

## **Supplementary Materials**

maxAlike: Maximum-likelihood based sequence reconstruction  
with application to improved primer design for unknown  
sequences

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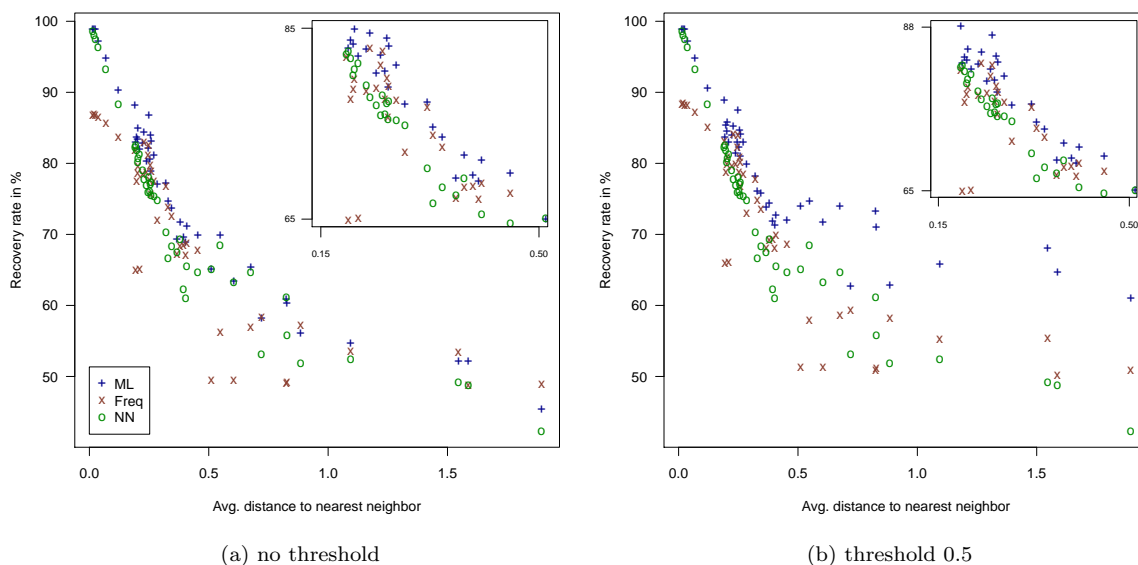


Figure 1: **Data set MZ44-2:** Recovery rates in percent for sequences reconstructed by *maxAlike* (*ML*), frequency-based consensus (*Freq*) and nearest neighbor (*NN*). Each point is one species plotted as its average distance to the phylogenetically nearest neighbor.

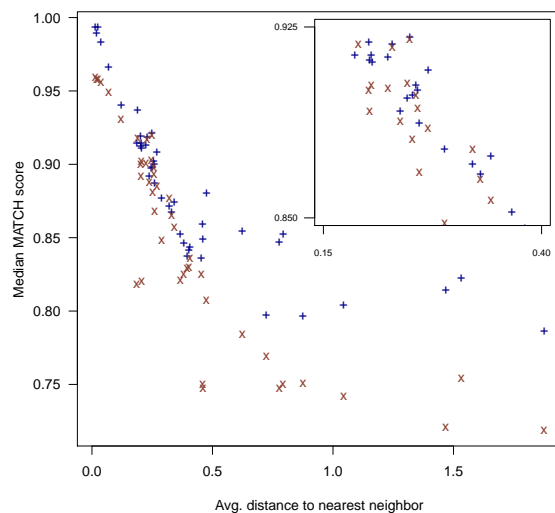


Figure 2: **Data set MZ44-1:** Median MATCH scores for *maxAlike* (*ML*) and nucleotide-frequency (*Freq*) PSSMs for each species compared to the average distance to its phylogenetically closest neighbor.

Table 1: **Data set MZ44-2:** Recovery rates in percent for reconstructed sequences by *maxAlike* probabilities and nucleotide frequencies (*Freq*), and the nearest neighbor sequence (*NN*) for each species. A value of, *e.g.*, 70 means that 70% of the nucleotides were predicted correctly. The  $T = .5$  columns show recovery rates for only those sites with a nucleotide probability/relative frequency above a 0.5 threshold, the “n/T” columns show the recovery rates for reconstructed sequences with highest probability/frequency nucleotides at each site (no threshold). The “Dist.” column shows the average distance to the nearest neighbor in the tree. “Total nt” shows the total number of reconstructed nucleotides for each species and the “%>  $T$ ” columns denote the percentage of sites exceeding the threshold. Bold-faced *maxAlike* values are significantly better ( $P \leq 0.05$ ) than both values of *Freq* with corresponding threshold and *NN*.

| Species | Dist. | Total nt | <i>maxAlike</i> |             |        | <i>Freq</i> |      |        | <i>NN</i> |
|---------|-------|----------|-----------------|-------------|--------|-------------|------|--------|-----------|
|         |       |          | $T = .5$        | n/T         | %> $T$ | $T = .5$    | n/T  | %> $T$ |           |
| hg18    | 0.014 | 1527282  | <b>98.9</b>     | <b>98.9</b> | 99     | 88.3        | 86.7 | 96     | 98.6      |
| gorGor1 | 0.019 | 1003671  | <b>98.9</b>     | <b>98.9</b> | 99     | 88.5        | 86.9 | 96     | 98.0      |
| panTro2 | 0.025 | 1502740  | <b>98.9</b>     | <b>98.9</b> | 99     | 88.2        | 86.7 | 96     | 97.4      |
| ponAbe2 | 0.037 | 1470478  | <b>97.1</b>     | <b>97.1</b> | 99     | 88.1        | 86.5 | 96     | 96.4      |
| rheMac2 | 0.069 | 1458335  | <b>94.7</b>     | <b>94.7</b> | 99     | 87.2        | 85.7 | 96     | 93.2      |
| calJac1 | 0.121 | 1419466  | <b>90.5</b>     | <b>90.3</b> | 99     | 85.1        | 83.6 | 96     | 88.3      |
| turTru1 | 0.191 | 1234224  | <b>88.8</b>     | <b>88.1</b> | 98     | 83.1        | 81.9 | 96     | 82.3      |
| rn4     | 0.194 | 1003434  | <b>83.6</b>     | <b>82.9</b> | 97     | 65.9        | 64.9 | 96     | 82.6      |
| bosTau4 | 0.198 | 1204893  | <b>85.4</b>     | <b>83.7</b> | 95     | 78.7        | 77.5 | 96     | 81.8      |
| felCat3 | 0.202 | 886473   | <b>84.5</b>     | <b>83.3</b> | 96     | 79.8        | 78.6 | 96     | 80.1      |
| vicPac1 | 0.204 | 969615   | <b>85.8</b>     | <b>84.9</b> | 97     | 80.8        | 79.6 | 96     | 80.7      |
| mm9     | 0.210 | 1063702  | <b>82.9</b>     | <b>82.0</b> | 96     | 66.1        | 65.1 | 96     | 81.3      |
| canFam2 | 0.223 | 1380923  | <b>83.9</b>     | <b>82.8</b> | 97     | 79.6        | 78.4 | 96     | 79.0      |
| micMur1 | 0.229 | 1061189  | <b>85.2</b>     | <b>84.4</b> | 97     | 84.1        | 82.9 | 96     | 77.8      |
| otoGar1 | 0.239 | 1012495  | <b>81.4</b>     | <b>80.3</b> | 96     | 79.8        | 78.7 | 96     | 76.9      |
| tarSyr1 | 0.246 | 1089058  | <b>82.9</b>     | <b>82.1</b> | 97     | 82.2        | 81.1 | 96     | 75.9      |
| equCab2 | 0.249 | 1430126  | <b>87.4</b>     | <b>86.8</b> | 98     | 83.9        | 82.6 | 96     | 78.0      |
| choHof1 | 0.253 | 837417   | <b>82.2</b>     | <b>80.5</b> | 95     | 78.7        | 77.6 | 96     | 76.0      |
| pteVam1 | 0.256 | 1119029  | <b>84.6</b>     | <b>83.9</b> | 98     | 80.9        | 79.7 | 96     | 77.1      |
| dasNov2 | 0.258 | 813500   | <b>81.0</b>     | <b>78.8</b> | 94     | 76.8        | 75.7 | 96     | 75.5      |
| myoLuc1 | 0.259 | 719138   | <b>84.0</b>     | <b>83.1</b> | 97     | 80.1        | 78.9 | 96     | 77.3      |
| loxAfr1 | 0.271 | 821203   | <b>82.9</b>     | <b>81.1</b> | 95     | 78.5        | 77.4 | 96     | 75.4      |
| proCap1 | 0.285 | 705318   | <b>79.8</b>     | <b>77.0</b> | 91     | 73.0        | 72.0 | 96     | 74.8      |
| tupBel1 | 0.321 | 862942   | <b>78.1</b>     | <b>77.2</b> | 96     | 77.7        | 76.7 | 96     | 70.3      |
| speTri1 | 0.330 | 902432   | <b>76.1</b>     | <b>74.6</b> | 95     | 74.8        | 73.8 | 96     | 66.7      |
| oryCun1 | 0.345 | 821638   | <b>75.8</b>     | <b>73.6</b> | 93     | 73.5        | 72.5 | 96     | 68.3      |
| ochPri2 | 0.367 | 634906   | <b>73.8</b>     | <b>69.3</b> | 85     | 68.0        | 67.2 | 96     | 67.5      |
| echTel1 | 0.380 | 436794   | <b>74.3</b>     | <b>71.7</b> | 90     | 69.2        | 68.3 | 96     | 69.3      |
| cavPor3 | 0.394 | 1190012  | <b>71.8</b>     | <b>69.6</b> | 91     | 69.3        | 68.4 | 96     | 62.2      |
| dipOrd1 | 0.403 | 641787   | <b>71.2</b>     | <b>68.9</b> | 91     | 68.0        | 67.1 | 96     | 61.0      |
| eriEur1 | 0.408 | 298529   | <b>72.7</b>     | <b>71.1</b> | 92     | 69.8        | 68.8 | 96     | 65.5      |
| sorAra1 | 0.454 | 357798   | <b>71.9</b>     | <b>69.8</b> | 90     | 68.6        | 67.7 | 96     | 64.6      |
| fr2     | 0.516 | 32708    | <b>73.9</b>     | 64.8        | 74     | 51.3        | 49.4 | 92     | 65.0      |
| taeGut1 | 0.548 | 11319    | <b>74.7</b>     | <b>69.8</b> | 82     | 57.9        | 56.2 | 94     | 68.4      |
| tetNig1 | 0.605 | 36333    | <b>71.7</b>     | 63.4        | 73     | 51.3        | 49.5 | 92     | 63.3      |
| galGal3 | 0.676 | 16777    | <b>73.9</b>     | <b>65.4</b> | 74     | 58.6        | 56.9 | 94     | 64.6      |
| monDom4 | 0.721 | 138532   | <b>62.7</b>     | 58.1        | 74     | 59.3        | 58.3 | 96     | 53.1      |
| oryLat2 | 0.825 | 32758    | <b>73.2</b>     | 60.9        | 66     | 50.9        | 49.0 | 92     | 61.1      |
| gasAcu1 | 0.828 | 35685    | <b>71.0</b>     | <b>60.3</b> | 69     | 51.1        | 49.2 | 92     | 55.8      |
| ornAna1 | 0.885 | 65067    | <b>62.8</b>     | 56.1        | 64     | 58.2        | 57.2 | 96     | 51.8      |
| anoCar1 | 1.094 | 25514    | <b>65.8</b>     | <b>54.7</b> | 58     | 55.2        | 53.5 | 93     | 52.4      |
| xenTro2 | 1.546 | 36683    | <b>68.0</b>     | 52.1        | 50     | 55.4        | 53.4 | 92     | 49.2      |
| danRer5 | 1.588 | 48724    | <b>64.6</b>     | <b>52.1</b> | 48     | 50.2        | 48.6 | 93     | 48.7      |
| petMar1 | 1.895 | 24080    | <b>60.8</b>     | 45.3        | 45     | 50.8        | 48.9 | 92     | 42.2      |

Table 2: **Data set MZ44-1, fasttree:** Recovery rates in percent for reconstructed sequences by *maxAlike* probabilities and nucleotide frequencies (*Freq*), and the nearest neighbor sequence (*NN*) for each species. The phylogenetic tree was estimated with *fasttree* for each alignment. A value of, *e.g.*, 70 means that 70% of the nucleotides were predicted correctly. The  $T = .5$  columns show recovery rates for only those sites with a nucleotide probability/relative frequency above a 0.5 threshold, the “n/T” columns show the recovery rates for reconstructed sequences with highest probability/frequency nucleotides at each site (no threshold). The “Dist.” column shows the average distance to the nearest neighbor in the tree. “Total nt” shows the total number of reconstructed nucleotides for each species and the “%>  $T$ ” columns denote the percentage of sites exceeding the threshold. Bold-faced *maxAlike* values are significantly better ( $P < 0.05$ ) than both values of *Freq* with corresponding threshold and *NN*.

| Species | Dist. | Total nt | <i>maxAlike</i> |             |        | <i>Freq</i> |      |        | <i>NN</i> |
|---------|-------|----------|-----------------|-------------|--------|-------------|------|--------|-----------|
|         |       |          | $T = .5$        | n/T         | %> $T$ | $T = .5$    | n/T  | %> $T$ |           |
| hg18    | 0.014 | 778915   | <b>99.1</b>     | <b>99.0</b> | 99     | 89.6        | 88.5 | 97     | 98.5      |
| gorGor1 | 0.018 | 529106   | <b>99.0</b>     | <b>98.9</b> | 99     | 89.6        | 88.5 | 97     | 97.9      |
| panTro2 | 0.022 | 761108   | <b>99.1</b>     | <b>99.1</b> | 99     | 89.6        | 88.5 | 97     | 97.6      |
| ponAbe2 | 0.031 | 747503   | <b>97.7</b>     | <b>97.6</b> | 99     | 89.4        | 88.3 | 97     | 96.8      |
| rheMac2 | 0.057 | 742551   | <b>95.6</b>     | <b>95.4</b> | 99     | 88.7        | 87.6 | 97     | 94.4      |
| calJac1 | 0.103 | 706152   | <b>91.8</b>     | <b>91.6</b> | 99     | 86.9        | 85.9 | 97     | 90.3      |
| rn4     | 0.157 | 491071   | <b>86.7</b>     | <b>86.1</b> | 98     | 72.1        | 71.3 | 97     | 85.9      |
| turTru1 | 0.163 | 603369   | <b>90.6</b>     | <b>90.0</b> | 98     | 85.9        | 85.1 | 98     | 85.3      |
| mm9     | 0.176 | 529087   | <b>86.0</b>     | <b>85.1</b> | 97     | 72.4        | 71.6 | 97     | 84.7      |
| bosTau4 | 0.179 | 660772   | <b>87.4</b>     | <b>86.4</b> | 97     | 82.4        | 81.5 | 97     | 84.0      |
| felCat3 | 0.181 | 437120   | <b>87.9</b>     | <b>86.8</b> | 97     | 83.6        | 82.8 | 97     | 83.8      |
| vicPac1 | 0.188 | 513867   | <b>88.2</b>     | <b>87.6</b> | 98     | 84.1        | 83.3 | 98     | 83.5      |
| equCab2 | 0.193 | 740967   | <b>89.6</b>     | <b>89.2</b> | 99     | 86.7        | 85.8 | 97     | 82.9      |
| canFam2 | 0.194 | 719372   | <b>87.5</b>     | <b>86.6</b> | 97     | 83.7        | 82.8 | 97     | 82.9      |
| micMur1 | 0.201 | 538561   | <b>88.3</b>     | <b>87.7</b> | 98     | 87.0        | 86.1 | 97     | 82.5      |
| pteVam1 | 0.219 | 585057   | <b>86.9</b>     | <b>86.3</b> | 98     | 83.7        | 82.9 | 98     | 81.1      |
| myoLuc1 | 0.221 | 405380   | <b>86.3</b>     | <b>85.6</b> | 98     | 83.0        | 82.1 | 97     | 80.9      |
| otoGar1 | 0.225 | 512069   | <b>85.2</b>     | <b>84.1</b> | 97     | 83.2        | 82.4 | 97     | 80.9      |
| tarSyr1 | 0.230 | 557130   | <b>86.3</b>     | <b>85.7</b> | 98     | 85.2        | 84.4 | 97     | 80.7      |
| loxAfr2 | 0.240 | 412845   | <b>86.1</b>     | <b>84.6</b> | 96     | 82.1        | 81.3 | 97     | 79.7      |
| choHof1 | 0.244 | 401781   | <b>85.2</b>     | <b>83.7</b> | 96     | 82.0        | 81.2 | 97     | 79.7      |
| dasNov2 | 0.254 | 403326   | <b>83.9</b>     | <b>82.1</b> | 95     | 80.2        | 79.5 | 97     | 78.9      |
| proCap1 | 0.262 | 374774   | <b>82.2</b>     | <b>80.5</b> | 95     | 77.1        | 76.3 | 97     | 78.1      |
| tupBel1 | 0.286 | 460919   | <b>83.0</b>     | <b>82.1</b> | 97     | 82.1        | 81.3 | 97     | 76.6      |
| oryCun1 | 0.309 | 401587   | <b>81.9</b>     | <b>79.7</b> | 94     | 79.1        | 78.4 | 98     | 75.1      |
| taeGut1 | 0.312 | 25380    | <b>80.9</b>     | <b>75.3</b> | 88     | 62.5        | 60.5 | 94     | 74.1      |
| speTri1 | 0.326 | 436541   | <b>82.1</b>     | <b>80.6</b> | 95     | 80.1        | 79.3 | 97     | 74.5      |
| ochPri2 | 0.334 | 340830   | <b>78.5</b>     | <b>75.6</b> | 91     | 74.0        | 73.3 | 98     | 73.3      |
| echTel1 | 0.336 | 269107   | <b>78.4</b>     | <b>76.3</b> | 93     | 74.1        | 73.4 | 98     | 73.2      |
| eriEur1 | 0.344 | 207241   | <b>78.4</b>     | <b>76.9</b> | 95     | 75.5        | 74.8 | 97     | 72.7      |
| fr2     | 0.346 | 69842    | <b>79.7</b>     | <b>72.3</b> | 84     | 52.3        | 50.6 | 94     | 72.0      |
| sorAra1 | 0.371 | 218295   | <b>77.4</b>     | <b>75.7</b> | 94     | 74.2        | 73.5 | 97     | 71.6      |
| tetNig1 | 0.383 | 69625    | <b>79.1</b>     | <b>71.4</b> | 83     | 52.1        | 50.5 | 94     | 70.6      |
| dipOrd1 | 0.384 | 349108   | <b>78.0</b>     | <b>75.7</b> | 93     | 74.8        | 74.1 | 97     | 70.6      |
| cavPor3 | 0.385 | 607860   | <b>77.6</b>     | <b>75.5</b> | 93     | 74.9        | 74.1 | 97     | 70.5      |
| galGal3 | 0.412 | 35632    | <b>77.7</b>     | <b>70.1</b> | 83     | 60.9        | 59.1 | 94     | 69.0      |
| oryLat2 | 0.417 | 67953    | <b>76.2</b>     | <b>68.1</b> | 80     | 52.2        | 50.4 | 94     | 66.9      |
| gasAcu1 | 0.424 | 72157    | <b>76.9</b>     | <b>68.9</b> | 81     | 52.0        | 50.5 | 94     | 67.2      |
| monDom4 | 0.528 | 169777   | <b>69.5</b>     | <b>64.8</b> | 83     | 64.3        | 63.4 | 97     | 62.1      |
| ornAna1 | 0.569 | 84772    | <b>68.6</b>     | <b>63.1</b> | 80     | 62.2        | 61.1 | 96     | 60.7      |
| anoCar1 | 0.607 | 39206    | <b>68.3</b>     | <b>59.9</b> | 74     | 56.7        | 55.2 | 94     | 57.9      |
| xenTro2 | 0.641 | 73195    | <b>68.2</b>     | <b>57.8</b> | 72     | 56.2        | 54.5 | 94     | 55.9      |
| danRer5 | 0.681 | 75481    | <b>67.9</b>     | <b>57.3</b> | 70     | 50.2        | 48.8 | 94     | 54.9      |
| petMar1 | 0.775 | 48471    | <b>63.1</b>     | <b>52.5</b> | 66     | 51.2        | 49.7 | 94     | 50.6      |

Table 3: **Data set MZ44-2, fasttree:** Recovery rates in percent for reconstructed sequences by *maxAlike* probabilities and nucleotide frequencies (*Freq*), and the nearest neighbor sequence (*NN*) for each species. The phylogenetic tree was estimated with *fasttree* for each alignment. A value of, *e.g.*, 70 means that 70% of the nucleotides were predicted correctly. The  $T = .5$  columns show recovery rates for only those sites with a nucleotide probability/relative frequency above a 0.5 threshold, the “n/T” columns show the recovery rates for reconstructed sequences with highest probability/frequency nucleotides at each site (no threshold). The “Dist.” column shows the average distance to the nearest neighbor in the tree. “Total nt” shows the total number of reconstructed nucleotides for each species and the “%>  $T$ ” columns denote the percentage of sites exceeding the threshold. Bold-faced *maxAlike* values are significantly better ( $P < 0.05$ ) than both values of *Freq* with corresponding threshold and *NN*.

| Species | Dist. | Total nt | <i>maxAlike</i> |             |        | <i>Freq</i> |      |        | <i>NN</i> |
|---------|-------|----------|-----------------|-------------|--------|-------------|------|--------|-----------|
|         |       |          | $T = .5$        | n/T         | %> $T$ | $T = .5$    | n/T  | %> $T$ |           |
| hg18    | 0.020 | 1519175  | <b>99.2</b>     | <b>99.1</b> | 99     | 88.2        | 86.8 | 96     | 98.1      |
| gorGor1 | 0.023 | 995246   | <b>99.1</b>     | <b>99.1</b> | 99     | 88.5        | 87.0 | 96     | 97.7      |
| panTro2 | 0.029 | 1494088  | <b>99.1</b>     | <b>99.1</b> | 99     | 88.2        | 86.7 | 96     | 97.3      |
| ponAbe2 | 0.037 | 1461934  | <b>97.7</b>     | <b>97.6</b> | 99     | 88.1        | 86.6 | 96     | 96.6      |
| rheMac2 | 0.067 | 1449917  | <b>95.2</b>     | <b>95.1</b> | 99     | 87.2        | 85.8 | 96     | 94.1      |
| calJac1 | 0.125 | 1411791  | <b>90.9</b>     | <b>90.6</b> | 99     | 85.1        | 83.7 | 96     | 89.4      |
| turTru1 | 0.201 | 1226989  | <b>89.5</b>     | <b>88.7</b> | 97     | 83.2        | 82.0 | 96     | 83.7      |
| rn4     | 0.223 | 997719   | <b>84.2</b>     | <b>83.1</b> | 96     | 65.8        | 64.9 | 96     | 82.9      |
| bosTau4 | 0.225 | 1197123  | <b>85.6</b>     | <b>84.5</b> | 97     | 78.7        | 77.6 | 96     | 82.2      |
| vicPac1 | 0.236 | 964234   | <b>86.5</b>     | <b>85.7</b> | 97     | 80.8        | 79.7 | 96     | 81.5      |
| felCat3 | 0.240 | 881632   | <b>85.8</b>     | <b>84.4</b> | 96     | 79.8        | 78.7 | 96     | 81.0      |
| mm9     | 0.243 | 1057341  | <b>83.7</b>     | <b>82.3</b> | 95     | 66.1        | 65.2 | 96     | 81.8      |
| equCab2 | 0.246 | 1422375  | <b>88.1</b>     | <b>87.5</b> | 98     | 83.9        | 82.7 | 96     | 80.7      |
| canFam2 | 0.259 | 1373387  | <b>85.1</b>     | <b>83.8</b> | 96     | 79.6        | 78.4 | 96     | 79.9      |
| micMur1 | 0.263 | 1054674  | <b>86.1</b>     | <b>85.0</b> | 97     | 84.1        | 83.0 | 96     | 79.6      |
| pteVam1 | 0.270 | 1114250  | <b>85.4</b>     | <b>84.6</b> | 97     | 80.9        | 79.8 | 96     | 79.2      |
| myoLuc1 | 0.275 | 715572   | <b>84.8</b>     | <b>83.8</b> | 97     | 80.1        | 78.9 | 96     | 79.0      |
| otoGar1 | 0.298 | 1006424  | <b>82.6</b>     | <b>81.2</b> | 95     | 79.8        | 78.8 | 96     | 77.6      |
| tarSyr1 | 0.304 | 1083090  | <b>83.7</b>     | <b>82.8</b> | 97     | 82.2        | 81.1 | 96     | 77.5      |
| loxAfr2 | 0.305 | 816200   | <b>84.1</b>     | <b>82.1</b> | 94     | 78.5        | 77.5 | 96     | 77.3      |
| choHof1 | 0.306 | 832965   | <b>83.4</b>     | <b>81.5</b> | 94     | 78.7        | 77.6 | 96     | 77.4      |
| dasNov2 | 0.318 | 808721   | <b>82.0</b>     | <b>79.9</b> | 93     | 76.8        | 75.8 | 96     | 76.6      |
| proCap1 | 0.331 | 701398   | <b>80.1</b>     | <b>78.1</b> | 93     | 73.0        | 72.1 | 96     | 75.8      |
| tupBel1 | 0.388 | 858134   | <b>79.2</b>     | <b>77.9</b> | 95     | 77.7        | 76.8 | 96     | 72.4      |
| oryCun1 | 0.432 | 817021   | <b>77.9</b>     | <b>74.9</b> | 90     | 73.5        | 72.6 | 96     | 70.2      |
| echTel1 | 0.436 | 433682   | <b>75.5</b>     | <b>72.9</b> | 90     | 69.2        | 68.3 | 96     | 70.0      |
| speTri1 | 0.449 | 897852   | <b>78.1</b>     | <b>75.7</b> | 92     | 74.8        | 73.8 | 96     | 69.5      |
| fr2     | 0.452 | 32587    | <b>77.3</b>     | <b>70.3</b> | 79     | 51.3        | 49.7 | 92     | 68.3      |
| ochPri2 | 0.464 | 629803   | <b>74.5</b>     | <b>71.0</b> | 86     | 68.0        | 67.2 | 96     | 68.6      |
| taeGut1 | 0.470 | 11319    | <b>76.7</b>     | <b>71.3</b> | 84     | 57.9        | 56.3 | 94     | 70.6      |
| eriEur1 | 0.477 | 296570   | <b>74.3</b>     | <b>72.1</b> | 90     | 69.8        | 68.9 | 96     | 68.1      |
| oryLat2 | 0.487 | 32637    | <b>75.1</b>     | <b>67.5</b> | 77     | 51.0        | 49.2 | 92     | 65.6      |
| sorAra1 | 0.510 | 355983   | <b>73.3</b>     | <b>70.8</b> | 89     | 68.6        | 67.8 | 96     | 66.9      |
| galGal3 | 0.511 | 16777    | <b>75.0</b>     | <b>69.1</b> | 80     | 58.6        | 57.3 | 94     | 67.0      |
| tetNig1 | 0.516 | 36848    | <b>75.4</b>     | <b>67.7</b> | 78     | 51.1        | 49.5 | 92     | 66.0      |
| cavPor3 | 0.531 | 1183223  | <b>73.9</b>     | <b>70.7</b> | 87     | 69.3        | 68.4 | 96     | 65.6      |
| gasAcu1 | 0.535 | 35564    | <b>74.4</b>     | <b>66.1</b> | 75     | 51.1        | 49.4 | 92     | 63.8      |
| dipOrd1 | 0.548 | 637432   | <b>73.6</b>     | <b>70.1</b> | 86     | 68.0        | 67.2 | 96     | 65.0      |
| anoCar1 | 0.748 | 25290    | <b>66.1</b>     | <b>59.2</b> | 71     | 55.2        | 53.9 | 93     | 56.6      |
| ornAna1 | 0.750 | 64598    | <b>64.7</b>     | <b>59.4</b> | 74     | 58.2        | 57.3 | 96     | 57.3      |
| xenTro2 | 0.760 | 36562    | <b>66.3</b>     | <b>57.5</b> | 67     | 55.5        | 53.7 | 92     | 54.7      |
| monDom4 | 0.769 | 137445   | <b>65.3</b>     | <b>60.6</b> | 75     | 59.3        | 58.4 | 96     | 57.7      |
| petMar1 | 0.868 | 23959    | <b>61.0</b>     | <b>52.5</b> | 61     | 50.9        | 49.2 | 92     | 50.2      |
| danRer5 | 0.870 | 48573    | <b>65.4</b>     | <b>57.3</b> | 66     | 50.2        | 48.9 | 93     | 54.2      |

Table 4: **Data set MZ44-1:** Median MATCH scores of the *maxAlike* predicted PSSMs (*ML*) and the frequency based PSSMs (*Freq*) The last column shows the difference of both scores, with \* denoting a significant difference ( $P < 0.05$ ).

| Species | <i>ML</i> | <i>Freq</i> | <i>ML - Freq</i> |
|---------|-----------|-------------|------------------|
| hg18    | 0.993     | 0.959       | 0.034*           |
| gorGor1 | 0.989     | 0.958       | 0.031*           |
| panTro2 | 0.993     | 0.958       | 0.034*           |
| ponAbe2 | 0.983     | 0.956       | 0.027*           |
| rheMac2 | 0.966     | 0.949       | 0.018*           |
| calJac1 | 0.940     | 0.931       | 0.009*           |
| rn4     | 0.914     | 0.818       | 0.097*           |
| turTru1 | 0.937     | 0.918       | 0.019*           |
| felCat3 | 0.919     | 0.900       | 0.020*           |
| bosTau4 | 0.912     | 0.892       | 0.019*           |
| vicPac1 | 0.914     | 0.902       | 0.012*           |
| mm9     | 0.911     | 0.820       | 0.091*           |
| canFam2 | 0.913     | 0.901       | 0.012*           |
| micMur1 | 0.918     | 0.917       | 0.001*           |
| otoGar1 | 0.892     | 0.888       | 0.004*           |
| tarSyr1 | 0.897     | 0.903       | -0.006           |
| equCab2 | 0.921     | 0.920       | 0.001*           |
| choHof1 | 0.898     | 0.881       | 0.017*           |
| pteVam1 | 0.902     | 0.898       | 0.004*           |
| myoLuc1 | 0.900     | 0.893       | 0.008*           |
| dasNov2 | 0.887     | 0.868       | 0.018*           |
| loxAfr2 | 0.908     | 0.885       | 0.023*           |
| proCap1 | 0.877     | 0.848       | 0.029*           |
| tupBel1 | 0.871     | 0.877       | -0.006           |
| speTri1 | 0.867     | 0.865       | 0.002*           |
| oryCun1 | 0.874     | 0.857       | 0.018*           |
| ochPri2 | 0.852     | 0.821       | 0.031*           |
| echTel1 | 0.846     | 0.825       | 0.021*           |
| cavPor3 | 0.837     | 0.829       | 0.008*           |
| dipOrd1 | 0.841     | 0.830       | 0.011*           |
| eriEur1 | 0.843     | 0.836       | 0.007*           |
| sorAra1 | 0.836     | 0.825       | 0.011*           |
| fr2     | 0.859     | 0.750       | 0.108*           |
| tetNig1 | 0.849     | 0.747       | 0.102*           |
| taeGut1 | 0.880     | 0.807       | 0.073*           |
| galGal3 | 0.854     | 0.784       | 0.070*           |
| monDom4 | 0.797     | 0.769       | 0.028*           |
| gasAcu1 | 0.847     | 0.747       | 0.100*           |
| oryLat2 | 0.852     | 0.750       | 0.102*           |
| ornAna1 | 0.796     | 0.751       | 0.045*           |
| anoCar1 | 0.804     | 0.742       | 0.063*           |
| danRer5 | 0.814     | 0.721       | 0.092*           |
| xenTro2 | 0.822     | 0.754       | 0.068*           |
| petMar1 | 0.786     | 0.719       | 0.066*           |

Table 5: **Data set MZ44-2:** Median MATCH scores of the *maxAlike* predicted PSSMs (*ML*) and the frequency based PSSMs (*Freq*) The last column shows the difference of both scores, with \* denoting a significant difference ( $P < 0.05$ ).

| Species | <i>ML</i> | <i>Freq</i> | <i>ML - Freq</i> |
|---------|-----------|-------------|------------------|
| hg18    | 0.992     | 0.951       | 0.042*           |
| gorGor1 | 0.989     | 0.952       | 0.037*           |
| panTro2 | 0.992     | 0.950       | 0.041*           |
| ponAbe2 | 0.980     | 0.947       | 0.033*           |
| rheMac2 | 0.961     | 0.938       | 0.023*           |
| calJac1 | 0.926     | 0.915       | 0.011*           |
| turTru1 | 0.923     | 0.896       | 0.028*           |
| rn4     | 0.885     | 0.757       | 0.128*           |
| bosTau4 | 0.890     | 0.859       | 0.031*           |
| felCat3 | 0.896     | 0.867       | 0.029*           |
| vicPac1 | 0.893     | 0.874       | 0.019*           |
| mm9     | 0.881     | 0.758       | 0.123*           |
| canFam2 | 0.884     | 0.864       | 0.020*           |
| micMur1 | 0.892     | 0.890       | 0.002*           |
| otoGar1 | 0.861     | 0.854       | 0.006*           |
| tarSyr1 | 0.867     | 0.875       | -0.008           |
| equCab2 | 0.901     | 0.897       | 0.004*           |
| choHof1 | 0.875     | 0.851       | 0.025*           |
| pteVam1 | 0.882     | 0.874       | 0.008*           |
| dasNov2 | 0.863     | 0.835       | 0.028*           |
| myoLuc1 | 0.879     | 0.866       | 0.013*           |
| loxAfr2 | 0.885     | 0.852       | 0.033*           |
| proCap1 | 0.849     | 0.807       | 0.042*           |
| tupBel1 | 0.827     | 0.835       | -0.008           |
| speTri1 | 0.820     | 0.816       | 0.004*           |
| oryCun1 | 0.828     | 0.805       | 0.023*           |
| ochPri2 | 0.804     | 0.760       | 0.044*           |
| echTel1 | 0.806     | 0.773       | 0.033*           |
| cavPor3 | 0.788     | 0.773       | 0.015*           |
| dipOrd1 | 0.784     | 0.764       | 0.020*           |
| eriEur1 | 0.791     | 0.778       | 0.012*           |
| sorAra1 | 0.786     | 0.768       | 0.018*           |
| fr2     | 0.825     | 0.716       | 0.109*           |
| taeGut1 | 0.817     | 0.724       | 0.093*           |
| tetNig1 | 0.803     | 0.705       | 0.098*           |
| galGal3 | 0.814     | 0.726       | 0.088*           |
| monDom4 | 0.726     | 0.695       | 0.031*           |
| oryLat2 | 0.824     | 0.715       | 0.108*           |
| gasAcu1 | 0.808     | 0.713       | 0.095*           |
| ornAna1 | 0.735     | 0.691       | 0.044*           |
| anoCar1 | 0.763     | 0.702       | 0.060*           |
| xenTro2 | 0.784     | 0.718       | 0.066*           |
| danRer5 | 0.763     | 0.675       | 0.089*           |
| petMar1 | 0.745     | 0.684       | 0.062*           |

Table 6: **Data set MZ44-1**: Average number of mismatches and difference of melting temperature  $T_m$  in °C (in brackets) between the predicted primer oligonucleotides and the actual primer complement in the target gene. \*: *maxAlike* reconstruction with  $T = .5$  is significantly lower ( $P < 0.05$ ); †: *maxAlike* reconstruction with n/T is significantly lower ( $P < 0.05$ ); °: *maxAlike* reconstruction with  $T = 0.5$  has significantly less cases of dinucleotide mismatches or mismatches at extreme positions ( $P < 0.05$ ); •: *maxAlike* reconstruction with n/T has significantly less cases of dinucleotide mismatches or mismatches at extreme positions ( $P < 0.05$ )

| Species | <i>maxAlike</i> |            | Freq          |               | NN               |
|---------|-----------------|------------|---------------|---------------|------------------|
|         | $T = .5$        | n/T        | $T = .5$      | n/T           |                  |
| hg18    | 0.2 (1.1)       | 0.2 (1.1)  | 2.0* (8.8*°)  | 2.3† (10.2†•) | 0.3*† (1.4*†°•)  |
| gorGor1 | 0.2 (1.1)       | 0.2 (1.2)  | 2.0* (8.6*°)  | 2.3† (10.1†•) | 0.4*† (1.6*†°•)  |
| panTro2 | 0.2 (1.1)       | 0.2 (1.1)  | 2.0* (8.8*°)  | 2.3† (10.2†•) | 0.5*† (2.1*†°•)  |
| ponAbe2 | 0.6 (2.7)       | 0.6 (2.8)  | 2.1* (8.8*°)  | 2.3† (10.2†•) | 0.7*† (3.4*†°•)  |
| rheMac2 | 1.0 (4.4)       | 1.0 (4.4)  | 2.2* (9.2*°)  | 2.4† (10.8†•) | 1.2*† (5.6*†°•)  |
| calJac1 | 1.7 (7.5)       | 1.7 (7.7)  | 2.5* (10.6*°) | 2.8† (11.6†•) | 2.1*† (9.5*†°•)  |
| rn4     | 2.6 (9.9)       | 2.7 (9.9)  | 5.2* (18.3*°) | 5.4† (19.0†•) | 2.7*† (9.9)      |
| turTru1 | 1.9 (7.7)       | 2.0 (8.3)  | 2.6* (10.5*°) | 2.9† (12.0†•) | 3.1*† (12.7*†°•) |
| felCat3 | 2.4 (10.0)      | 2.7 (10.9) | 3.0* (12.2*°) | 3.3† (13.3†•) | 3.2*† (13.0*†°•) |
| bosTau4 | 2.4 (9.7)       | 2.7 (10.7) | 3.2* (12.7*°) | 3.5† (13.5†•) | 3.2*† (13.1*†°•) |
| vicPac1 | 2.3 (8.9)       | 2.4 (9.6)  | 2.9* (11.4*°) | 3.2† (12.3†•) | 3.3*† (13.1*†°•) |
| mm9     | 2.7 (10.2)      | 2.9 (10.7) | 5.1* (17.4*°) | 5.4† (18.8†•) | 3.1*† (10.6*°)   |
| canFam2 | 2.5 (10.0)      | 2.7 (10.8) | 3.0* (11.5*°) | 3.3† (12.6†•) | 3.5*† (14.1*†°•) |
| micMur1 | 2.3 (9.1)       | 2.4 (9.7)  | 2.4* (9.8*°)  | 2.7† (10.6†•) | 3.7*† (14.5*†°•) |
| otoGar1 | 2.9 (11.4)      | 3.2 (12.2) | 3.2* (12.5*°) | 3.4† (13.4†•) | 3.9*† (14.6*†°•) |
| tarSyr1 | 2.8 (11.2)      | 2.9 (11.9) | 2.9* (11.6*°) | 3.1† (13.3†•) | 4.0*† (16.5*†°•) |
| equCab2 | 2.0 (8.5)       | 2.1 (9.0)  | 2.4* (10.0*°) | 2.7† (11.1†•) | 3.7*† (14.6*†°•) |
| choHof1 | 2.9 (12.6)      | 3.3 (13.9) | 3.5* (13.2*°) | 3.7† (14.1†•) | 4.3*† (16.5*†°•) |
| pteVam1 | 2.6 (11.1)      | 2.8 (11.7) | 3.1* (12.0*°) | 3.3† (13.5†•) | 4.1*† (16.8*†°•) |
| myoLuc1 | 2.7 (11.0)      | 2.8 (11.7) | 3.0* (12.2*°) | 3.3† (13.4†•) | 3.8*† (15.8*†°•) |
| dasNov2 | 3.1 (12.2)      | 3.6 (14.1) | 3.8* (14.4*°) | 4.0† (15.7†•) | 4.3*† (16.6*†°•) |
| loxAfr2 | 2.7 (11.4)      | 3.1 (12.6) | 3.4* (13.0*°) | 3.6† (13.8†•) | 4.3*† (16.7*†°•) |
| proCap1 | 3.2 (12.6)      | 3.8 (15.0) | 4.3* (15.1*°) | 4.6† (16.3†•) | 4.4*† (17.1*†°•) |
| tupBel1 | 3.3 (12.1)      | 3.5 (12.7) | 3.4 (11.9)    | 3.6† (12.6†)  | 4.8*† (17.5*†°•) |
| speTri1 | 3.5 (12.3)      | 3.8 (13.3) | 3.7* (13.1*°) | 4.0† (14.3†)  | 5.3*† (18.8*†°•) |
| oryCun1 | 3.4 (12.2)      | 3.9 (13.6) | 3.8* (13.5*°) | 4.0† (14.3†)  | 5.0*† (17.4*†°•) |
| ochPri2 | 3.4 (11.0)      | 4.5 (15.9) | 4.6* (15.0*°) | 4.9† (15.5†•) | 5.0*† (17.3*†°•) |
| echTel1 | 3.8 (14.0)      | 4.4 (15.5) | 4.7* (16.4*°) | 4.9† (17.1†•) | 5.1*† (18.6*†°•) |
| cavPor3 | 4.1 (14.6)      | 4.7 (16.4) | 4.7* (15.8*°) | 4.9† (16.3†)  | 6.0*† (20.2*†°•) |
| dipOrd1 | 4.1 (13.2)      | 4.6 (15.0) | 4.7* (15.5°)  | 5.0† (16.5†)  | 5.9*† (18.3*†°•) |
| eriEur1 | 4.0 (13.9)      | 4.5 (15.6) | 4.5* (15.5*°) | 4.8† (16.1†)  | 5.6*† (19.5*†°•) |
| sorAra1 | 4.1 (12.2)      | 4.7 (15.0) | 4.8* (15.7*°) | 5.1† (16.9†•) | 5.8*† (17.9*†°•) |
| fr2     | 2.3 (9.1)       | 4.7 (14.8) | 7.0* (23.2*°) | 7.4† (23.6†)  | 4.8*† (14.3*°)   |
| tetNig1 | 2.6 (8.0)       | 4.7 (13.4) | 7.1* (21.9*°) | 7.5† (22.1†)  | 4.7* (10.2°)     |
| taeGut1 | 2.6 (9.7)       | 3.9 (10.2) | 5.7* (17.8*°) | 6.0† (22.2†•) | 4.1* (10.6°)     |
| galGal3 | 2.6 (11.0)      | 5.1 (14.6) | 6.3* (19.8*°) | 6.7† (17.3†•) | 5.3* (16.9*°)    |
| monDom4 | 4.2 (13.4)      | 6.1 (19.1) | 6.1* (20.3*°) | 6.4† (21.1)   | 7.1*† (24.9†°•)  |
| gasAcu1 | 2.4 (11.2)      | 5.3 (18.8) | 7.2* (22.2*°) | 7.5† (24.4†)  | 6.1*† (18.3*†°)  |
| oryLat2 | 2.2 (10.9)      | 5.4 (19.0) | 7.2* (25.5*°) | 7.6† (22.0†)  | 5.7*† (22.0*°)   |
| ornAna1 | 3.8 (11.8)      | 6.5 (20.2) | 6.5* (23.2°)  | 6.9† (23.7†)  | 7.4*† (20.3°)    |
| anoCar1 | 2.7 (n/a)       | 6.7 (19.0) | 7.2* (n/a)    | 7.5† (n/a)    | 7.5*† (21.6)     |
| danRer5 | 2.2 (10.7)      | 6.8 (24.1) | 7.8* (24.6°)  | 7.9† (27.6)   | 7.8*† (22.7°)    |
| xenTro2 | 1.8 (n/a)       | 6.7 (22.0) | 6.7* (20.7)   | 7.1† (20.3)   | 7.8*† (27.1†)    |
| petMar1 | n/a (n/a)       | 7.2 (19.2) | 6.9 (21.4)    | 7.2 (23.2)    | 8.3† (n/a)       |



Table 7: **Data set MZ44-2:** Average number of mismatches and difference of melting temperature  $T_m$  in °C (in brackets) between the predicted primer oligonucleotides and the actual primer complement in the target gene. \*: *maxAlike* reconstruction with  $T = .5$  is significantly lower ( $P < 0.05$ ); †: *maxAlike* reconstruction with n/T is significantly lower ( $P < 0.05$ ); °: *maxAlike* reconstruction with  $T = 0.5$  has significantly less cases of dinucleotide mismatches or mismatches at extreme positions ( $P < 0.05$ ); •: *maxAlike* reconstruction with n/T has significantly less cases of dinucleotide mismatches or mismatches at extreme positions ( $P < 0.05$ )

| Species | <i>maxAlike</i> |            | Freq          |               | NN               |
|---------|-----------------|------------|---------------|---------------|------------------|
|         | $T = .5$        | n/T        | $T = .5$      | n/T           |                  |
| hg18    | 0.2 (1.2)       | 0.2 (1.2)  | 2.4* (10.6*°) | 2.8† (12.4†•) | 0.3*† (1.5*†°•)  |
| gorGor1 | 0.2 (1.2)       | 0.2 (1.2)  | 2.3* (10.2*°) | 2.7† (12.1†•) | 0.4*† (1.8*†°•)  |
| panTro2 | 0.2 (1.2)       | 0.2 (1.2)  | 2.4* (10.6*°) | 2.8† (12.4†•) | 0.5*† (2.3*†°•)  |
| ponAbe2 | 0.6 (2.9)       | 0.6 (2.9)  | 2.4* (10.7*°) | 2.9† (12.6†•) | 0.8*† (3.6*†°•)  |
| rheMac2 | 1.1 (5.1)       | 1.1 (5.2)  | 2.6* (11.5*°) | 3.0† (13.3†•) | 1.4*† (6.5*†°•)  |
| calJac1 | 2.0 (8.8)       | 2.0 (9.1)  | 3.0* (13.2*°) | 3.4† (14.7†•) | 2.4*† (11.0*†°•) |
| turTru1 | 2.2 (9.5)       | 2.4 (10.2) | 3.3* (13.7*°) | 3.7† (15.1†•) | 3.6*† (15.6*†°•) |
| rn4     | 3.2 (13.1)      | 3.6 (13.8) | 6.8* (25.8*°) | 7.2† (28.0†•) | 3.6*† (13.8°)    |
| bosTau4 | 2.9 (12.6)      | 3.3 (14.1) | 4.2* (17.4*°) | 4.6† (18.9†•) | 3.8*† (16.0*†°•) |
| felCat3 | 3.0 (12.6)      | 3.4 (14.0) | 3.9* (16.6*°) | 4.3† (17.7†•) | 4.0*† (16.7*†°•) |
| vicPac1 | 2.8 (11.8)      | 3.1 (12.6) | 3.8* (15.6*°) | 4.1† (17.2†•) | 4.0*† (16.9*†°•) |
| mm9     | 3.2 (13.3)      | 3.8 (13.8) | 6.8* (24.5*°) | 7.2† (27.5†•) | 3.9*† (13.6°)    |
| canFam2 | 3.2 (13.5)      | 3.5 (14.5) | 4.0* (16.9*°) | 4.4† (18.0†•) | 4.4*† (18.9*†°•) |
| micMur1 | 3.0 (12.2)      | 3.2 (13.1) | 3.1* (12.8*°) | 3.5† (14.6†•) | 4.6*† (19.4*†°•) |
| otoGar1 | 3.7 (15.6)      | 4.1 (16.9) | 4.0* (16.7*°) | 4.4† (18.2†•) | 4.8*† (20.5*†°•) |
| tarSyr1 | 3.5 (14.8)      | 3.7 (16.0) | 3.6* (15.4*°) | 4.0† (17.0†•) | 5.0*† (21.7*†°•) |
| equCab2 | 2.5 (10.5)      | 2.7 (11.2) | 3.1* (13.0*°) | 3.6† (14.5†•) | 4.5*† (18.9*†°•) |
| choHof1 | 3.5 (14.7)      | 4.1 (16.4) | 4.3* (17.3*°) | 4.7† (18.7†•) | 5.0*† (19.8*†°•) |
| pteVam1 | 3.2 (13.4)      | 3.4 (14.4) | 3.8* (15.9*°) | 4.2† (17.6†•) | 4.8*† (20.7*†°•) |
| dasNov2 | 3.8 (16.1)      | 4.3 (17.7) | 4.6* (19.4*°) | 5.0† (20.6†•) | 5.1*† (20.4*†°•) |
| myoLuc1 | 3.1 (13.2)      | 3.4 (14.1) | 3.9* (15.9*°) | 4.3† (17.2†•) | 4.6*† (19.4*†°•) |
| loxAfr2 | 3.3 (13.7)      | 3.9 (15.6) | 4.3* (17.8*°) | 4.7† (19.0†•) | 5.1*† (20.8*†°•) |
| proCap1 | 3.9 (16.5)      | 4.7 (19.6) | 5.3* (22.4*°) | 5.7† (24.0†•) | 5.2*† (20.6*†°•) |
| tupBel1 | 4.3 (18.2)      | 4.7 (19.2) | 4.4* (18.3°)  | 4.8† (20.1†)  | 6.1*† (26.7*†°•) |
| speTri1 | 4.6 (18.2)      | 5.2 (20.0) | 5.0* (19.0°)  | 5.4† (20.1•)  | 6.8*† (28.0*†°•) |
| oryCun1 | 4.6 (17.7)      | 5.3 (20.3) | 5.2* (20.1*°) | 5.6† (21.5†•) | 6.5*† (24.9*†°•) |
| ochPri2 | 5.2 (18.5)      | 6.1 (23.5) | 6.2* (24.5°)  | 6.6† (24.3†•) | 6.6*† (27.0*†°•) |
| echTel1 | 4.6 (18.2)      | 5.7 (22.7) | 6.1* (22.7*°) | 6.4† (25.1†•) | 6.3*† (25.5*†°•) |
| cavPor3 | 5.2 (19.8)      | 6.2 (24.1) | 6.1* (23.2*°) | 6.5† (25.1†•) | 7.7*† (29.2*†°•) |
| dipOrd1 | 5.3 (19.5)      | 6.4 (22.6) | 6.4* (22.9°)  | 6.8† (24.3†•) | 8.0*† (30.2*†°•) |
| eriEur1 | 5.2 (21.1)      | 5.9 (23.6) | 6.1* (22.8*°) | 6.5† (24.5†•) | 7.2*† (29.4*†°•) |
| sorAra1 | 5.3 (19.2)      | 6.3 (24.7) | 6.3* (25.3*°) | 6.7† (26.2†•) | 7.4*† (32.8*†°•) |
| fr2     | 1.9 (8.5)       | 5.9 (14.5) | 8.2* (n/a)    | 9.0† (n/a)    | 6.1* (16.0°)     |
| taeGut1 | 2.9 (6.2)       | 5.2 (8.1)  | 7.5* (n/a)    | 8.3† (n/a)    | 6.0* (9.3°)      |
| tetNig1 | 2.6 (8.7)       | 6.2 (12.7) | 8.2* (n/a)    | 8.8† (n/a)    | 6.4* (11.8°)     |
| galGal3 | 3.5 (9.0)       | 6.3 (10.6) | 7.5* (n/a)    | 8.1† (n/a)    | 6.9 (8.5°)       |
| monDom4 | 4.1 (9.6)       | 8.1 (21.7) | 7.9* (23.4°)  | 8.4† (22.9)   | 9.5*† (22.0°•)   |
| oryLat2 | 1.4 (n/a)       | 6.6 (23.1) | 8.2* (n/a)    | 9.0† (n/a)    | 6.9* (19.4)      |
| gasAcu1 | 2.1 (9.5)       | 6.8 (16.9) | 8.4* (n/a)    | 9.0† (n/a)    | 7.6*† (15.4°)    |
| ornAna1 | 3.8 (n/a)       | 8.1 (23.2) | 7.9* (26.6)   | 8.3 (24.5)    | 9.3*† (20.1•)    |
| anoCar1 | n/a (n/a)       | 7.9 (22.6) | 8.2 (n/a)     | 8.7† (n/a)    | 9.0† (n/a)       |
| xenTro2 | n/a (n/a)       | 8.0 (n/a)  | 7.8 (35.3)    | 8.5† (28.4)   | 9.4† (n/a)       |
| danRer5 | n/a (n/a)       | 8.1 (29.1) | 9.1 (n/a)     | 9.7† (n/a)    | 9.8† (n/a)       |
| petMar1 | n/a (n/a)       | 9.0 (n/a)  | 8.6 (n/a)     | 9.2 (n/a)     | 10.1† (n/a)      |

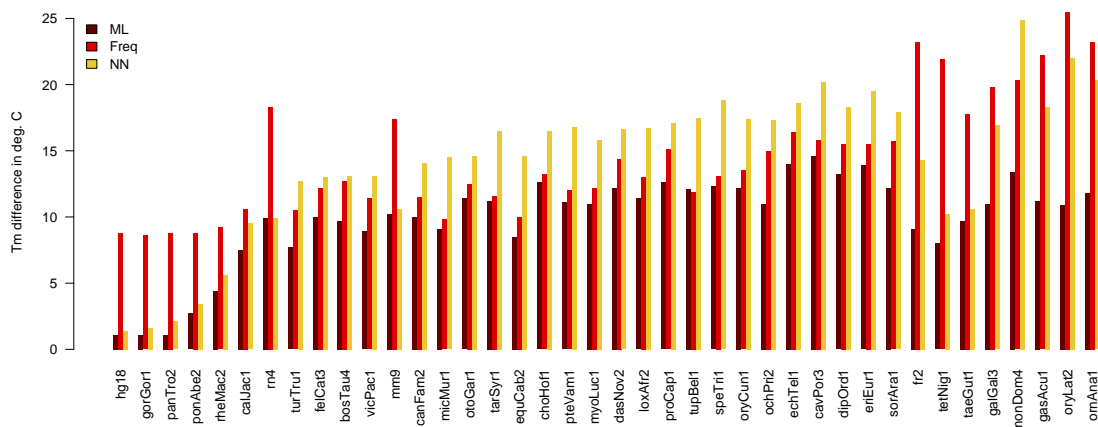


Figure 3: **Data set MZ44-1:** Average differences of the predicted and actual melting temperature  $T_m$  of the oligonucleotide duplex for primers derived from *maxAlike* (threshold 0.5) and *Freq* (threshold 0.5) reconstructed sequences and nearest neighbor (NN) sequence for each species, sorted by average distance to its phylogenetically nearest neighbor.

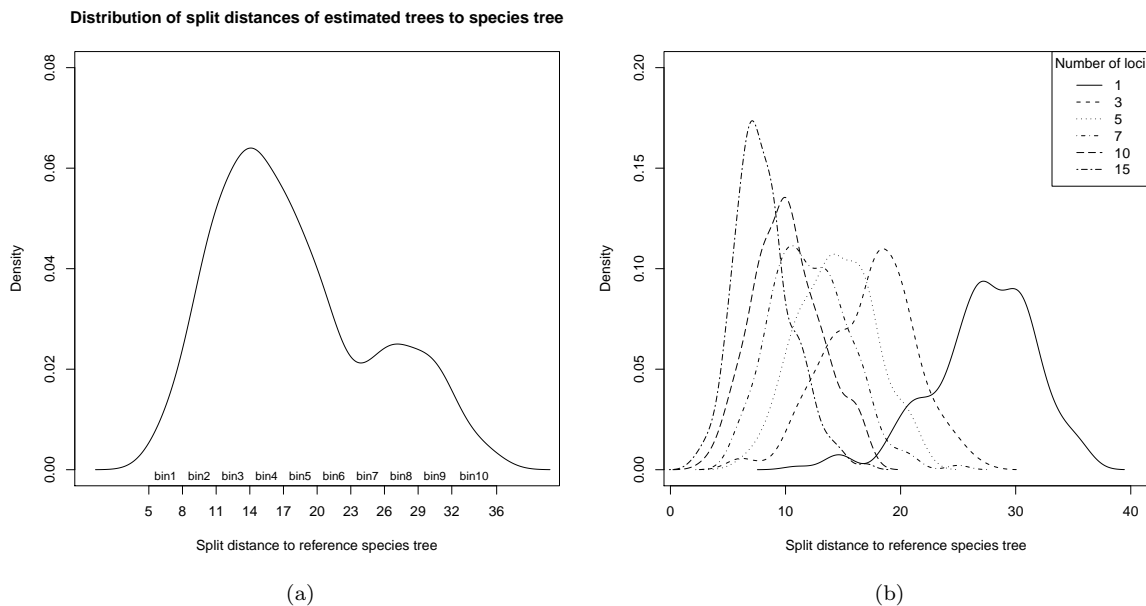


Figure 4: **Split distances of estimated trees to reference species tree:** We created 200 alignments from randomly chosen single loci of the full genome alignments that contain all 44 species (average alignment length is 178nt). Additional 200 alignments were created from each 3, 5, 7, 10, and 15 concatenated randomly selected loci from this set. A phylogenetic tree was estimated from each alignment and its split distance to the reference species tree was calculated. **(a)** Overall distribution of split distances from trees estimated from alignments containing 1, 3, 5, and 7 genomic loci. The x-axis shows the intervals used for binning the trees into 10 bins, *e.g.*, the first bin contains trees in the distance range from 5 to 8, etc. **(b)** Distributions of the split distances for each group of trees based on alignments composed of 1 to 15 loci

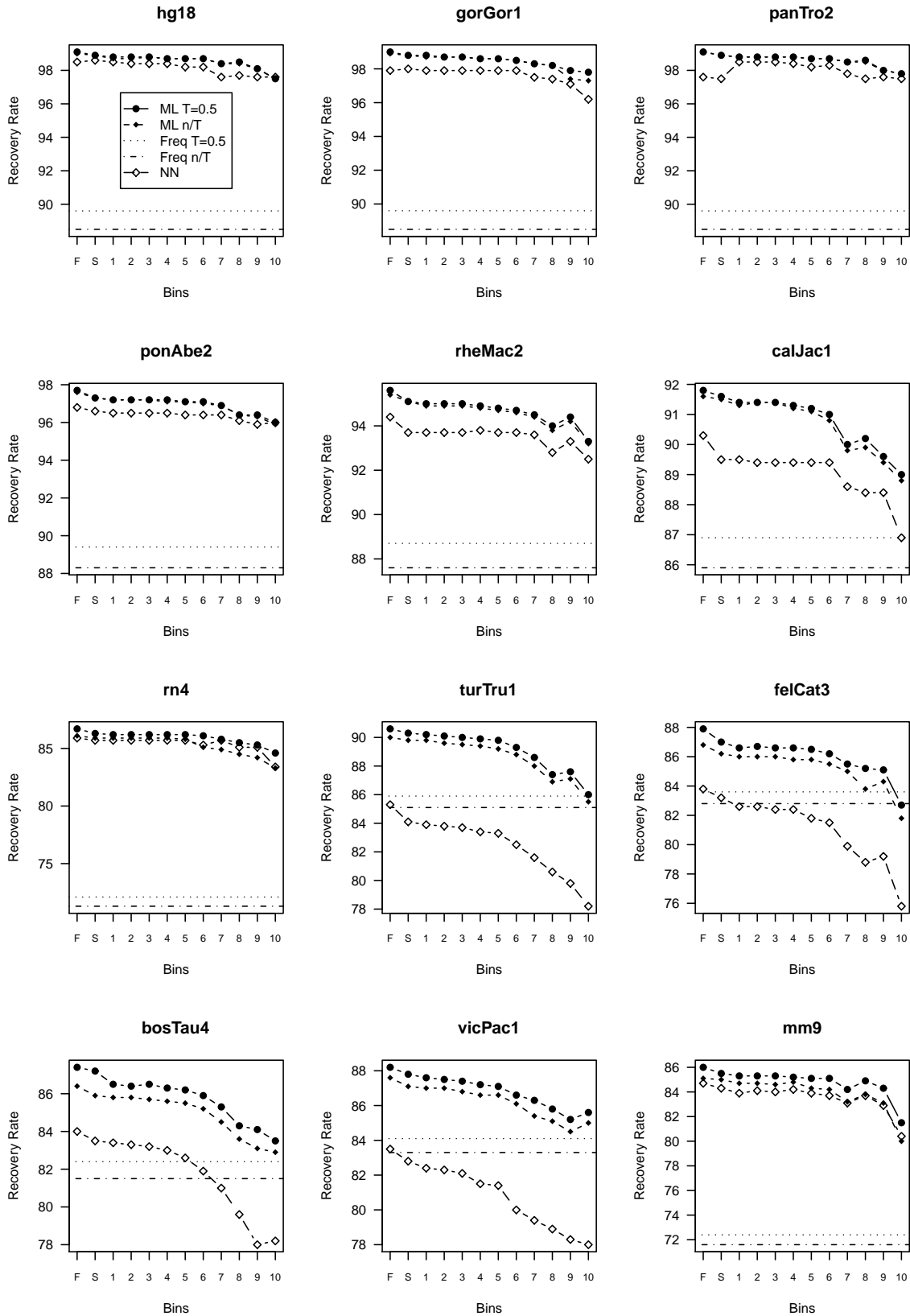


Figure 5: **MZ44-1: Recovery rates of estimated trees:** Shown are the total recovery rates for each species for the five methods with different input trees, using the tree estimated from the same alignment (column F), using the reference species tree (S), and using trees from the 10 bins of trees estimated from random genomic loci.

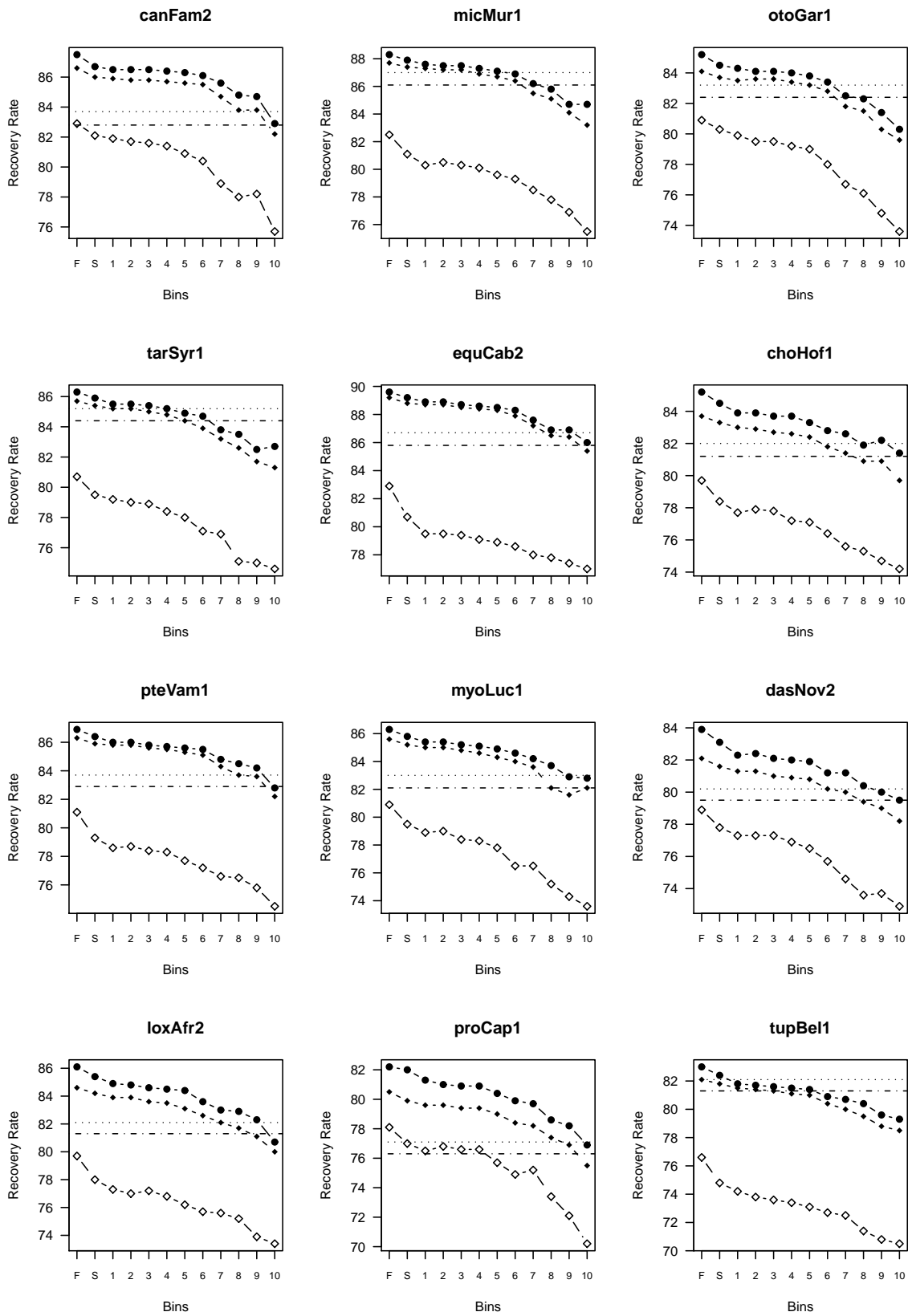


Figure 5: (continued)

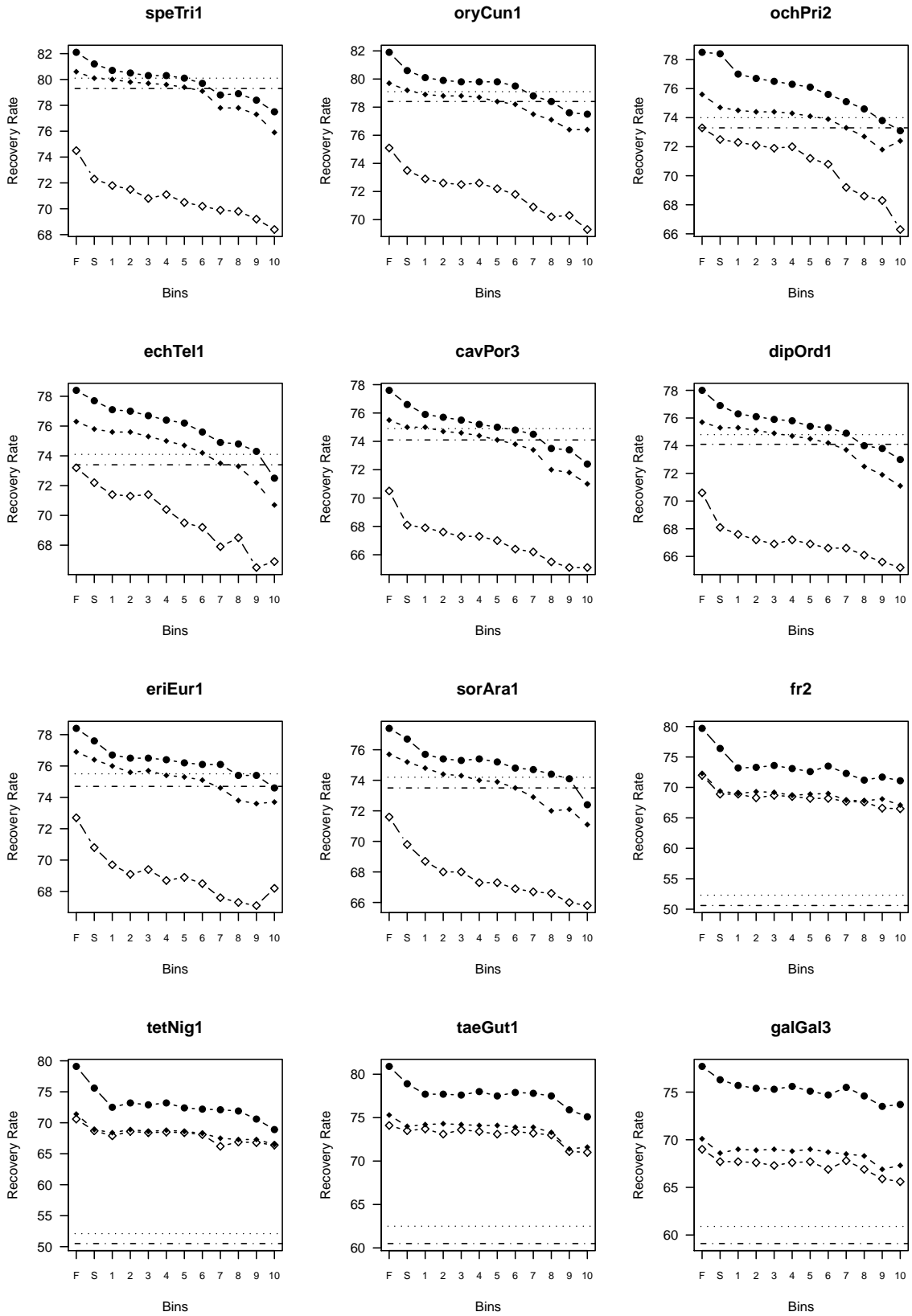


Figure 5: (continued)

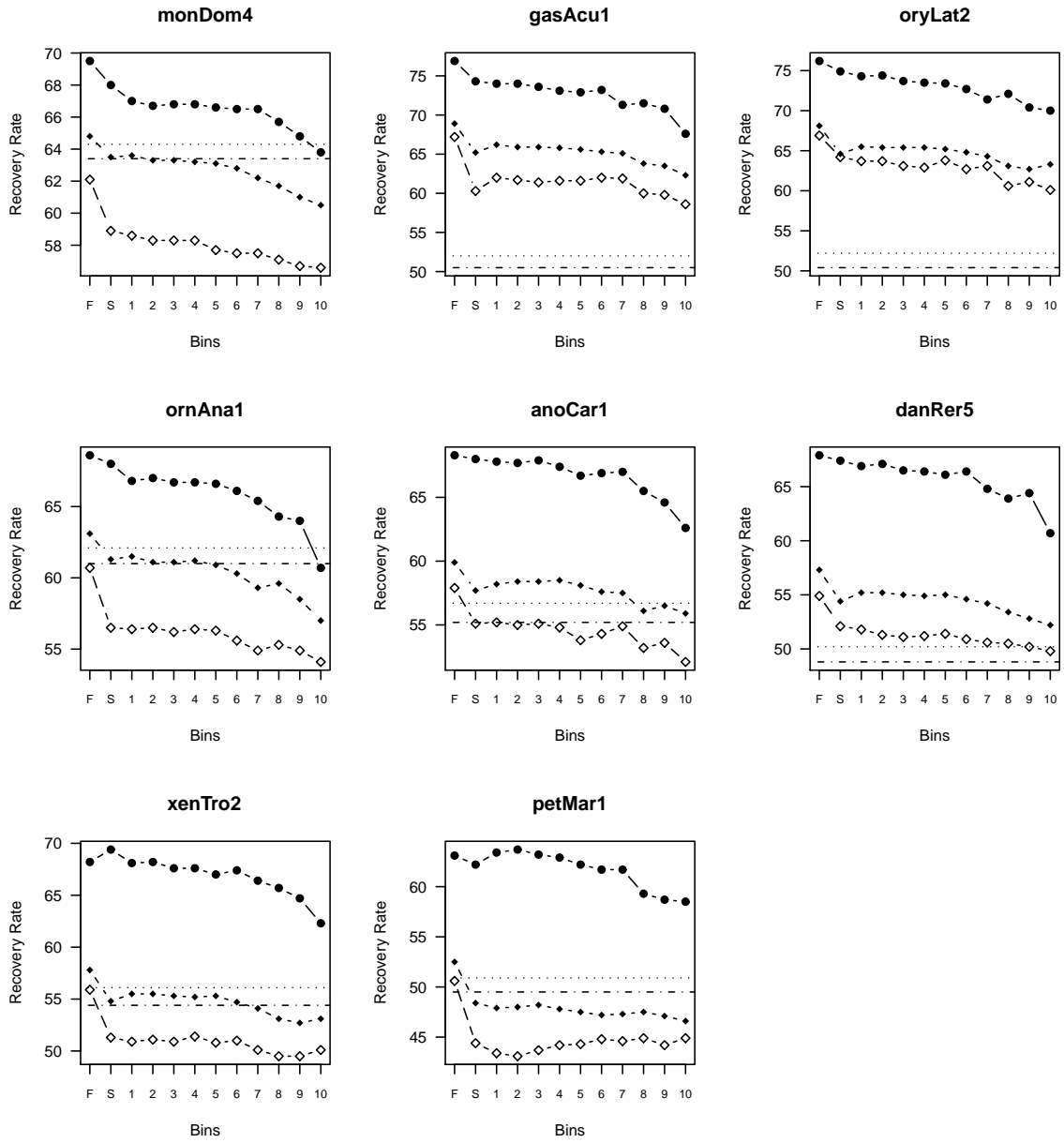
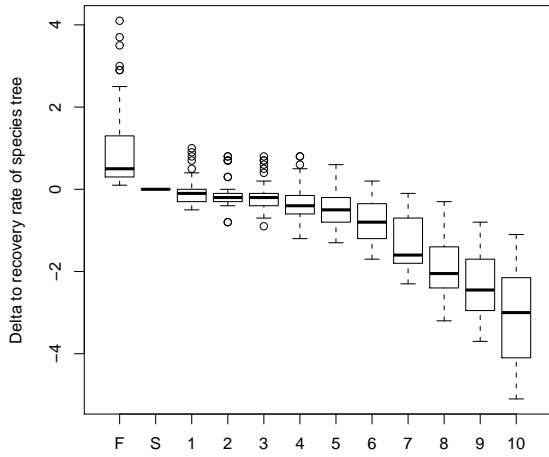
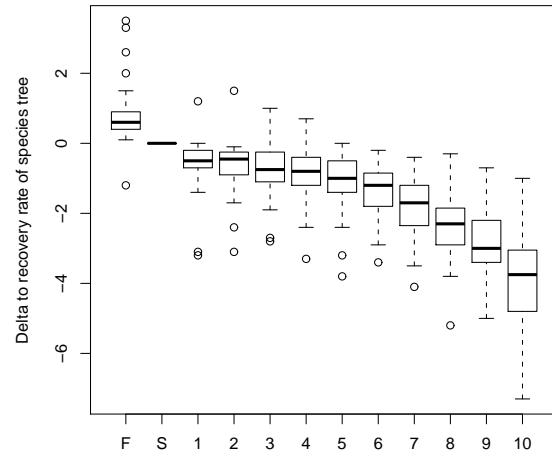


Figure 5: (continued)

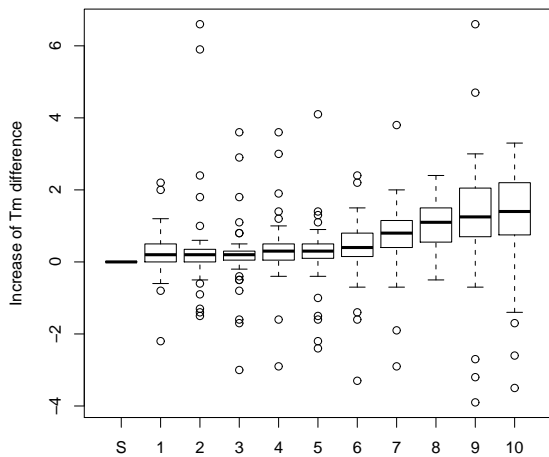


(a)

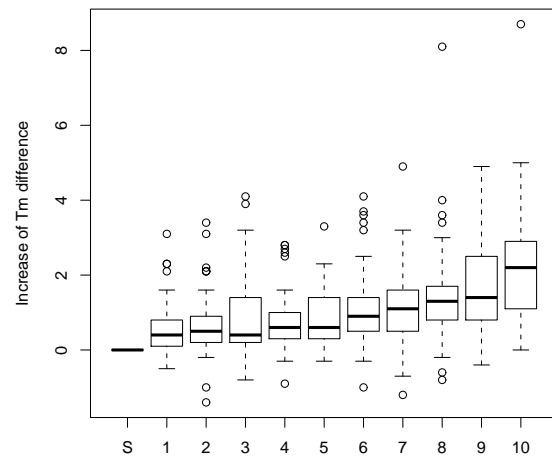


(b)

Figure 6: **MZ44-1: Change of total recovery rates of estimated trees:** Shown is distribution of change of total recovery rates of all species for different sets of input trees: (F) Gene tree (S) Reference species tree (1-10) Bins with trees estimated from other genomic loci, increasing bin number corresponds to higher topological distance to reference tree. (a) no threshold (b) threshold 0.5



(a)



(b)

Figure 7: **MZ44-1: Increase of the  $T_m$  difference of estimated trees:** Shown is the distribution of the increase of the  $T_m$  difference compared to the species tree for all species. (S) Species tree (1-10) Bins with trees estimated from other genomic loci, increasing bin number corresponds to higher topological distance to reference tree. (a) no threshold (b) threshold 0.5

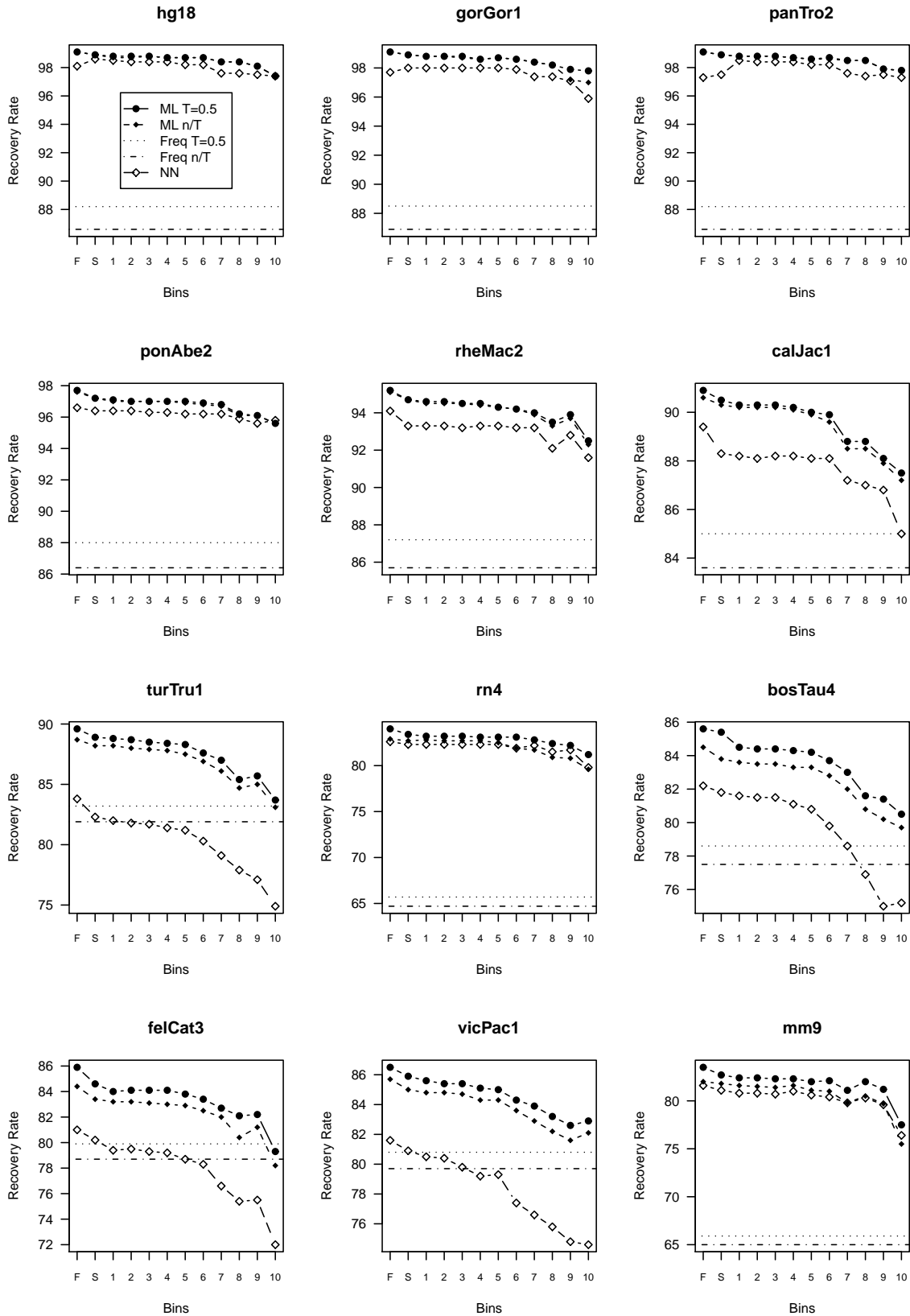


Figure 8: **MZ44-2: Recovery rates of estimated trees:** Shown are the total recovery rates for each species for the five methods with different input trees, using the tree estimated from the same alignment (column F), using the reference species tree (S), and using trees from the 10 bins of trees estimated from random genomic loci.



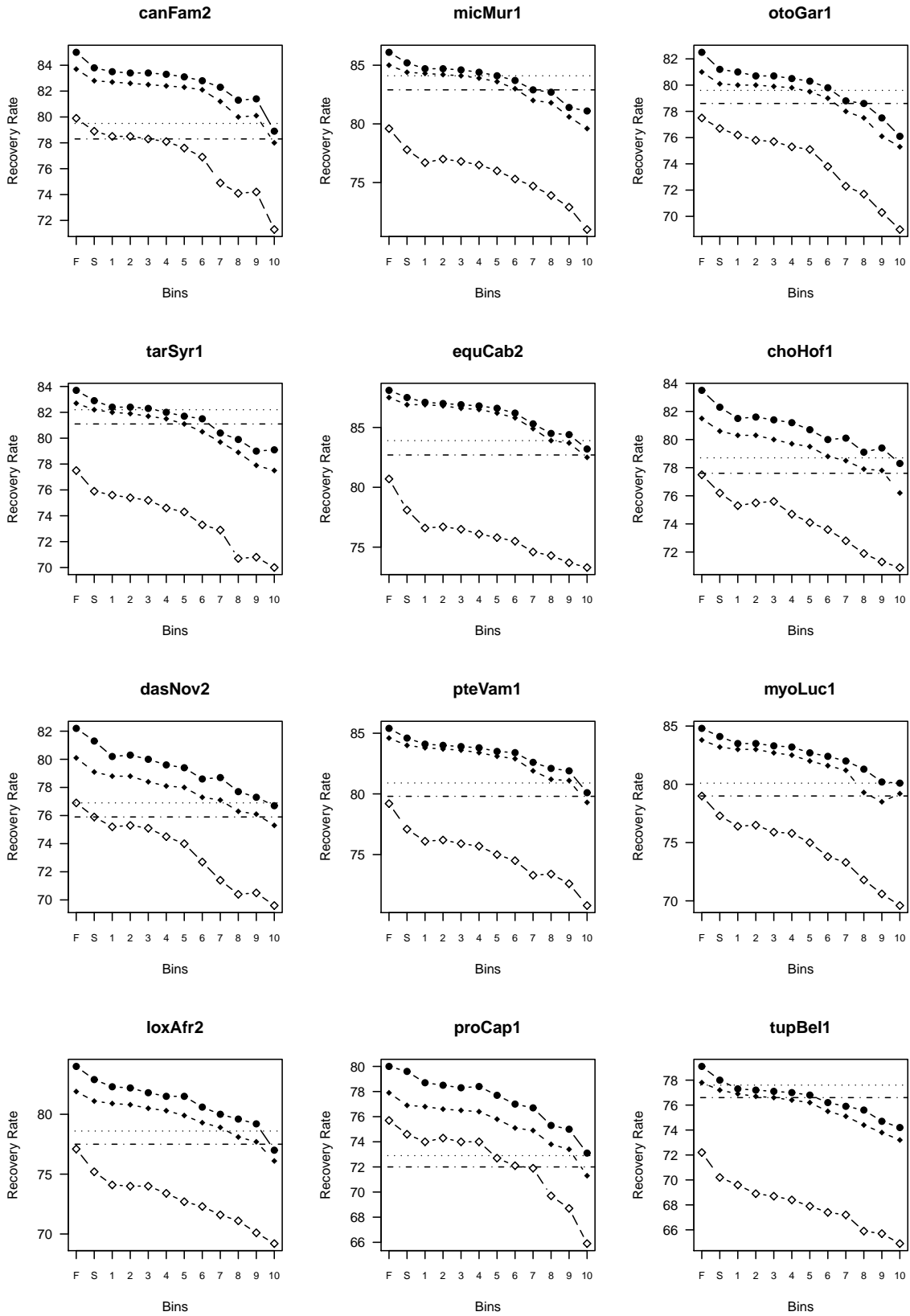


Figure 8: (continued)

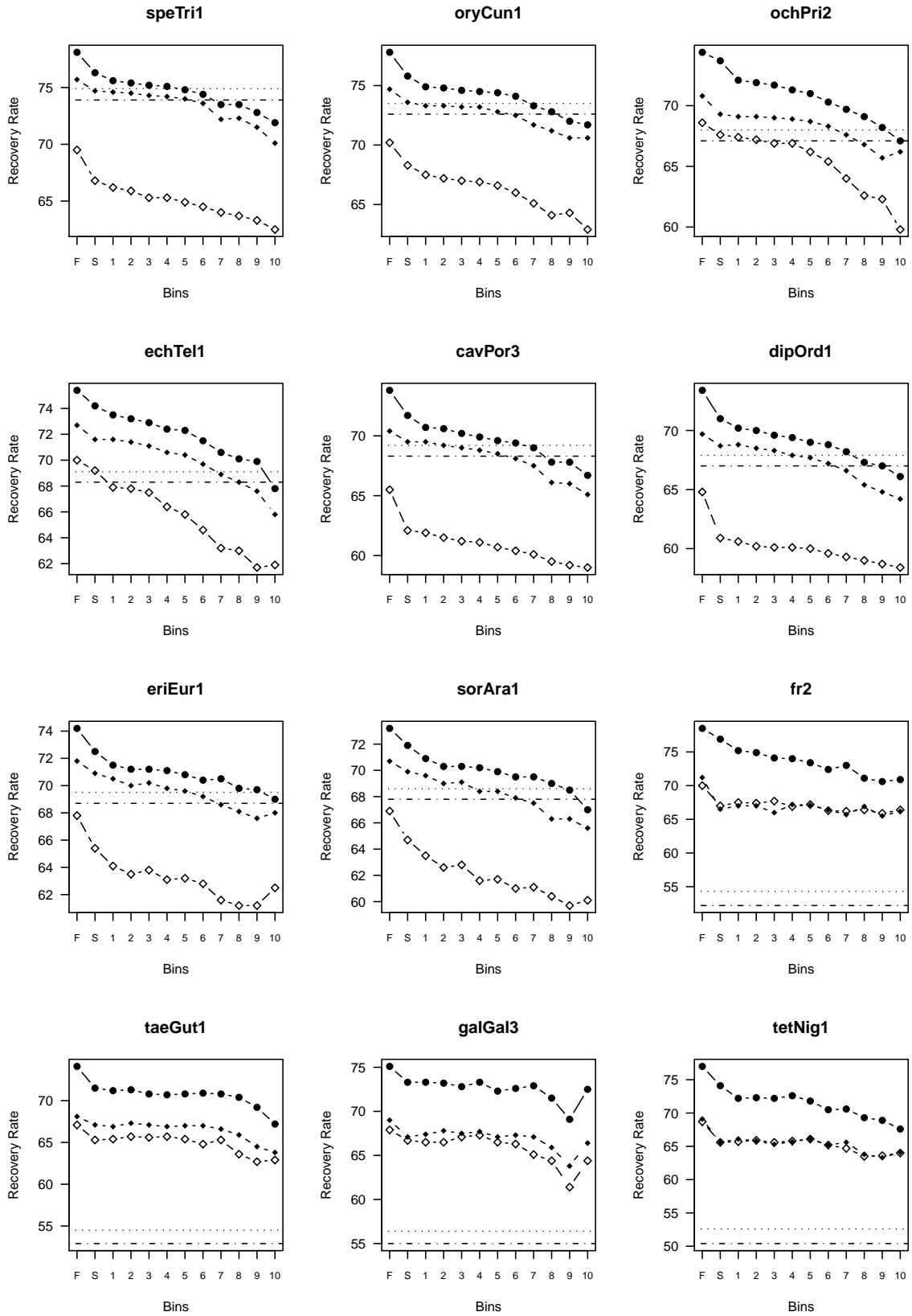


Figure 8: (continued)

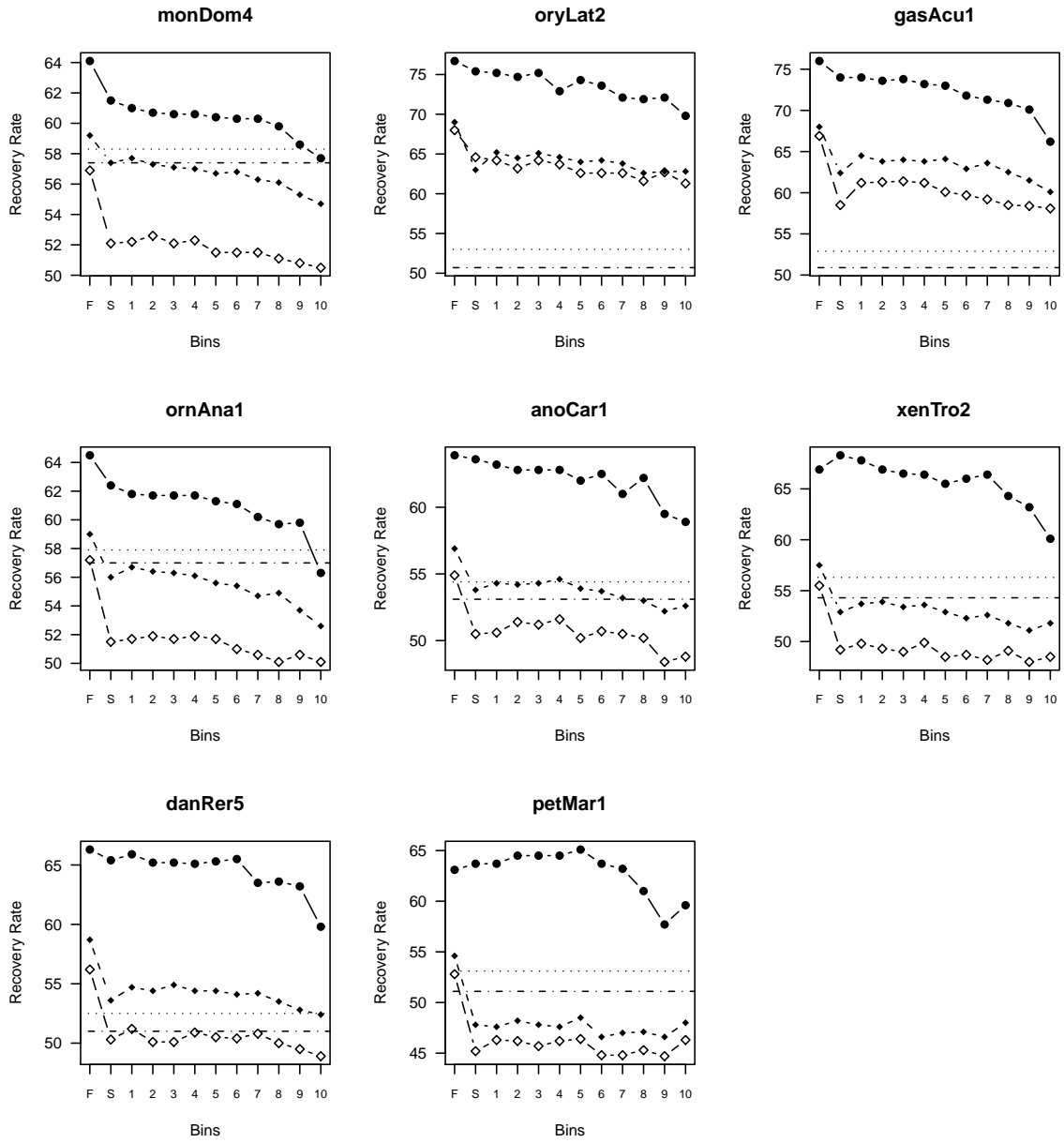
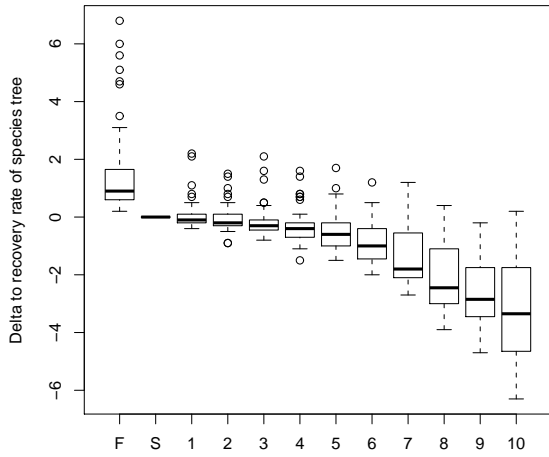
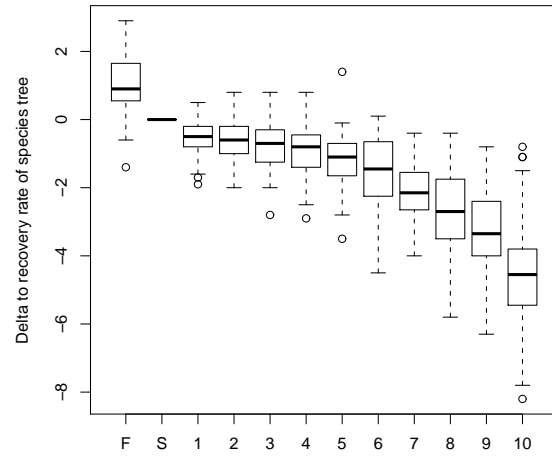


Figure 8: (continued)

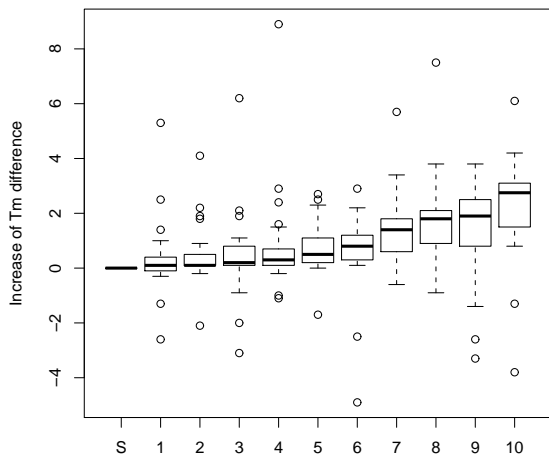


(a)

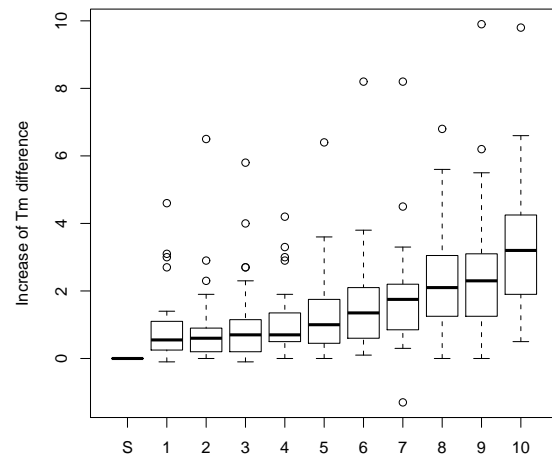


(b)

Figure 9: **MZ44-2: Change of total recovery rates of estimated trees:** Shown is distribution of change of total recovery rates of all species for different sets of input trees: (F) Gene tree (S) Reference species tree (1-10) Bins with trees estimated from other genomic loci, increasing bin number corresponds to higher topological distance to reference tree. (a) no threshold (b) threshold 0.5



(a)



(b)

Figure 10: **MZ44-2: Increase of the  $T_m$  difference of estimated trees:** Shown is the distribution of the increase of the  $T_m$  difference compared to the species tree for all species. (S) Species tree (1-10) Bins with trees estimated from other genomic loci, increasing bin number corresponds to higher topological distance to reference tree. (a) no threshold (b) threshold 0.5

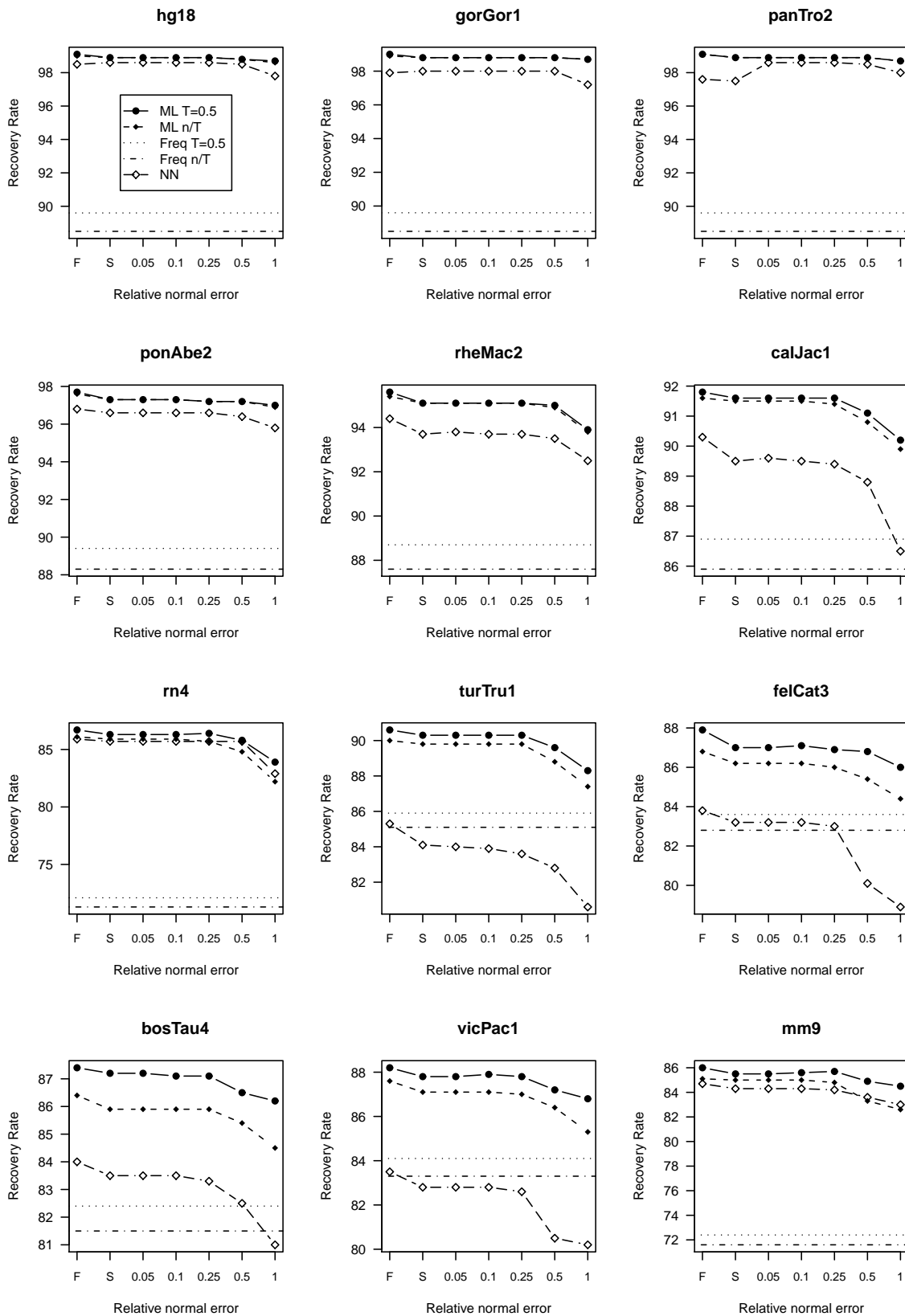


Figure 11: **MZ44-1: Recovery rates of trees with distorted branch lengths:** Shown are the total recovery rates for each species for the five methods with different input trees, using the tree estimated from the same alignment (column F), using the reference species tree (S), and using trees from the 5 bins of trees with distorted branch lengths.

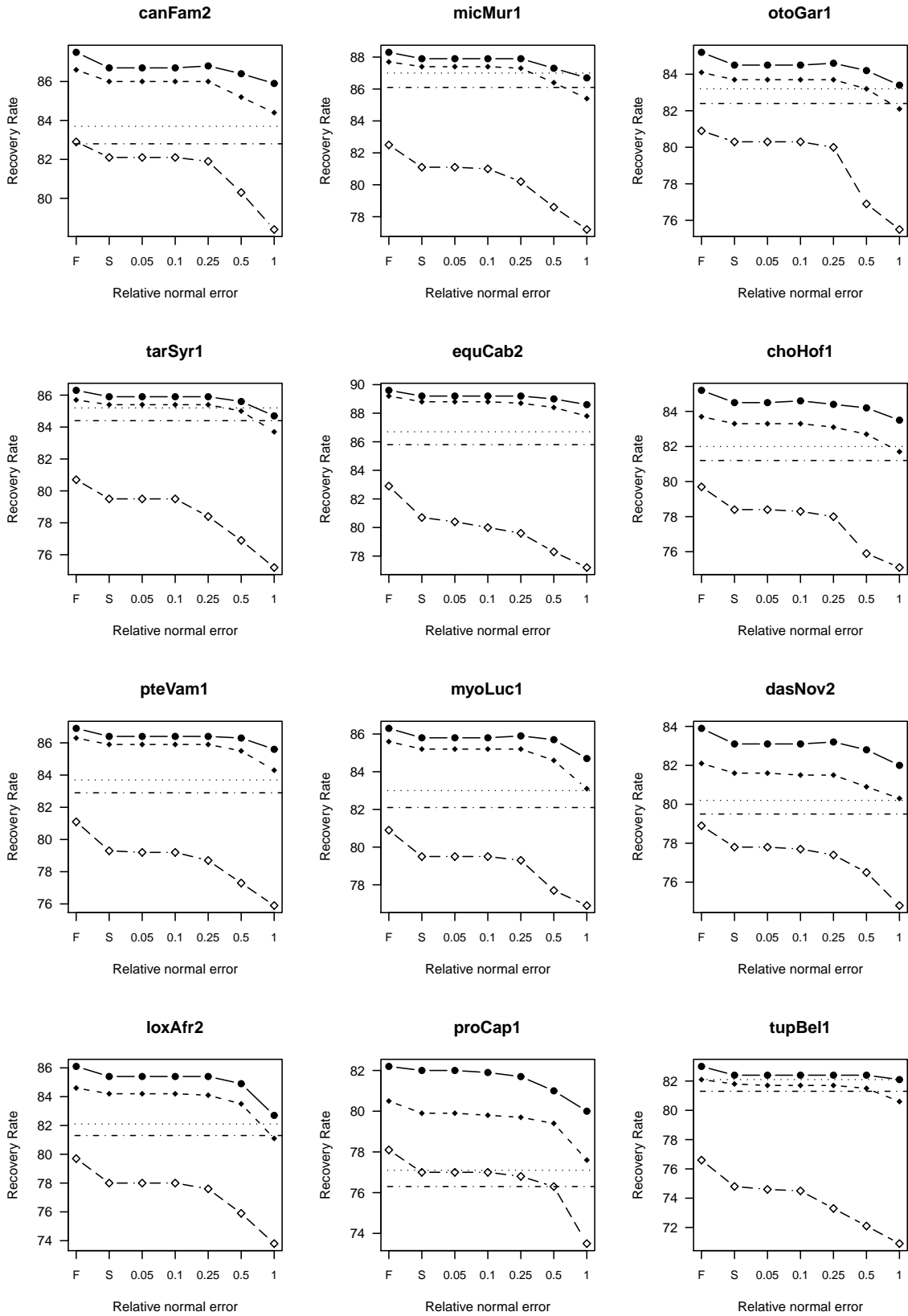


Figure 11: (continued)

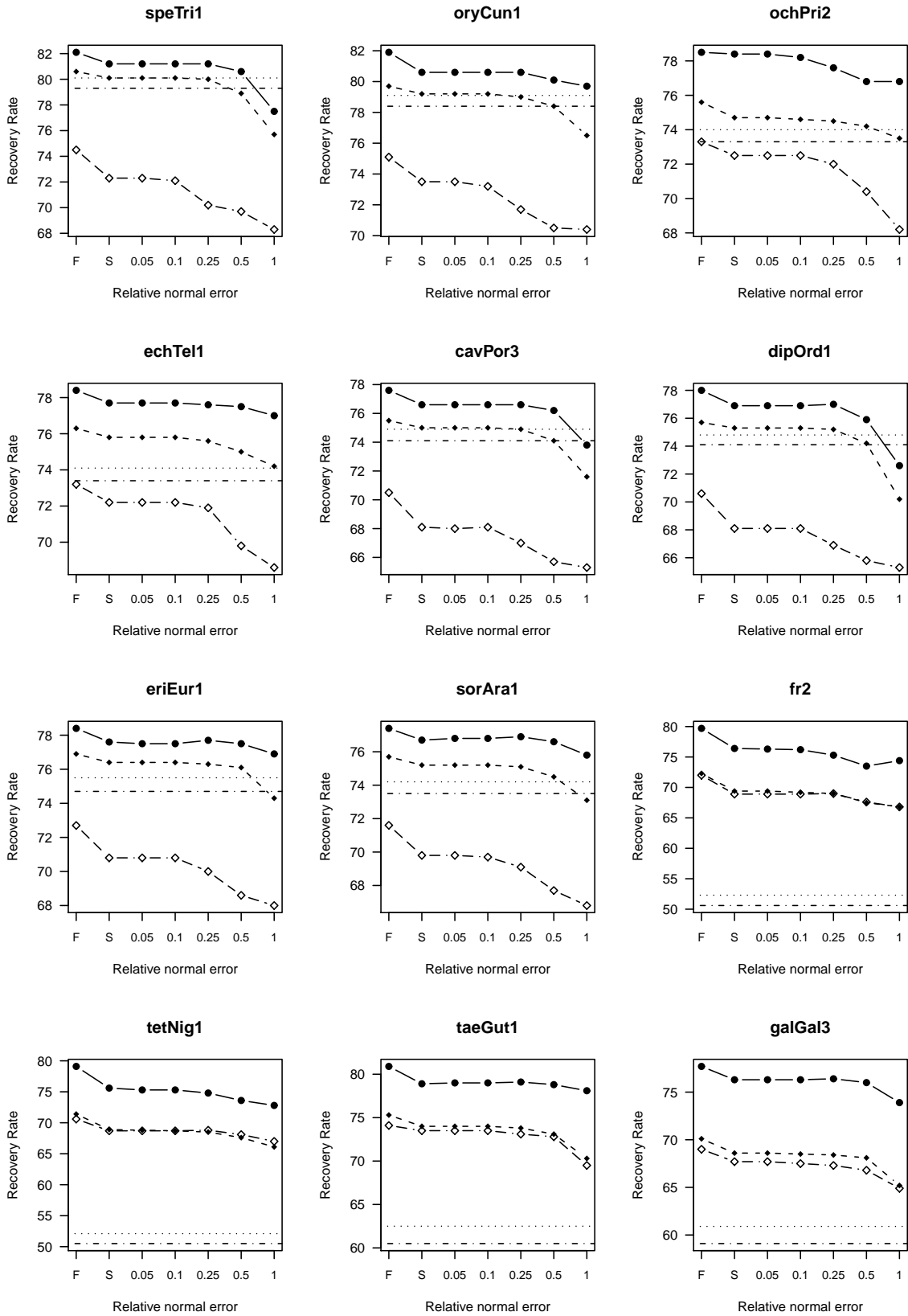


Figure 11: (continued)

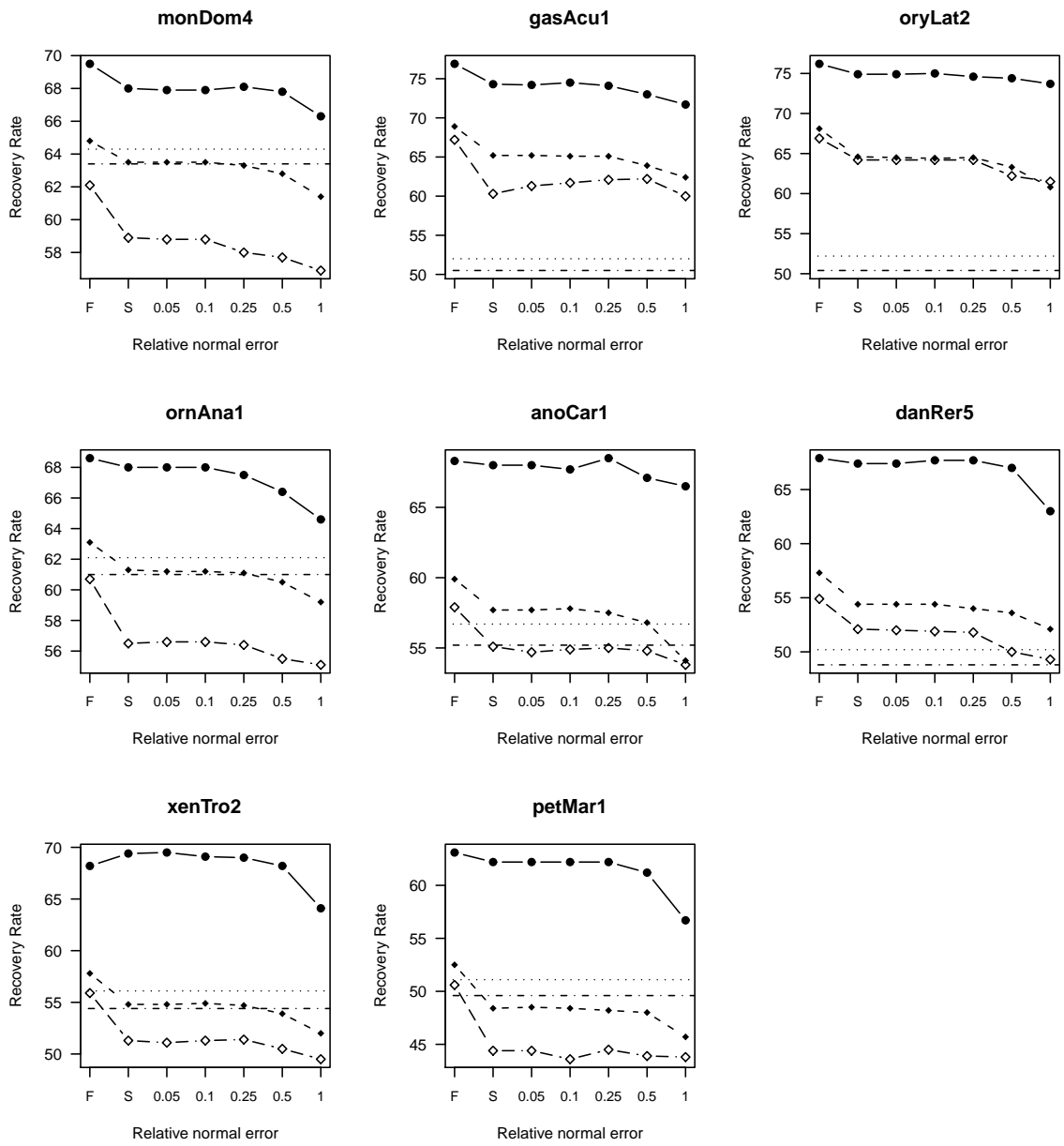


Figure 11: (continued)



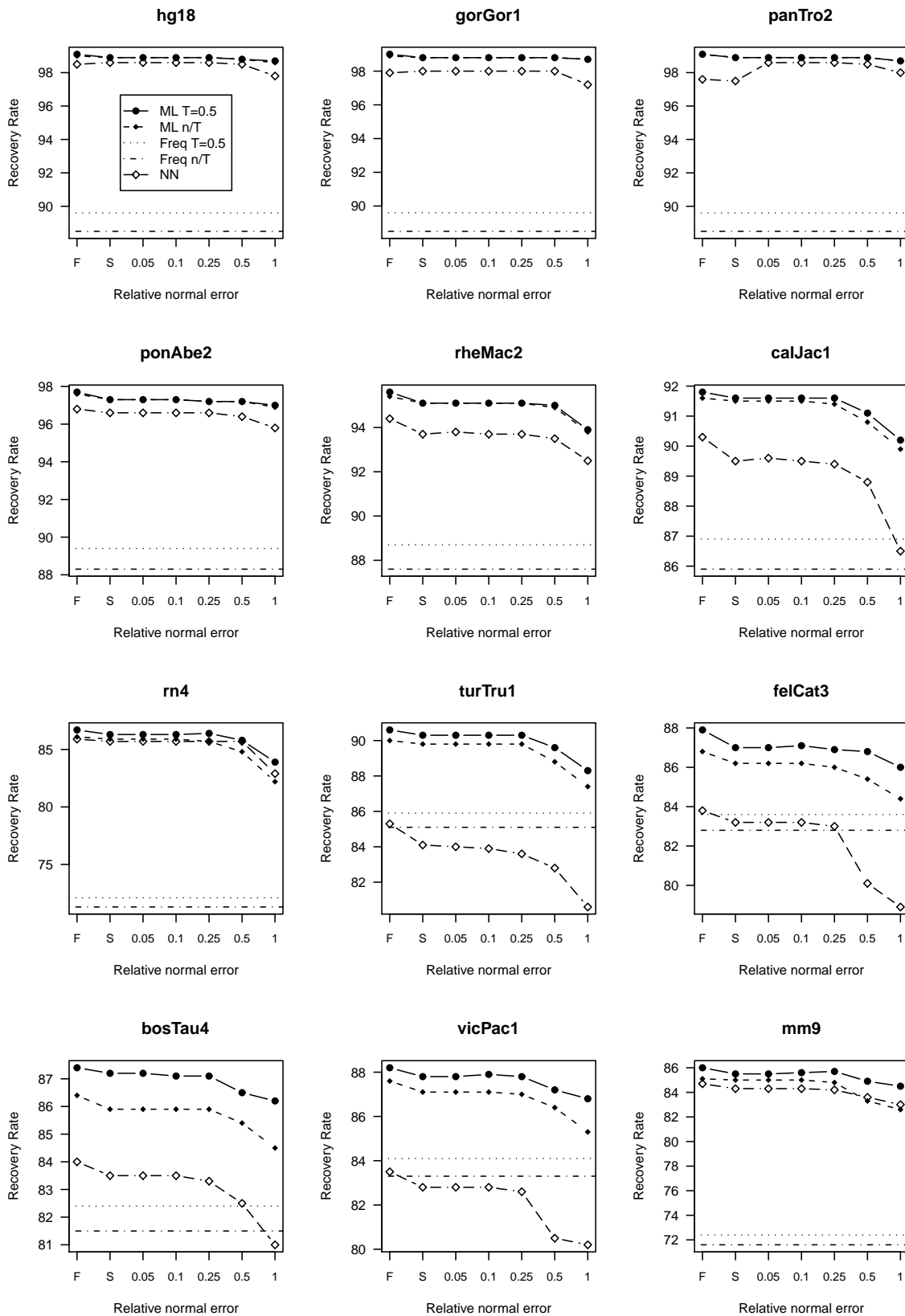


Figure 12: **MZ44-2: Recovery rates of trees with distorted branch lengths:** Shown are the total recovery rates for each species for the five methods with different input trees, using the tree estimated from the same alignment (column F), using the reference species tree (S), and using trees from the 5 bins of trees with distorted branch lengths.

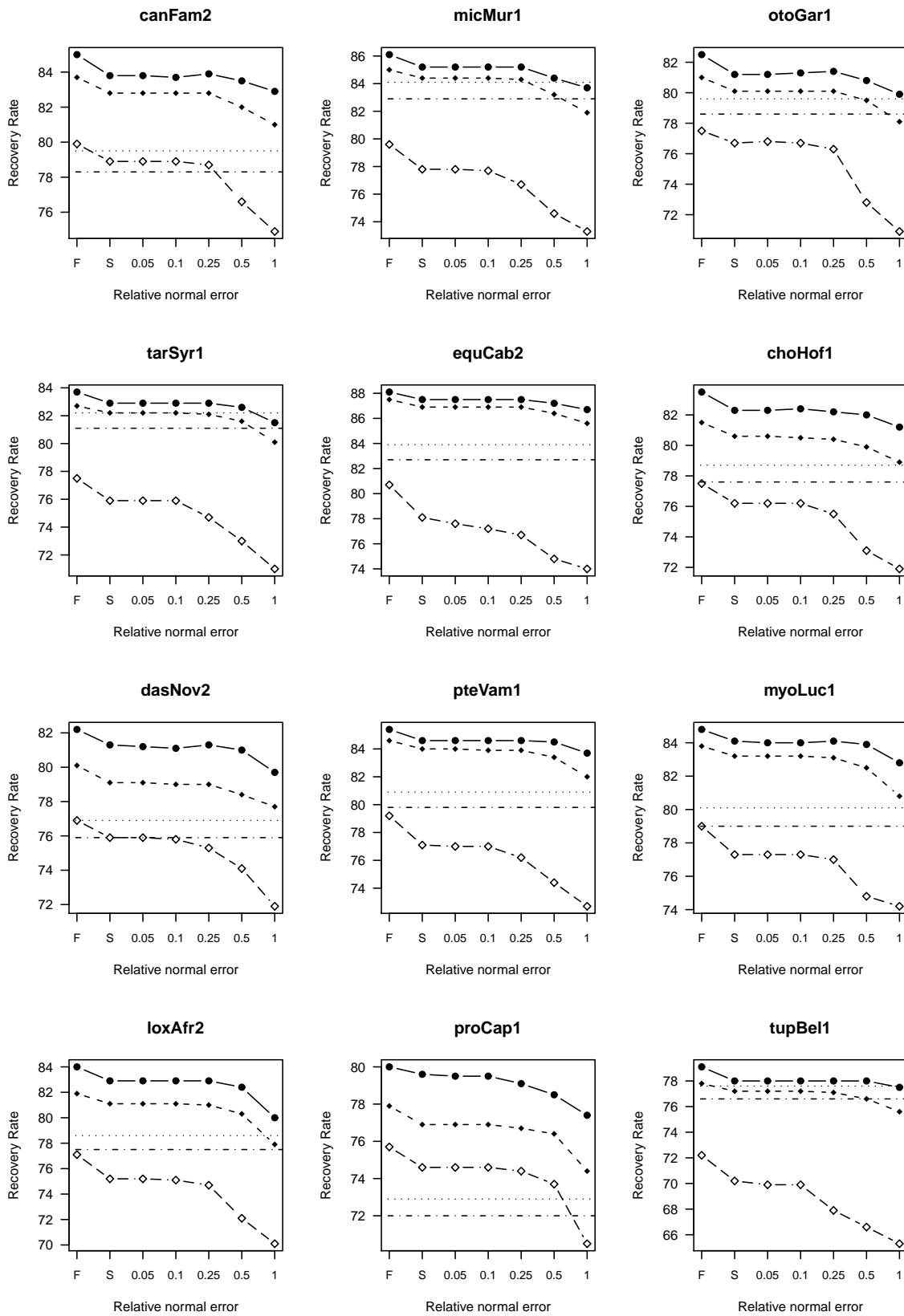


Figure 12: (continued)

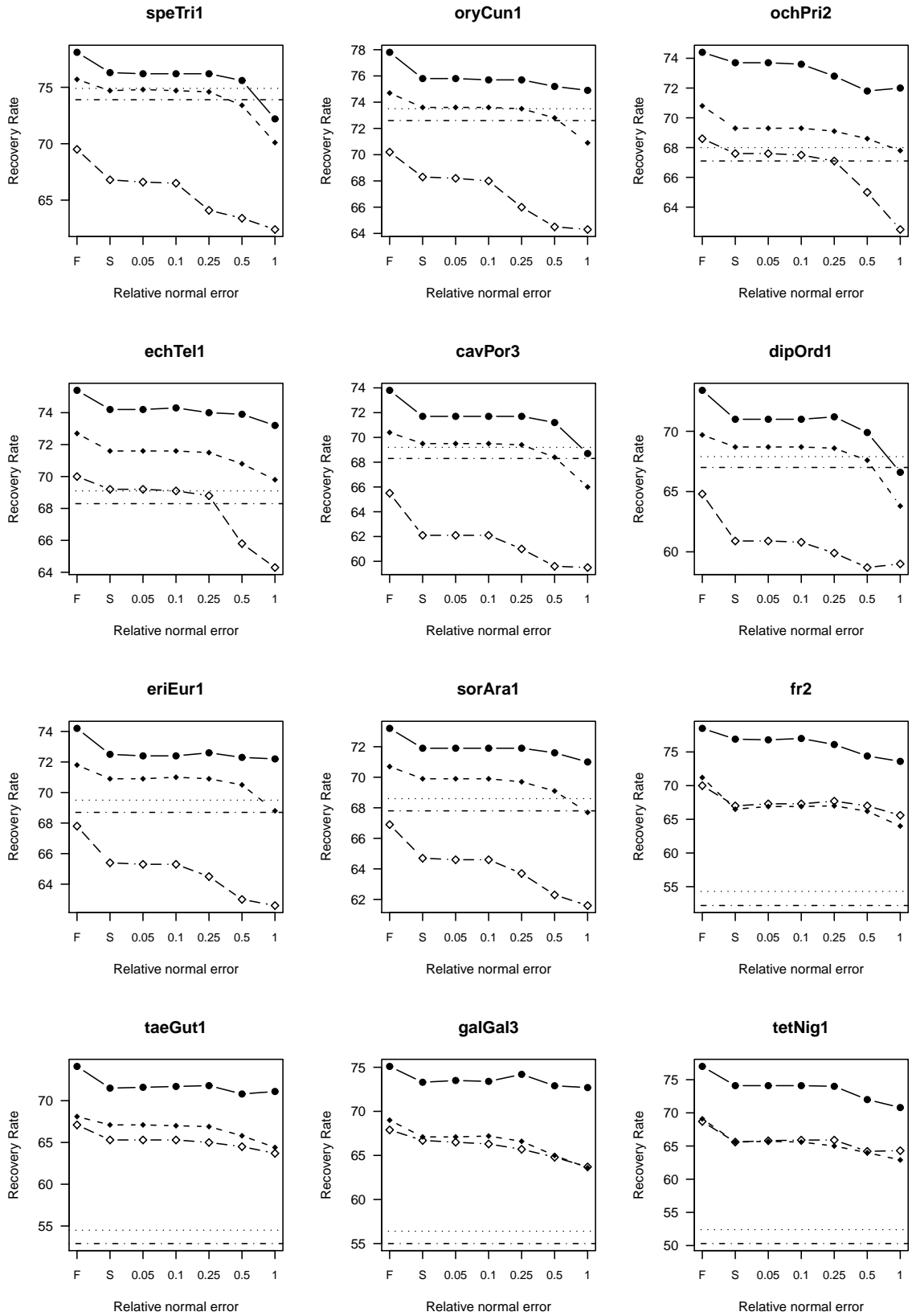


Figure 12: (continued)

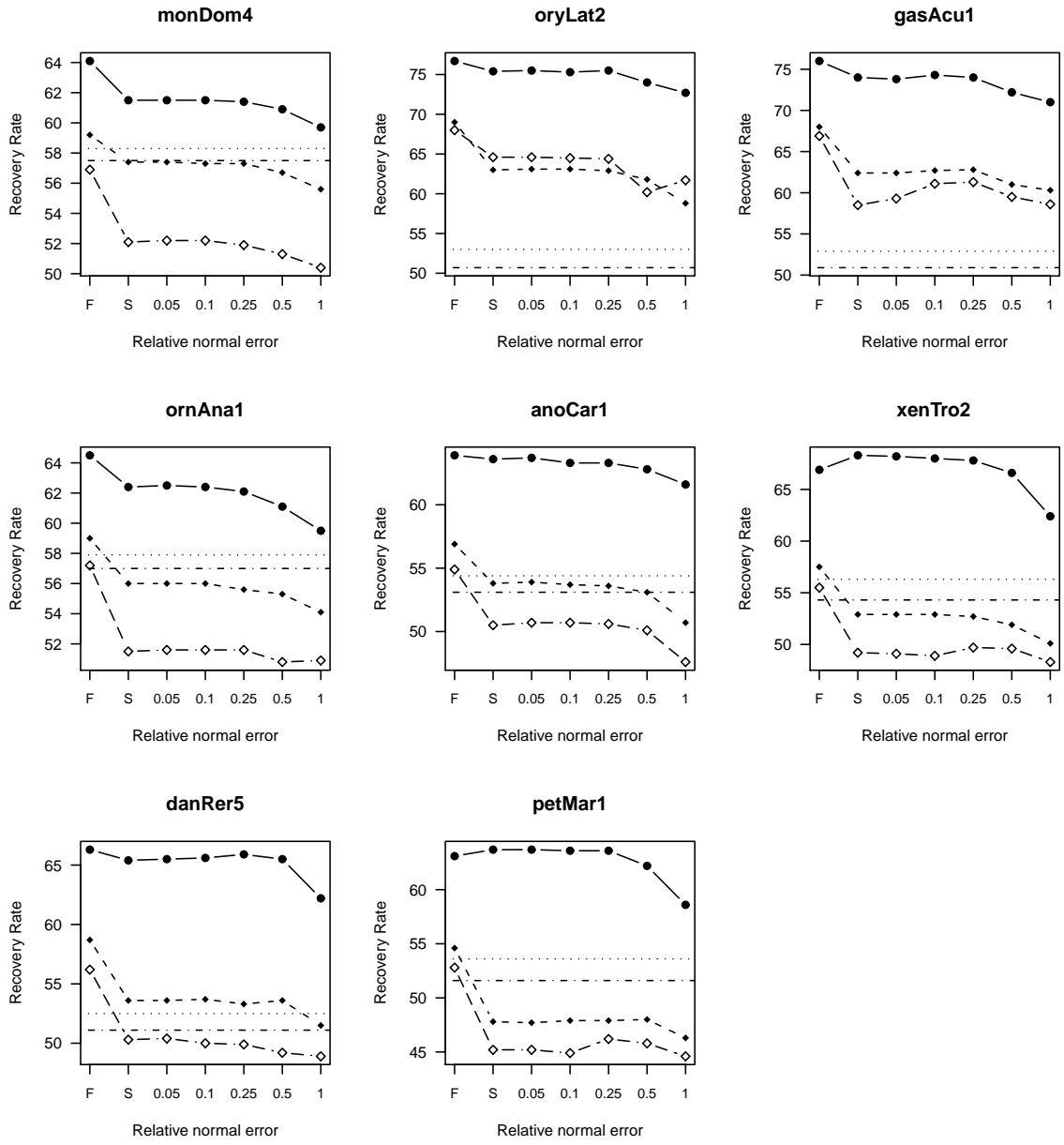


Figure 12: (continued)

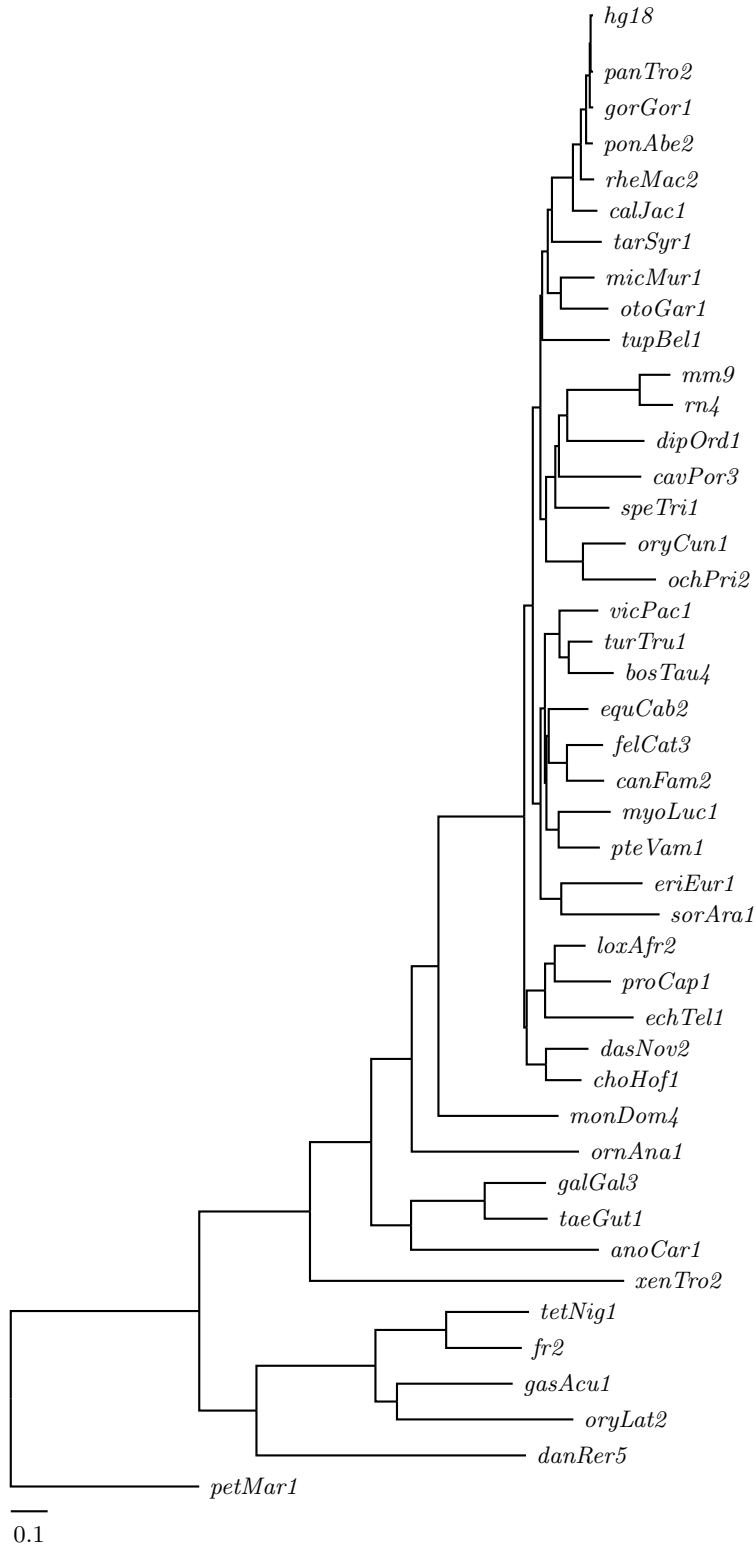


Figure 13: Species tree of the multiz-44 full genome alignments