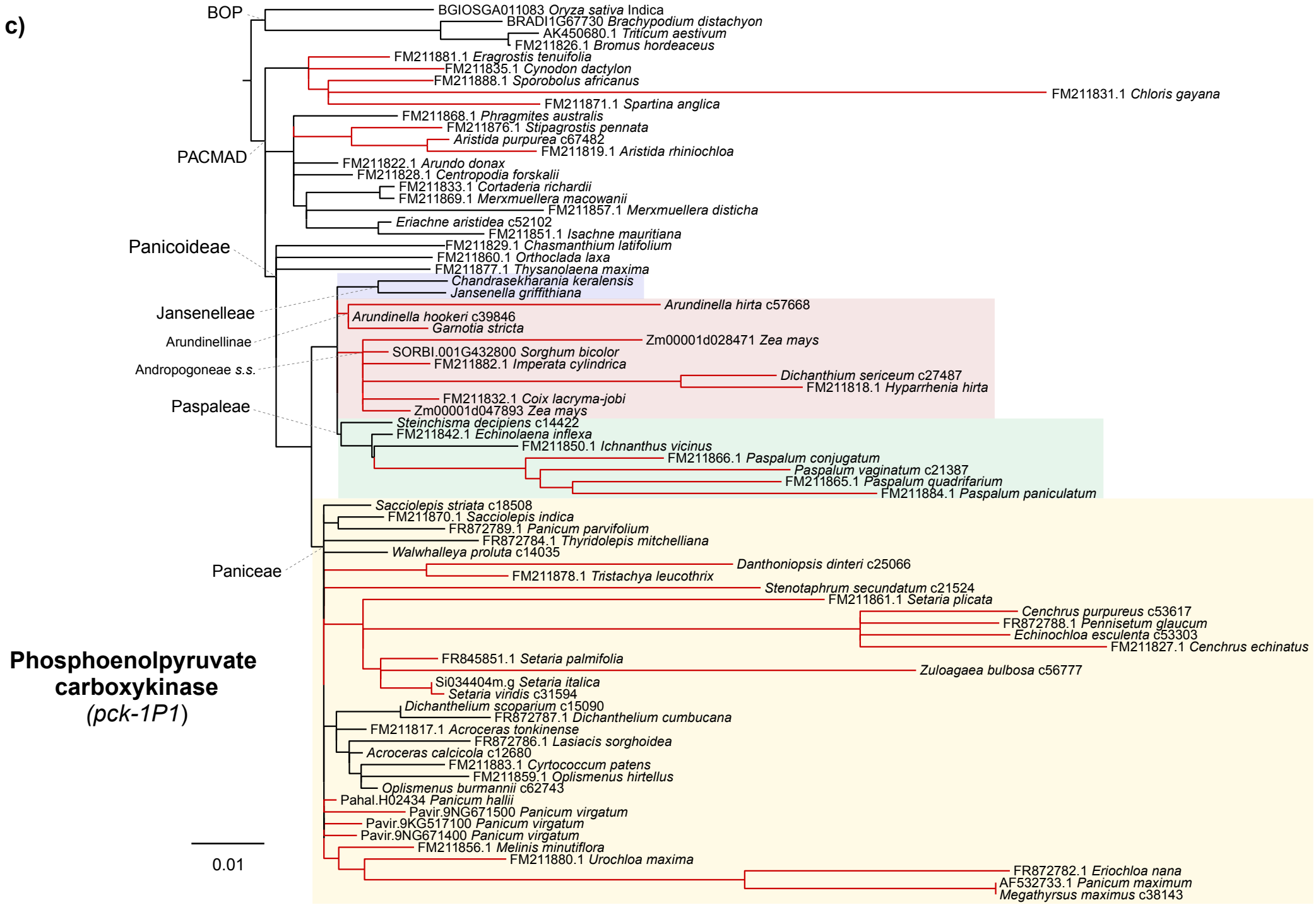
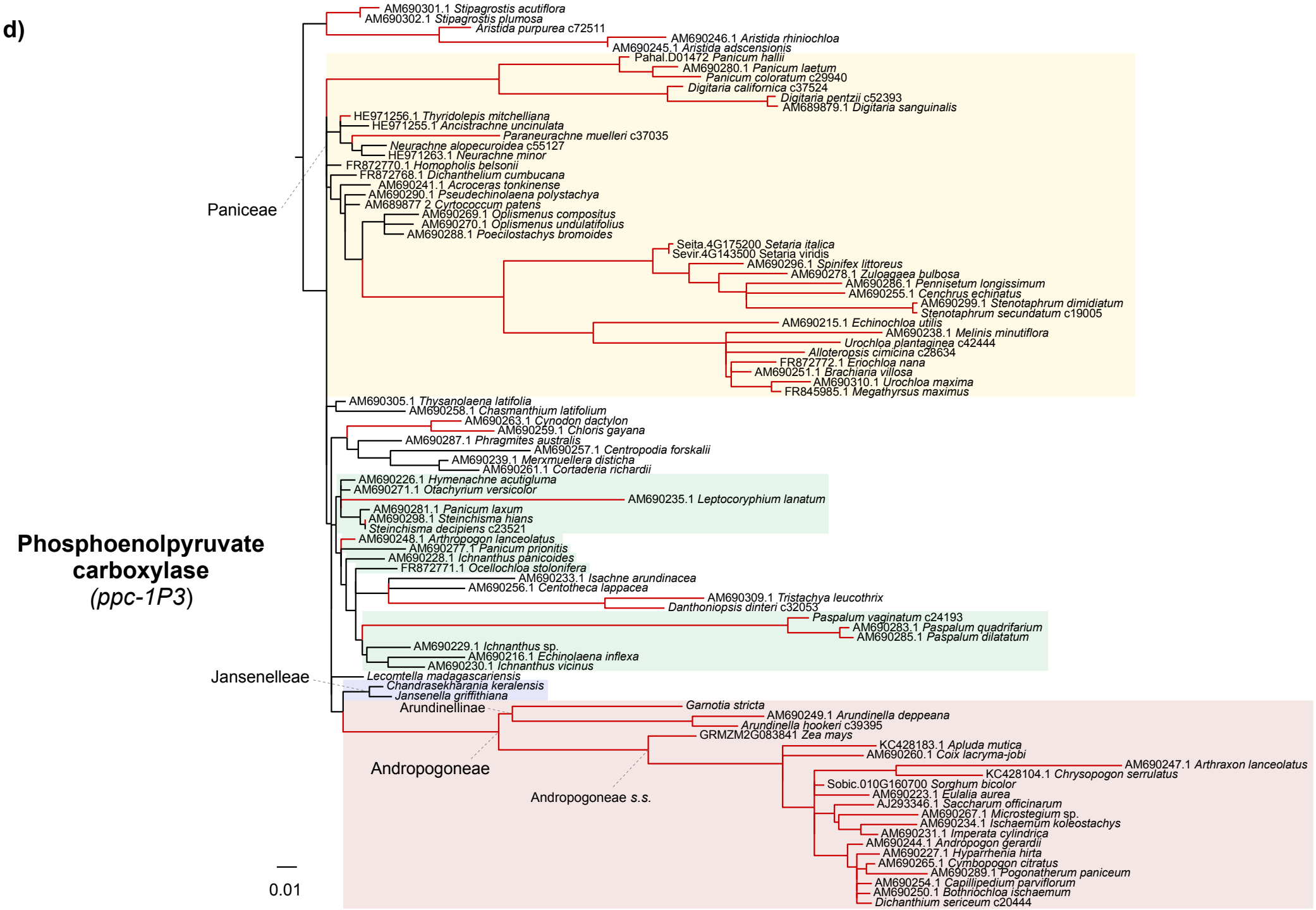
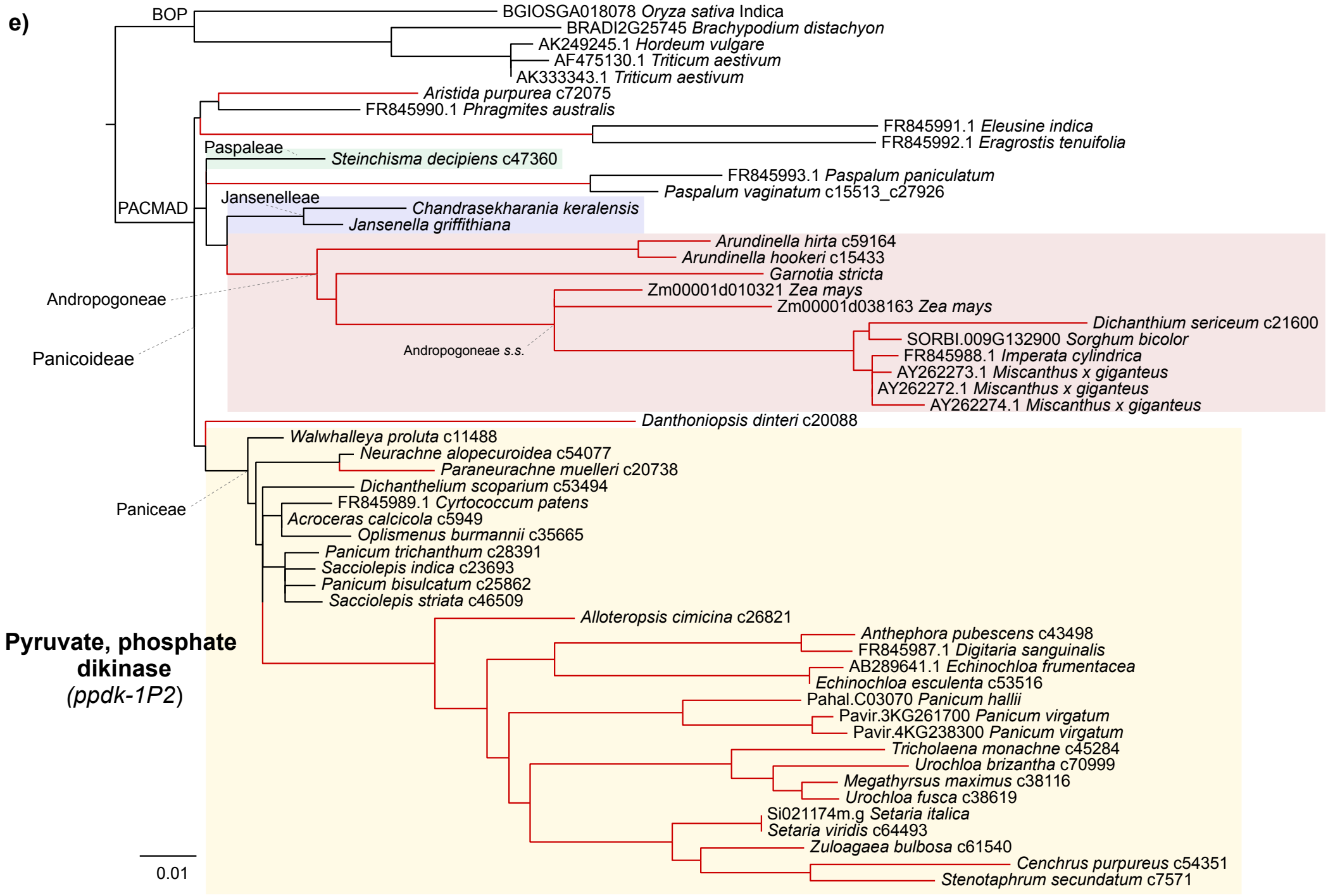
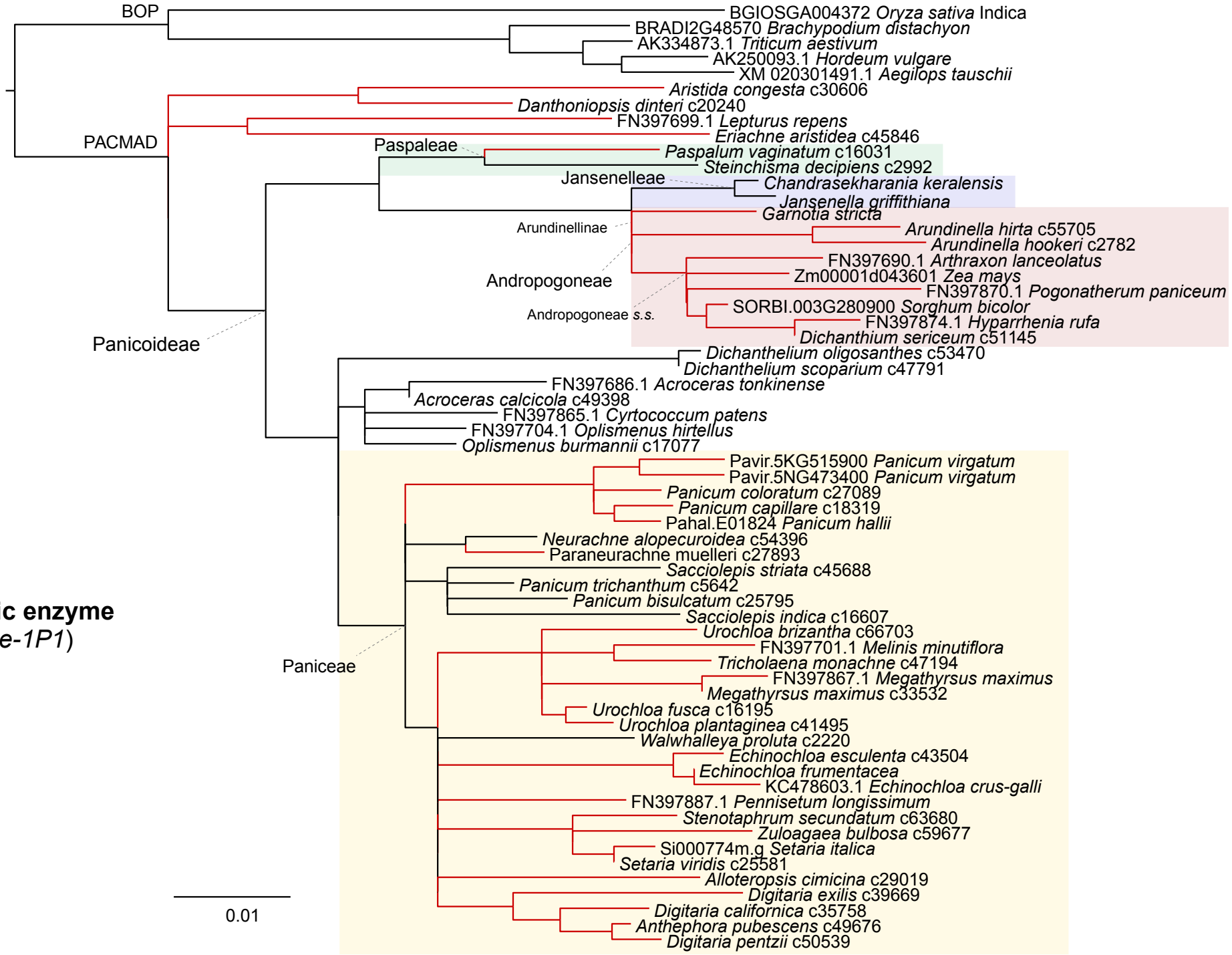

c)
BOP
PACMAD
Panicoideae
Jansenelleae
Arundinellinae
Andropogoneae s.s.
Paspaleae
Paniceae
BGIOSGA011083 Oryza sativa Indica
BRADI1G67730 Brachypodium distachyon
AK450680.1 Triticum aestivum
FM211826.1 Bromus hordeaceus
FM211881.1 Eragrostis tenuifolia
FM211835.1 Cynodon dactylon
FM211888.1 Sporobolus africanus
FM211831.1 Chloris gayana
FM211871.1 Spartina anglica
FM211868.1 Phragmites australis
FM211876.1 Stipagrostis pennata
Aristida purpurea c67482
FM211819.1 Aristida rhiniochloa
FM211822.1 Arundo donax
FM211828.1 Centropodia forskalii
FM211833.1 Cortaderia richardii
FM211869.1 Merxmuellera macowanii
FM211857.1 Merxmuellera disticha
Eriachne aristidea c52102
FM211851.1 Isachne mauritiana
FM211829.1 Chasmanthium latifolium
FM211860.1 Orthoclada laxa
FM211877.1 Thysanolaena maxima
Chandrasekharania keralensis
Jansenella griffithiana
Arundinella hirta c57668
Arundinella hookeri c39846
Garnotia stricta
Zm00001d028471 Zea mays
SORBI.001G432800 Sorghum bicolor
FM211882.1 Imperata cylindrica
Dichanthium sericeum c27487
FM211818.1 Hyparrhenia hirta
FM211832.1 Coix lacryma-jobi
Zm00001d047893 Zea mays
Steinchisma decipiens c14422
FM211842.1 Echinolaena inflexa
FM211850.1 Ichnanthus vicinus
FM211866.1 Paspalum conjugatum
Paspalum vaginatum c21387
FM211865.1 Paspalum quadrifarium
FM211884.1 Paspalum paniculatum
Sacciolepis striata c18508
FM211870.1 Sacciolepis indica
FR872789.1 Panicum parvifolium
FR872784.1 Thyridolepis mitchelliana
Walwhalleya proluta c14035
Danthoniopsis dinteri c25066
FM211878.1 Tristachya leucothrix
Stenotaphrum secundatum c21524
FM211861.1 Setaria plicata
Cenchrus purpureus c53617
FR872788.1 Pennisetum glaucum
Echinochloa esculenta c53303
FM211827.1 Cenchrus echinatus
FR845851.1 Setaria palmifolia
Zuloagaea bulbosa c56777
Si034404m.g Setaria italica
Setaria viridis c31594
Dichanthelium scoparium c15090
FR872787.1 Dichanthelium cumbucana
FM211817.1 Acroceras tonkinense
FR872786.1 Lasiacis sorghoidea
Acroceras calcicola c12680
FM211883.1 Cyrtococcum patens
FM211859.1 Oplismenus hirtellus
Oplismenus burmannii c62743
Pahal.H02434 Panicum hallii
Pavir.9NG671500 Panicum virgatum
Pavir.9KG517100 Panicum virgatum
Pavir.9NG671400 Panicum virgatum
FM211856.1 Melinis minutiflora
FM211880.1 Urochloa maxima
FR872782.1 Eriochloa nana
AF532733.1 Panicum maximum
Megathyrsus maximus c38143
0.01
Phosphoenolpyruvate carboxykinase
(pck-1P1)

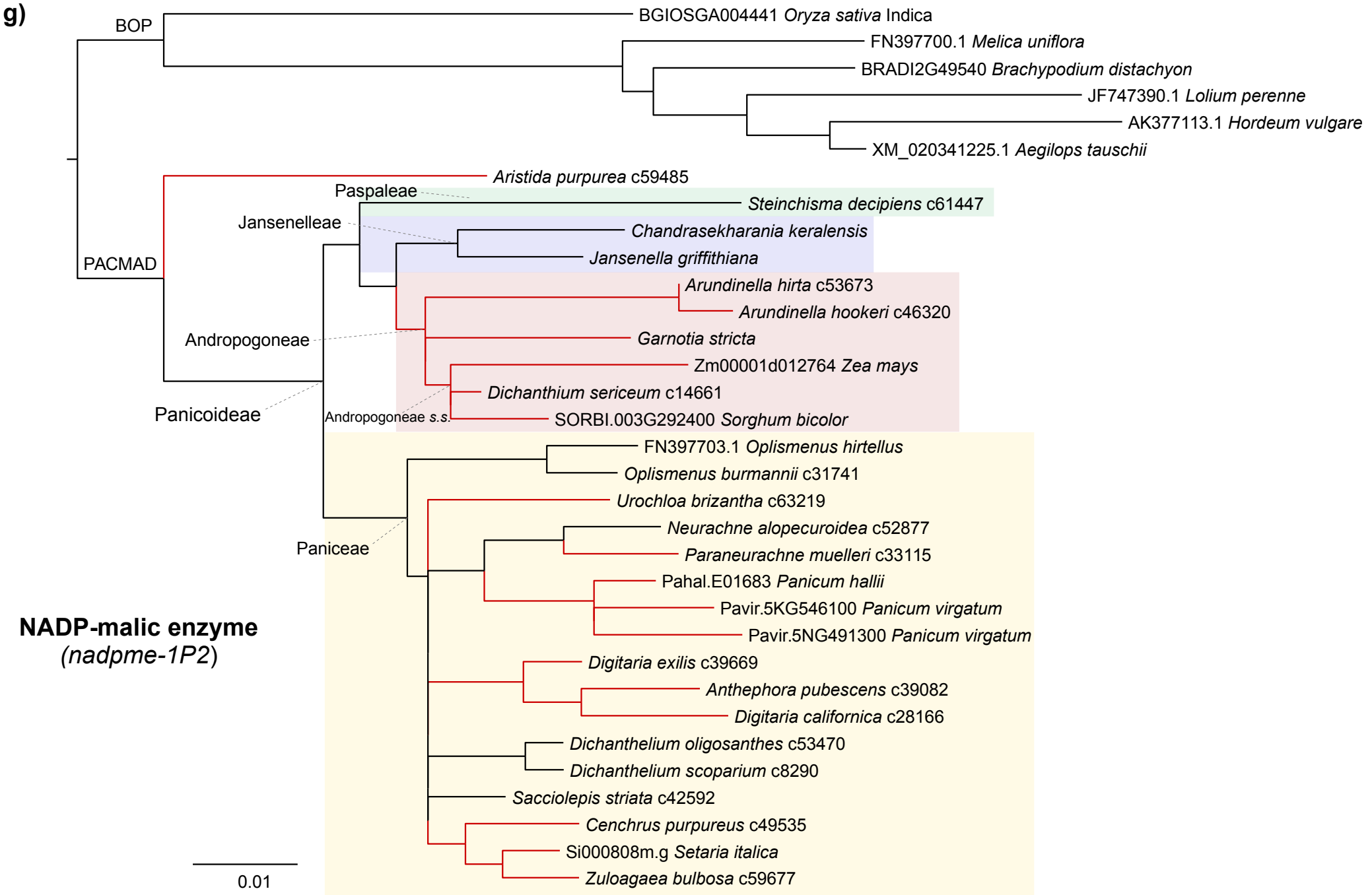

d)
Phosphoenolpyruvate carboxylase (ppc-1P3)
Paniceae
Jansenelleae
Arundinellinae
Andropogoneae
Andropogoneae s.s.
0.01
AM690301.1 Stipagrostis acutiflora
AM690302.1 Stipagrostis plumosa
Aristida purpurea c72511
AM690246.1 Aristida rhiniochloa
AM690245.1 Aristida adscensionis
Pahal.D01472 Panicum hallii
AM690280.1 Panicum laetum
Panicum coloratum c29940
Digitaria californica c37524
Digitaria pentzii c52393
AM689879.1 Digitaria sanguinalis
HE971256.1 Thyridolepis mitchelliana
HE971255.1 Ancistrachne uncinulata
Paraneurachne muelleri c37035
Neurachne alopecuroidea c55127
HE971263.1 Neurachne minor
FR872770.1 Homopholis belsonii
FR872768.1 Dichanthelium cumbucana
AM690241.1 Acroceras tonkinense
AM690290.1 Pseudechinolaena polystachya
AM689877 2 Cyrtococcum patens
AM690269.1 Oplismenus compositus
AM690270.1 Oplismenus undulatifolius
AM690288.1 Poecilostachys bromoides
Seita.4G175200 Setaria italica
Sevir.4G143500 Setaria viridis
AM690296.1 Spinifex littoreus
AM690278.1 Zuloagaea bulbosa
AM690286.1 Pennisetum longissimum
AM690255.1 Cenchrus echinatus
AM690299.1 Stenotaphrum dimidiatum
Stenotaphrum secundatum c19005
AM690215.1 Echinochloa utilis
AM690238.1 Melinis minutiflora
Urochloa plantaginea c42444
Alloteropsis cimicina c28634
FR872772.1 Eriochloa nana
AM690251.1 Brachiaria villosa
AM690310.1 Urochloa maxima
FR845985.1 Megathyrsus maximus
AM690305.1 Thysanolaena latifolia
AM690258.1 Chasmanthium latifolium
AM690263.1 Cynodon dactylon
AM690259.1 Chloris gayana
AM690287.1 Phragmites australis
AM690257.1 Centropodia forskalii
AM690239.1 Merxmuellera disticha
AM690261.1 Cortaderia richardii
AM690226.1 Hymenachne acutigluma
AM690271.1 Otachyrium versicolor
AM690235.1 Leptocoryphium lanatum
AM690281.1 Panicum laxum
AM690298.1 Steinchisma hians
Steinchisma decipiens c23521
AM690248.1 Arthropogon lanceolatus
AM690277.1 Panicum prionitis
AM690228.1 Ichnanthus panicoides
FR872771.1 Ocellochloa stolonifera
AM690233.1 Isachne arundinacea
AM690256.1 Centotheca lappacea
AM690309.1 Tristachya leucothrix
Danthoniopsis dinteri c32053
Paspalum vaginatum c24193
AM690283.1 Paspalum quadrifarium
AM690285.1 Paspalum dilatatum
AM690229.1 Ichnanthus sp.
AM690216.1 Echinolaena inflexa
AM690230.1 Ichnanthus vicinus
Lecomtella madagascariensis
Chandrasekharania keralensis
Jansenella griffithiana
Garnotia stricta
AM690249.1 Arundinella deppeana
Arundinella hookeri c39395
GRMZM2G083841 Zea mays
KC428183.1 Apluda mutica
AM690260.1 Coix lacryma-jobi
AM690247.1 Arthraxon lanceolatus
KC428104.1 Chrysopogon serrulatus
Sobic.010G160700 Sorghum bicolor
AM690223.1 Eulalia aurea
AJ293346.1 Saccharum officinarum
AM690267.1 Microstegium sp.
AM690234.1 Ischaemum koleostachys
AM690231.1 Imperata cylindrica
AM690244.1 Andropogon gerardii
AM690227.1 Hyparrhenia hirta
AM690265.1 Cymbopogon citratus
AM690289.1 Pogonatherum paniceum
AM690254.1 Capillipedium parviflorum
AM690250.1 Bothriochloa ischaemum
Dichanthium sericeum c20444

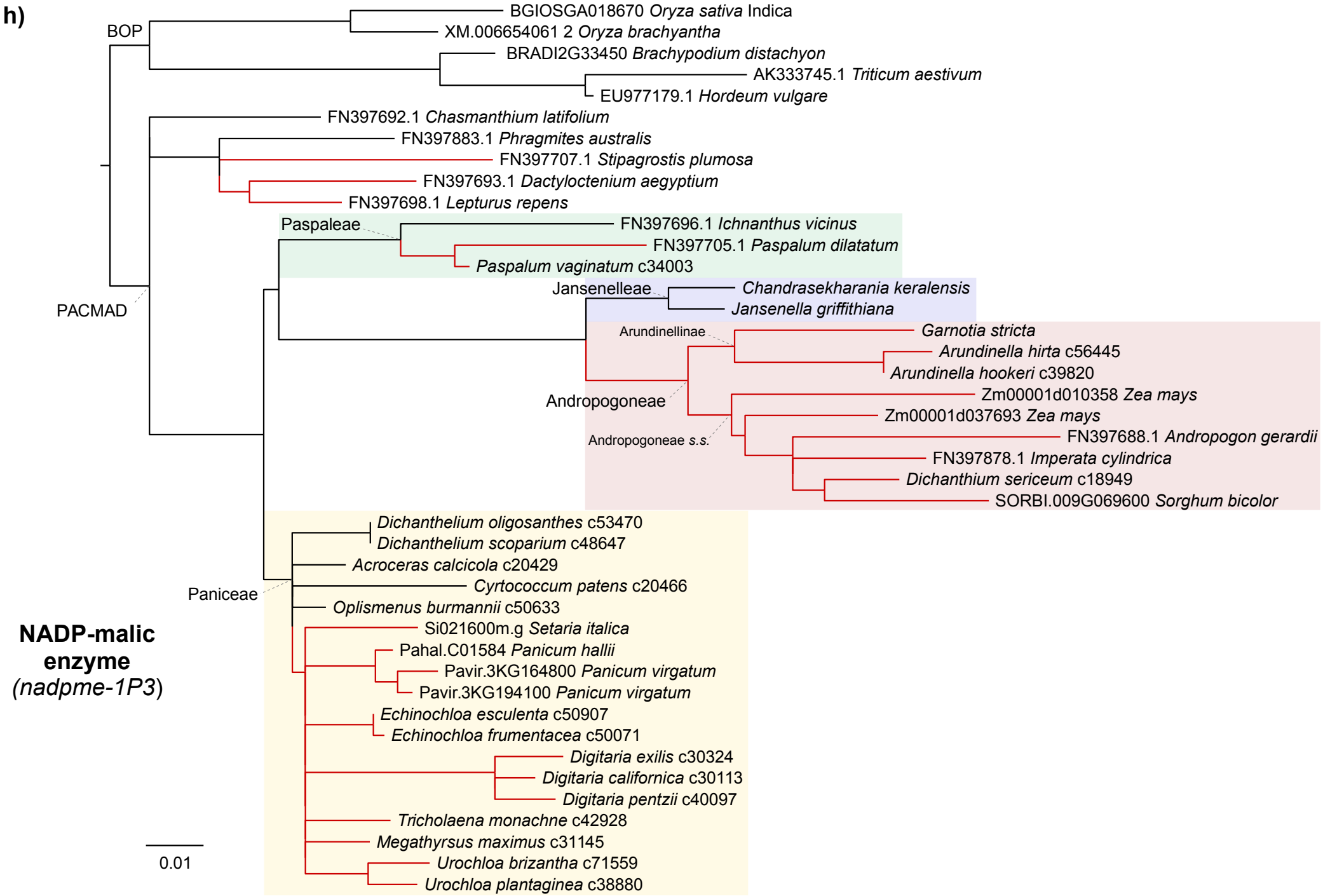

e)
BOP
BGIOSGA018078 Oryza sativa Indica
BRADI2G25745 Brachypodium distachyon
AK249245.1 Hordeum vulgare
AF475130.1 Triticum aestivum
AK333343.1 Triticum aestivum
Aristida purpurea c72075
FR845990.1 Phragmites australis
FR845991.1 Eleusine indica
FR845992.1 Eragrostis tenuifolia
Paspaleae
Steinchisma decipiens c47360
FR845993.1 Paspalum paniculatum
Paspalum vaginatum c15513_c27926
Jansenelleae
PACMAD
Chandrasekharania keralensis
Jansenella griffithiana
Arundinella hirta c59164
Arundinella hookeri c15433
Garnotia stricta
Andropogoneae
Zm00001d010321 Zea mays
Zm00001d038163 Zea mays
Dichanthium sericeum c21600
Andropogoneae s.s.
SORBI.009G132900 Sorghum bicolor
FR845988.1 Imperata cylindrica
AY262273.1 Miscanthus x giganteus
AY262272.1 Miscanthus x giganteus
AY262274.1 Miscanthus x giganteus
Panicoideae
Danthoniopsis dinteri c20088
Walwhalleya proluta c11488
Neurachne alopecuroidea c54077
Paraneurachne muelleri c20738
Dichanthelium scoparium c53494
Paniceae
FR845989.1 Cyrtococcum patens
Acroceras calcicola c5949
Oplismenus burmannii c35665
Panicum trichanthum c28391
Sacciolepis indica c23693
Panicum bisulcatum c25862
Sacciolepis striata c46509
Alloteropsis cimicina c26821
Anthephora pubescens c43498
FR845987.1 Digitaria sanguinalis
AB289641.1 Echinochloa frumentacea
Echinochloa esculenta c53516
Pahal.C03070 Panicum hallii
Pavir.3KG261700 Panicum virgatum
Pavir.4KG238300 Panicum virgatum
Tricholaena monachne c45284
Urochloa brizantha c70999
Megathyrsus maximus c38116
Urochloa fusca c38619
Si021174m.g Setaria italica
Setaria viridis c64493
Zuloagaea bulbosa c61540
Cenchrus purpureus c54351
Stenotaphrum secundatum c7571
Pyruvate, phosphate dikinase
(ppdk-1P2)
0.01

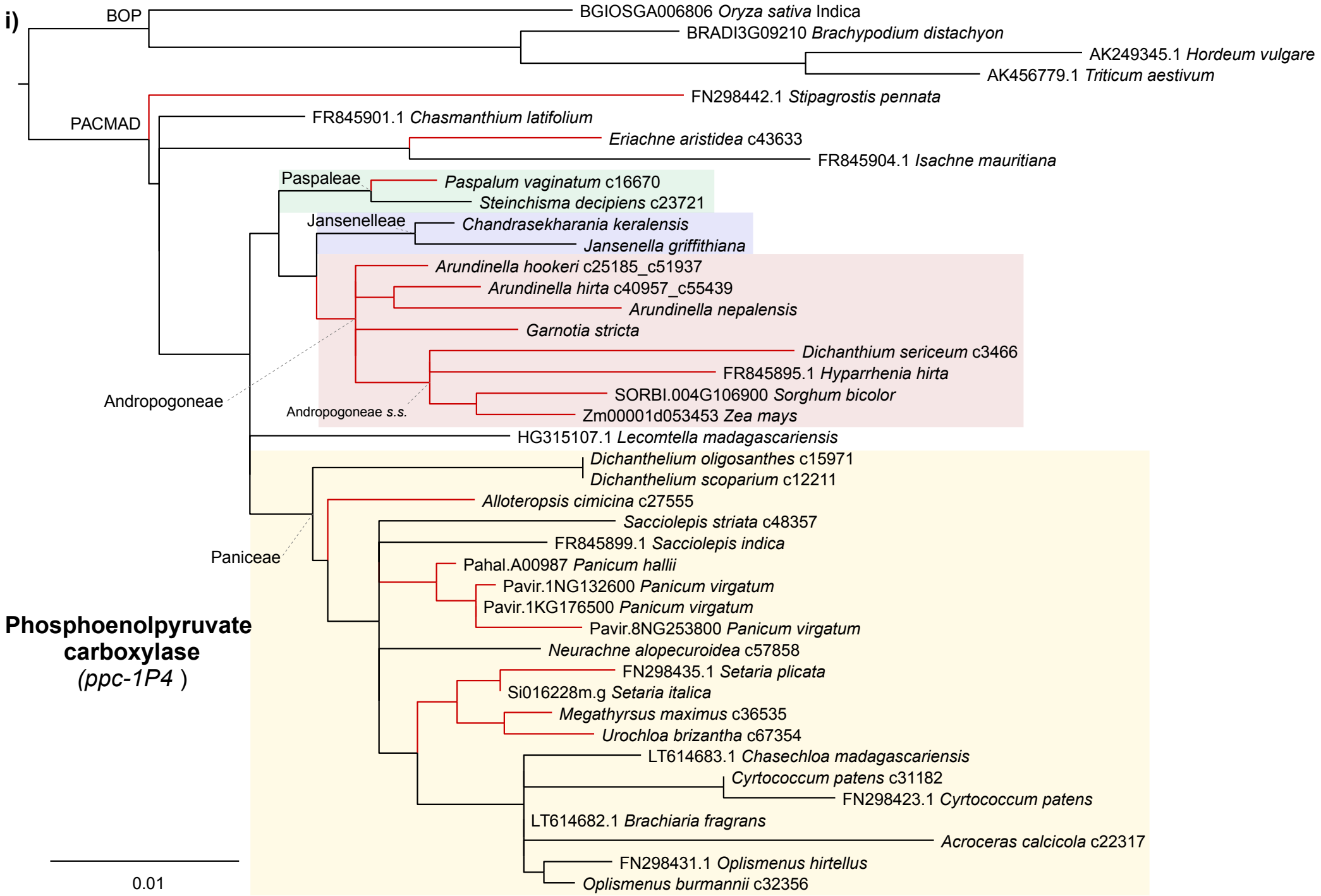
f)



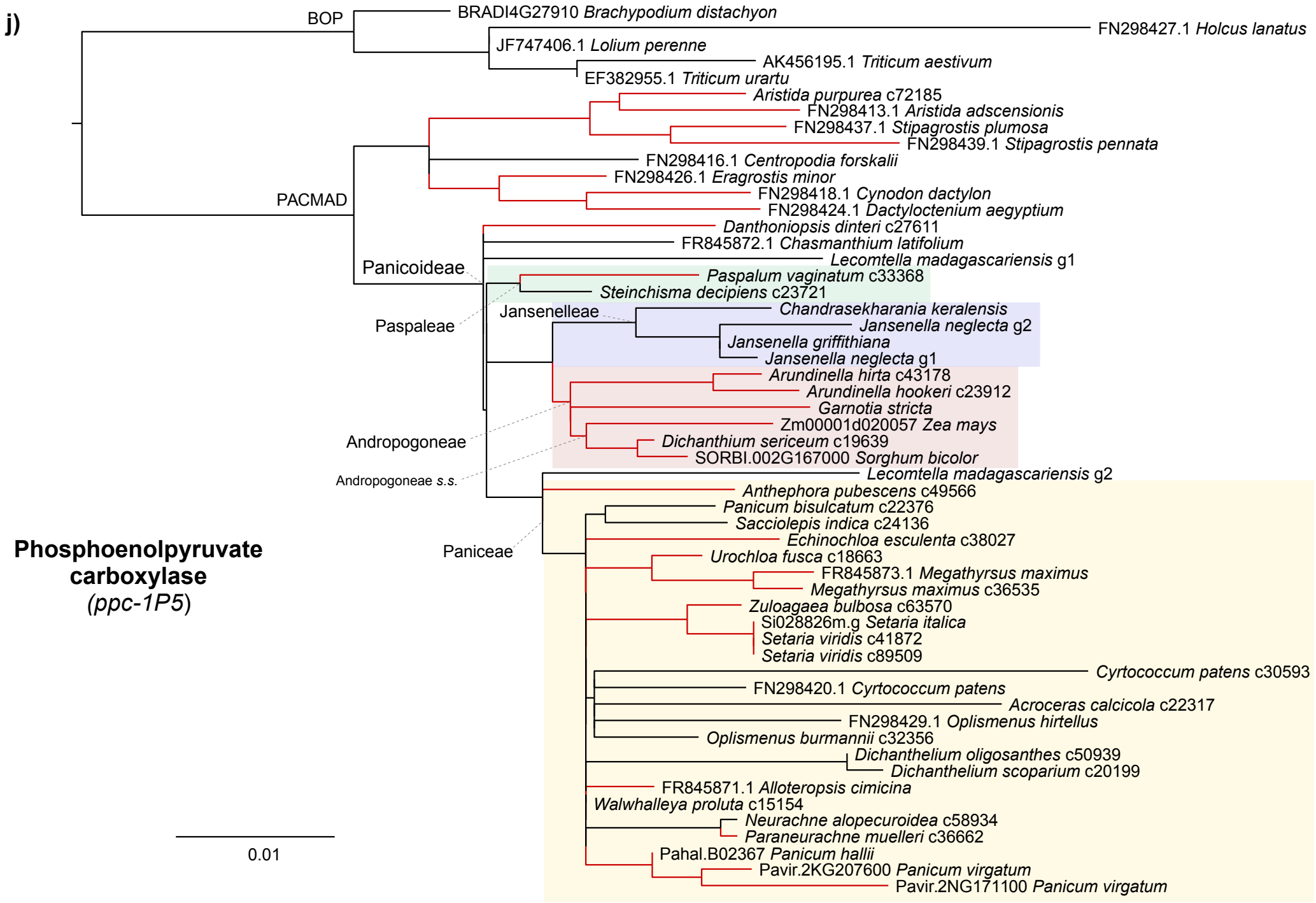
NADP-malic enzyme
(nadpme-1P1)

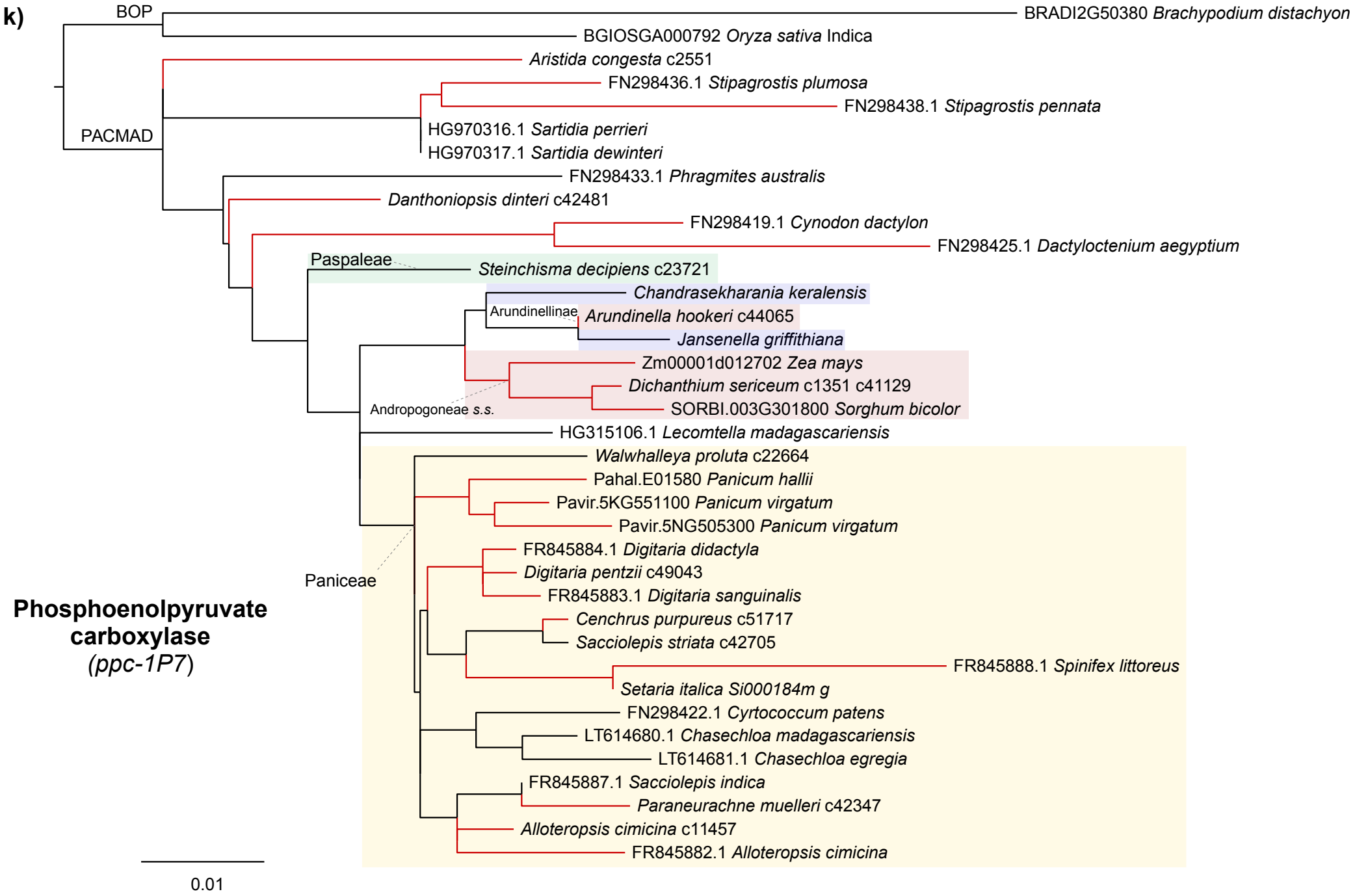

g)
BOP
PACMAD
BGIOSGA004441 Oryza sativa Indica
FN397700.1 Melica uniflora
BRADI2G49540 Brachypodium distachyon
JF747390.1 Lolium perenne
AK377113.1 Hordeum vulgare
XM_020341225.1 Aegilops tauschii
Aristida purpurea c59485
Paspaleae
Steinchisma decipiens c61447
Jansenelleae
Chandrasekharania keralensis
Jansenella griffithiana
Arundinella hirta c53673
Arundinella hookeri c46320
Andropogoneae
Garnotia stricta
Zm00001d012764 Zea mays
Dichanthium sericeum c14661
Panicoideae
Andropogoneae s.s.
SORBI.003G292400 Sorghum bicolor
FN397703.1 Oplismenus hirtellus
Oplismenus burmannii c31741
Urochloa brizantha c63219
Neurachne alopecuroidea c52877
Paraneurachne muelleri c33115
Paniceae
Pahal.E01683 Panicum hallii
Pavir.5KG546100 Panicum virgatum
Pavir.5NG491300 Panicum virgatum
Digitaria exilis c39669
Anthephora pubescens c39082
Digitaria californica c28166
Dichanthelium oligosanthes c53470
Dichanthelium scoparium c8290
Sacciolepis striata c42592
Cenchrus purpureus c49535
Si000808m.g Setaria italica
Zuloagaea bulbosa c59677
NADP-malic enzyme
(nadpme-1P2)
0.01


h)
BOP
PACMAD
BGIOSGA018670 Oryza sativa Indica
XM.006654061 2 Oryza brachyantha
BRADI2G33450 Brachypodium distachyon
AK333745.1 Triticum aestivum
EU977179.1 Hordeum vulgare
FN397692.1 Chasmanthium latifolium
FN397883.1 Phragmites australis
FN397707.1 Stipagrostis plumosa
FN397693.1 Dactyloctenium aegyptium
FN397698.1 Lepturus repens
Paspaleae
FN397696.1 Ichnanthus vicinus
FN397705.1 Paspalum dilatatum
Paspalum vaginatum c34003
Jansenelleae
Chandrasekharania keralensis
Jansenella griffithiana
Arundinellinae
Garnotia stricta
Arundinella hirta c56445
Arundinella hookeri c39820
Andropogoneae
Zm00001d010358 Zea mays
Zm00001d037693 Zea mays
Andropogoneae s.s.
FN397688.1 Andropogon gerardii
FN397878.1 Imperata cylindrica
Dichanthium sericeum c18949
SORBI.009G069600 Sorghum bicolor
Paniceae
Dichanthelium oligosanthes c53470
Dichanthelium scoparium c48647
Acroceras calcicola c20429
Cyrtococcum patens c20466
Oplismenus burmannii c50633
Si021600m.g Setaria italica
Pahal.C01584 Panicum hallii
Pavir.3KG164800 Panicum virgatum
Pavir.3KG194100 Panicum virgatum
Echinochloa esculenta c50907
Echinochloa frumentacea c50071
Digitaria exilis c30324
Digitaria californica c30113
Digitaria pentzii c40097
Tricholaena monachne c42928
Megathyrsus maximus c31145
Urochloa brizantha c71559
Urochloa plantaginea c38880
NADP-malic enzyme (nadpme-1P3)
0.01

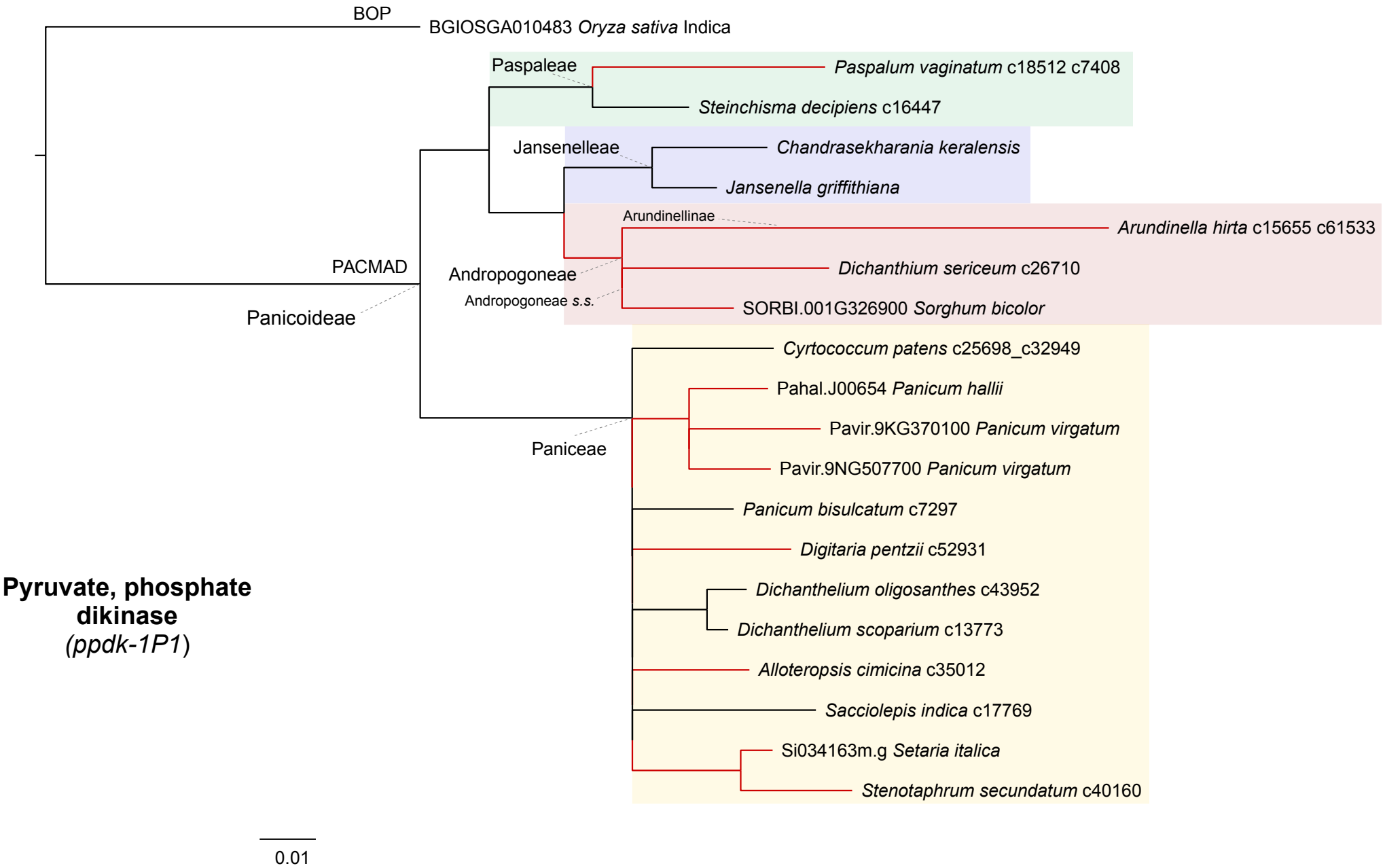


Phosphoenolpyruvate carboxylase
(ppc-1P4)

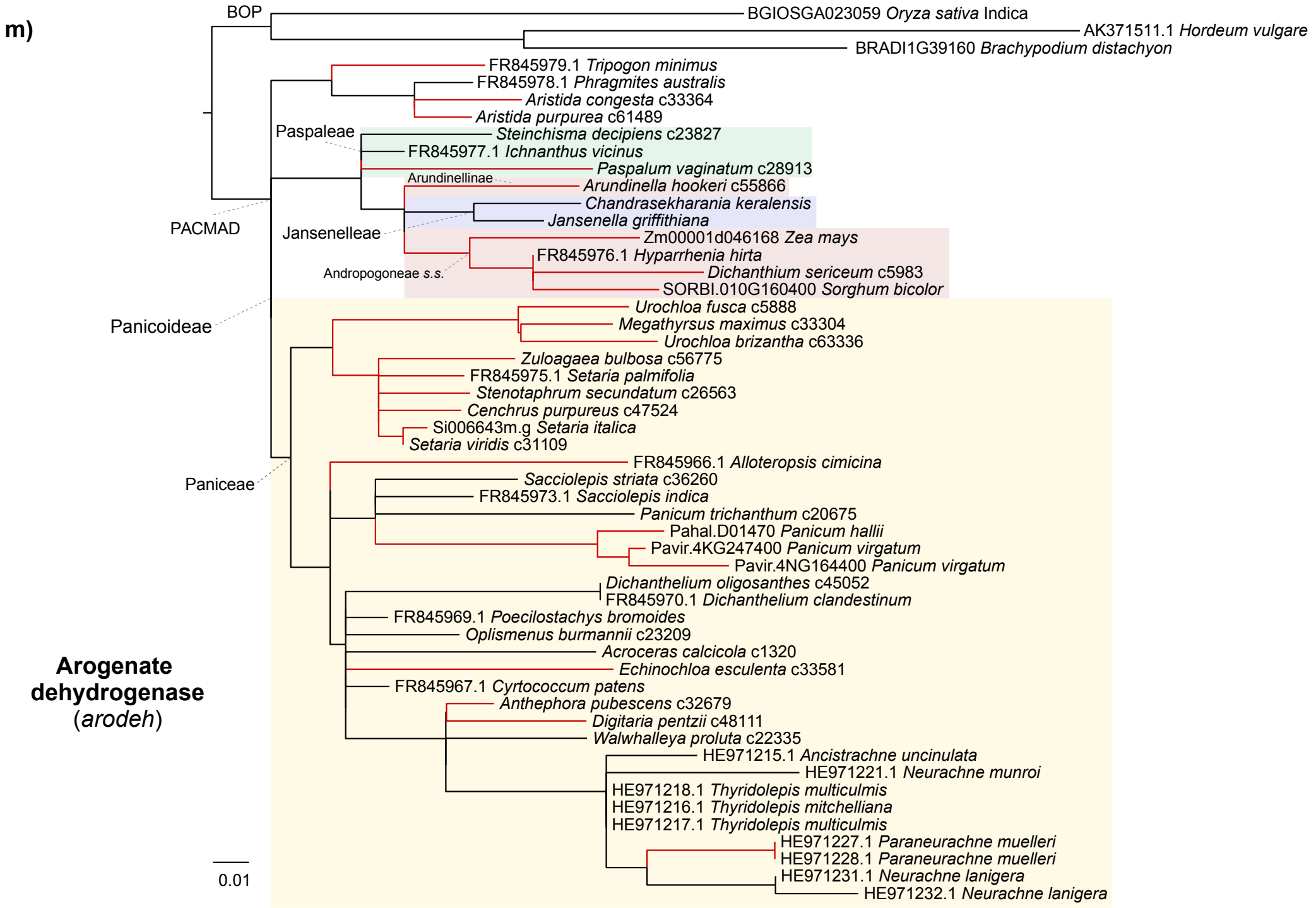

j)
BOP
PACMAD
Panicoideae
Paspaleae
Andropogoneae
Andropogoneae s.s.
Paniceae
Jansenelleae
BRADI4G27910 Brachypodium distachyon
FN298427.1 Holcus lanatus
JF747406.1 Lolium perenne
AK456195.1 Triticum aestivum
EF382955.1 Triticum urartu
Aristida purpurea c72185
FN298413.1 Aristida adscensionis
FN298437.1 Stipagrostis plumosa
FN298439.1 Stipagrostis pennata
FN298416.1 Centropodia forskalii
FN298426.1 Eragrostis minor
FN298418.1 Cynodon dactylon
FN298424.1 Dactyloctenium aegyptium
Danthoniopsis dinteri c27611
FR845872.1 Chasmanthium latifolium
Lecomtella madagascariensis g1
Paspalum vaginatum c33368
Steinchisma decipiens c23721
Chandrasekharania keralensis
Jansenella neglecta g2
Jansenella griffithiana
Jansenella neglecta g1
Arundinella hirta c43178
Arundinella hookeri c23912
Garnotia stricta
Zm00001d020057 Zea mays
Dichanthium sericeum c19639
SORBI.002G167000 Sorghum bicolor
Lecomtella madagascariensis g2
Anthephora pubescens c49566
Panicum bisulcatum c22376
Sacciolepis indica c24136
Echinochloa esculenta c38027
Urochloa fusca c18663
FR845873.1 Megathyrsus maximus
Megathyrsus maximus c36535
Zuloagaea bulbosa c63570
Si028826m.g Setaria italica
Setaria viridis c41872
Setaria viridis c89509
Cyrtococcum patens c30593
FN298420.1 Cyrtococcum patens
Acroceras calcicola c22317
FN298429.1 Oplismenus hirtellus
Oplismenus burmannii c32356
Dichanthelium oligosanthes c50939
Dichanthelium scoparium c20199
FR845871.1 Alloteropsis cimicina
Walwhalleya proluta c15154
Neurachne alopecuroidea c58934
Paraneurachne muelleri c36662
Pahal.B02367 Panicum hallii
Pavir.2KG207600 Panicum virgatum
Pavir.2NG171100 Panicum virgatum
Phosphoenolpyruvate carboxylase
(ppc-1P5)
0.01

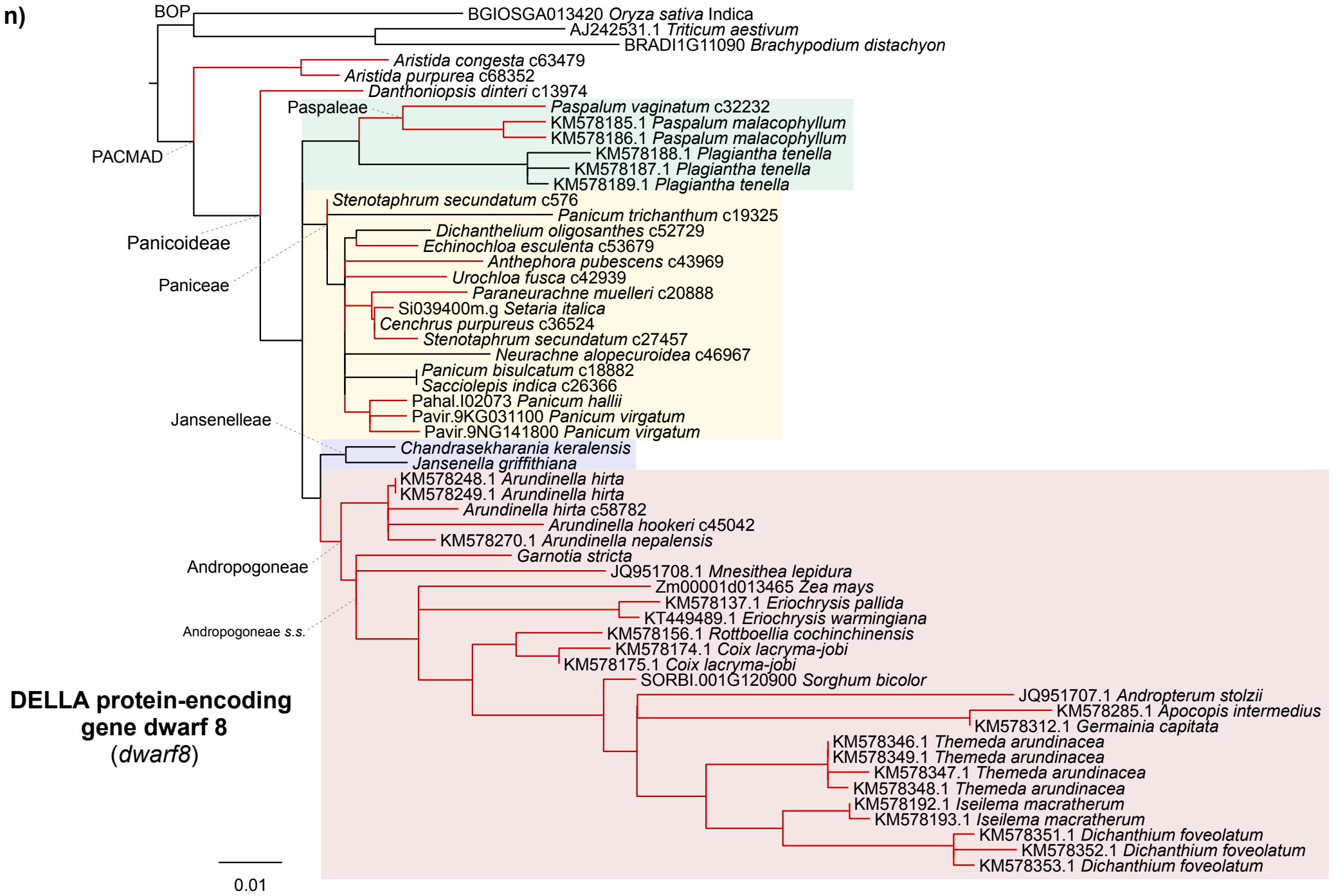

k)
BOP
PACMAD
BRADI2G50380 Brachypodium distachyon
BGIOSGA000792 Oryza sativa Indica
Aristida congesta c2551
FN298436.1 Stipagrostis plumosa
FN298438.1 Stipagrostis pennata
HG970316.1 Sartidia perrieri
HG970317.1 Sartidia dewinteri
FN298433.1 Phragmites australis
Danthoniopsis dinteri c42481
FN298419.1 Cynodon dactylon
FN298425.1 Dactyloctenium aegyptium
Paspaleae
Steinchisma decipiens c23721
Chandrasekharania keralensis
Arundinellinae
Arundinella hookeri c44065
Jansenella griffithiana
Zm00001d012702 Zea mays
Dichanthium sericeum c1351 c41129
Andropogoneae s.s.
SORBI.003G301800 Sorghum bicolor
HG315106.1 Lecomtella madagascariensis
Walwhalleya proluta c22664
Pahal.E01580 Panicum hallii
Pavir.5KG551100 Panicum virgatum
Pavir.5NG505300 Panicum virgatum
FR845884.1 Digitaria didactyla
Digitaria pentzii c49043
FR845883.1 Digitaria sanguinalis
Paniceae
Cenchrus purpureus c51717
Sacciolepis striata c42705
FR845888.1 Spinifex littoreus
Setaria italica Si000184m g
FN298422.1 Cyrtococcum patens
LT614680.1 Chasechloa madagascariensis
LT614681.1 Chasechloa egregia
FR845887.1 Sacciolepis indica
Paraneurachne muelleri c42347
Alloteropsis cimicina c11457
FR845882.1 Alloteropsis cimicina
Phosphoenolpyruvate carboxylase (ppc-1P7)
0.01

I)



Pyruvate, phosphate dikinase
(ppdk-1P1)


m)
BOP
BGIOSGA023059 Oryza sativa Indica
AK371511.1 Hordeum vulgare
BRADI1G39160 Brachypodium distachyon
FR845979.1 Tripogon minimus
FR845978.1 Phragmites australis
Aristida congesta c33364
Aristida purpurea c61489
Paspaleae
Steinchisma decipiens c23827
FR845977.1 Ichnanthus vicinus
Paspalum vaginatum c28913
Arundinellinae
Arundinella hookeri c55866
Chandrasekharania keralensis
Jansenella griffithiana
PACMAD
Jansenelleae
Zm00001d046168 Zea mays
FR845976.1 Hyparrhenia hirta
Andropogoneae s.s.
Dichanthium sericeum c5983
SORBI.010G160400 Sorghum bicolor
Panicoideae
Urochloa fusca c5888
Megathyrsus maximus c33304
Urochloa brizantha c63336
Zuloagaea bulbosa c56775
FR845975.1 Setaria palmifolia
Stenotaphrum secundatum c26563
Cenchrus purpureus c47524
Si006643m.g Setaria italica
Setaria viridis c31109
FR845966.1 Alloteropsis cimicina
Paniceae
Sacciolepis striata c36260
FR845973.1 Sacciolepis indica
Panicum trichanthum c20675
Pahal.D01470 Panicum hallii
Pavir.4KG247400 Panicum virgatum
Pavir.4NG164400 Panicum virgatum
Dichanthelium oligosanthes c45052
FR845970.1 Dichanthelium clandestinum
FR845969.1 Poecilostachys bromoides
Oplismenus burmannii c23209
Acroceras calcicola c1320
Echinochloa esculenta c33581
FR845967.1 Cyrtococcum patens
Anthephora pubescens c32679
Digitaria pentzii c48111
Walwhalleya proluta c22335
HE971215.1 Ancistrachne uncinulata
HE971221.1 Neurachne munroi
HE971218.1 Thyridolepis multiculmis
HE971216.1 Thyridolepis mitchelliana
HE971217.1 Thyridolepis multiculmis
HE971227.1 Paraneurachne muelleri
HE971228.1 Paraneurachne muelleri
HE971231.1 Neurachne lanigera
HE971232.1 Neurachne lanigera
Arogenate dehydrogenase (arodeh)
0.01


n)
BOP
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BRADI1G11090 Brachypodium distachyon
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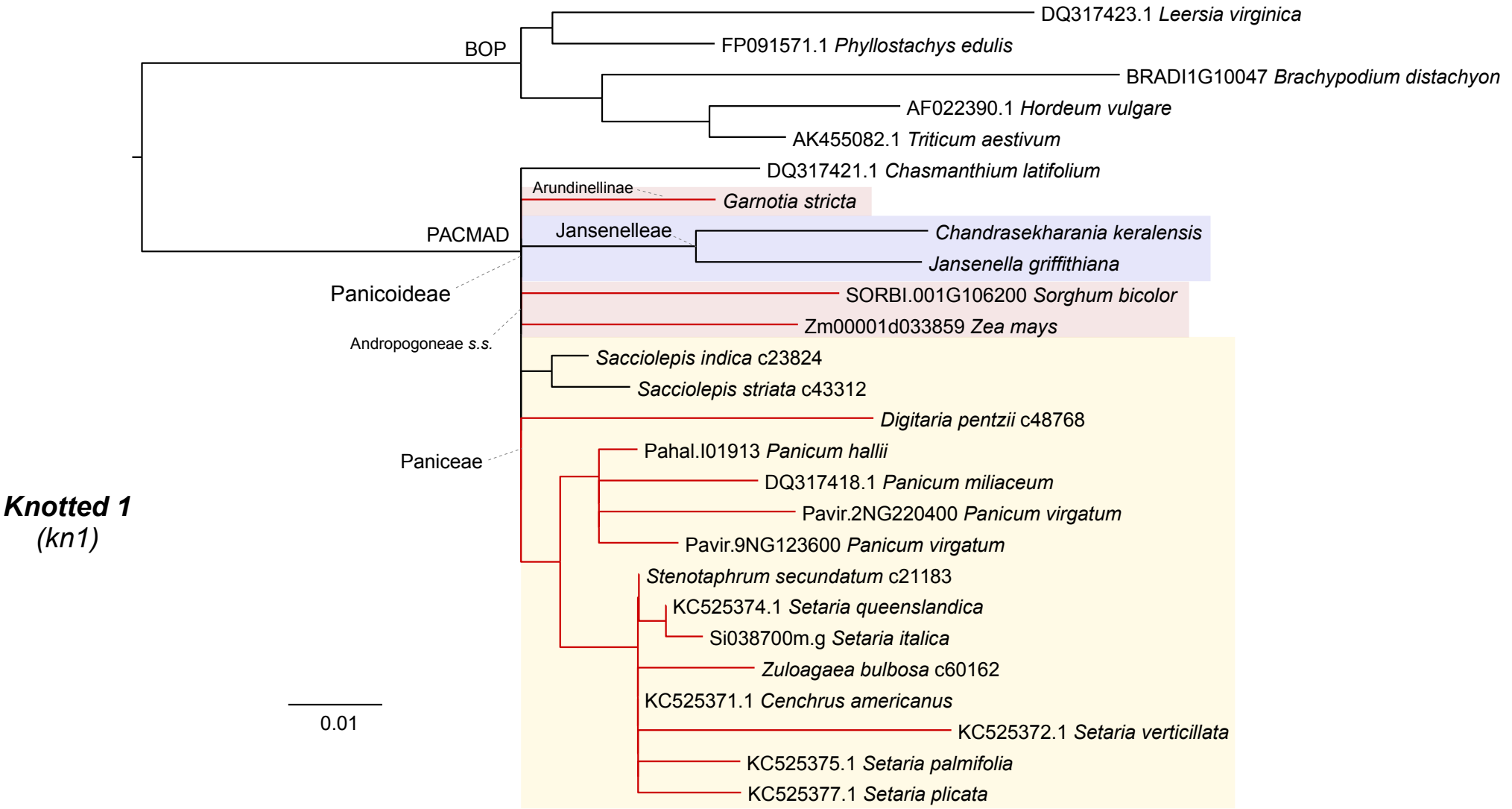
Aristida purpurea c68352
Danthoniopsis dinteri c13974
Paspaleae
Paspalum vaginatum c32232
KM578185.1 Paspalum malacophyllum
KM578186.1 Paspalum malacophyllum
KM578188.1 Plagiantha tenella
KM578187.1 Plagiantha tenella
KM578189.1 Plagiantha tenella
PACMAD
Stenotaphrum secundatum c576
Panicum trichanthum c19325
Dichanthelium oligosanthes c52729
Echinochloa esculenta c53679
Anthephora pubescens c43969
Urochloa fusca c42939
Paraneurachne muelleri c20888
Si039400m.g Setaria italica
Cenchrus purpureus c36524
Stenotaphrum secundatum c27457
Neurachne alopecuroidea c46967
Panicum bisulcatum c18882
Sacciolepis indica c26366
Pahal.I02073 Panicum hallii
Pavir.9KG031100 Panicum virgatum
Pavir.9NG141800 Panicum virgatum
Panicoideae
Paniceae
Jansenelleae
Chandrasekharania keralensis
Jansenella griffithiana
KM578248.1 Arundinella hirta
KM578249.1 Arundinella hirta
Arundinella hirta c58782
Arundinella hookeri c45042
KM578270.1 Arundinella nepalensis
Garnotia stricta
JQ951708.1 Mnesithea lepidura
Zm00001d013465 Zea mays
KM578137.1 Eriochrysis pallida
KT449489.1 Eriochrysis warmingiana
KM578156.1 Rottboellia cochinchinensis
KM578174.1 Coix lacryma-jobi
KM578175.1 Coix lacryma-jobi
SORBI.001G120900 Sorghum bicolor
Andropogoneae
Andropogoneae s.s.
JQ951707.1 Andropterum stolzii
KM578285.1 Apocopis intermedius
KM578312.1 Germainia capitata
KM578346.1 Themeda arundinacea
KM578349.1 Themeda arundinacea
KM578347.1 Themeda arundinacea
KM578348.1 Themeda arundinacea
KM578192.1 Iseilema macratherum
KM578193.1 Iseilema macratherum
KM578351.1 Dichanthium foveolatum
KM578352.1 Dichanthium foveolatum
KM578353.1 Dichanthium foveolatum
0.01
DELLA protein-encoding gene dwarf 8 (dwarf8)

o)



Knotted 1
(kn1)

p)
BOP
PACMAD
AF137294.1 Brachypodium pinnatum
LN554540.1 Brachypodium sylvaticum
LN554533.1 Stipa sibirica
U61217.1 Stipa viridula
AF137299.1 Danthonia spicata
U61197.1 Danthonia unispicata
AF137292.1 Aristida longiseta
Aristida congesta c38413
AF137330.1 Thysanolaena maxima
EU272465.1 Zeugites pittieri
AF137297.1 Chasmanthium latifolium
EU272423.1 Chasmanthium laxum
EU272424.1 Danthoniopsis dinteri
EU272433.1 Gynerium sagittatum
HG315105.1 Lecomtella madagascariensis
Panicoideae
EU272425.1 Dichanthelium sabulorum
EU276387.1 Dichanthelium koolauense
FR845923.1 Dichanthelium clandestinum
FR845918.1 Alloteropsis cimicina
Walwhalleya proluta c10522
EU272454.1 Sacciolepis indica
Sacciolepis striata c49001
EU272438.1 Lasiacis sorghoidea
Acroceras calcicola c13157
FR845919.1 Cyrtococcum patens
FR845921.1 Poecilostachys bromoides
Oplismenus burmannii c5308
Neurachne alopecuroidea c58194
Paniceae
Pahal.I04301 Panicum hallii
Pavir.9KG418200 Panicum virgatum
Pavir.9NG674300 Panicum virgatum
AF443820.1 Urochloa mutica
EU272439.1 Melinis repens
EU272464.1 Urochloa acuminata
Urochloa brizantha c70509
EU272455.1 Setaria palmifolia
Si033968m.g Setaria italica
Setaria viridis c52244
EU272460.1 Stenotaphrum secundatum
Cenchrus purpureus c55238
EU272420.1 Cenchrus ciliaris
Paspaleae
Paspalum vaginatum c25483
EU272437.1 Ichnanthus pallens
EU272449.1 Panicum prionitis
EU272418.1 Axonopus fissifolius
EU272462.1 Streptostachys ramosa
EU272415.1 Anthaenantia lanata
EU272436.1 Hymenachne donacifolia
EU272458.1 Steinchisma hians
Steinchisma decipiens c25841
EU272414.1 Altoparadisium chapadense
EU272417.1 Arthropogon villosus
EU272435.1 Homolepis isocalycia
EU272440.1 Mesosetum chaseae
EU272463.1 Tatianyx arnacites
Jansenelleae
Chandrasekharania keralensis
Jansenella griffithiana
Arundinellinae
Garnotia stricta
AF137293.1 Arundinella hirta
Arundinella hookeri c36358
Arundinella nepalensis
Andropogoneae
AF443813.1 Microstegium nudum
Zm00001d047632 Zea mays
AF443806.1 Coix aquatica
AF443818.1 Tripsacum dactyloides
Zm00001d028905 Zea mays
AF443807.1 Cymbopogon flexuosus
AF443816.1 Saccharum officinarum
AF137326.1 Sorghum halepense
SORBI.001G394400 Sorghum bicolor
Andropogoneae s.s.
AF443799.1 Apluda mutica
JN560782.2 Dimeria lawsonii
AF443810.1 Heteropogon contortus
AF137296.1 Capillipedium parviflorum
AF443800.1 Bothriochloa odorata
AF443808.1 Dichanthium aristatum
Dichanthium sericeum c18384
JN560778.2 Andropogon gayanus
AF443798.1 Andropogon ternarius
AF443817.1 Schizachyrium scoparium
AF443811.1 Hyparrhenia hirta
JN560781.2 Diheteropogon amplectens
0.01
phytochrome B
(phyB)

q)
BOP
BGIOSGA022241 Oryza sativa Indica
EF600042.1 Austrostipa aristiglumis
BRADI1G50090 Brachypodium distachyon
AB154358.1 Hordeum bogdanii
EF600043.1 Elymus scaber
KC572688.1 Secale cereale
AF409085.1 Triticum aestivum
JX679018.1 Aegilops longissima
AY508650.1 Dactyloctenium radulans
FJ429112.1 Arundo donax
AF353521.1 Merxmuellera rangei
FJ429124.1 Lophatherum gracile
PACMAD
FJ429117.1 Chasmanthium laxum
FJ429122.1 Chasmanthium curvifolium
FJ429123.1 Gynerium sagittatum
FJ429130.1 Thysanolaena latifolia
FJ429115.1 Centotheca lappacea
FJ429119.1 Cyperochloa hirsuta
FJ429121.1 Danthoniopsis petiolata
Panicoideae
AF079251.1 Danthoniopsis dinteri
FJ429113.1 Arundoclaytonia dissimilis
FJ429131.1 Tristachya biseriata
HE971237.1 Thyridolepis multiculmis
HE971247.1 Neurachne munroi
HE971250.1 Paraneurachne muelleri
FR845940.1 Acroceras tonkinense
Paniceae
FR845952.1 Sacciolepis indica
FR845941.1 Cyrtococcum patens
JN378832.1 Coelachyrum piercei
Si006103m.g Setaria italica
FR845944.1 Ottochloa nodosa
AB668982.1 Echinochloa esculenta
Tricholaena monachne c43505
FR845945.1 Dichanthelium clandestinum
FR845939.1 Alloteropsis cimicina
KC477404.1 Panicum sumatrense
Pavir.4NG035000 Panicum virgatum
Pahal.J00308 Panicum hallii
Pavir.J384900 Panicum virgatum
Garnotia stricta
AF318769.1 Phacelurus digitatus
AF079236.1 Arundinella hirta
AF079237.1 Arundinella nepalensis
Paspaleae
AF318770.1 Paspalum simplex
Paspalum vaginatum c13389
Jansenelleae
JN560729.1 Hemarthria compressa
Chandrasekharania keralensis
Jansenella griffithiana
Zm00001d033937 Zea mays
Zm00001d045462 Zea mays
AF079241.1 Chrysopogon gryllus
AF079242.1 Coix aquatica
EU041672.1 Coix lacryma-jobi
AF079256.1 Schizachyrium scoparium
AF079235.1 Andropogon gerardii
JN560722.1 Andropogon gayanus
SORBI.010G022600 Sorghum bicolor
AF079250.1 Cymbopogon schoenanthus
AF446084.1 Saccharum officinarum
KC469416.1 Bothriochloa bladhii
KC469417.1 Bothriochloa bladhii
AF079239.1 Capillipedium parviflorum
AF079252.1 Dichanthium aristatum
KC469418.1 Dichanthium annulatum
KC469419.1 Dichanthium annulatum
AF446083.1 Miscanthus japonicus
AF446081.1 Cleistachne sorghoides
EF600040.1 Sorghum nitidum
AF079257.1 Sorghastrum nutans
KC469414.1 Sorghastrum nutans
AF446082.1 Microstegium nudum
JN560726.1 Dimeria lawsonii
Granule-bound starch synthase 1
(waxy)
0.01

Table S5. Summary of multigene coalescent species trees inferred from nuclear data under different filtering stringencies[a].

				Clades[f]						
N species[b]	Trim[c]	Set of trees[d]	Ref. species[e]	Androp. + Jans.	Jans.	Androp.	Arundin.	Androp. *s.s.*	Paniceae	Paspaleae
37	50%	bs ≥ 50	*Setaria*	0.94 (1)	0.92 (1)	0.84 (1)	Paraphyl.	0.94 (1)	0.8 (1)	0.93 (1)
			Sorghum	0.91 (1)	0.94 (1)	0.94 (1)	0.48 (1)	0.94 (1)	0.94 (1)	0.91 (1)
		All	*Setaria*	0.92 (1)	0.92 (1)	0.77 (1)	0.42 (0.96)	0.89 (1)	0.77 (1)	0.91 (1)
			Sorghum	0.88 (1)	0.95 (1)	0.72 (1)	0.49 (1)	0.9 (1)	0.88 (1)	0.89 (1)
	70%	bs ≥ 50	*Setaria*	-	-	-	Paraphyl.	-	-	-
			Sorghum	0.92 (1)	0.94 (1)	0.76 (1)	0.39 (0.45)	0.94 (1)	0.95 (1)	0.91 (1)
		All	*Setaria*	0.94 (1)	0.92 (1)	0.68 (1)	0.47 (0.89)	0.87 (1)	0.8 (1)	0.93 (1)
			Sorghum	0.89 (1)	0.97 (1)	0.67 (1)	0.45 (0.96)	0.89 (1)	0.89 (1)	0.92 (1)
	No	bs ≥ 50	*Setaria*	0.95 (1)	0.93 (1)	0.78 (1)	Paraphyl.	0.92 (1)	0.86 (1)	0.92 (1)
			Sorghum	0.95 (1)	0.95 (1)	0.8 (1)	0.47 (1)	0.8 (1)	0.94 (1)	0.9 (1)
		All	*Setaria*	0.93 (1)	0.91 (1)	0.76 (1)	Paraphyl.	0.9 (1)	0.8 (1)	0.89 (1)
			Sorghum	0.9 (1)	0.95 (1)	0.91 (1)	0.5 (1)	0.91 (1)	0.89 (1)	0.88 (1)
66	50%	bs ≥ 50	*Setaria*	-	-	-	-	-	-	-
			Sorghum	-	-	-	-	-	-	-
		All	*Setaria*	0.97 (1)	0.96 (1)	0.83 (1)	0.37 (0.57)	0.92 (1)	0.93 (1)	0.87 (1)
			Sorghum	0.94 (1)	0.98 (1)	0.76 (1)	0.66 (1)	0.93 (1)	0.93 (1)	0.92 (1)
	70%	bs ≥ 50	*Setaria*	-	-	-	-	-	-	-
			Sorghum	-	-	-	-	-	-	-
		All	*Setaria*	0.99 (1)	0.99 (1)	0.6 (0.86)	0.48 (0.57)	0.99 (1)	0.56 (0.48)	0.76 (0.88)
			Sorghum	0.91 (0.99)	0.99 (1)	0.57 (0.67)	0.72 (0.91)	0.86 (0.98)	0.98 (1)	0.49 (0.43)
	No	bs ≥ 50	*Setaria*	-	-	-	-	-	-	-
			Sorghum	1 (1)	0.98 (1)	0.8 (1)	0.39 (0.55)	1 (1)	1 (1)	0.82 (1)
		All	*Setaria*	0.97 (1)	0.95 (1)	0.81 (1)	Paraphyl.	0.93 (1)	0.93 (1)	0.84 (1)
			Sorghum	0.96 (1)	0.97 (1)	0.78 (1)	0.6 (1)	0.96 (1)	0.93 (1)	0.84 (1)

[a] Values are the proportion of quartets supporting the group, with local posterior probabilities between parentheses. Paraphyl. = paraphyletic groups. Missing values refer to datasets with less than 50 genes recovered after filtering; [b] Number of species in the dataset; [c] Alignment trimming threshold: 50% and 70% = sites available for less than 50% and 70% of sequences, respectively, were removed; No = no trimming; [d] Set of gene trees used for inferring the multigene coalescent species tree: All = all gene trees retained after filtering; bs ≥ 50 = only gene trees retained after filtering with at least 50% of branches with bootstrap support value ≥ 50%; [e] Reference species used for read mapping of genomic datasets: *Setaria* = *Setaria italica, Sorghum* = *Sorghum bicolor*; [f] Selected groups of grasses: Androp. = Andropogoneae, Jans. = Jansenelleae, Arundin. = Arundinellinae.

Table S6. Summary of branch model comparisons performed using the species tree topology.

		Branch models[a]							
Scenarios of adaptive evolution			**Single episode** (Preceding C_4 evolution)		**Single episode** (During C_4 evolution, at the base of Andropogoneae)		**Two episodes** (During C_4 evolution, at the base of Arundinellinae and Andropogoneae *s.s.*)		dN/dS[d]
Gene[b]	N[c]	Null model	Internal branch only	Sustained	Internal branch only	Sustained	Internal branch only	Sustained	
Core C_4 genes									
nadpmdh-1P1 (NADP-MDH)	10	**2.67**	4.67	3.71	4.64	0.00	4.32	0.17	0.11
nadpme-1P4 (NADP-ME)	22	89.56	77.97*	38.10*	91.47	**0.00***	90.78	1.23*	0.09 – 0.33
pck-1P1 (PCK)	11	**3.15**	5.15	0.00	4.59	1.93	5.14	1.67	0.03
ppc-1P3 (PEPC)	11	51.04	51.47	11.72*	38.93*	**0.00***	46.32	28.06*	0.03 – 0.13
ppdk-1P2 (PPDK)	9	47.65	49.66	39.12	44.00	35.36*	**0.00***	39.61	0.11 – 0.41
Paralogs of core C_4 genes									
nadpme-1P1	8	**1.39**	2.72	0.00	3.19	1.77	3.38	2.02	0.06
nadpme-1P2	8	**5.12**	0.00	7.09	7.10	6.41	6.88	6.45	0.08
nadpme-1P3	9	**9.63**	11.14	3.84	0.00	4.75	9.27	8.80	0.05
ppc-1P4	12	**0.00**	1.66	1.88	0.25	2.00	0.16	1.82	0.05
ppc-1P5	11	**0.00**	0.31	1.93	0.79	1.97	1.63	1.78	0.07
ppc-1P7	9	**8.93**	10.70	5.01	7.74	0.00	-	-	0.08
ppdk-1P1	4	-	-	-	-	-	-	-	0.16
Other nuclear genes									
arodeh	6	**1.27**	3.27	3.27	0.00	2.94	-	-	0.10
dwarf8	11	**8.34**	10.34	6.88	10.04	0.76	10.19	0.00	0.07
knotted1	7	**2.04**	3.99	2.61	3.08	4.04	0.00	4.03	0.08
phyB	12	**9.35**	11.30	0.00	3.63	7.45	10.22	9.40	0.09
waxy	14	**0.00**	1.98	0.69	2.00	1.01	1.22	1.01	0.04

[a] dAIC values relative to the best-fit model for each gene are shown. The best-fit model is highlighted in bold. Asterisks indicate significant likelihood ratio tests (LRT) against the null model after Bonferroni correction. Two hypotheses of potential enzyme adaptation were tested for each scenario, the first assuming a shift in selective pressure only in the internal branch of the group specified, the second assuming a sustained shift from the internal branch including all descendant branches. The tree topology inferred from whole genome nuclear data (66 species dataset; Fig. S3) was used for these tests. Missing values correspond to trees in which Andropogoneae was not monophyletic, trees in which Arundinellinae was not represented or only a single representative of Andropogoneae *s.s.* was represented; [b] C_4 gene annotation following Moreno-Villena et al. (2018); [c] Number of sequences in the alignment; [d] dN/dS ratios of background and foreground branches, respectively, estimated for the best-fit model, except in cases where the null was the best-fit model, for which there was a single dN/dS estimate for all branches.