Australian Journal of Botany 66, 298–312

doi:10.1071/BT17179_AC

Supplementary material for

Consequences of season of prescribed burning on two spring-flowering terrestrial orchids and their endophytic fungi

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Table S1 Genetic distances of ITS sequences of *Serendipita* isolates from *Glossodia major*. Bottom left-hand matrix shows distance; top right-hand matrix shows corresponding standard error.

	GU1SP1A.seq	GB2SU2S.seq	GU1W2L.seq	GUIC2C.seq	GU1WIS.seq	GU1SU2D.seq	GU1SU1E.seq	GU2C3C.seq	GU1SP3K.seq	GU1C3A.seq	GU1W1D.seq	GU1A2D.seq	GB2A2G.seq	GU1SU3F.seq	GU1C1J.seq
GU1SP1A.seq		0.003	0.005	0.006	0.006	0.006	0.006	0.020	0.021	0.022	0.023	0.023	0.023	0.025	0.031
GB2SU2S.seq	0.005		0.003	0.007	0.007	0.007	0.007	0.019	0.020	0.021	0.023	0.022	0.023	0.024	0.030
GU1W2L.seq	0.010	0.005		0.008	0.008	0.008	0.008	0.019	0.020	0.021	0.023	0.022	0.023	0.024	0.030
GUIC2C.seq	0.013	0.018	0.023		0.000	0.000	0.000	0.021	0.022	0.023	0.024	0.023	0.024	0.026	0.032
GU1WIS.seq	0.013	0.018	0.023	0.000		0.000	0.000	0.021	0.022	0.023	0.024	0.023	0.024	0.026	0.032
GU1SU2D.seq	0.013	0.018	0.023	0.000	0.000		0.000	0.021	0.022	0.023	0.024	0.023	0.024	0.026	0.032
GU1SU1E.seq	0.013	0.018	0.023	0.000	0.000	0.000		0.021	0.022	0.023	0.024	0.023	0.024	0.026	0.032
GU2C3C.seq	0.113	0.107	0.107	0.119	0.119	0.119	0.119		0.004	0.006	0.008	0.007	0.008	0.011	0.016
GU1SP3K.seq	0.119	0.113	0.113	0.122	0.122	0.122	0.122	0.008		0.006	0.007	0.006	0.006	0.009	0.015
GU1C3A.seq	0.128	0.122	0.122	0.131	0.131	0.131	0.131	0.015	0.013		0.006	0.002	0.005	0.008	0.014
GU1W1D.seq	0.137	0.132	0.132	0.141	0.141	0.141	0.141	0.026	0.018	0.015		0.006	0.006	0.007	0.014
GU1A2D.seq	0.131	0.125	0.125	0.134	0.134	0.134	0.134	0.018	0.015	0.003	0.013		0.004	0.007	0.014
GB2A2G.seq	0.140	0.134	0.134	0.144	0.144	0.144	0.144	0.026	0.018	0.010	0.015	0.008		0.008	0.014
GU1SU3F.seq	0.148	0.142	0.142	0.151	0.151	0.151	0.151	0.039	0.031	0.023	0.018	0.021	0.023		0.015
GU1C1J.seq	0.205	0.199	0.199	0.209	0.209	0.209	0.209	0.085	0.076	0.068	0.068	0.065	0.068	0.077	

The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al*. 2004). The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 395 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*. 2016).

Fig. S1 Annual rainfall and mean maximum and minimum temperatures in 2008-2014 at Bendigo Airport (081123) (Australian Government Bureau of Meteorology 2017).

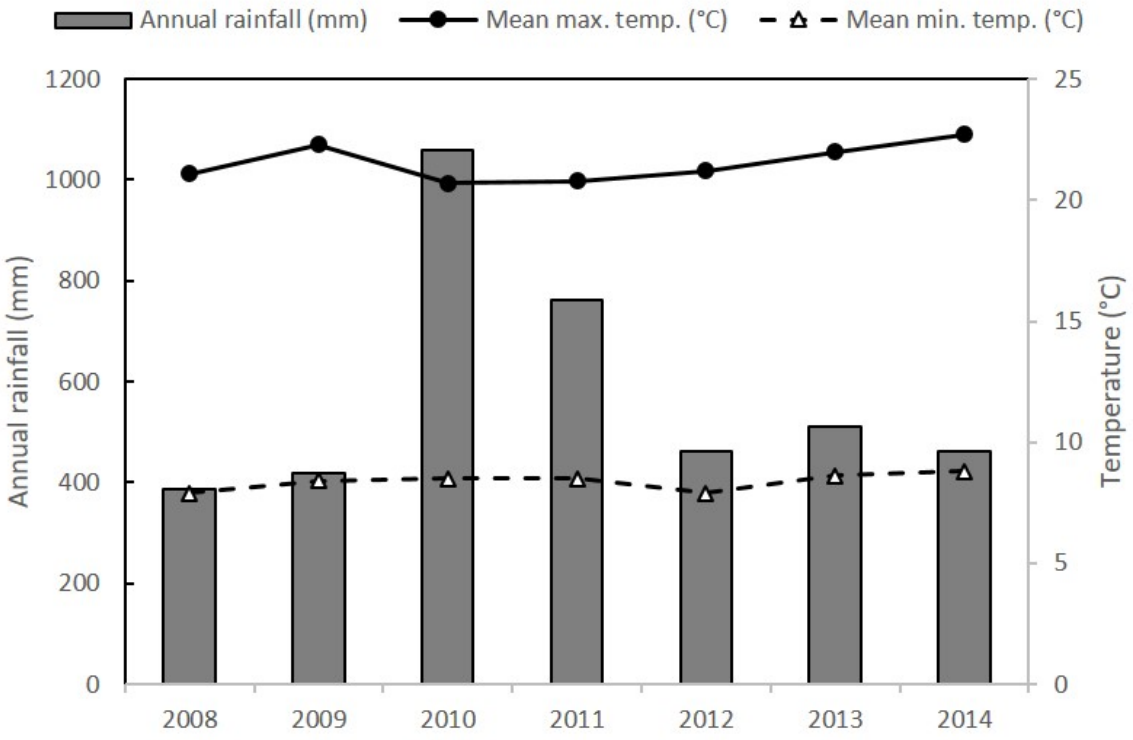


Fig. S2 RFLP patterns of *G. major* isolates from pre-burn (a) and post-burn (b) plots cleaved with *Taq*I restriction endonuclease and pre-burn (c) and post-burn (d) plots cleaved with *Hin*6I restriction endonuclease. Fragments were measured against GeneRuler™ ladder (first lanes) with a negative control (last lane). Pre-burn isolates were (left to right) from: control (C1D, C1J, C2C, C2E, C3A, C3D), spring (SP1A, SP1C, SP2E, SP2H, SP3I, SP3K), summer (SU1C, SU1E, SU2D, SU2G, SU3F, SU3H), autumn (A1L, A1N, A2A, A2D, A3C, A3X) and winter (W1D, W1S, W2A, W2L, W3P, W3T) burn quadrats. Post-burn isolates were: control (C1A, C1E, C2D, C2J, C3C, C3H), spring (SP1A, SP1C, SP2C, SP2E, SP3A, SP3H), summer (SU1E, SU1K, SU2S, SU2P, SU3A, SU3H) and autumn unburnt (A1C, A1X, A2G, A2H, A3A, A3C).

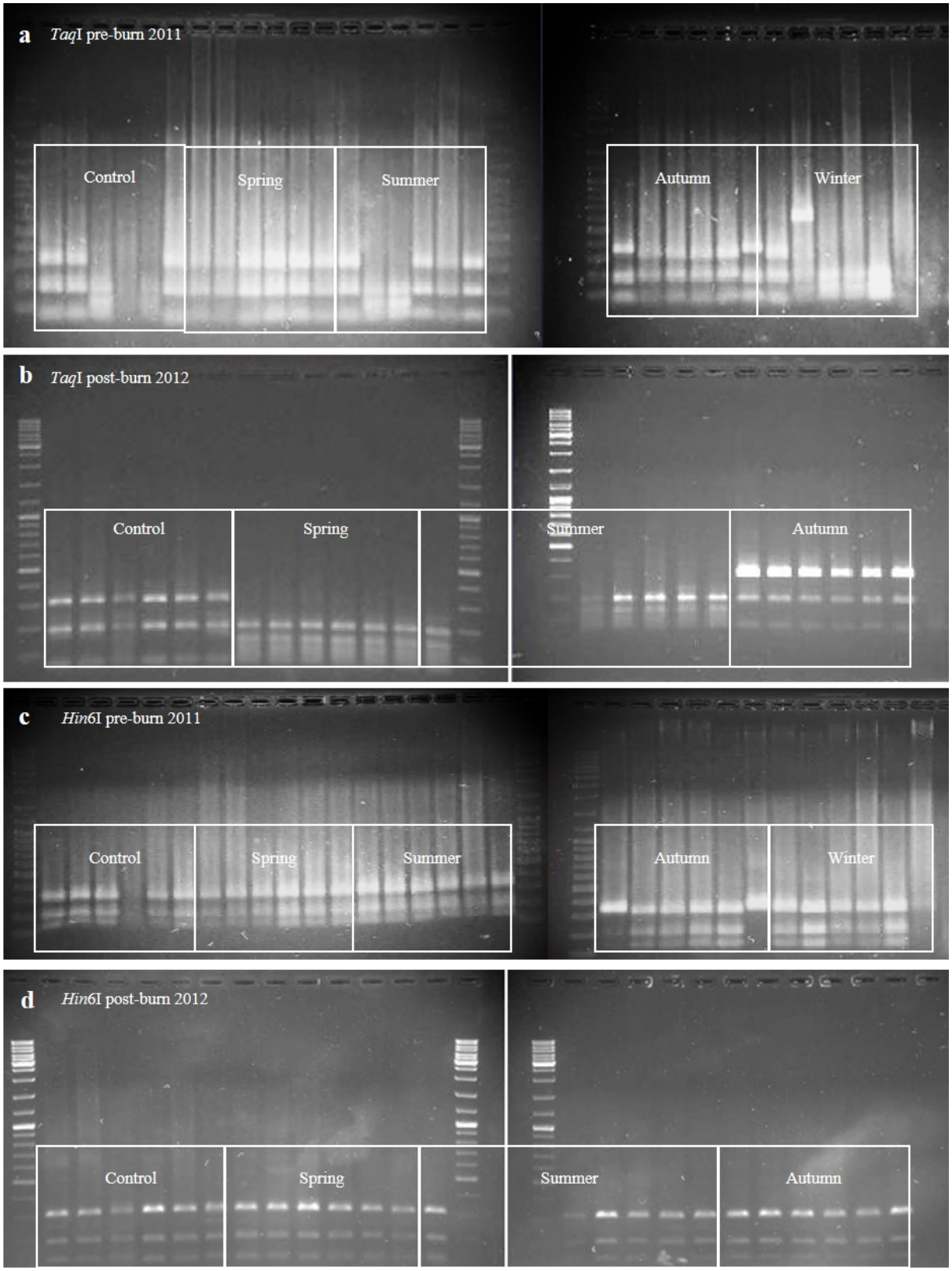
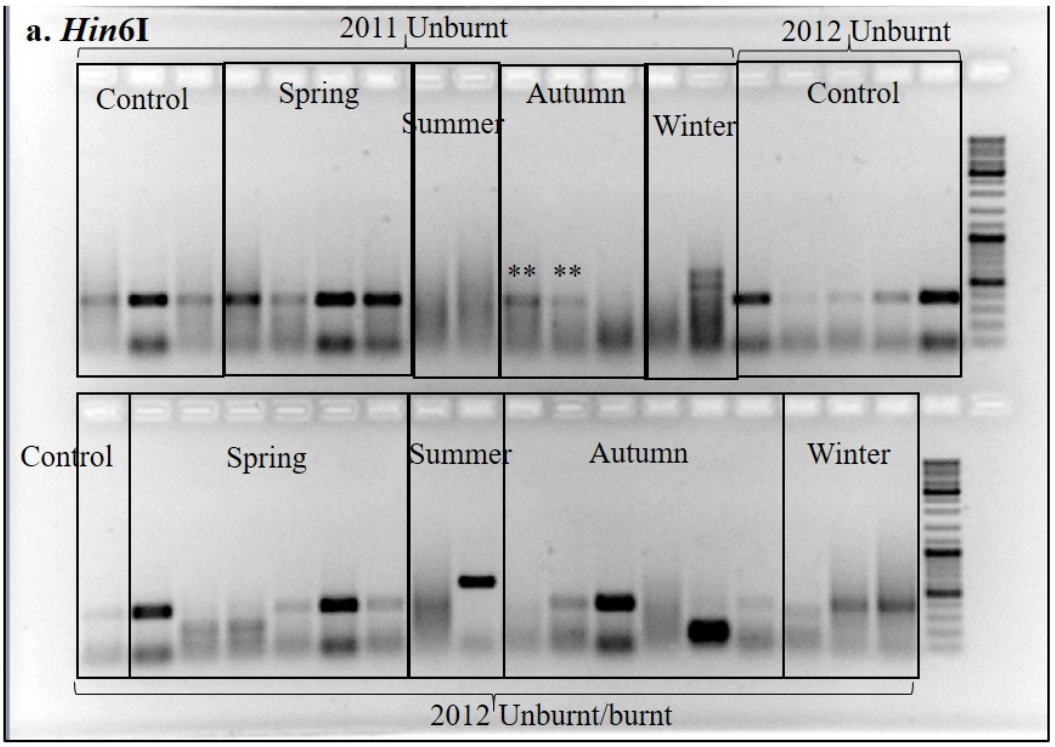


Fig. S3 RFLP patterns of *Thelymitra pauciflora* isolates from 2011 pre-burn and 2012 post-burn plots cleaved with (a) *Hin*6I restriction endonuclease and (b) *Taq*I restriction endonuclease. Fragments were measured against GeneRuler™ ladder (last lanes). Pre-burn 2011 isolates were (left to right, top-bottom row) from: control (TU1C1I, TU1C2K, TU1C2O), spring (TU1SP1A, TU1SP2L, TU1SP3S, TU1SP3W), summer (TU1SU3G, TU1SU3H), autumn (TU1A2L**, TU1A3B) and winter (TU1W2A, TU1W2H) burn quadrats. Post-burn 2012 isolates were: control (TU2C1G, TU2C1S, TU2C2A, TU2C2N, TU2C3H, TU2C3J), spring (TB2SP1D, TB2SP1K, TB2SP2H, TB2SP2P, TB2SP3O, TB2SP3R), summer (TB2SU1G, TB2SU3L), autumn (TB2A1H, TB2A1K, TB2A2A, TB2A2C, TB2A3B, TB2A3H) and winter (TB2W1C, TB2W1E, TB2W1Q). **In (a), the 2011 asterisked sample autumn burn 22 was loaded twice and so the subsequent samples were displaced right.



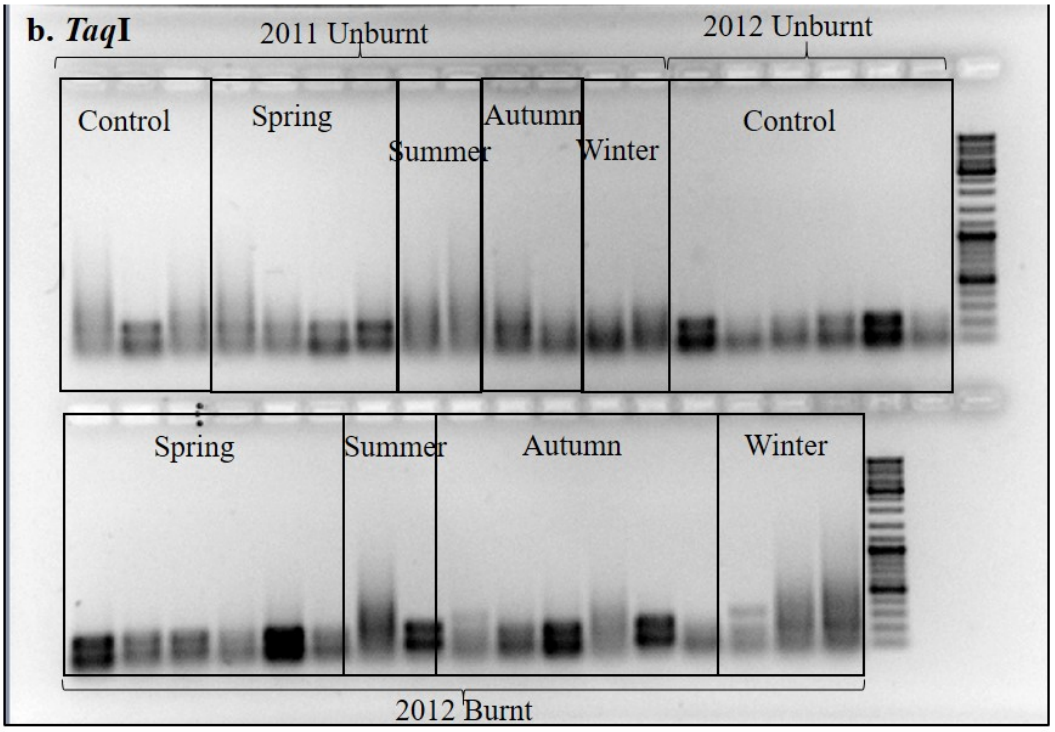
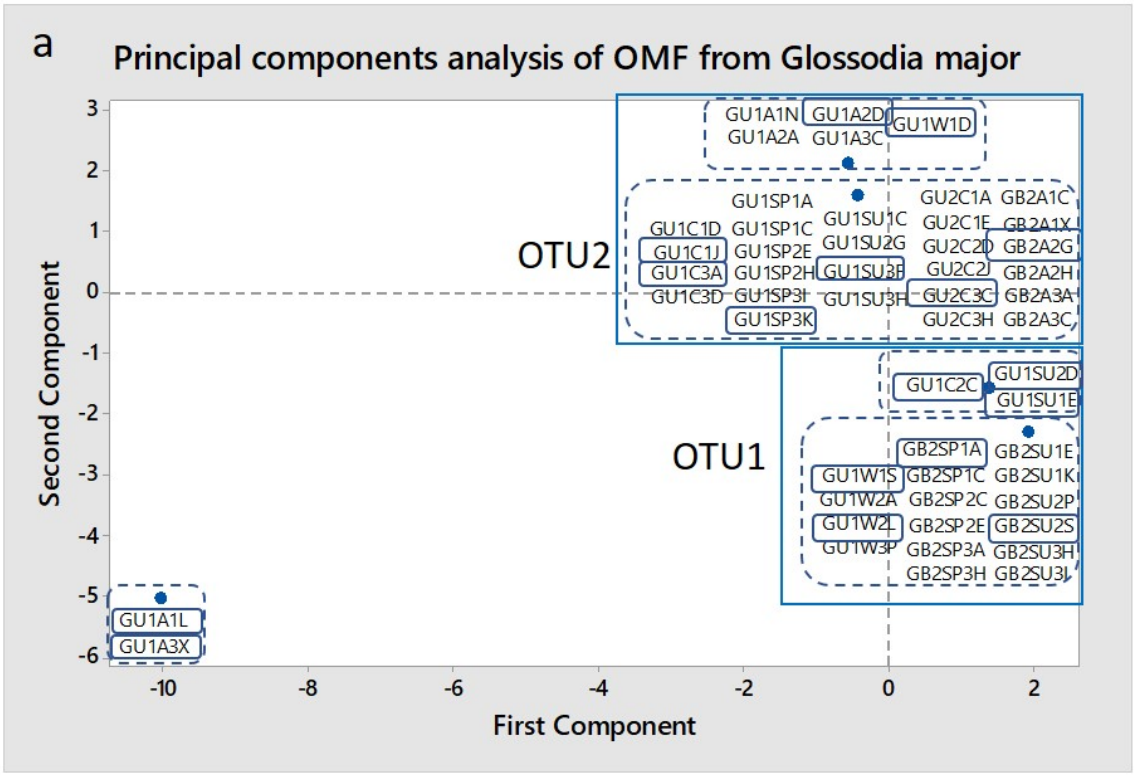


Fig. S4 Principal components analysis of ITS-RFLP patterns of (a) *Glossodia major* and (b) *Thelymitra pauciflora* isolates from 2011-2012. Isolates were labelled as follows: 1. Orchid (G=*G. major*, T=*Th. pauciflora*), 2. Treatment (u=pre-burn, b=post-burn), 3. Year of collection (1=2011, 2=2012), 4. Season of burn (C=control, SP=spring, SU=summer, A=autumn, W=winter), 5. Plant replicate (1=plant 1, 2=plant 2, 3=plant 3), 6. Isolate identification (A-Z). Smallest rectangles with solid lines show sequenced isolates. Intermediate rectangles with dotted lines group isolates with identical RFLP patterns (same data point). Largest rectangles with solid lines indicate boundaries of clusters in which sequenced isolates were ≥97% similar.



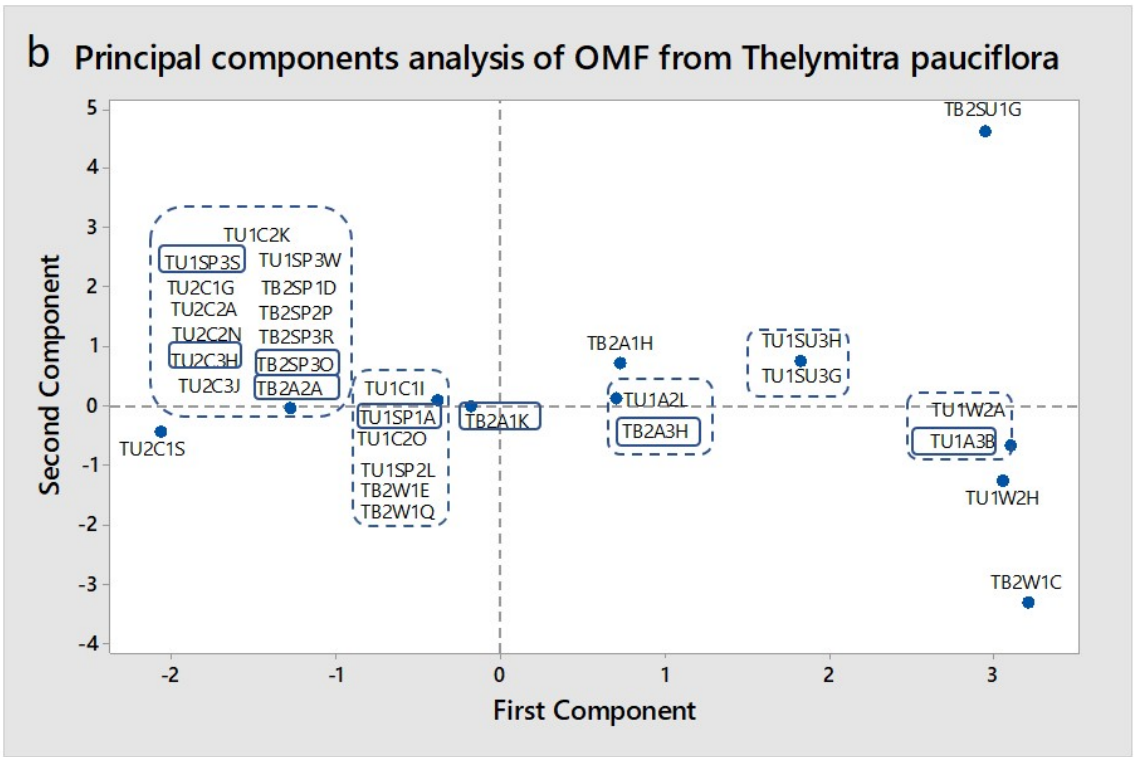


Fig. S5 Relationships of ITS sequences from 2011-2012 isolates from *Glossodia major* to those in GenBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the greatest log likelihood (-2386.26) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 57 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 346 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016). Key: JHW signifies that this was isolated by J.H. Warcup; Sp. x signifies that this sequence was classified as: sp. x (Sommer *et al.* 2012), P-OTUx signifies that this sequence clustered in OTU x (Phillips *et al.* 2016) and W-X signifies that this sequence clustered in Group X (Whitehead *et al.* 2017).

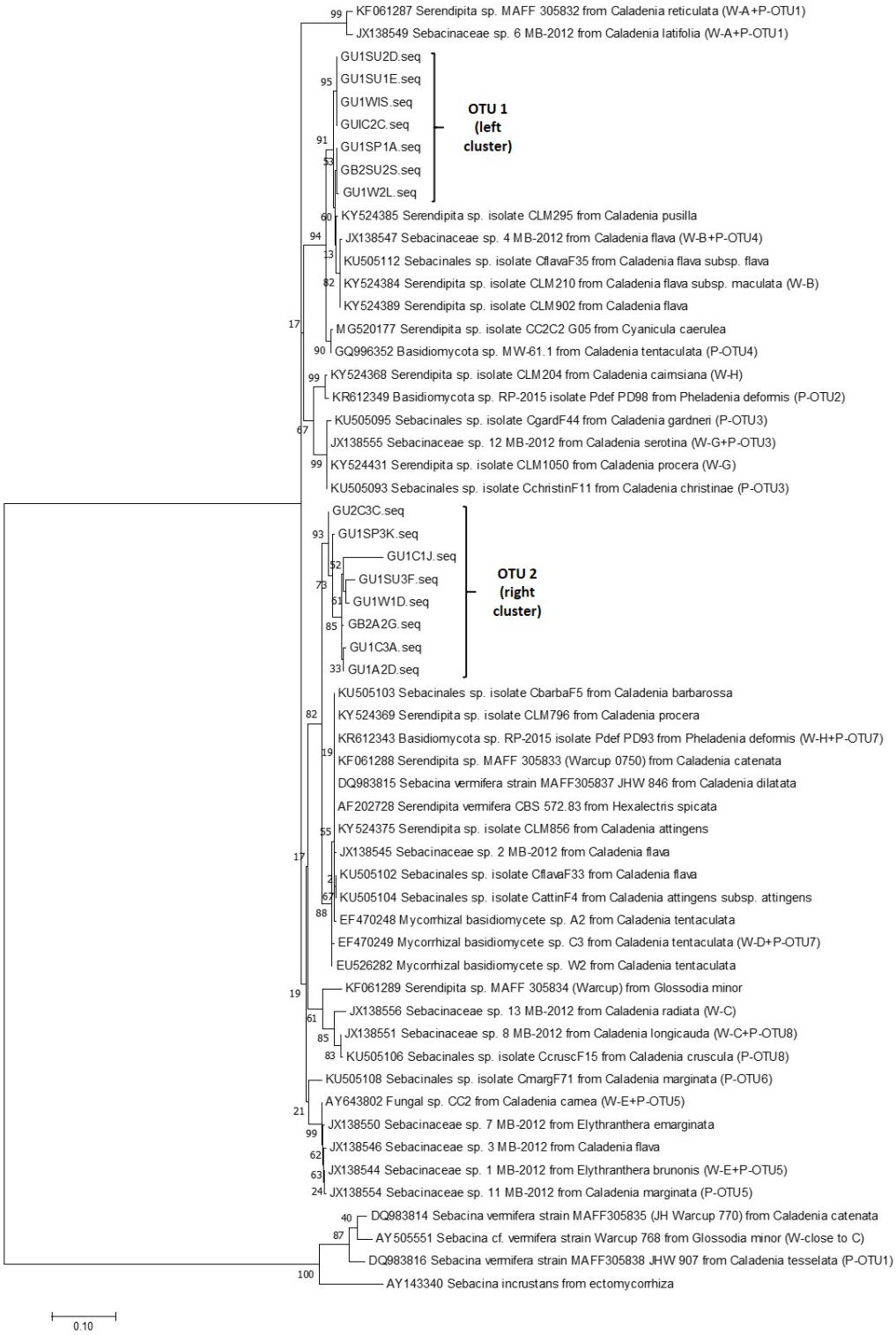


Fig. S6 Relationships of ITS sequence from 2011-2012 isolates GU1A1L and GU1A3X from *Glossodia major* to those in GenBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the greatest log likelihood (-1377.64) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 45 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 286 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016).

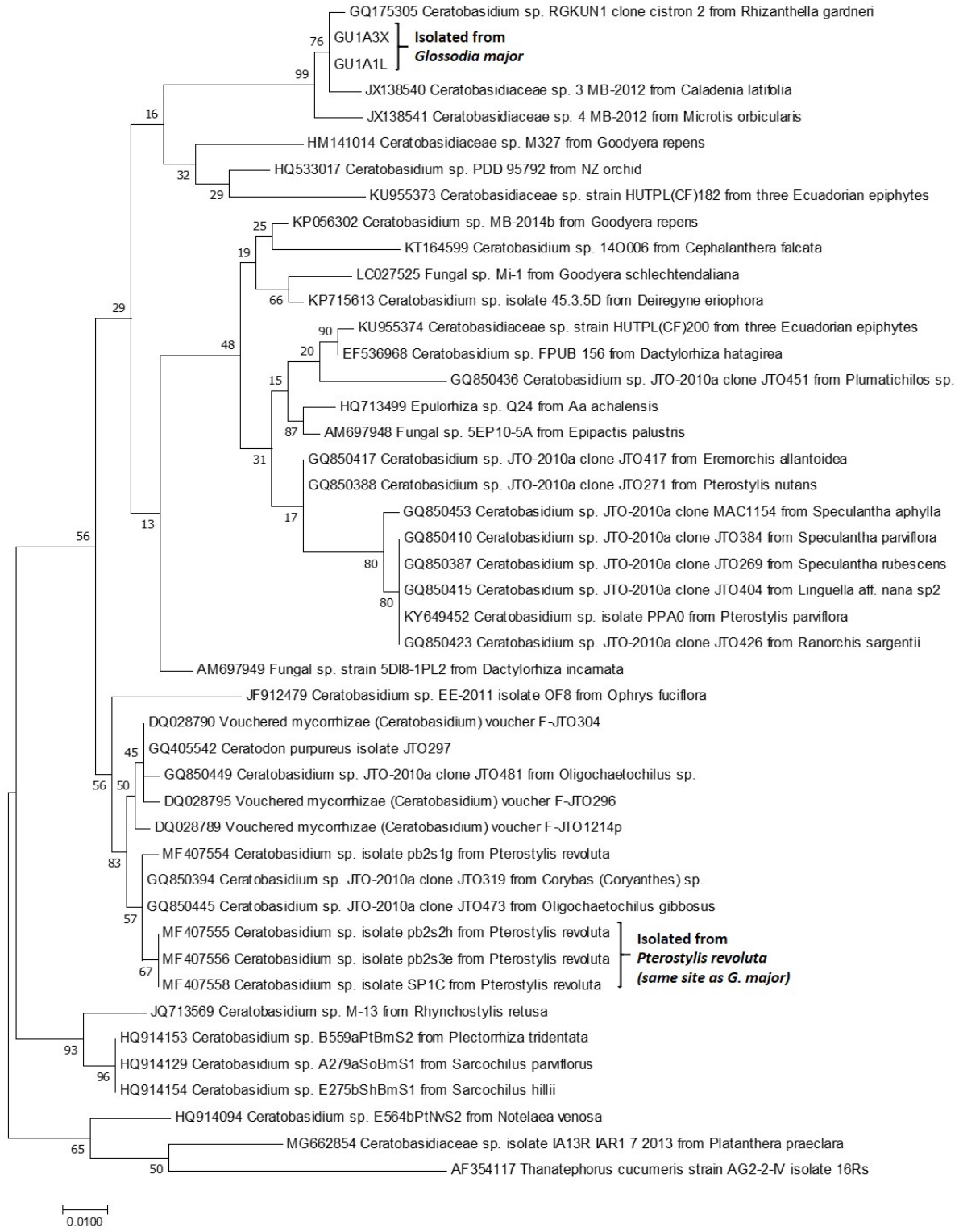
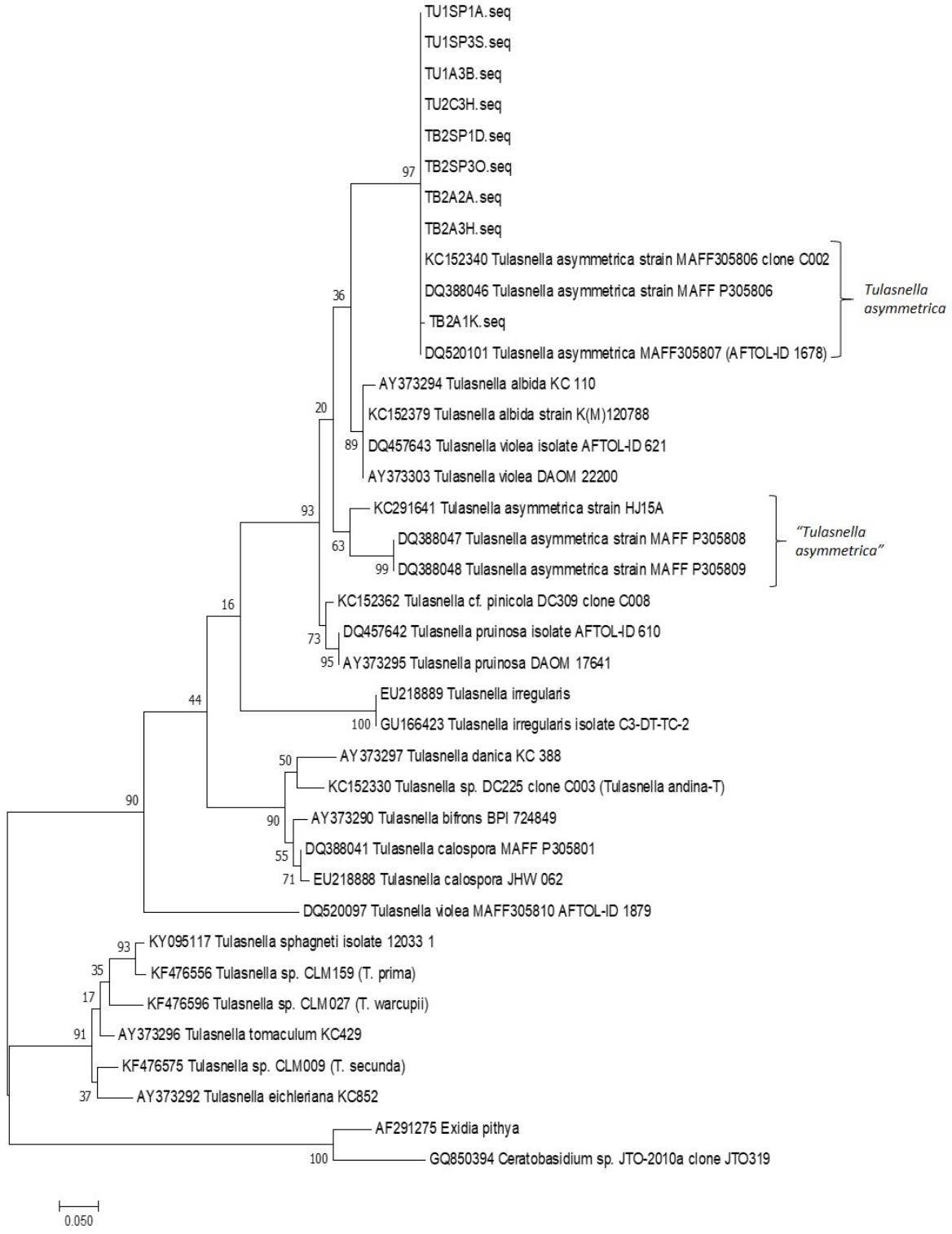


Fig. S7 Relationships of ITS sequence from 2011-2012 isolates from *Thelymitra pauciflora* to those in GenBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree with the greatest log likelihood (-1823.52) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 201 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016).



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