

Supplementary Table 1. Forward and reverse primers used in PCR amplification and cycle sequencing for individual loci used in this study.

Locus	Forward primer (5'-3')	Reverse primer (5'-3')
16S	TTATTCACCTGTTTATCAAAACAT	TATAGATAGAAACCAATCT
CO1	ATAATTTTTTTTTATAGTTATAC	GGAAAAAAGTTATATTAACWC
ArgK	GACAGCAARTCTCTGCTGAAGAA	GGTYTTGGCATCGTTGTGGTAGATAC
EF1- α (short)	GYATCGACAARCGTACSATYG	ACRGCVACKGTYTGHCKCATGTC
EF1- α (long)	GGGYAAAGGWCCTTCAARTATGC	AATCAGCACCTTTAGGTGG
Pol-II	AAYAARCCVGTATGGGTATTGTRCA	AGRTANGARTTCTCRACGAATCCTCT

Supplementary Table 2. Best-fit models of sequence evolution used for each locus in Bayesian phylogenetic analyses. Likelihood Ratio Tests (LRT) between nested models of sequence evolution were implemented to determine the best-fit model in the software package *Modeltest* v3.7

Locus	Model test (hLRT)	Number of parsimony-informative sites (bp)
16S	TVM+I+G	199
CO1	GTR+I+G	423
ArgK	TrN+G	79
EF1- α (coding)	TrN+G	412
EF1- α (intron)	HKY+G	412
Pol-II	TrN+I+G	182

Supplementary Table 3. Collection data, taxonomic information, and voucher *GenBank* accession numbers for specimens and sequences used in the present study.

ID#	Voucher	Taxon	Country	Collector	Date	16S	CO1	Ef1-alpha	ArgK	RNApol-2
EU53a	TA321	<i>Aglae caerulea</i>	Colombia	Ramírez	Apr-15-03	EU162926	EU163091	EU163173	EU163007	EU162842
MP145	outg37	<i>Apis andreniformis</i>	China	Roubik		EU162957	EU163123	EU163210	EU163042	EU162879
MP110	outg10	<i>Apis cerana</i>	Thailand	Roubik		EU162939	EU163106	EU163190	EU163023	EU162859
MP112	outg12	<i>Apis cerana</i>	Thailand	Roubik		EU162941	EU163108	EU163192	EU163025	EU162861
MP140		<i>Apis cerana</i>	Thailand			EU162955		EU163208	EU163040	EU162877
MP111	outg11	<i>Apis dorsata</i>	Thailand	Roubik		EU162940	EU163107	EU163191	EU163024	EU162860
MP143	outg35	<i>Apis florea</i>	China	Roubik		EU162956	EU163122	EU163209	EU163041	EU162878
MP113	outg13	<i>Apis koschevnikovi</i>	Thailand	Roubik		EU162942	EU163109	EU163193	EU163026	EU162862
MP114	outg14	<i>Apis koschevnikovi</i>	Thailand	Roubik		EU162943	EU163110	EU163194	EU163027	EU162863
MP147		<i>Apis mellifera</i>	USA			AF250955.1	AY114457.1	AF015267.1	NM0010116031	DQ069332.1
MP146	SR2066	<i>Bombus transversalis</i>	Peru	Ramirez		EU162958	EU163124	EU163211	EU163043	EU162880
MP138	Outg34	<i>Bombus vagans</i>	USA	Ramirez		EU162953	EU163120	EU163206	EU163038	EU162875
MP124	outg24	<i>Centris</i> sp.	Colombia	Ramirez		EU162948		EU163200	EU163032	EU162869
MP106	outg7	<i>Cephalotrigona zexmeniae</i>	Panama	Nieh	Aug-26-99	EU162935	EU163102	EU163186	EU163019	EU162855
MP89	SR2119	<i>Cephalotrigona</i> sp.	Ecuador	Ramirez	Apr-8-05	EU162995	EU163161	EU163248	EU163080	EU162916
MP125	outg25	<i>Epicharis</i> sp.	Colombia	Ramirez			EU163115	EU163201	EU163033	EU162870
Eu99	SR699	<i>Eufriesea caerulescens</i>	Mexico	Ramírez	Jul-17-03		EU163095	EU163178	EU163011	EU162847
EU15b	CS84	<i>Euglossa asarophora</i>	Costa Rica	Skov	Aug-11-02		EU163090	EU163171	EU163005	EU162840
EU22c	SR346	<i>Euglossa decorata</i> (dark)	Colombia	Ramírez	Jan-10-03	EU162925		EU163172	EU163006	EU162841
EU8	CS155	<i>Euglossa mixta</i>	Costa Rica	Skov	Aug-16-02		EU163094	EU163177		EU162846
EU70	Yurr 912	<i>Euglossa villosa</i>	Guatemala	Ramirez	Sep-10-03		EU163092	EU163175	EU163009	EU162844
EU54	SK02	<i>Eulaema peruviana</i>	Peru	Skov	Apr-1-03			EU163174	EU163008	EU162843
EU71	male#1	<i>Exaerete azteca</i>	Mexico	Skov	Jul-26-03	EU162927	EU163093	EU163176	EU163010	EU162845

MP107	outg8	<i>Friesella schrottkyi</i>	Brazil	Nieh	Aug-19-00	EU162936	EU163103	EU163187	EU163020	EU162856
MP108	outg9	<i>Frieseomelitta silvestrii</i>	Brazil	Nieh	Aug-12-00	EU162937	EU163104	EU163188	EU163021	EU162857
MP118	outg18	<i>Geotrigona kraussi</i>	Panama	Roubik	94	EU162945	EU163112	EU163196		EU162865
MP115	outg15	<i>Lestrimelitta danuncia</i>	Panama	Roubik	92	EU162944	EU163111	EU163195	EU163028	EU162864
MP33	126	<i>Melipona aff. costaricaensis</i>	Panama	Roubik	Jan-Feb 00	EU162968	EU163134	EU163221	EU163053	EU162890
MP94	SR1960	<i>Melipona amazonica</i>	Perú	Ramirez	Mar-4-05	EU163000	EU163166	EU163253	EU163085	
MP82	379	<i>Melipona asilvae</i>	Brazil	Nieh	Aug-26-00	EU162991	EU163157	EU163244	EU163076	EU162912
MP18	101	<i>Melipona beecheii</i>	Mexico	Roubik	Feb-98	EU162960	EU163126	EU163213	EU163045	EU162882
MP83	403	<i>Melipona bicolor bicolor</i>	Brazil	Nieh	Aug-26-00	EU162992	EU163158	EU163245	EU163077	EU162913
MP43	187	<i>Melipona captiosa</i>	French Guiana	Roubik	Sept-12-15-00	EU162976	EU163142	EU163229	EU163061	EU162898
MP37	134	<i>Melipona compressipes compressipes</i>	Brazil	Camargo et al	Jun-2-00	EU162971	EU163137	EU163224	EU163056	EU162893
MP20	114	<i>Melipona compressipes interrupta</i>	French Guiana	Roubik	Apr-94	EU162962	EU163128	EU163215	EU163047	EU162884
MP44	190	<i>Melipona compressipes interrupta</i>	French Guiana	Roubik	Sept-12-15-00	EU162977	EU163143	EU163230	EU163062	EU162899
MP14	84	<i>Melipona costaricaensis</i>	Costa Rica	Roubik	Sep-12-99	EU162954	EU163121	EU163207	EU163039	EU162876
MP21	110	<i>Melipona costaricaensis</i>	Panama	Roubik	Mar-97	EU162963	EU163129	EU163216	EU163048	EU162885
MP32	124	<i>Melipona aff. crinita</i>	Panama	Roubik	Jan-Feb 00	EU162967	EU163133	EU163220	EU163052	EU162889
MP92	SR1622	<i>Melipona aff. crinita</i>	Perú	Ramirez	Mar-3-05	EU162998	EU163164	EU163251	EU163083	EU162919
MP13	81	<i>Melipona fallax</i>	Costa Rica	Roubik	Sep-12-99	EU162952	EU163119	EU163205	EU163037	EU162874
MP19	103	<i>Melipona favosa</i>	French Guiana	Roubik	May-94	EU162961	EU163127	EU163214	EU163046	EU162883
MP42	186	<i>Melipona fuliginosa</i>	French Guiana	Roubik	Sept-12-15-00	EU162975	EU163141	EU163228	EU163060	EU162897
MP15	95	<i>Melipona fulva</i>	French Guiana	Roubik	May-94	EU162959	EU163125	EU163212	EU163044	EU162881

MP35	129	<i>Melipona fuscopilosa</i>	Brazil	Camargo et al	Jun-2-00	EU162970	EU163136	EU163223	EU163055	EU162892
MP128	R16	<i>Melipona grandis</i>	Peru	Ramirez		EU162950	EU163117	EU163203	EU163035	EU162872
MP22	115	<i>Melipona grandis</i>	Ecuador	Roubik	Nov-98	EU162964	EU163130	EU163217	EU163049	EU162886
MP97	SR1982	<i>Melipona grandis</i>	Perú	Ramirez	Mar-14-05	EU163003	EU163169	EU163256	EU163088	EU162923
MP95	SR2118	<i>Melipona illota</i>	Ecuador	Ramirez	Apr-3-05	EU163001	EU163167	EU163254	EU163086	EU162921
MP46		<i>Melipona illustris</i>	Brazil		Jul-99	EU162979	EU163145	EU163232	EU163064	EU162901
MP45	191	<i>Melipona lateralis</i>	French Guiana	Roubik	Sept-12-15-00	EU162978	EU163144	EU163231	EU163063	EU162900
MP81	303	<i>Melipona mandacaia</i>	Brazil	Nieh	Aug-7-00	EU162990	EU163156	EU163243	EU163075	EU162911
MP79	286	<i>Melipona marginata</i>	Brazil	Nieh	Aug-8-00	EU162987	EU163153	EU163240	EU163072	EU162908
MP12	77	<i>Melipona melanopleura</i>	Costa Rica	Roubik	Sep-12-99	EU162946	EU163113	EU163197	EU163029	EU162866
MP34	128	<i>Melipona melanoventer</i>	Brazil	Roubik	Feb-25-00	EU162969	EU163135	EU163222	EU163054	EU162891
MP39	172	<i>Melipona micheneri</i>	Panama	Nieh	Jul-00	EU162973	EU163139	EU163226	EU163058	EU162895
MP98	SMR0036	<i>Melipona n. sp.</i>	Colombia	Madriñan	May-05-05	EU163004	EU163170	EU163257	EU163089	EU162924
MP48	193	<i>Melipona nebulosa</i>	Ecuador	Roubik	Nov-7-15-98	EU162980	EU163146	EU163233	EU163065	
MP41	184	<i>Melipona ogliviei</i>	French Guiana	Roubik	Sept-12-15-00	EU162974	EU163140	EU163227	EU163059	EU162896
MP1	1	<i>Melipona panamica</i>	Panama	Nieh	Aug-7-99	EU162928	EU163096	EU163179	EU163012	EU162848
MP23	118	<i>Melipona quadrifasciata anthidioides</i>	Brazil	Roubik	Oct-98	EU162965	EU163131	EU163218	EU163050	EU162887
MP76	257	<i>Melipona quadrifasciata anthidioides</i>	Brazil	Nieh	8-Aug-00	EU162984	EU163150	EU163237	EU163069	EU162905
MP80	300	<i>Melipona quinquefasciata</i>	Brazil	Nieh	Aug-10-00	EU162989	EU163155	EU163242	EU163074	EU162910
MP24	120	<i>Melipona rufiventris</i>	Ecuador	Roubik	Nov-98	EU162966	EU163132	EU163219	EU163051	EU162888
MP49	195	<i>Melipona rufiventris flavolineata</i>	Brazil	Venturieri	Jan-00	EU162981	EU163147	EU163234	EU163066	EU162902
MP72	411	<i>Melipona rufiventris flavolineata</i>	Bolivia	Boni	2-Oct-02	EU162983	EU163149	EU163236	EU163068	EU162904

MP77	267	<i>Melipona rufiventris rufiventris</i>	Brazil	Nieh	8-Aug-00	EU162985	EU163151	EU163238	EU163070	EU162906
MP78	277	<i>Melipona scutellaris</i>	Brazil	Nieh	8-Aug-00	EU162986	EU163152	EU163239	EU163071	EU162907
MP38	137	<i>Melipona seminigra atrofulva</i>	Brazil	Camargo et al	Jun-2-00	EU162972	EU163138	EU163225	EU163057	EU162894
MP86	414	<i>Melipona solani</i>	Mexico	Nieh	Mar-1-02	EU162994	EU163160	EU163247	EU163079	EU162915
MP127	T3W2	<i>Melipona</i> sp.	Costa Rica	Archibald		EU162949	EU163116	EU163202	EU163034	EU162871
MP93	SR1780	<i>Melipona</i> sp.	Perú	Ramirez	Mar-16-05	EU162999	EU163165	EU163252	EU163084	EU162920
MP70	409	<i>Melipona</i> sp.	Peru	Rarioet	1-Nov-01	EU162982	EU163148	EU163235	EU163067	EU162903
MP85	454	<i>Melipona</i> sp.	Colombia	Arias	Mar-30-03	EU162993	EU163159	EU163246	EU163078	EU162914
MP90	SR1977	<i>Melipona</i> sp.	Ecuador	Ramirez	Mar-27-05	EU162996	EU163162	EU163249	EU163081	EU162917
MP96	SR2120	<i>Melipona</i> sp. (male)	Ecuador	Ramirez	Apr-10-05	EU163002	EU163168	EU163255	EU163087	EU162922
MP91	SR1976	<i>Melipona</i> sp. (queen)	Ecuador	Ramirez	Mar -27-05	EU162997	EU163163	EU163250	EU163082	EU162918
MP8.1	54	<i>Melipona triplaris</i>	Panama	Nieh	Aug-28-99	EU162988	EU163154	EU163241	EU163073	EU162909
MP129	outg29	<i>Meliponula bocandei</i>	Kenya	Martins	May-15-00	EU162951	EU163118	EU163204	EU163036	EU162873
MP123	outg23	<i>Meliwillea bivea</i>	Panama	Roubik	Mar-01-02		EU163114	EU163199	EU163031	EU162868
MP104	outg5	<i>Nannotrigona perilampoides</i>	Panama	Nieh	Aug-28-99	EU162933	EU163100	EU163184	EU163017	EU162853
MP120	outg20	<i>Nogueirapis mirandula</i>	Panama	Roubik	94	EU162947		EU163198	EU163030	EU162867
MP101	outg2	<i>Plebeia franklii</i>	Panama	Nieh	Aug-28-99	EU162930	EU163098	EU163181	EU163014	EU162850
MP105	outg6	<i>Scaptotrigona polysticta</i>	Brazil	Nieh	Aug-12-00	EU162934	EU163101	EU163185	EU163018	EU162854
MP102	outg3	<i>Scaptotrigona barrocoloradensis</i>	Panama	Nieh	Aug-28-99	EU162931	EU163099	EU163182	EU163015	EU162851
MP103	outg4	<i>Scaptotrigona barrocoloradensis</i>	Panama	Nieh	Aug-28-99	EU162932		EU163183	EU163016	EU162852
MP100	outg1	<i>Tetragona angustula</i>	Panama	Nieh	Aug-28-99	EU162929	EU163097	EU163180	EU163013	EU162849
MP109	SR2086	<i>Trigona</i> sp.	Ecuador	Ramirez	Mar-27-05	EU162938	EU163105	EU163189	EU163022	EU162858

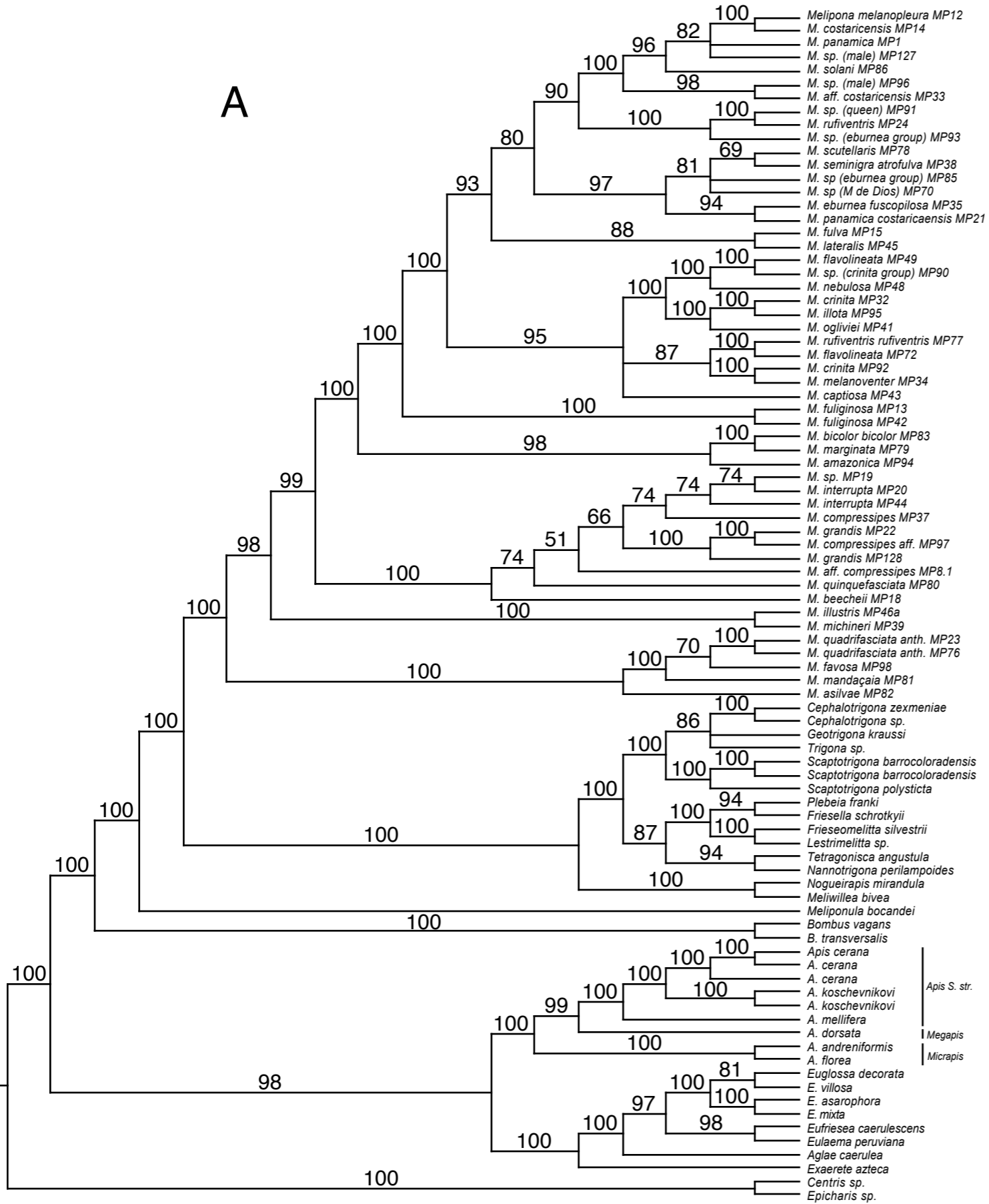
Supplementary Figure 1. Bayesian phylogenetic trees estimated with (a) a single model of nucleotide substitution (GTR+ Γ +I) and (b) partitioned models of sequence evolution for each locus. Phylogenies were estimated in the software package *MrBayes* v3.1.1. Posterior probabilities correspond to node frequencies obtained using a 50% majority-rule consensus.

Supplementary Figure 2. Bayesian phylogenetic trees estimated with partitioned models of sequence evolution by codons, where first, second and third position were estimated separately. The phylogeny was estimated in the software package *MrBayes* v3.1.1. Posterior probabilities correspond to tree frequencies obtained using a 50% majority-rule consensus.

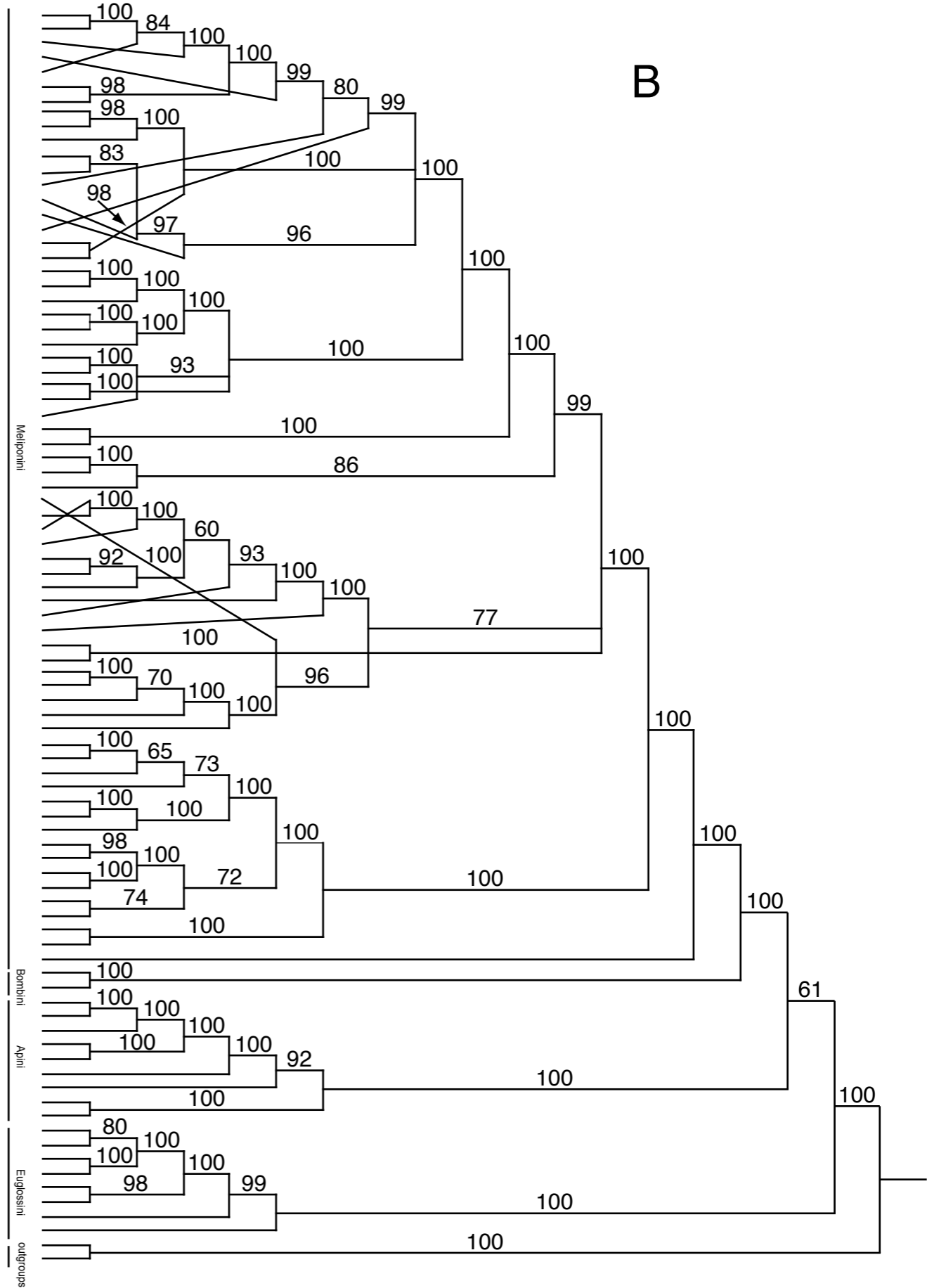
Supplementary Figure 3. Cross-validation test to identify inconsistent fossil calibrations (Near *et al.*, 2005). (a) Histogram of mean age deviation (Dx) of calibrated nodes showing difference between molecular and fossil ages. (b) Sum of the squared differences (Dx) between fossil ages and molecular ages. (c) Effect of removing individual fossil calibration on the average squared deviation of all five fossil calibrations; none of the changes in square deviation were statistically significant based on a one-tailed *F*-test.

Supplementary Figure 1

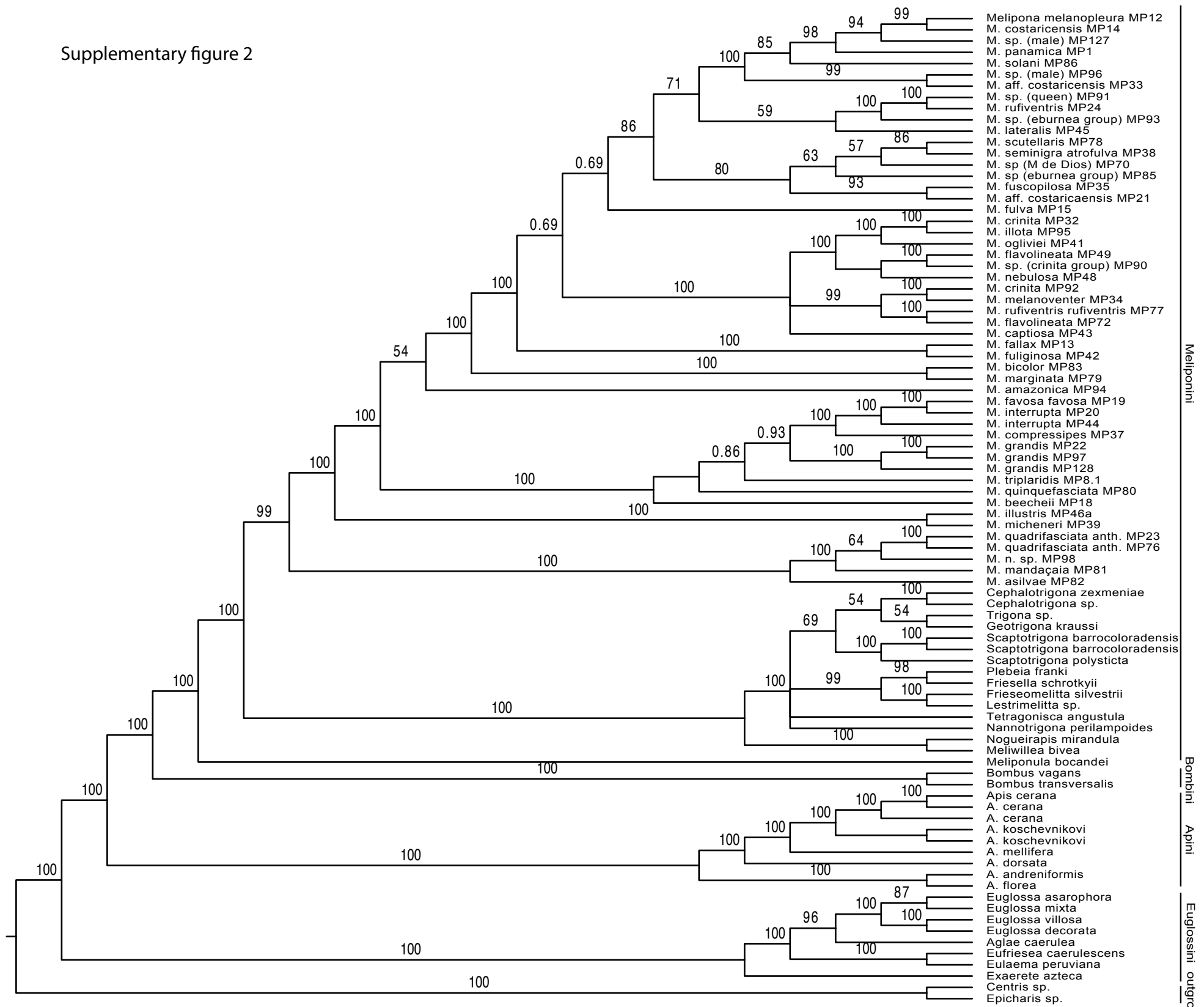
A



B



Supplementary figure 2



Supplementary Figure 3

