

Extra: Tutorial on creating a phylogenetic tree in MEGA software

Website:

<https://www.megasoftware.net/>



Molecular Evolutionary Genetics Analysis



ALIGN



DATA



MODELS



DISTANCE



DIVERSITY



PHYLOGENY



USER TREE



ANCESTORS



SELECTION



RATES



CLOCKS



DISEASE



TIMETREE



DATA MONKEY

RECENT PUBLICATIONS



HELP DOCS



EXAMPLES



CITATION



REPORT BUG



UPDATES



MEGA LINKS



TOOLBAR



PREFERENCES

ANALYZE
PROTOTYPE

fx1

Load FASTA file



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ANALYZE
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fx1

Molecular Evolutionary Genetics Analysis



ALIGN



Open a File/Session...

⌘ O

F4

Concatenate Sequence Alignments

Explore Active Data

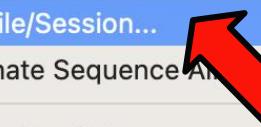
Export Data...

Save Data Session to File...

Select Genetic Code Table...

Select Genes and Domains...

Select Taxa and Groups...



PHYLOGENY



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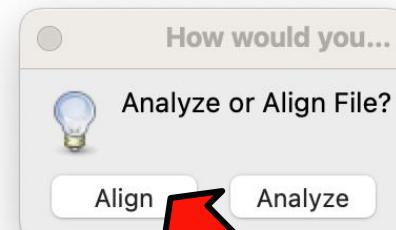
DISEASE



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REPORT BUG



UPDATES



MEGA LINKS



TOOLBAR



PREFERENCES

ANALYZE
PROTOTYPE

fx1

M11: Alignment Explorer (australia_0423.fasta)



DNA Sequences Translated Protein Sequences

Species/Abbrv

1. 5a.2a A/India/Pun-NIV319324/2021

2. 5a.2a.1 A/Alagoas/28876/2022

3. A/Chungbuk/871/2023

4. A/Ulsan/867/2023

5. A/Netherlands/10512/2023

6. A/Catalonia/NSVH102069383/2023

7. A/Wisconsin/37/2023

8. A/Manitoba/RV00381/2023

9. A/Denmark/516/2023

10. A/BosniaandHerzegovina/UKC-Tuzla-C

11. A/Saskatchewan/SKFLU297285/2023

12. A/Washington/23/2023

13. A/Romania/547395/2023

14. A/Lisboa/15/2023

15. A/Arkhangelsk/CRIE/608/2023

16. A/Norway/04936/2023

17. A/Amazonas/2023-004064-IEC/2023

18. A/Moscow oblast/CRIE/616/2023

19. A/Arizona/24/2023

20. A/Iowa/25/2023

21. A/Melilla/1041/2023

22. A/Ceuta/565/2023

23. A/Catalonia/NSVH198267435/2023

24. A/South Dakota/21/2023

25. A/Mato Grosso do Sul/IAL/C10477/2022

26. A/Montana/24/2023

27. A/District Of Columbia/08/2023

28. A/Minnesota/16/2023

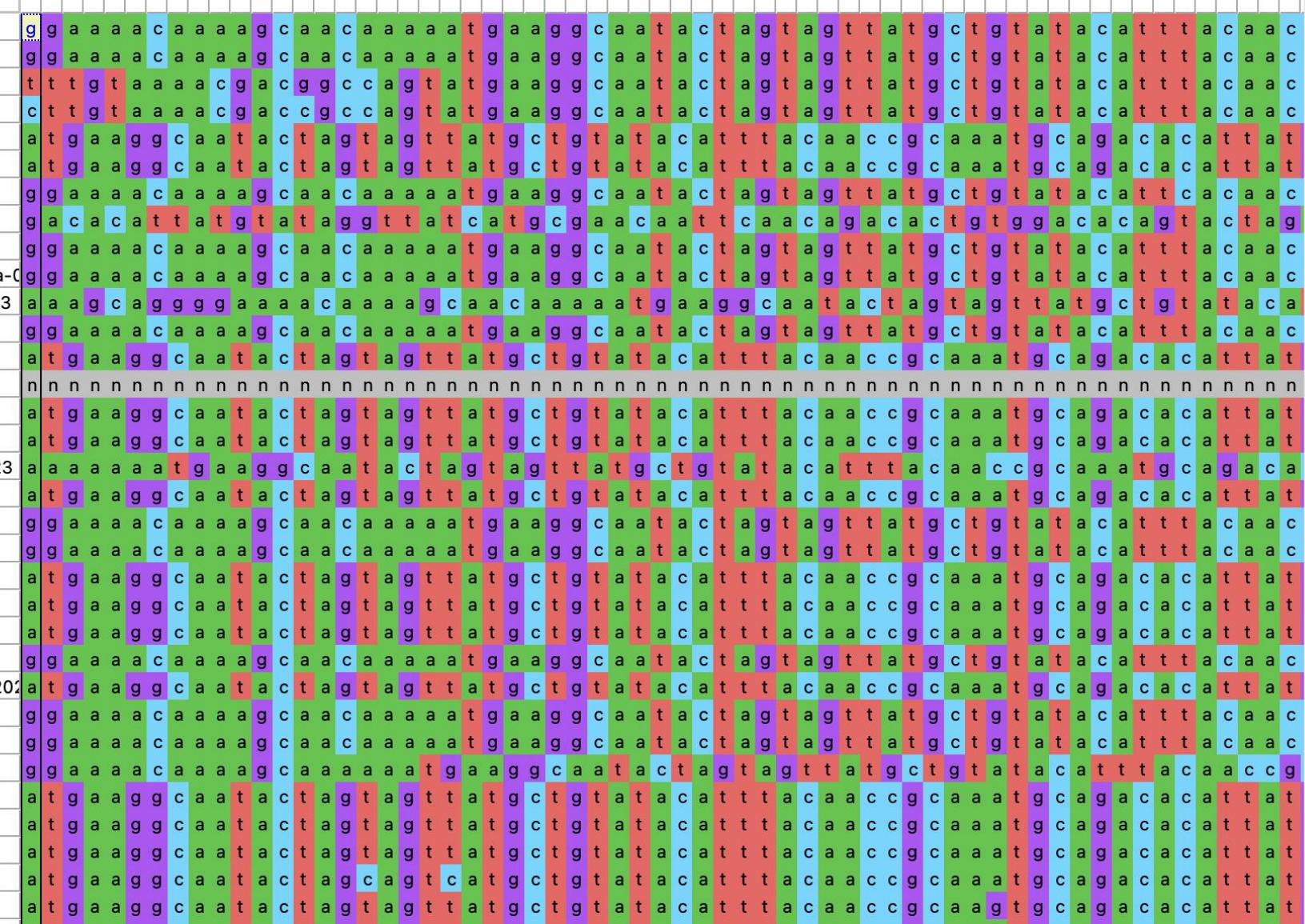
29. A/Victoria/380B/2023(2)

30. A/Victoria/380C/2023(2)

31. A/Victoria/453/2023

32. A/Victoria/454/2023

33. A/Victoria/488/2023



Site # 1



with



w/o gaps

Selected genetic code: Standard

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

W Align by ClustalW

W Align by ClustalW (Codons)

Align by MUSCLE

Align by MUSCLE (Codons)

Mark/Unmark Site ⌘M

Align Marked Sites ⌘L

Unmark All Sites

Delete Gap-Only Sites

Auto-Fill Gaps

ustralia_0423.fasta

Selected Protein Sequences

Species/Abbrv

1. 5a.2a A/India/Pun-NIV319324/2021

2. 5a.2a.1 A/Alagoas/28876/2022

3. A/Chungbuk/871/2023

4. A/Ulsan/867/2023

5. A/Netherlands/10512/2023

6. A/Catalonia/NSVH102069383/2023

7. A/Wisconsin/37/2023

8. A/Manitoba/RV00381/2023

9. A/Denmark/516/2023

10. A/Bosnia and Herzegovina/UKC-Tuzla-0

11. A/Saskatchewan/SKFLU297285/2023

12. A/Washington/23/2023

13. A/Romania/547395/2023

14. A/Lisboa/15/2023

15. A/Arkhangelsk/CRIE/608/2023

16. A/Norway/04936/2023

17. A/Amazonas/2023-004064-IEC/2023

18. A/Moscow oblast/CRIE/616/2023

19. A/Arizona/24/2023

20. A/Iowa/25/2023

21. A/Melilla/1041/2023

22. A/Ceuta/565/2023

23. A/Catalonia/NSVH198267435/2023

24. A/South Dakota/21/2023

25. A/Mato Grosso do Sul/IAL/C10477/2023

26. A/Montana/24/2023

27. A/District Of Columbia/08/2023

28. A/Minnesota/16/2023

29. A/Victoria/380B/2023(2)

30. A/Victoria/380C/2023(2)

31. A/Victoria/453/2023

32. A/Victoria/454/2023

33. A/Victoria/488/2023

Site # 1 with w/o gaps Selected genetic code: Standard

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

M11: Alignment Explorer (australia_0423.fasta)

DNA Sequences Translated Protein Sequences

Species/Abbrv	Sequence
1. 5a.2a A/India/Pun-N	ggaaaaacaaaaagcaacaaaaatgaaaggcaatactagttatgttatacattacaaccgcaaatg
2. 5a.2a.1 A/Alagoas/2	ggaaaaacaaaaagcaacaaaaatgaaaggcaatactagttatgttatacattacaaccgcaaatg
3. A/Chungbuk/871/20	tttgtaaaaa
4. A/Ulsan/867/2023	cttgtaaaaa
5. A/Netherlands/1051	atgaaggca
6. A/Catalonia/NSVH10	atgaaggca
7. A/Wisconsin/37/202	ggaaaaacaaa
8. A/Manitoba/RV0038	gacacattt
9. A/Denmark/516/202	ggaaaaacaaa
10. A/BosniaandHerze	ggaaaaacaaa
11. A/Saskatchewan/SK	aaggcaggg
12. A/Washington/23/2	ggaaaaacaaa
13. A/Romania/547395	atgaaggca
14. A/Lisboa/15/2023	nnnnnnnnnn
15. A/Arkhangelsk/CRII	atgaaggca
16. A/Norway/04936/2	atgaaggca
17. A/Amazonas/2023-	aaaaaaaatg
18. A/Moscow oblast/C	atgaaggca
19. A/Arizona/24/2023	ggaaaaacaaa
20. A/Iowa/25/2023	ggaaaaacaaa
21. A/Melilla/1041/2023	atgaaggca
22. A/Ceuta/565/2023	atgaaggca
23. A/Catalonia/NSVH1	atgaaggcaatactagttatgttatacattacaaccgcaat
24. A/South Dakota/21	ggaaaaacaaaaagcaacaaaaatgaaaggcaatactagttatgttatacattacaaccgcaaatg
25. A/Mato Grosso do	atgaaggcaatactagttatgttatacattacaaccgcaat
26. A/Montana/24/202	ggaaaaacaaaaagcaacaaaaatgaaaggcaatactagttatgttatacattacaaccgcaat
27. A/District Of Colum	ggaaaaacaaaaagcaacaaaaatgaaaggcaatactagttatgttatacattacaaccgcaat
28. A/Minnesota/16/20	ggaaaaacaaaaagcaaaaaatgaaaggcaatactagttatgttatacattacaaccgcaat
29. A/Victoria/380B/20	atgaaggcaataactagttatgttatacattacaaccgcaat
30. A/Victoria/380C/20	atgaaggcaataactagttatgttatacattacaaccgcaat
31. A/Victoria/453/202	atgaaggcaataactagttatgttatacattacaaccgcaat
32. A/Victoria/454/202	atgaaggcaataactagttatgttatacattacaaccgcaat
33. A/Victoria/488/202	atgaaggcaataactagttatgttatacattacaaccgcaat

MUSCLE Alignment Options

Option	Setting
GAP PENALTIES	
Gap Open	<input checked="" type="checkbox"/> -400.00
Gap Extend	<input checked="" type="checkbox"/> 0.00
MEMORY/ITERATIONS	
Max Memory in MB	<input checked="" type="checkbox"/> 2048
Max Iterations	<input checked="" type="checkbox"/> 16
ADVANCED OPTIONS	
Cluster Method (Iterations 1,2)	<input checked="" type="checkbox"/> UPGMA
Cluster Method (Other Iterations)	<input checked="" type="checkbox"/> UPGMA
Min Diag Length (Lambda)	<input checked="" type="checkbox"/> 24

Buttons: ? Help, Reset, Cancel, OK

Site # 1 with w/o gaps Selected genetic code: Standard

M11: Alignment Explorer (australia_0423.fasta)



DNA Sequences Trans

Translated Protein Sequences

Trim sequences to obtain uniform length

Site # 1769

with w/o gaps

Selected genetic code: Standard

M11: Alignment Explorer (australia_0423.fasta)



DNA Sequences Translated Protein Sequences

1. Go to at the beginning of the alignment
 2. Select the positions to be deleted

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

Species/Abbrv

1. 5a.2a A/India/Pun-NIV319
2. 5a.2a.1 A/Alagoas/28876/
3. A/Chungbuk/871/2023
4. A/Ulsan/867/2023
5. A/Netherlands/10512/2022
6. A/Catalonia/NSVH102069/
7. A/Wisconsin/37/2023
8. A/Manitoba/RV00381/2022
9. A/Denmark/516/2023
10. A/Bosnia and Herzegovina/
11. A/Saskatchewan/SKFLU2/
12. A/Washington/23/2023
13. A/Romania/547395/2023
14. A/Lisboa/15/2023
15. A/Arkhangelsk/CRIE/608
16. A/Norway/04936/2023
17. A/Amazonas/2023-004064-IEC/2023
18. A/Moscow oblast/CRIE/616/2023
19. A/Arizona/24/2023
20. A/Iowa/25/2023
21. A/Melilla/1041/2023
22. A/Ceuta/565/2023
23. A/Catalonia/NSVH198267435/2023
24. A/South Dakota/21/2023
25. A/Mato Grosso do Sul/IAL/C10477/2022
26. A/Montana/24/2023
27. A/District Of Columbia/08/2023
28. A/Minnesota/16/2023
29. A/Victoria/380B/2023(2)
30. A/Victoria/380C/2023(2)
31. A/Victoria/453/2023
32. A/Victoria/454/2023
33. A/Victoria/488/2023

Delete

Allow Base Editing

Modify All Bases To Upper Case

Site # 28 with w/o gaps Selected genetic code: Standard

Segment Explorer (australia_0423.fasta)

Sequences Translated Protein Sequences

M11: Alignment Explorer (australia_0423.fasta)



DNA Sequences Translated Protein Sequences

Species/Abbrv																			*	*	*	*	*
1. 5a.2a A/India/Pun-NIV319324/2021	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	T	G	C	A	A
2. 5a.2a.1 A/Alagoas/28876/2022	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	T	G	C	A	A
3. A/Chungbuk/871/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	T	G	C	A	A
4. A/Ulsan/867/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	T	G	C	A	A
5. A/Netherlands/10512/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	T	G	C	A	A
6. A/Catalonia/NSVH102069383/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
7. A/Wisconsin/37/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
8. A/Manitoba/RV00381/2023	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	A	C
9. A/Denmark/516/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	C	A
10. A/BosniaandHerzegovina/UKC-Tuzla-0	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
11. A/Saskatchewan/SKFLU297285/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
12. A/Washington/23/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
13. A/Romania/547395/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
14. A/Lisboa/15/2023	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
15. A/Arkhangelsk/CRIE/608/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
16. A/Norway/04936/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	A
17. A/Amazonas/2023-004064-IEC/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
18. A/Moscow oblast/CRIE/616/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
19. A/Arizona/24/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
20. A/Iowa/25/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
21. A/Melilla/1041/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
22. A/Ceuta/565/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
23. A/Catalonia/NSVH198267435/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
24. A/South Dakota/21/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
25. A/Mato Grosso do Sul/IAL/C10477/202	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
26. A/Montana/24/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
27. A/District Of Columbia/08/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
28. A/Minnesota/16/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
29. A/Victoria/380B/2023(2)	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
30. A/Victoria/380C/2023(2)	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
31. A/Victoria/453/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
32. A/Victoria/454/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T
33. A/Victoria/488/2023	A	T	G	A	G	G	C	A	A	T	A	C	T	A	G	T	T	A	C	A	C	A	T

Site # 1

with w/o gaps

Selected genetic code: Standard

M11: Alignment Explorer (australia_0423.mas)



DNA Sequences Translated Protein Sequences

Species/Abbrv	*	*	*	*	*	*	*	*	*	*	*	*	*
41. A/Tasmania/30/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
42. A/Brisbane/13/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
43. A/South Australia/85/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
44. A/Canberra/43/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
45. A/South Australia/86/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
46. A/South Australia/90/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
47. A/Canberra/50/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
48. A/Canberra/49/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
49. A/South Australia/83/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
50. A/Tasmania/25/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
51. A/Victoria/380A/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
52. A/Victoria/380B/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
53. A/Canberra/47/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
54. A/Victoria/382/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
55. A/Victoria/473/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
56. A/Brisbane/24/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
57. A/Victoria/490/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
58. A/Darwin/53/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
59. A/Victoria/445/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
60. A/Sydney/83/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
61. A/Darwin/51/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
62. A/Victoria/409/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
63. A/Sydney/75/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
64. A/Sydney/81/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
65. A/Sydney/79/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
66. A/Victoria/424/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
67. A/Tasmania/53/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
68. A/Tasmania/54/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
69. A/Victoria/380C/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
70. A/Victoria/540/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
71. A/Victoria/537/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
72. A/Victoria/380/2023	G	T	G	T	A	G	A	A	T	A	T	G	T
73. A/Sydney/101/2023	G	T	G	T	A	G	A	A	T	A	T	G	T

1. Go to at the end of the alignment
 2. Select the positions to be deleted

Site # 1741

with w/o gaps

Selected genetic code: Standard

M11: Alignment Explorer (australia_0423.mas)



DNA Sequences Translated Protein Sequences

Save aligned sequences

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

Create New Open a Recently Used File Close Phylogenetic Analysis Save Session Export Alignment DNA Sequences Protein Sequences Translate/Untranslate Genetic Code Reverse Complement Reverse Complement Complement Quit MEGA Format FASTA Format NEXUS/PAUP Format

M11: Alignment Explorer (australia_0423.mas)

DNA Sequences Translated Protein Sequences

Site # 1701 with w/o gaps Selected genetic code: Standard

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

M11: Alignment Explorer (australia_0423.mas)

DNA Sequences Translated Protein Sequences

Species/Abbrv	Sequence
1. 5a.2a A/India/Pun-NIV319324/2021	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
2. 5a.2a.1 A/Alagoas/28876/2022	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
3. A/Chungbuk/871/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
4. A/Ulsan/867/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
5. A/Netherlands/10512/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
6. A/Catalonia/NSVH102069383/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
7. A/Wisconsin/37/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
8. A/Manitoba/RV00381/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
9. A/Denmark/516/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
10. A/Bosnia and Herzegovina/UKC-Tuzla-0	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
11. A/Saskatchewan/SKFLU297285/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
12. A/Washington/23/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
13. A/Romania/547395/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
14. A/Lisboa/15/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
15. A/Arkhangelsk/CRIE/608/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
16. A/Norway/04936/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
17. A/Amazonas/2023-004064-IEC/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
18. A/Moscow oblast/CRIE/616/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
19. A/Arizona/24/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
20. A/Iowa/25/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
21. A/Melilla/1041/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
22. A/Ceuta/565/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
23. A/Catalonia/NSVH198267435/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
24. A/South Dakota/21/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
25. A/Mato Grosso do Sul/IAL/C10477/2022	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
26. A/Montana/24/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
27. A/District Of Columbia/08/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
28. A/Minnesota/16/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
29. A/Victoria/380B/2023(2)	GAGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
30. A/Victoria/380C/2023(2)	GAGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
31. A/Victoria/453/2023	GGGCAAGTCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
32. A/Victoria/454/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA
33. A/Victoria/488/2023	GGGCAATCAGCTTCTGGATGTCCTAATGGGTCTCTACAGTGTAGAAATATGTATTAA

Save file as

Save As:

Tags:

Where:

Format: FASTA Files

Cancel Save

Site # 1701 with w/o gaps Selected genetic code: Standard

Nucleotide substitution models

Jukes and Cantor Model of Nucleotide Substitution

	A	C	G	T
A	-3 α	α	α	α
C	α	-3 α	α	α
G	α	α	-3 α	α
T	α	α	α	-3 α

Relative rate matrix

$$\Rightarrow P(t) = e^{Qt} = \begin{bmatrix} P_{AA} & P_{AC} & P_{AG} & P_{AT} \\ P_{CA} & P_{CC} & P_{CG} & P_{CT} \\ P_{GA} & P_{GC} & P_{GG} & P_{GT} \\ P_{TA} & P_{TC} & P_{TG} & P_{TT} \end{bmatrix}$$

Probability matrix
(function of time)

- Four nucleotides assumed to be equally frequent ($f=0.25$)
- All 12 substitution rates assumed to be equal
- Under this model the corrected distance is: $D_{JC} = \frac{3}{4} \ln(1 - \frac{4}{3} D_{OBS})$
- For instance: $D_{OBS} = 0.42 \Rightarrow D_{JC} = 0.64$

Other Models of Nucleotide Substitution

	<i>A</i>	<i>C</i>	<i>G</i>	<i>T</i>
<i>A</i>	$1 - \alpha - 2\beta$	β	α	β
<i>C</i>	β	$1 - \alpha - 2\beta$	β	α
<i>G</i>	α	β	$1 - \alpha - 2\beta$	β
<i>T</i>	β	α	β	$1 - \alpha - 2\beta$

	<i>A</i>	<i>C</i>	<i>G</i>	<i>T</i>
<i>A</i>	$1 - \alpha - 2\gamma$	γ	α	γ
<i>C</i>	δ	$1 - \beta - 2\delta$	δ	β
<i>G</i>	β	γ	$1 - \beta - 2\gamma$	γ
<i>T</i>	δ	α	δ	$1 - \alpha - 2\delta$

⋮

	<i>A</i>	<i>C</i>	<i>G</i>	<i>T</i>
<i>A</i>	$1 - \alpha_{12} - \alpha_{13} - \alpha_{14}$	α_{12}	α_{13}	α_{14}
<i>A</i>	α_{21}	$1 - \alpha_{21} - \alpha_{23} - \alpha_{24}$	α_{23}	α_{24}
<i>A</i>	α_{31}	α_{32}	$1 - \alpha_{31} - \alpha_{32} - \alpha_{34}$	α_{34}
<i>A</i>	α_{41}	α_{42}	α_{43}	$1 - \alpha_{41} - \alpha_{42} - \alpha_{43}$

Search for the best-fit nucleotide substitution model



Molecular Evolutionary Genetics Analysis



ALIGN



DATA



MODELS



DISTANCE



DIVERSITY



PHYLOGENY



USER TREE



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SELECTION



RATES



CLOCKS



DISEASE



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Find Best DNA/Protein Models (ML)...



Disparity Index Test of Pattern Heterogeneity



Estimate Substitution Matrix (ML)...



Estimate Transition/Transversion Bias (ML)...



TREE



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PREFERENCES

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PROTOTYPE

M



Molecular Evolutionary Genetics Analysis



M11: Analysis Preferences

Model Selection (ML)

Option	Setting
Tree to Use →	Automatic (Neighbor-joining tree)
User Tree File →	Not Applicable
Statistical Method →	Maximum Likelihood
Substitutions Type →	Nucleotide
Genetic Code Table →	Not Applicable
Gaps/Missing Data Treatment →	Use all sites
Site Coverage Cutoff (%) →	Not Applicable
Select Codon Positions →	<input checked="" type="checkbox"/> 1st <input checked="" type="checkbox"/> 2nd <input checked="" type="checkbox"/> 3rd <input checked="" type="checkbox"/> Noncoding Sites
Branch Swap Filter →	None
Number of Threads →	4

Help

Cancel

OK

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RATES



CLOCKS



DISEASE



M11: Progress

PROGRESS ●

DETAILS STOP

STATUS/OPTIONS

RUN STATUS

Start time	13-6-23 14:57:57
Operation Run Time	00:00:14
Status	Making initial tree
Thread-1	GTR



TIMETREE



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MEGA Caption Expert: Find Best-Fit Substitution Model (ML)



Results

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	InL	(+I)	(+G)	R	f(A)	f(T)	f(C)	f(G)
HKY+G	148	10202.576	8763.176	-4233.410	n/a	0.40	4.49	0.352	0.236	0.190	0.221
HKY+I	148	10210.708	8771.307	-4237.476	0.44	n/a	4.48	0.352	0.236	0.190	0.221
HKY+G+I	149	10214.214	8765.090	-4233.365	0.13	0.52	4.49	0.352	0.236	0.190	0.221
TN93+G	149	10214.286	8765.162	-4233.401	n/a	0.39	4.49	0.352	0.236	0.190	0.221
TN93+I	149	10222.444	8773.320	-4237.480	0.44	n/a	4.48	0.352	0.236	0.190	0.221
TN93+G+I	150	10225.979	8767.132	-4233.383	0.12	0.51	4.49	0.352	0.236	0.190	0.221
T92+G	146	10227.438	8807.484	-4257.569	n/a	0.39	4.49	0.294	0.294	0.206	0.206
HKY	147	10228.007	8798.329	-4251.989	n/a	n/a	4.47	0.352	0.236	0.190	0.221
T92+I	146	10235.660	8815.706	-4261.680	0.44	n/a	4.48	0.294	0.294	0.206	0.206
GTR+G	152	10236.716	8758.422	-4227.023	n/a	0.39	4.47	0.352	0.236	0.190	0.221
T92+G+I	147	10239.091	8809.414	-4257.531	0.14	0.54	4.49	0.294	0.294	0.206	0.206
TN93	148	10239.686	8800.285	-4251.964	n/a	n/a	4.47	0.352	0.236	0.190	0.221
GTR+I	152	10244.968	8766.675	-4231.150	0.44	n/a	4.48	0.352	0.236	0.190	0.221
GTR+G+I	153	10248.342	8760.325	-4226.972	0.14	0.54	4.47	0.352	0.236	0.190	0.221



MEGA Caption Expert: Find Best-Fit Substitution Model (ML)



Results

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameter	f(T)	f(C)	f(G)
HKY+G	148	0.236	0.190	0.221
HKY+I	148	0.236	0.190	0.221
HKY+G+I	149	0.236	0.190	0.221
TN93+G	149	0.236	0.190	0.221
TN93+I	149	0.236	0.190	0.221
TN93+G+I	150	0.236	0.190	0.221
T92+G	146	0.294	0.206	0.206
HKY	147	0.236	0.190	0.221
T92+I	146	0.294	0.206	0.206
GTR+G	152	0.236	0.190	0.221
T92+G+I	147	0.294	0.206	0.206
TN93	148	0.236	0.190	0.221
GTR+I	152	0.236	0.190	0.221
GTR+G+I	153	0.236	0.190	0.221

M11: Select Output Format

SELECT OUTPUT FORMAT

- XLSX: MS Excel workbook (2007+)
- XLS: MS Excel Workbook (all versions)
- ODS: Open/Libre Office Workbook
- CSV: Comma-Separated-Values

 Save to Disk Display Results

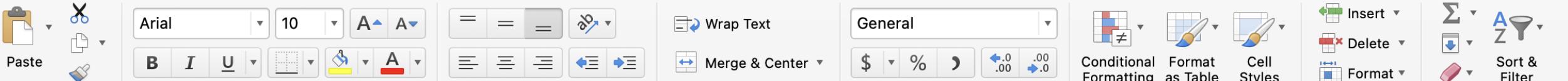
OUTPUT FILENAME

australia_aligned_0423_model_selection.txt

DIRECTORY

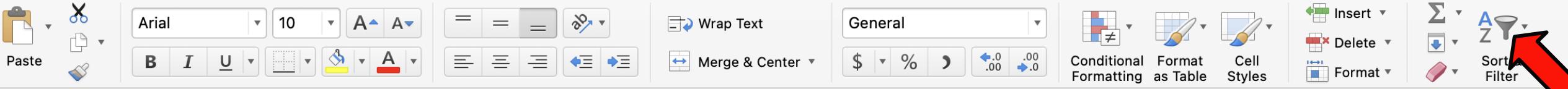
/Users/clydedapat/Desktop/2023_Timor-Leste/





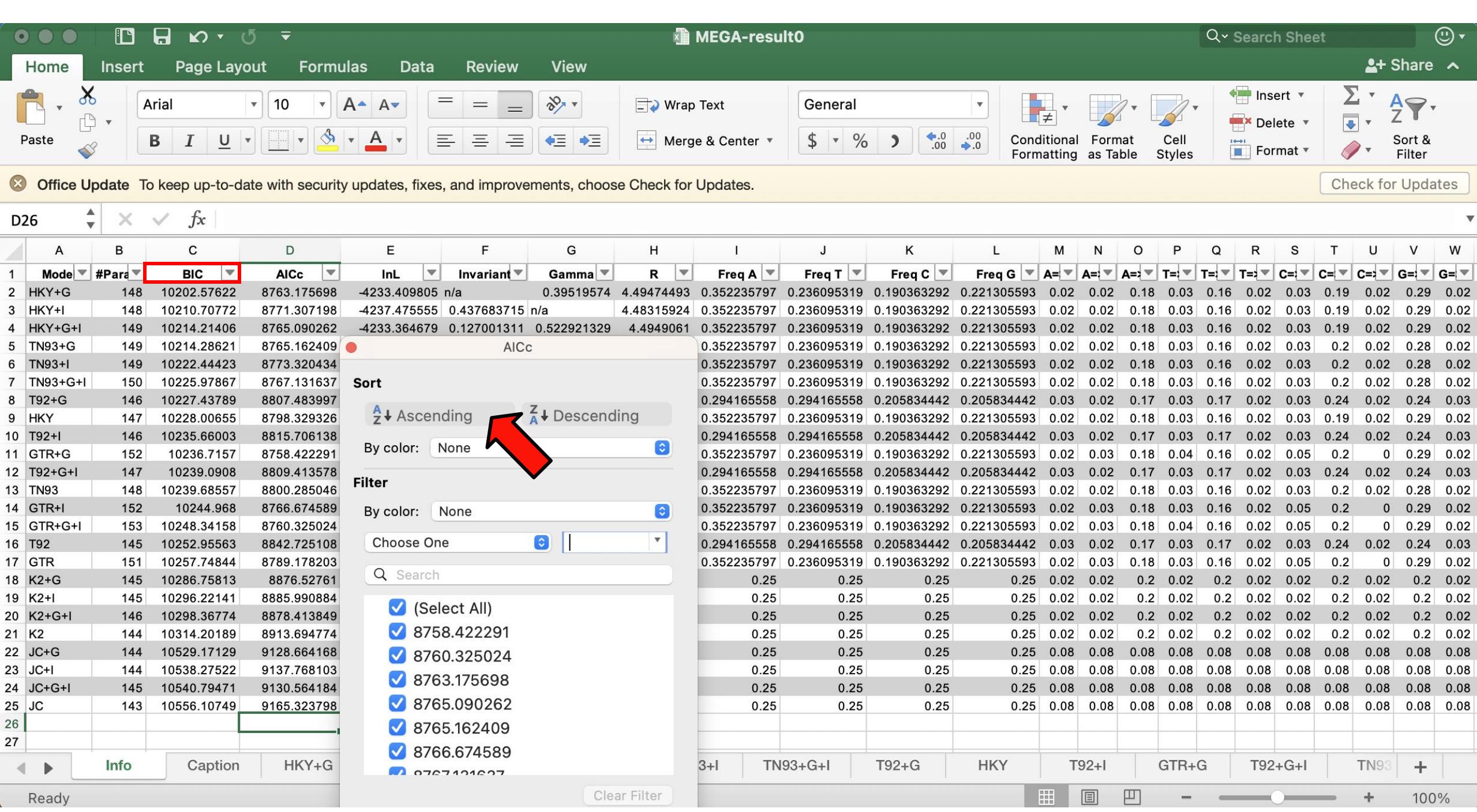
Office Update To keep up-to-date with security updates, fixes, and improvements, choose Check for Updates.

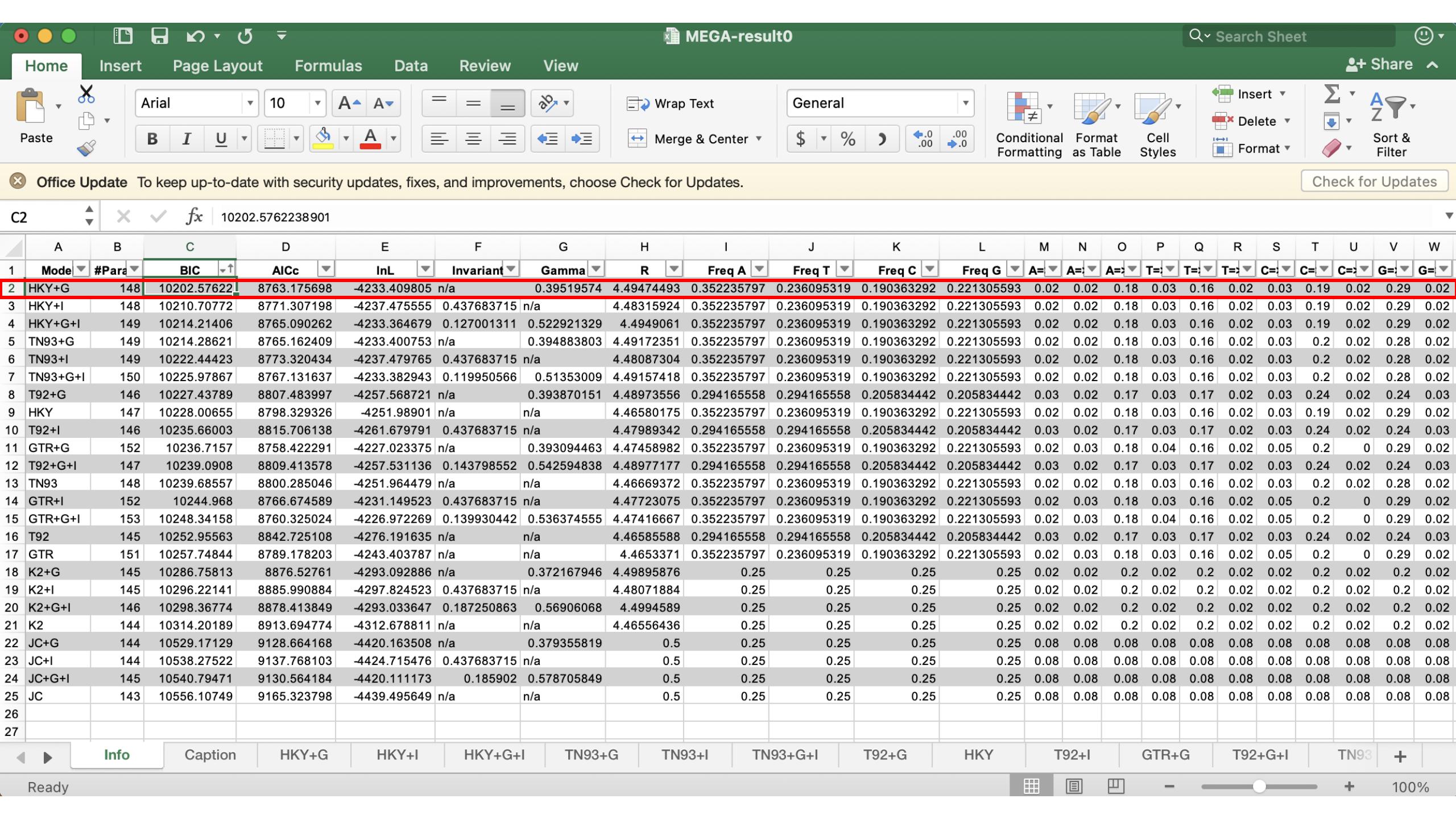
Check for Updates



 **Office Update** To keep up-to-date with security updates, fixes, and improvements, choose Check for Updates.

Check for Updates





MEGA-result0

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A1 Table. Maximum Likelihood fits of 24 different nucleotide substitution models

1 Table. Maximum Likelihood fits of 24 different nucleotide substitution models

NOTE.-- Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value ($\ln L$), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (R) are shown for each model, as well. They are followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. This analysis involved 73 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1701 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2][3]

2 Abbreviations: TR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor./div>

3 1. Nei M. and Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

4 2. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution <https://doi.org/10.1093/molbev/msab120>.

5 3. Stecher G., Tamura K., and Kumar S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Molecular Biology and Evolution 37:1237-1239.

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14

Info Caption HKY+G HKY+I HKY+G+I TN93+G TN93+I TN93+G+I T92+G HKY T92+I GTR+G T92+G+I TN93 +

Ready 100%

MEGA-result0

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Check for Updates

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	From\To	A	T	C	G											
2	A	-	0.021569852	0.017391739	0.18086829											
3	T	0.032180537	-	0.155579815	0.020218651											
4	C	0.032180537	0.192955614	-	0.020218651											
5	G	0.287874723	0.021569852	0.017391739	-											
6																
7																
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26																
27																

HKY+G HKY+I HKY+G+I TN93+G TN93+I TN93+G+I T92+G HKY T92+I GTR+G T92+G+I TN93 +

Ready 100%

Construct a maximum likelihood tree



Molecular Evolutionary Genetics Analysis



ALIGN



DATA



MODELS



DISTANCE



DIVERSITY



PHYLOGENY



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ANCESTORS



SELECTION



RATES



CLOCKS



DISEASE



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PROTOTYPE



Molecular Evolutionary Genetics Analysis



ALIGN



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MODELS



DISTANCE



DIVERSITY



Construct/Test Maximum Likelihood Tree...



1,2



+



RATES



CLOCKS



DISEASE



Construct/Test Maximum Likelihood Tree...

Construct/Test Neighbor-Joining Tree...

Construct/Test Minimum-Evolution Tree...

Construct/Test UPGMA Tree...

Construct/Test Maximum Parsimony Tree(s)

Open Tree Session



TIMETREE



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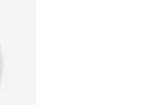
SELECTION



RATES



CLOCKS



DISEASE



Use the active file?



Would you like to use the currently active data (australia_aligned_0423.mas)?

 Remember to reuse currently active data.

Yes



No

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fx1

M11: Analysis Preferences

Phylogeny Reconstruction

Option	Setting
Statistical Method →	Maximum Likelihood
Test of Phylogeny →	Bootstrap method
No. of Bootstrap Replications →	100
Substitutions Type →	Nucleotide
Genetic Code Table →	Not Applicable
Model/Method →	Hasegawa-Kishino-Yano model
Rates among Sites →	Gamma Distributed (G)
No of Discrete Gamma Categories →	5
Gaps/Missing Data Treatment →	Use all sites
Site Coverage Cutoff (%) →	Not Applicable
Select Codon Positions →	<input checked="" type="checkbox"/> 1st <input checked="" type="checkbox"/> 2nd <input checked="" type="checkbox"/> 3rd <input checked="" type="checkbox"/> Noncoding Sites
ML Heuristic Method →	Nearest-Neighbor-Interchange (NNI)
Initial Tree for ML →	Make initial tree automatically (Default - NJ/BioNJ)
Initial Tree File →	Not Applicable
Branch Swap Filter →	None
Number of Threads →	4

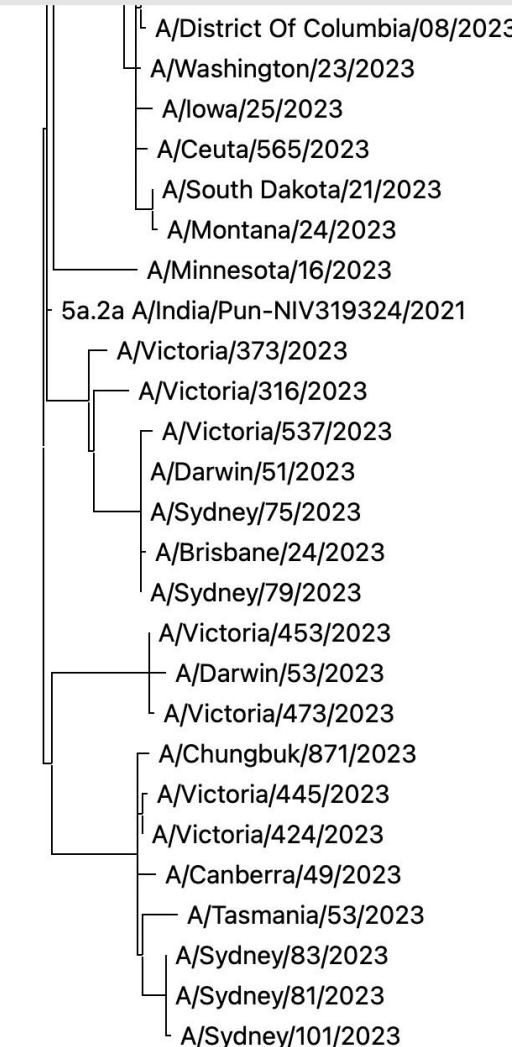
Help Cancel OK

Extra: Using MEGA to visualise phylogenetic tree



- Taxon Names
- Layout
- Subtree
- Branch Lengths
- Statistics/Frequency/Info
- Distance Scale
- Divergence Times
- Time Scale
- Ancestors Site #
- Collapse/Expand Lineages
- Compute
- Display Caption

Original Tree Bootstrap Tree



H

0.0020



◀ ▶ ⌂ ⌃ ⌄ ⌅ ⌆ ⌇ ⌈ ⌉ ⌊ ⌋ ⌍ ⌎

Taxon Names
Font.. 11 Font Size
 Hide Overlapping Taxa
 Show Taxon Markers Edit Markers...
Arrange Taxa
 For Balanced Shape
 By Input Order

▶ Layout

▶ Subtree

▶ Branch Lengths

▶ Statistics/Frequency/Info

▶ Distance Scale

▶ Divergence Times

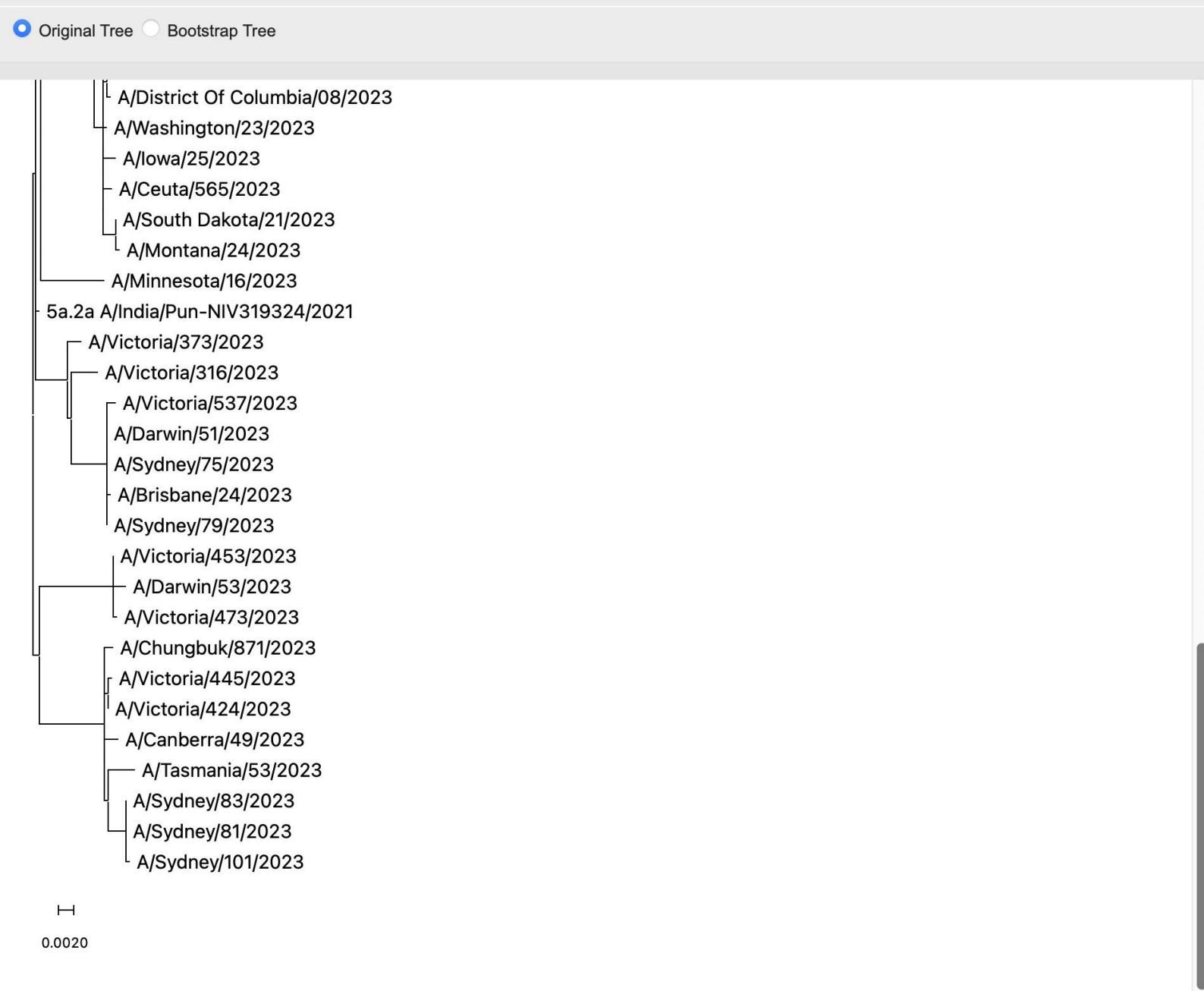
▶ Time Scale

▶ Ancestors Site # 1

▶ Collapse/Expand Lineages

▶ Compute

▶ Display Caption





► Taxon Names

▼ Layout

- Tree Style
- Toggle Scaling of the Tree
- Auto-size Tree
- Drag to Resize Tree
- Root on Midpoint
- Root on Outgroup

Tree Width

Tree Height

► Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Divergence Times

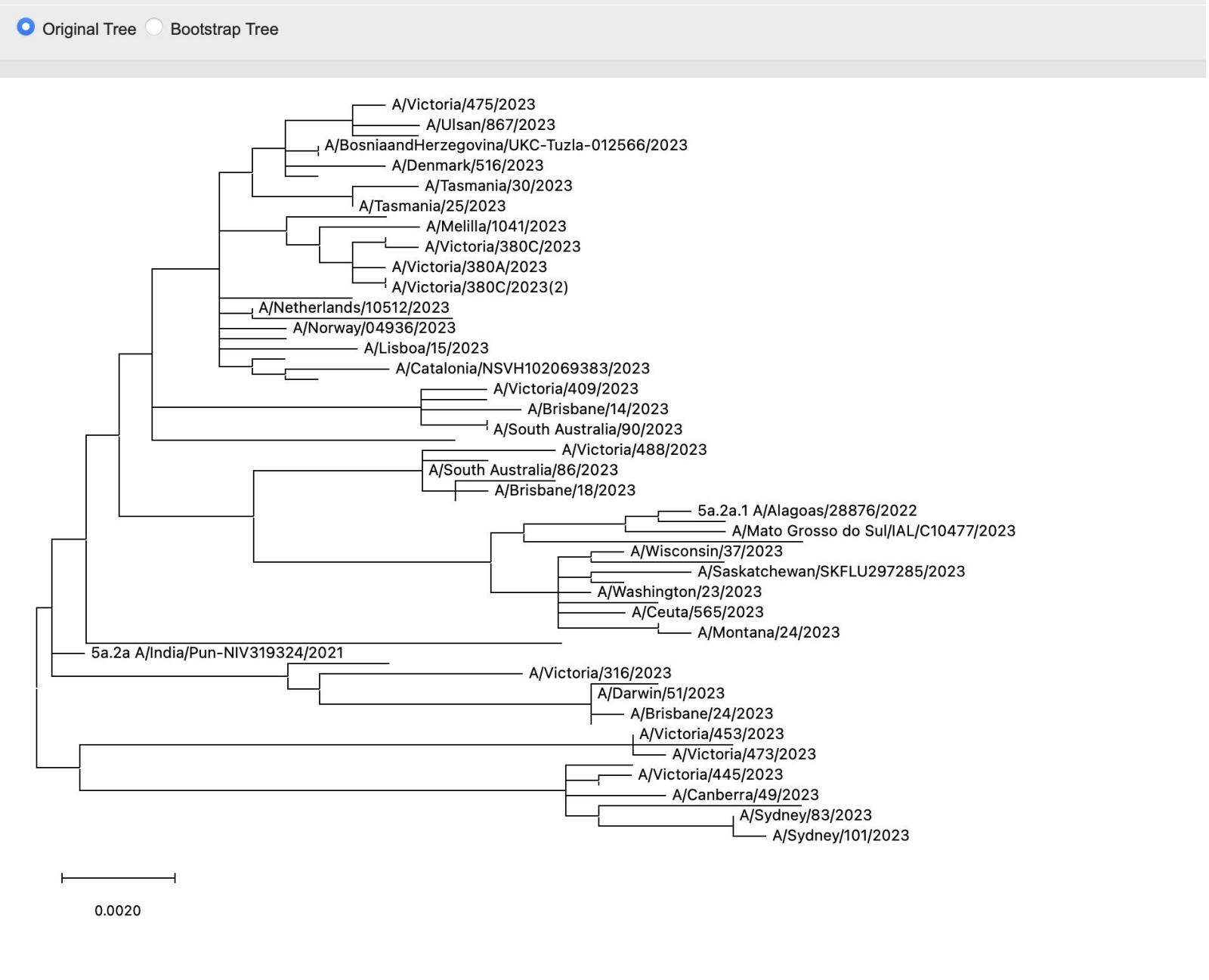
Time Scale

Ancestors Site #

Collapse/Expand Lineages

Compute

Display Caption





◀ ▶

Taxon Names

Layout

Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Line Width

Caption

Font Size

Scale Length

Tick Interval

Divergence Times

Time Scale

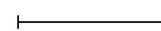
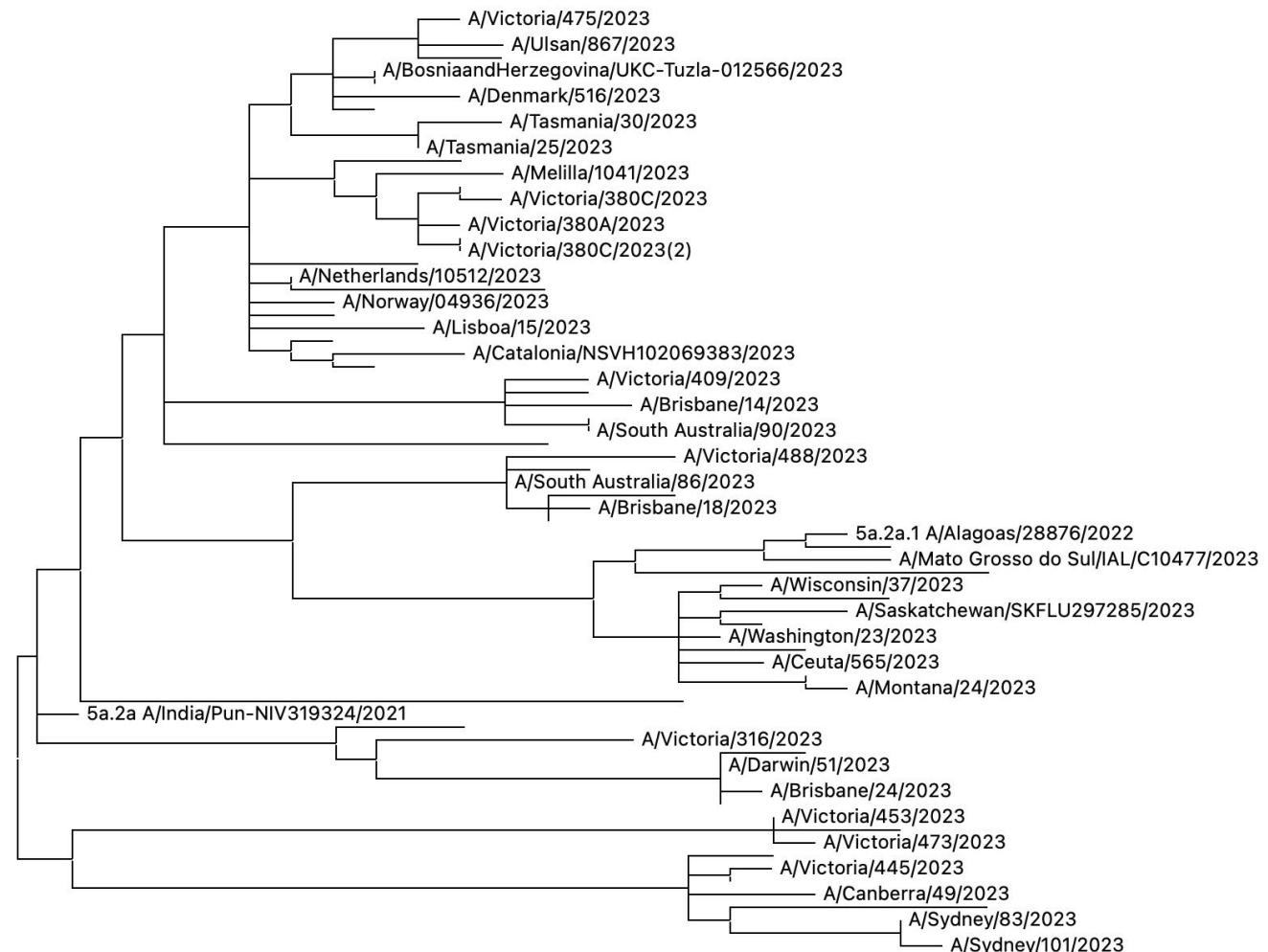
Ancestors Site #

Collapse/Expand Lineages

Compute

Display Caption

Original Tree Bootstrap Tree



0.0020



◀ ▶

Taxon Names

Layout

Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Divergence Times

Time Scale

Ancestors Site # 1 ▲ ▶ ▶

Collapse/Expand Lineages

Collapse Nodes

By Groups

By Cluster Size
10 ▲ ▼

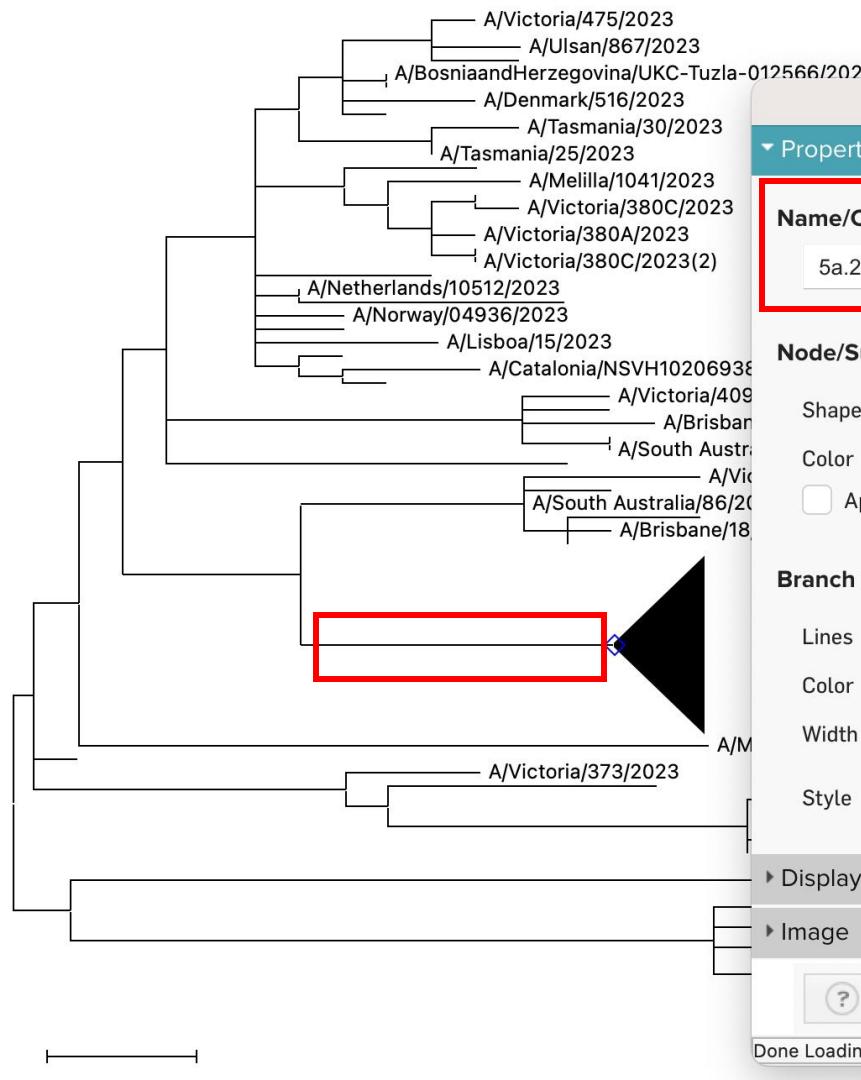
By Sequence Difference
0.000002 ▲ ▼

Display Cluster Size

▶ Compute

Display Caption

Original Tree Bootstrap Tree



Subtree Drawing Options

Property

Name/Caption

5a.2a.1

Font

Node/Subtree Marker

Shape

None ▾

Color

█

Apply to Taxon Markers

Branch Line

Lines

█ ▾

Color

█

Width

1 pt ▾

Style

Solid ▾

Display

Image

? Help

Cancel

OK

Done Loading





File Print View Options Help

Original Tree Bootstrap Tree

Taxon Names

Layout

Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Divergence Times

Time Scale

Ancestors Site # 1

Collapse/Expand Lineages

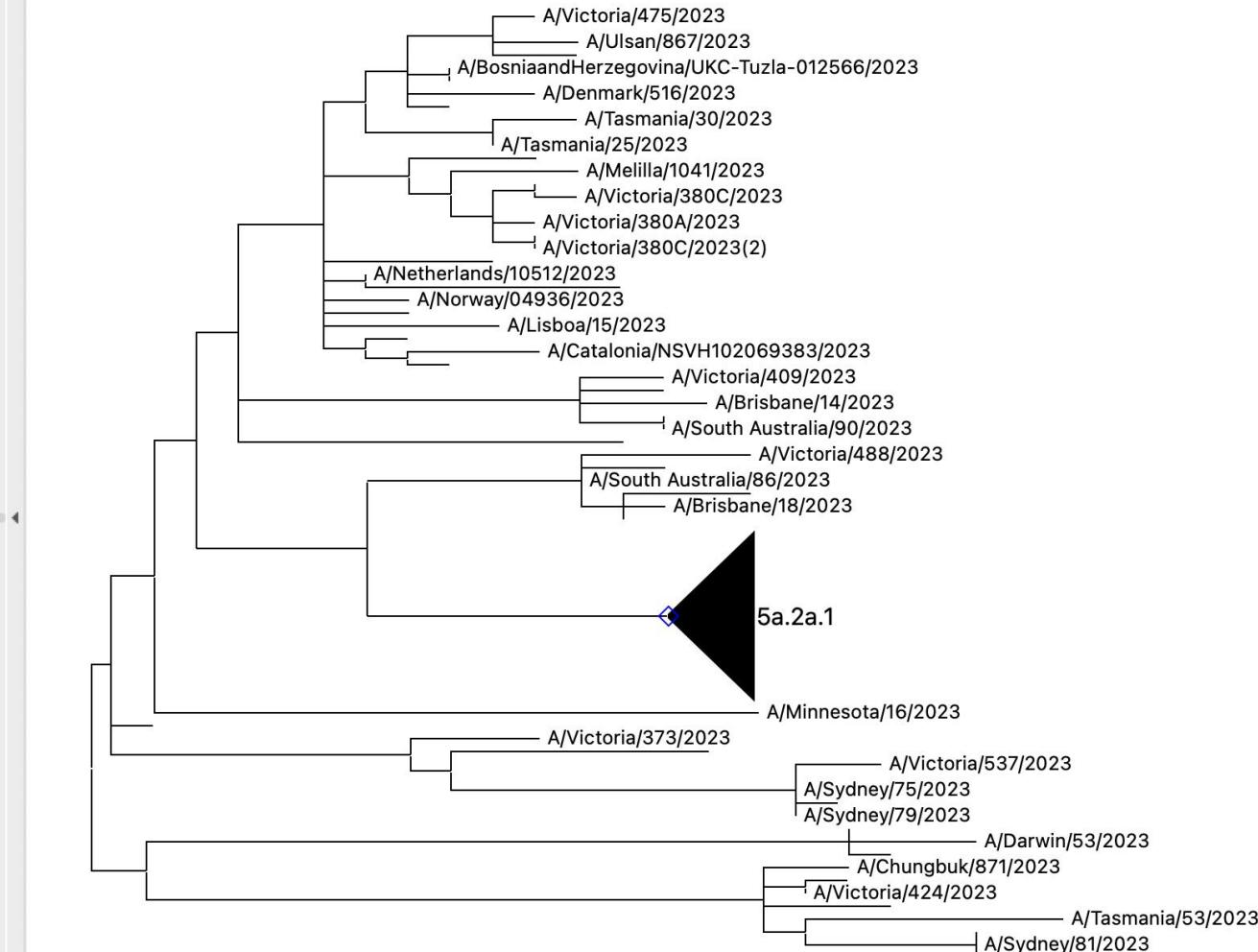
Collapse Nodes By Groups By Cluster Size 10

By Sequence Difference 0.000002

Display Cluster Size

Compute

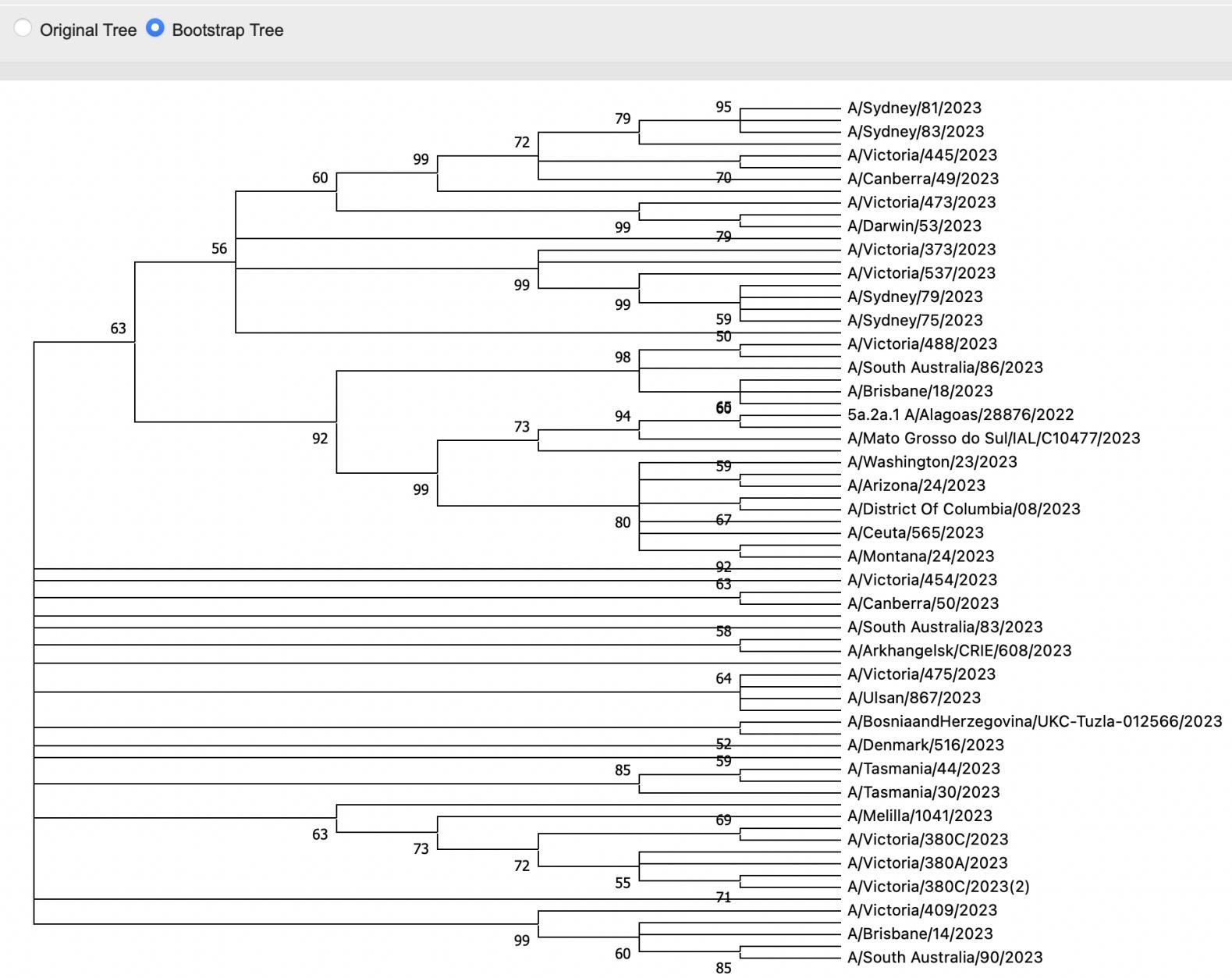
Display Caption

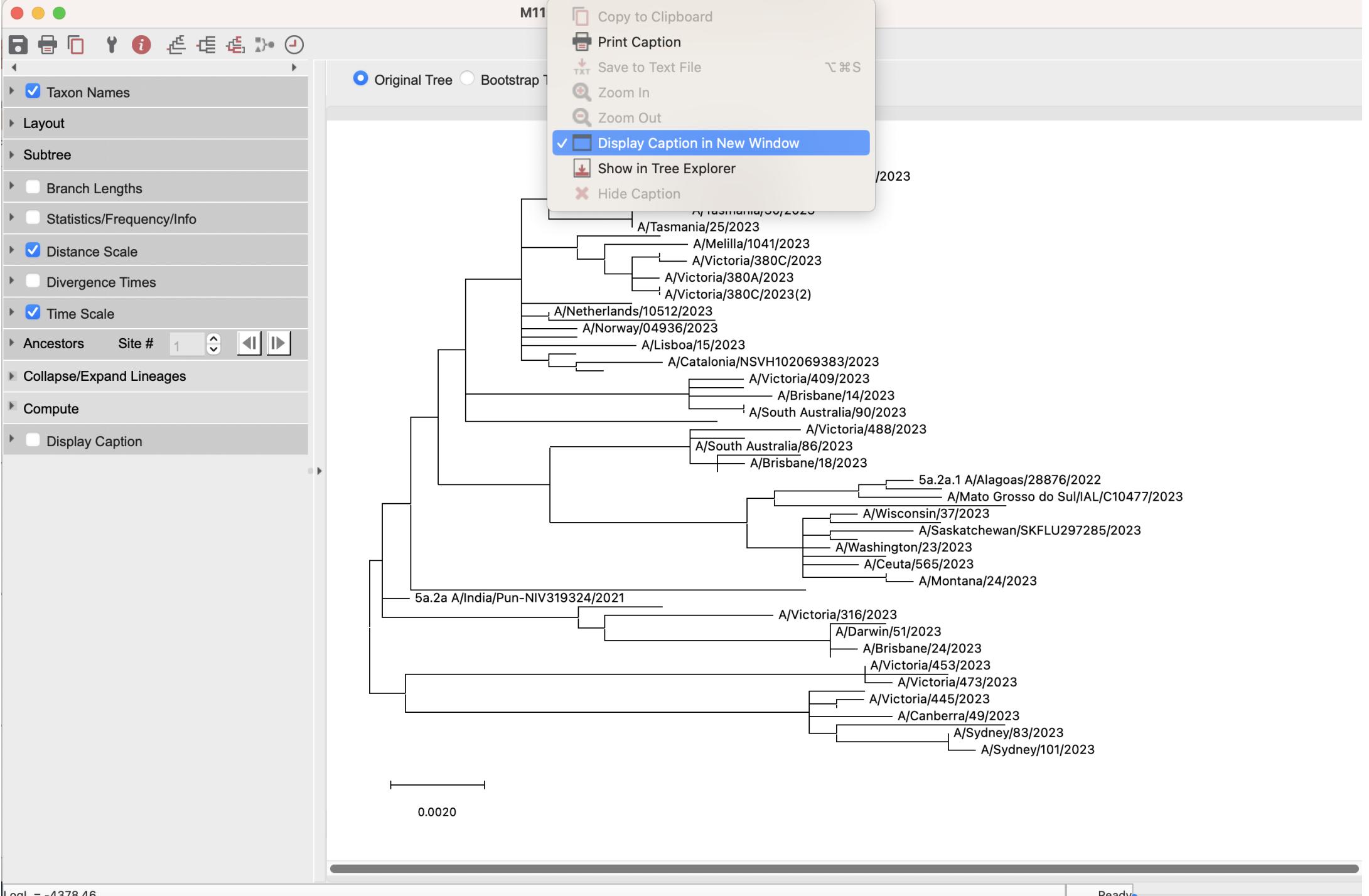


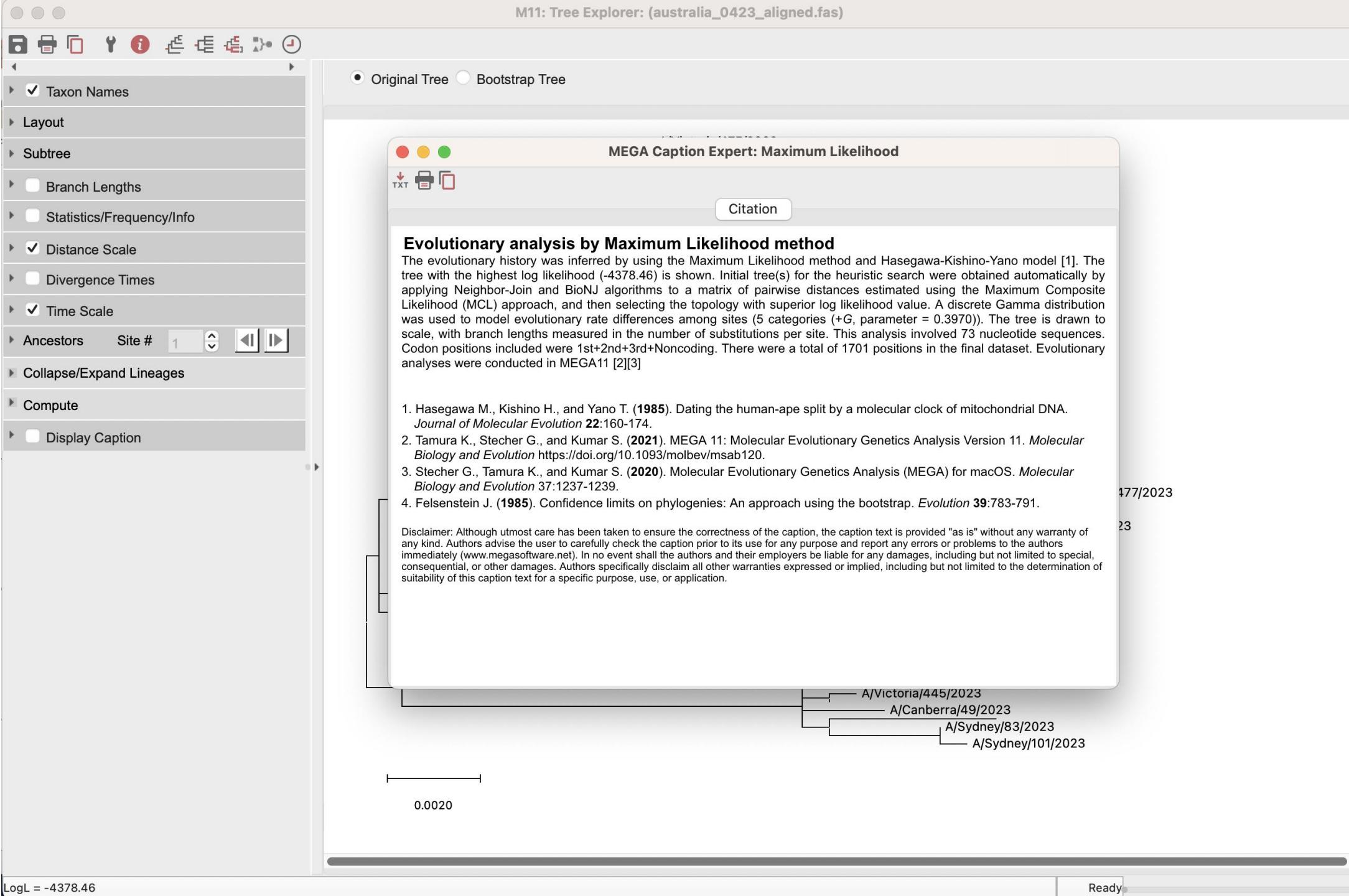
0.0020



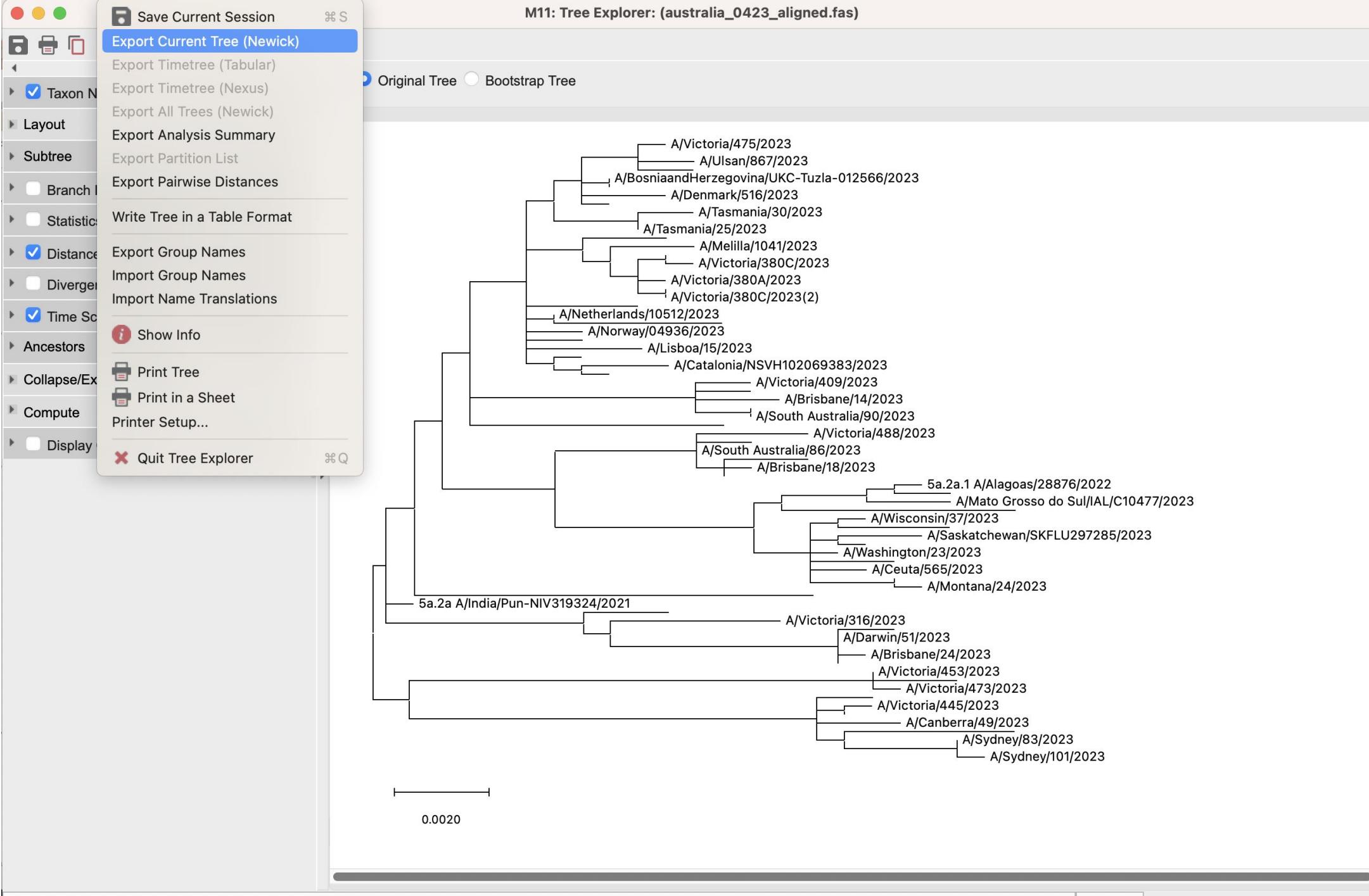
- Taxon Names
- Layout
- Subtree
- Branch Lengths
- Statistics/Frequency/Info
- Distance Scale
- Divergence Times
- Time Scale
- Ancestors Site #
- Collapse/Expand Lineages
- Compute
- Display Caption







Export tree file





◀ ▶

Taxon Names

Layout

Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Divergence Times

Time Scale

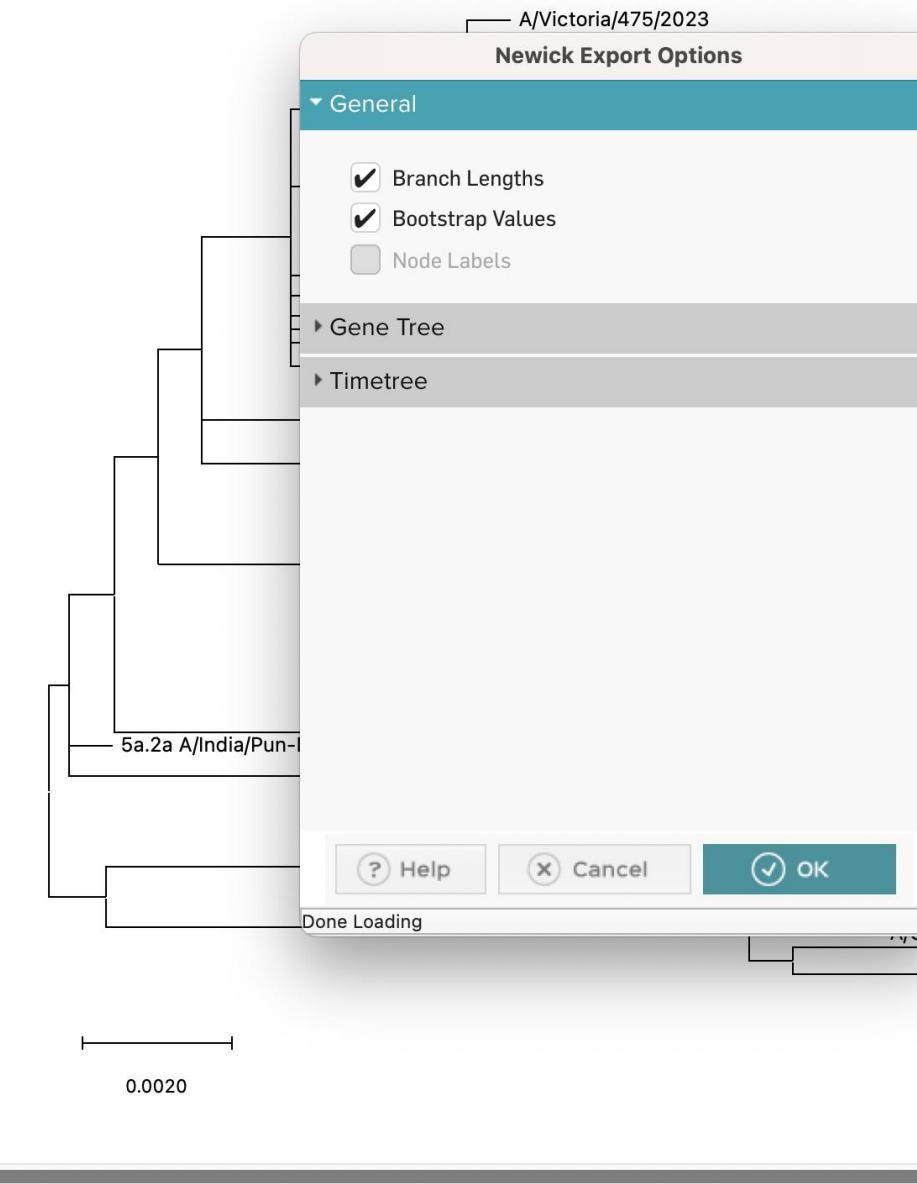
Ancestors Site # 1

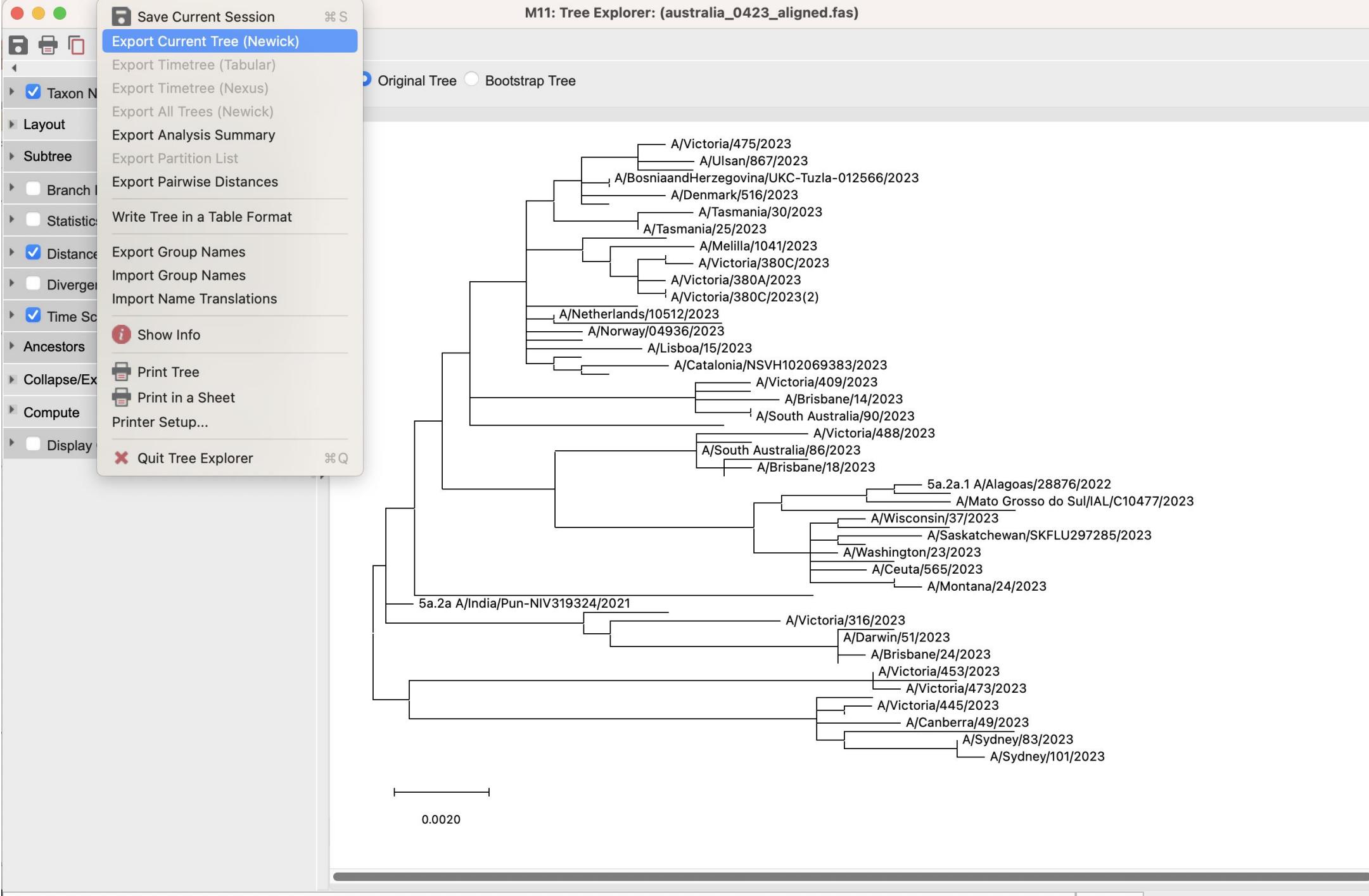
Collapse/Expand Lineages

Compute

Display Caption

● Original Tree ○ Bootstrap Tree







◀ ▶

Taxon Names

Layout

Subtree

Branch Lengths

Statistics/Frequency/Info

Distance Scale

Divergence Times

Time Scale

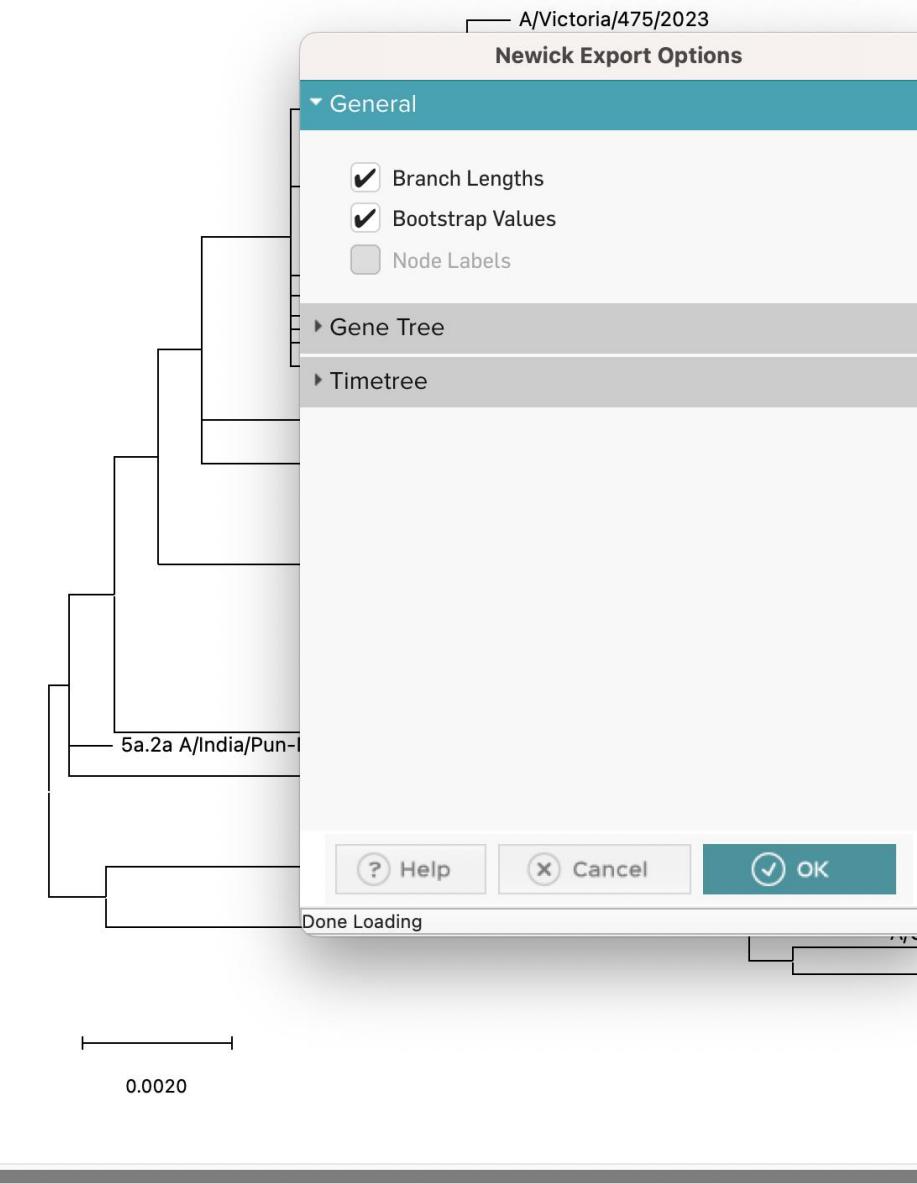
Ancestors Site # 1

Collapse/Expand Lineages

Compute

Display Caption

● Original Tree ○ Bootstrap Tree





- Taxon Names
- Layout
- Subtree
- Branch Lengths
- Statistics/Frequency/Info
- Distance Scale
- Divergence Times
- Time Scale
- Ancestors Site #
- Collapse/Expand Lineages
- Compute
- Display Caption

Original Tree Bootstrap Tree

M11: Text File Editor and Format Converter



Newick Export.nwk

```
1 | ((((((((((((A/Victoria/475/2023:0.00058942,A/Victoria/490/2023:0.00000206)0.4400:0.00000411,A/Ulsan/867/z023:
```

Import tree file in FigTree



Node Clade Taxa

← →

Filter

Selection Mode

Prev/Next

Layout



Zoom:

Expansion:

Fish Eye:

Root Length:

Curvature:

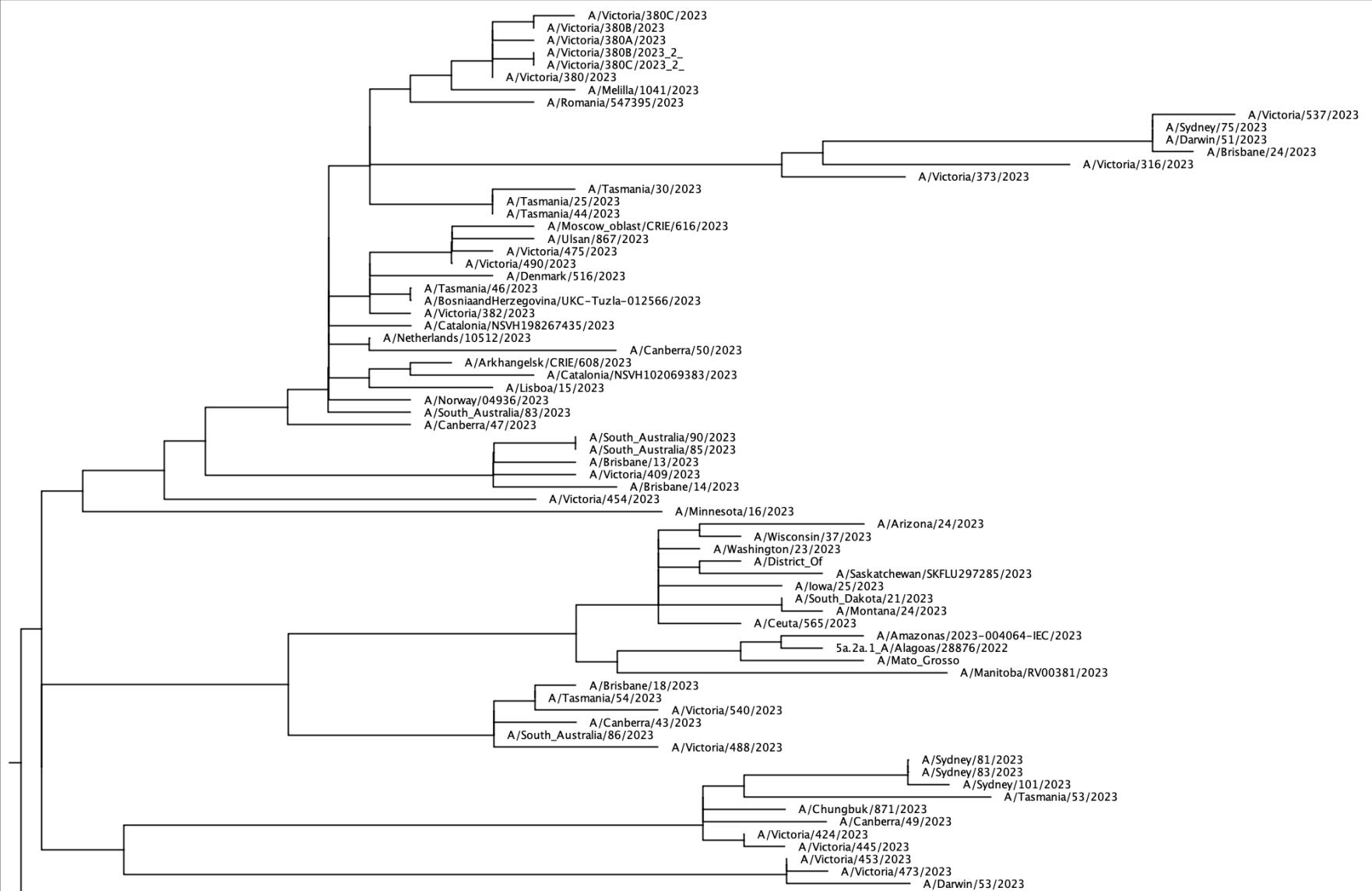
 Align Tip Labels

Current Tree: 1 / 1

Appearance

Trees

Time Scale

 Tip Labels Tip Shapes Node Labels Node Shapes Node Bars Branch Labels Scale Bar Scale Axis Legend

0.002

Extra: NJ methods

Neighbour Joining method

Neighbor joining takes as input a distance matrix specifying the distance between each pair of taxa.

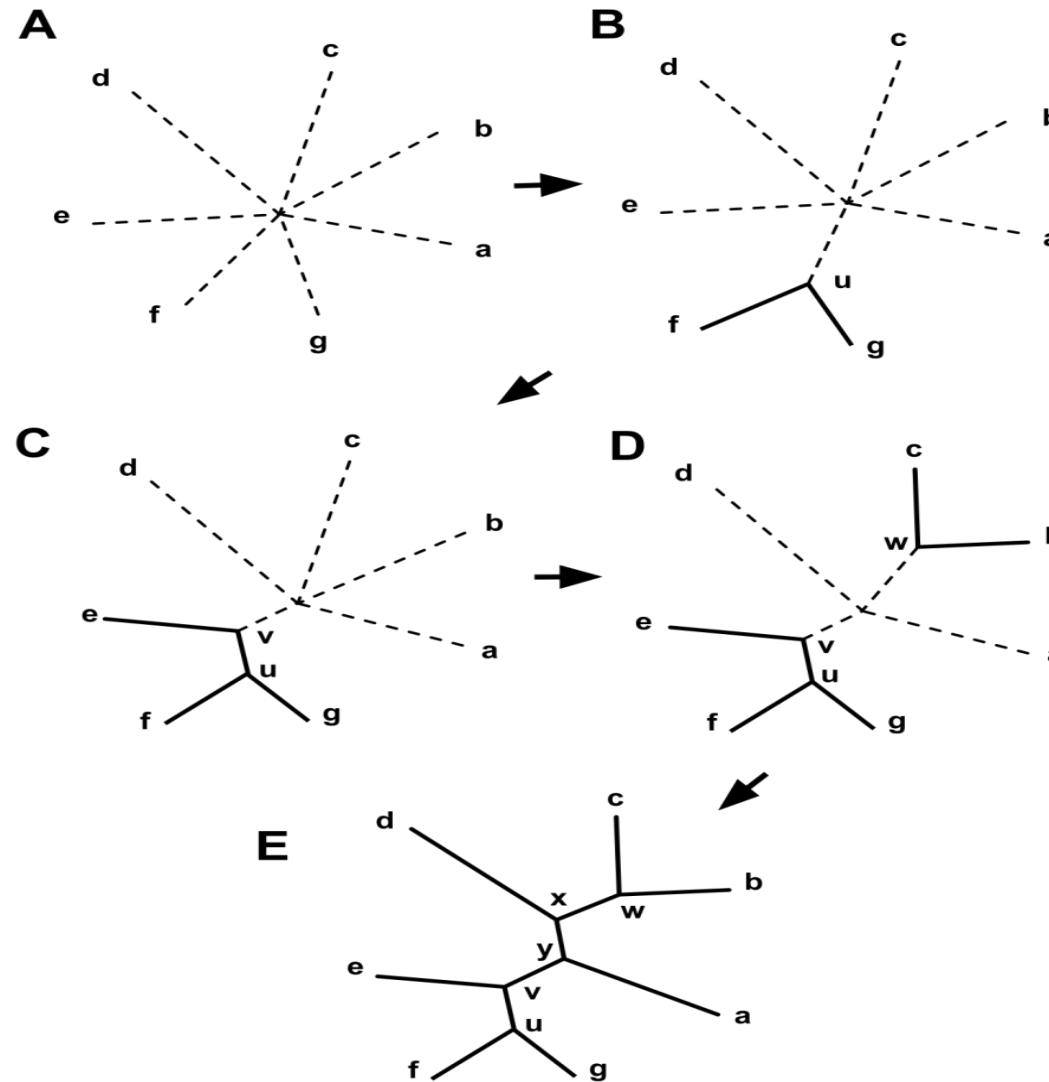
Seq A	ATC AT CGA TTT GCA
Seq B	TTC CCT CGA TAT GCA
Seq C	ATG AT GGAT AT GCA
Seq D	TTCAACGAATTGCA
Seq E	ATG AT GGAT TT GCA
Seq F	TTCAACGAAATGCA



	Matrix:	# Differences	Decimal Places:	0	Style:	Heatmap and Numbers
1_V1A_B/BRISBANE/60/20...	18	39	39	38	37	38
B/VICTORIA/502/2015e	18	41	41	40	39	40
B/Brisbane/22/2019_aug	39	41	0	1	10	21
B/New_Caledonia/10/2019...	39	41	0	1	10	21
B/Washington/02/2019_jan	38	40	1	1	9	20
V1A.2_B/Florida/39/2018_j...	37	39	10	10	9	17
B/Perth/1036/2019_jun	37	39	10	10	9	17
B/Perth/1036/2019_jun	38	40	21	21	20	17
B/Canberra/38/2019_jun	35	37	24	24	23	23
B/Perth/32/2019_jun	35	37	24	24	23	0
B/Darwin/69/2019_jun	37	39	26	26	25	2
B/Malaysia/RP0467/2019_f...	32	34	21	21	20	5
B/Perth/18/2019_apr	28	30	29	29	28	27

Neighbour Joining method

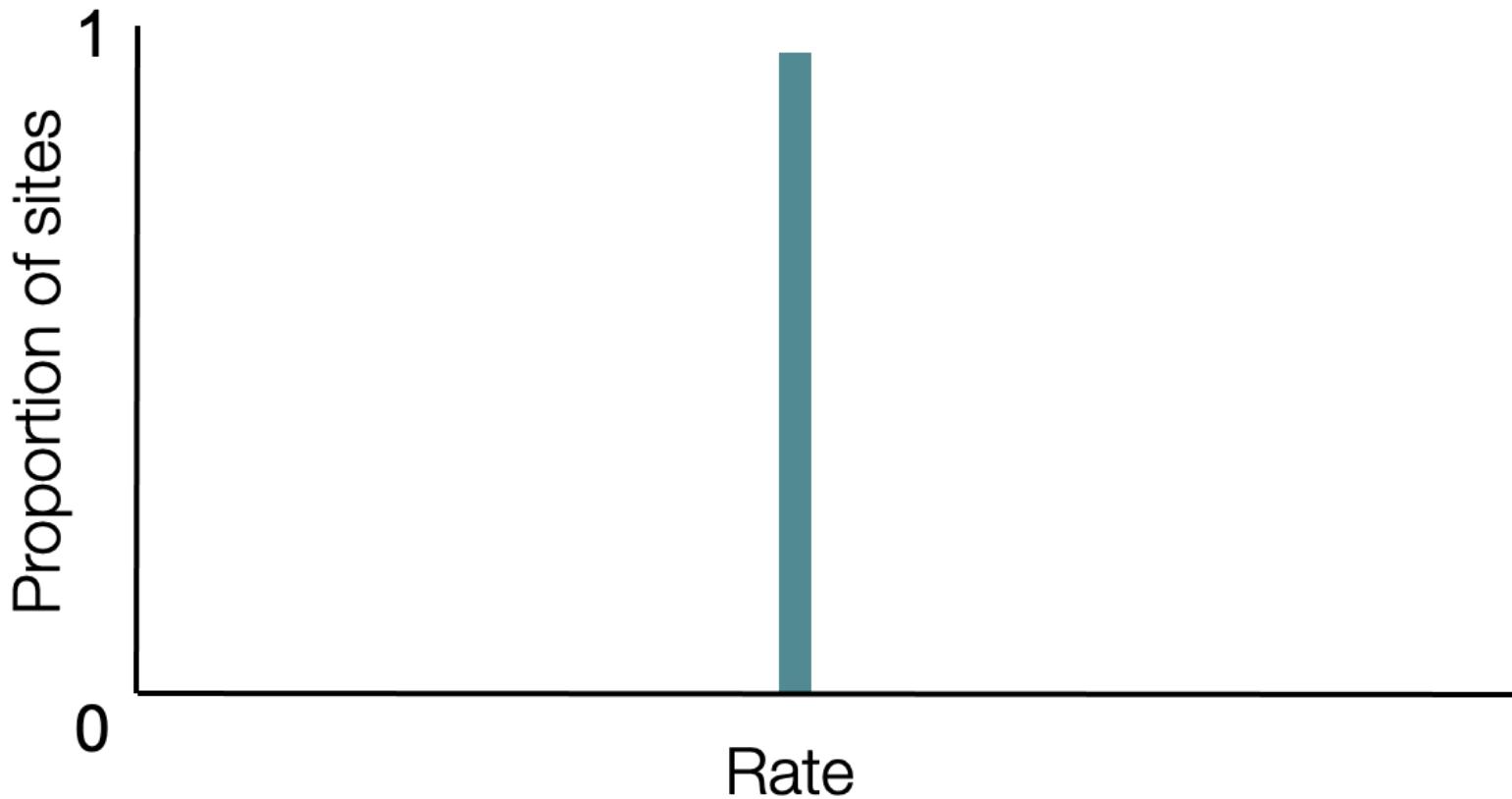
Connects 2 taxa
with smallest
distance



ML Rate variation

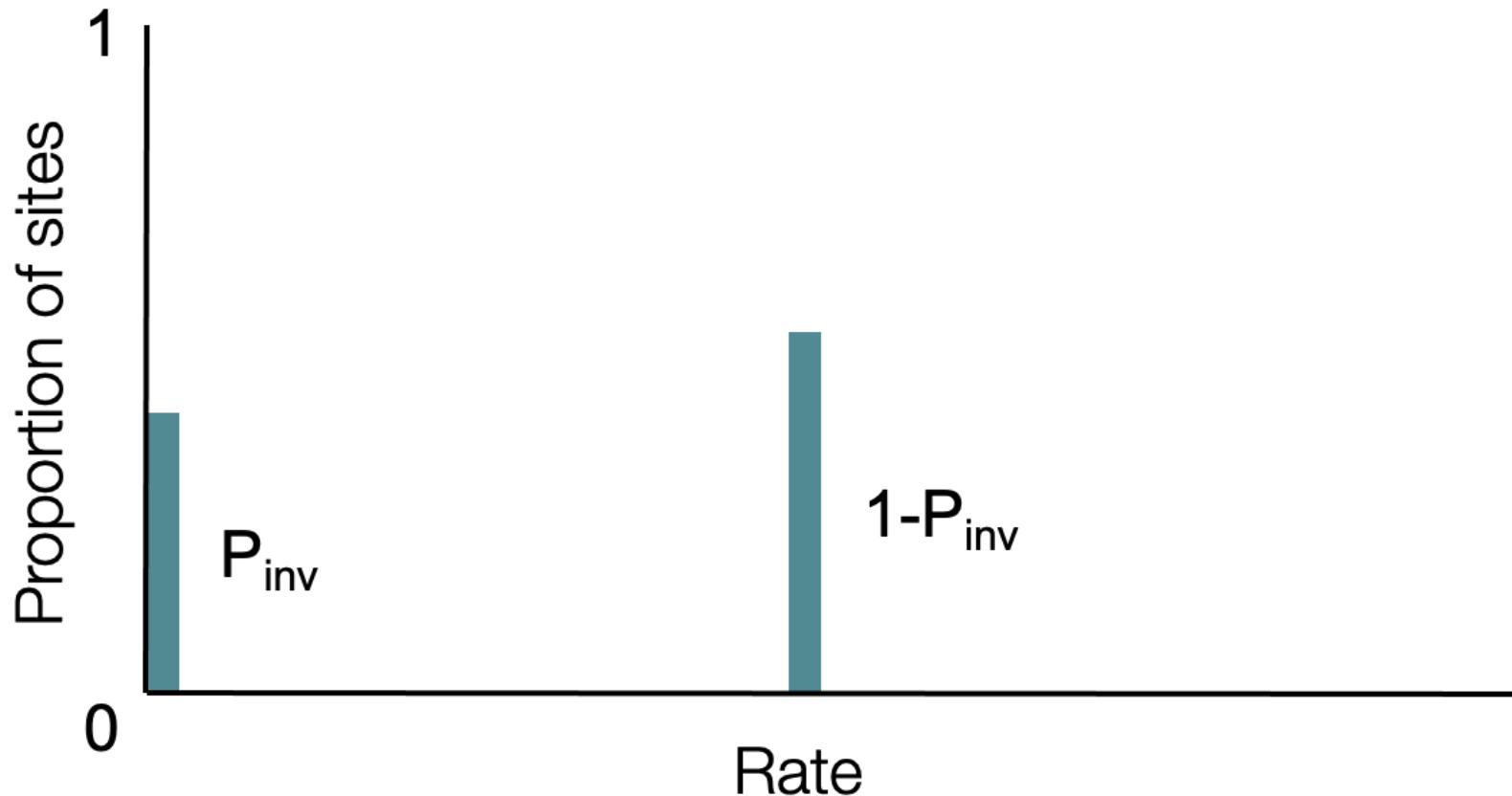
Rate variation across sites

Equal rates among sites (e.g., JC, GTR, HKY models)



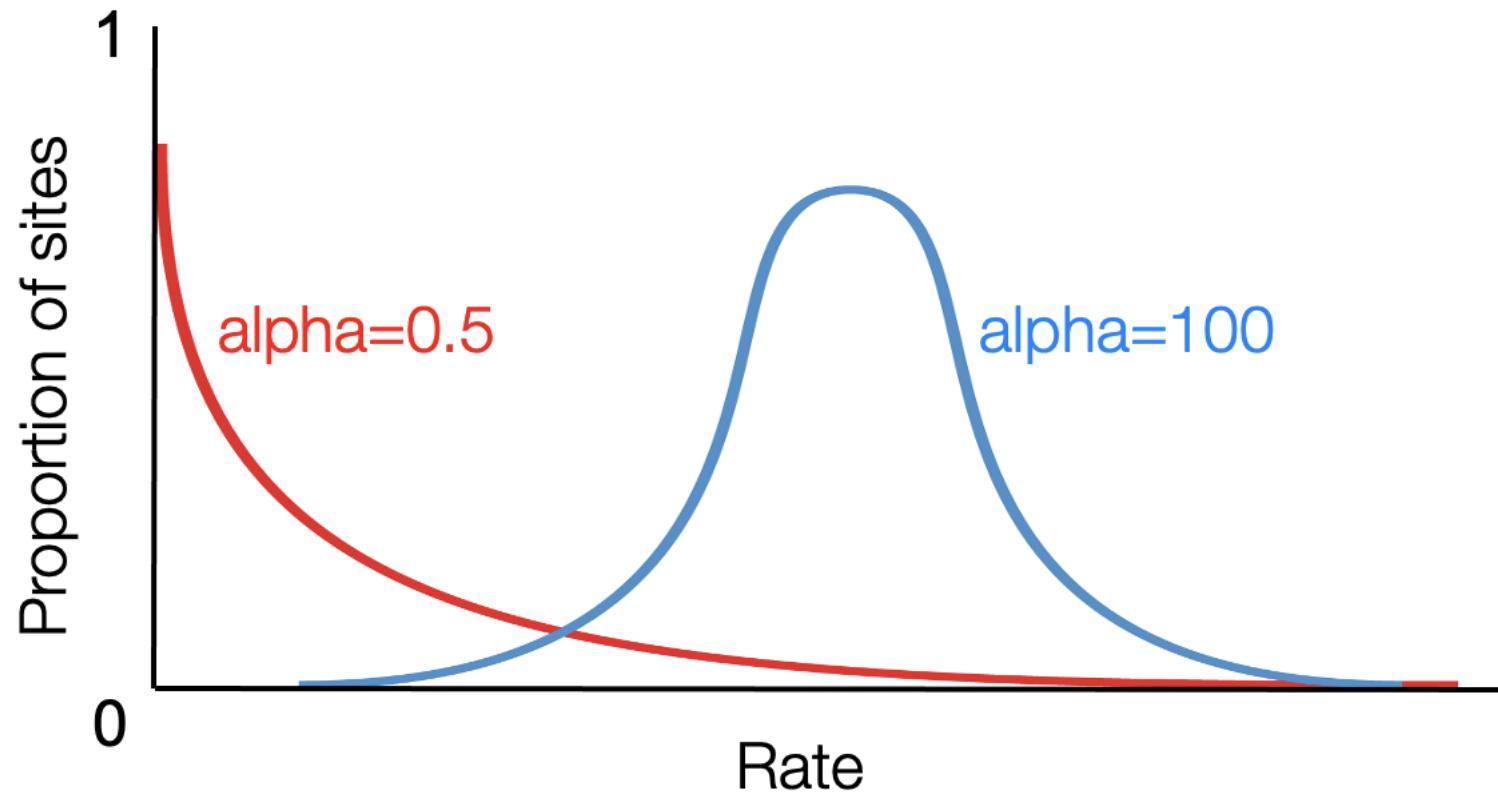
Rate variation across sites

- Proportion of invariable sites (e.g., JC+I, GTR+I, HKY+I models)



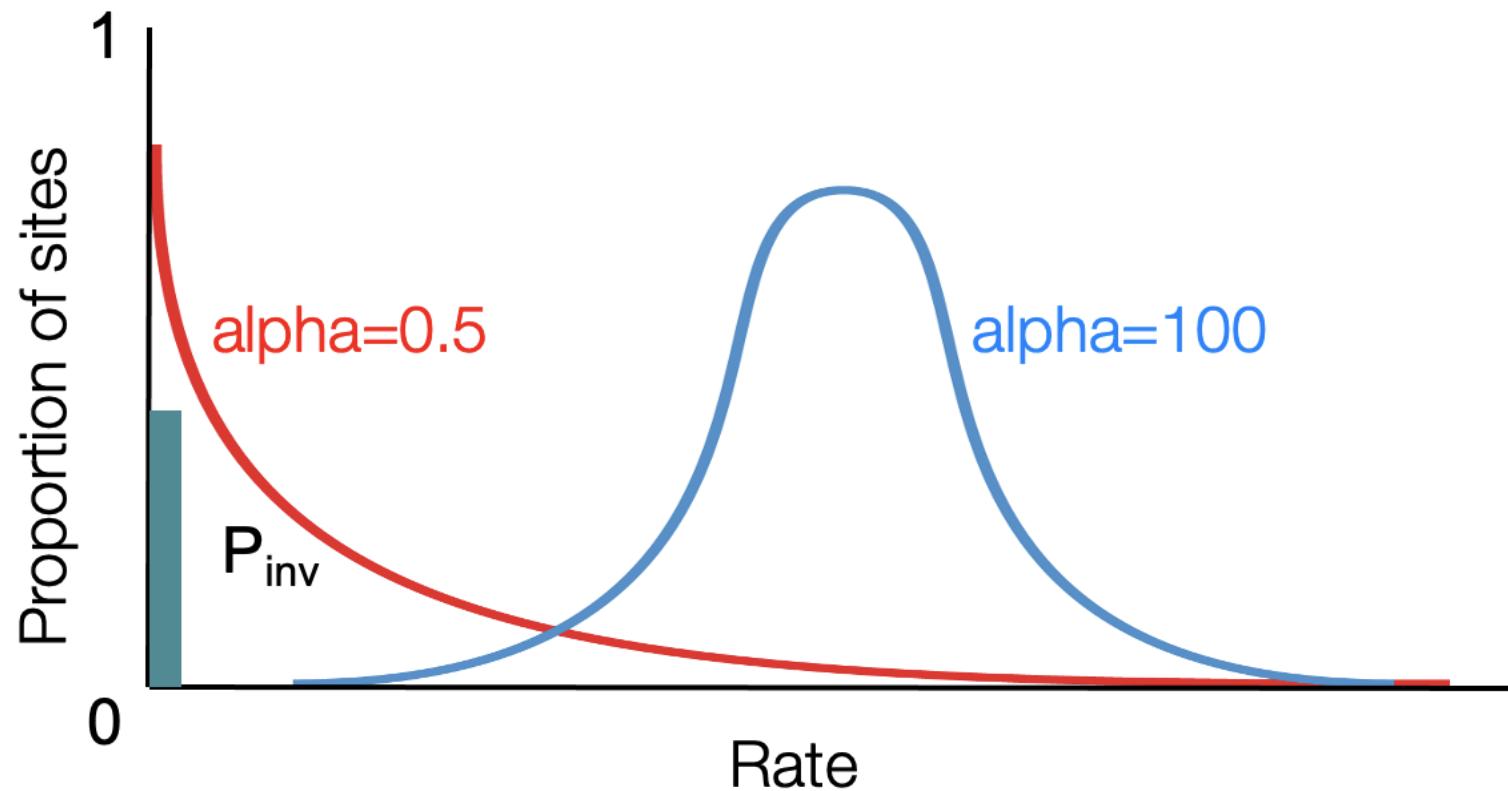
Rate variation across sites

- Gamma-distributed rate variation among sites
(e.g., JC+G, GTR+G, HKY+G models)

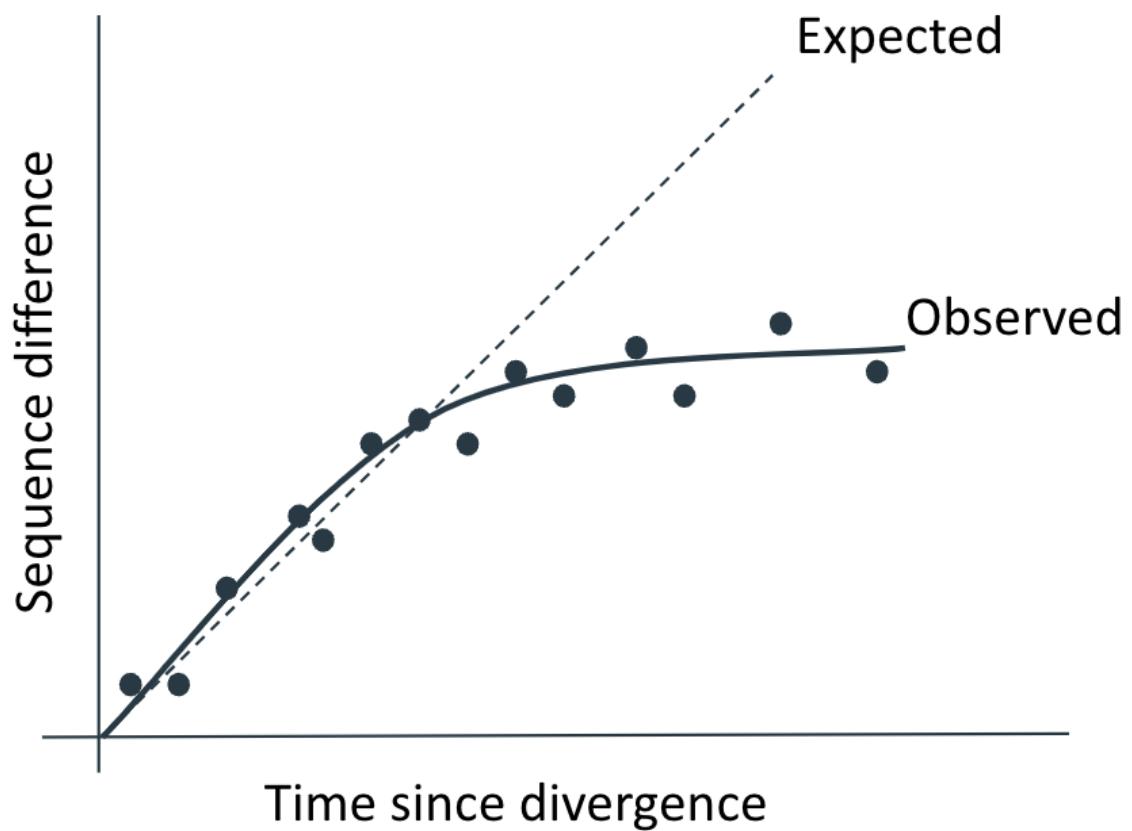


Rate variation across sites

- Gamma-distributed rate variation among sites and a proportion of invariable sites (e.g., JC+G+I, GTR+G+I, HKY+G+I models)

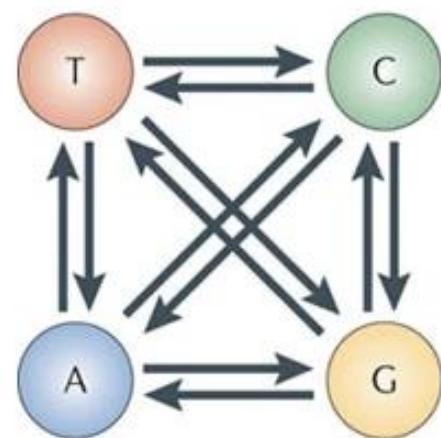
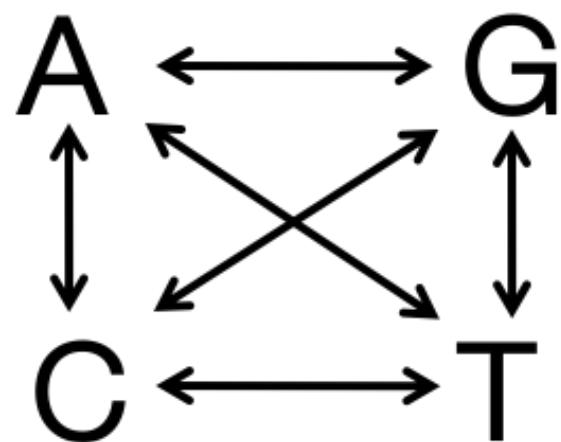


Multiple substitutions



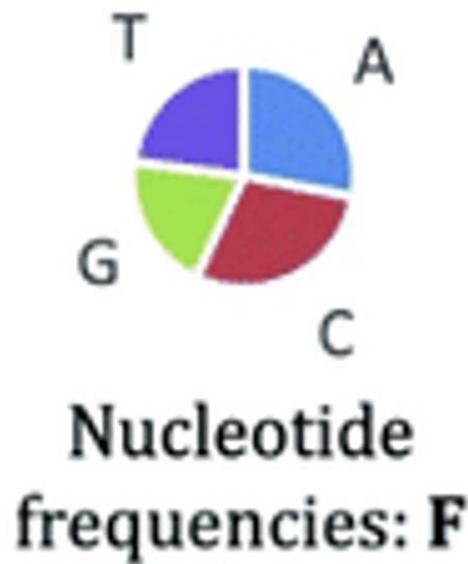
ACCGGGTC
↓ ↓ ↓
TCCC**C**GTC
↓ ↓
GCCGGTA

Rate Matrix



Base Frequencies

$$\pi_A + \pi_C + \pi_G + \pi_T = 1$$



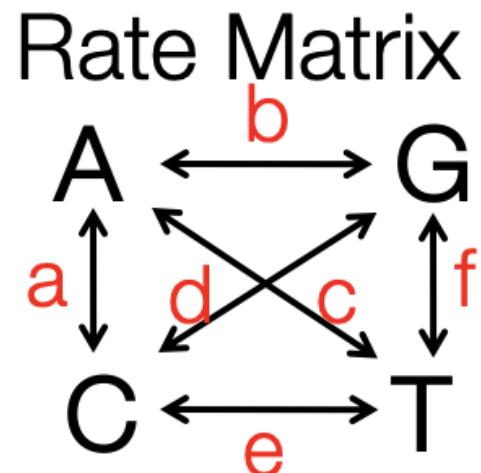
Site Rates

$$+ I + G$$

- C **G** A A A C A **T** G
A A **G** **A** G - - A **T** A
T A **G** **A** G C - T **T** G
A A **G** **A** A A A A **T** G



- C G A A A C A T G
A A G A G - - A T A
T A G A G C - T T G
A A G A A A A A T G



Base Frequencies

$$\pi_A + \pi_C + \pi_G + \pi_T = 1$$

Site Rates

$$+ I + G$$

JC

$$a=b=c=d=e=f$$

$$\pi_A = \pi_C = \pi_G = \pi_T$$

No I or G

0 free
parameters

HKY

$$a=c=d=f, b=e$$

$$\pi_A, \pi_C, \pi_G, \pi_T$$

No I or G

4 free
parameters

GTR

$$a, b, c, d, e, f$$

$$\pi_A, \pi_C, \pi_G, \pi_T$$

No I or G

8 free
parameters

GTR+I+G

$$a, b, c, d, e, f$$

$$\pi_A, \pi_C, \pi_G, \pi_T$$

I, G

10 free
parameters

