

Multiple Geographical Origins of Environmental Sex Determination enhanced the diversification of Darwin's Favourite Orchids

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Appendix S1:

Including: Extended Materials and Methods.

Including: Supplementary tables (S1-S7) and figures (S1-S14).

Extended Materials and Methods

Phylogenetic incongruence analysis

PACo is a co-phylogenetic approach that is capable of assessing similarities between two trees by comparison of Euclidean distance matrices derived from phylogenies. Additionally, it determines the contribution of every association (i.e. pair of terminals of the analysed phylogenies) to the co-phylogenetic pattern observed, thus it has the potential to statistically identify specific potential conflicting terminals. PACo requires as input data a set of phylograms (e.g., posterior distribution of trees, or bootstrap replicate trees) derived from every genomic compartment (i.e. nucleus and plastids) and a binary association matrix, in which same taxa present in both datasets are linked. We executed PACo using 1000 trees from the Bayesian posterior distribution of trees obtained from MrBayes analysis computed from concatenated nuclear and plastid datasets, respectively. Plastids are maternally inherited in orchids^{1,2}, hence they reflect maternal evolutionary relationships between species. Contrastingly, nuclear loci show bi-parental inheritance³, thus revealing maternal and paternal evolutionary histories – potentially revealing a more comprehensive perspective on the evolutionary relations between species. Therefore, conflicting plastid sequences were excluded from the dataset, and non-conflicting sequences of each locus were re-aligned and concatenated.

Ancestral area estimation (AAE)

We relied on BIOGEOBEARS (Biogeography with Bayesian and Likelihood Evolutionary Analysis in R script) to carry AAE analyses. Unlike previous programs such as LAGRANGE⁴, ‘BIOGEOBEARS’ evaluates several biogeographical models altogether testing the role and contribution of evolutionary processes (i.e., range expansions, local extinctions, founder-event speciation, vicariance, and speciation in

the same biogeographic region) that were taken into account to explain extant, observed distributions in a joint statistical framework. Thus, it enables model testing, because distinct geographical and phylogenetic datasets can have biogeographic histories best modelled by different approaches⁵. We divided the geographical range of Catasetinae and outgroup taxa in eight biogeographic regions (1) Central America, comprising southern Mexico through Panama and the Antilles; (2) Guiana Shield, encompasses areas above 500 m in Colombia, Venezuela, Brazil, Guyana, Suriname and French Guiana; (3) Amazonia, encompassing lowlands and pre-montane forest below 800 m in Colombia, Ecuador, Peru, Brazil, Venezuela, Guyana, Suriname, and French Guiana⁶; (4) Chocó, comprising lowlands below 500 m of the western Andes in Colombia and Ecuador; (5) Northern Andes, includes elevated areas above 800 m from Southernmost Peru to Northern Colombia and Northeast Venezuela; (6) Central Andes (comprising areas above 800 m in Northern Peru to Northern Chile and Northeast Argentina); (7) South-eastern South America (encompassing part of the Brazilian shield, South-eastern Bolivia, Paraguay, Uruguay, and Northern Argentina); and (8) Africa and Australasia (representing distribution ranges of outgroup taxa).

Molecular clock dating

We selected the crown group of Catasetinae and the tree root (i.e., MRCA of Vandae +Cymbidiidae), respectively. We set the tree root (i.e., MRCA of Vandae +Cymbidiidae) to 34.05 ± 8 Myrs and the crown group of Catasetinae to 19.88 ± 6 Myrs, using a normal distribution. For strict molecular clock calibration, we placed a single constraint only at the tree root (34.05 ± 8 Myrs), using a normal distribution.

For each clock model, we ran two MCMC analyses with 20 million generations each, sampled every 1000th generation. For the relaxed molecular clock

analyses, we estimated the coefficient of variation (CV) to inform us on the rate heterogeneity among branches (CV approaching 0 indicates that the among-branch variation in substitution rate is much smaller magnitude than the mean rate, hence, a strict clock model cannot be rejected). Parameter convergence was confirmed using TRACER 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>). All dating analyses were performed at the CIPRES Science Gateway computing facility⁷.

Trait-dependent diversification analyses

We relied on the Binary State Speciation and Extinction (BiSSE) model⁸ to estimate diversification rates associated with all three traits (i.e., ESD, epiphytism, and euglossine-bee pollination). BiSSE model built upon the premise that speciation and extinction rates depend on the state of a particular character. We tested eight models with different configurations of speciation, extinction, and transition rates between characters: (i) equal rates model (no effect of the trait on diversification, M_1); (ii) free speciation rates in lineages with and without a trait, and equal rates of extinction and transition (M_2); (iii) free extinction rates in lineages with and without a trait, and equal rates of speciation and transition (M_3); (iv) free transition rates in lineages with and without a trait, and equal rates of speciation and extinction (M_4); (v) free speciation and extinction rates in lineages with and without a trait, and equal rates of transition (M_5); (vi) free speciation and transition rates in lineages with and without a trait, and equal rates of extinction (M_6); (vii) free extinction and transition rates in lineages with and without a trait, and equal rates of speciation (M_7); and (viii) all parameters are free (M_8).

We estimated the speciation, extinction, and transition rates corresponding to the best fitting model, according to the AICc criterion. We ran Bayesian MCMC analyses on the consensus tree, using exponential priors with parameters obtained from the best fitting model, a 1000-step burn-in, and 10,000-step chain⁸. Convergence occurred within the few first steps and parameter estimates were very stable along the chain. We summarised the posterior distributions of samples to assess variation in net diversification, turnover, and transition rates across character states after the burn-in phase. We used a global sampling of 139 species (~32% of the extant diversity of Catasetinae + Cyrtopodiinae), and we accounted for missing taxa by accommodating sampling fractions for every trait as follows: (i) ESD: 41% of the know species with ESD sampled vs. 44% of the know species with alternative matting systems sampled; (ii) euglossine-bee pollination: 31% of the know species with this trait sampled vs. 33% of the know species with alternative pollination syndromes trait sampled; (iii) epiphytism: 40% of the know epiphyte species sampled vs. 45% of the known species with alternative plant habits sampled. Information on occurrence of epiphytism and euglossine-bee pollination syndrome was obtained from the literature^{9,10}. We took phylogenetic and dating uncertainties into account by running these models over a series of 500 dated trees randomly selected from the BEAST dating analysis. We then selected the best-fitting model based on the corrected Akaike Informative Criterion (AICc).

Because BiSSE may be subject to Type I errors biases¹¹, we tested whether diversification rates associated to character states are not artefacts. To do so, we reshuffled the coding states onto the phylogeny and ran the BiSSE models. We executed 10,000 iterations, estimating the AIC of the best model at each iteration to

get the null distribution of AIC (associated with random states) and compared it with the AIC value obtained with the observed dataset.

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Supplementary tables

Table S1. Species names and voucher information for material use in this study. Taxa sequenced and newly produced sequences are shown in bold face.

Species names	ITS	ETS	<i>Xdh</i>	<i>matK</i>	<i>trnL-F</i>	<i>ycf1</i>
<i>Acrolophia bolusii</i>	KF318909	-	-	KF358114	-	-
<i>Acrolophia capensis</i>	KF318958	-	-	KF358074	-	-
<i>Acrolophia cochlearis</i>	KF318947	-	-	KF358059	-	-
<i>Acrolophia lamellata</i>	KF318964	-	-	KF358063	-	-
<i>Acrolophia lunata</i>	KF318965	-	-	KF358129	-	-
<i>Acrolophia micrantha</i>	LN831482	-	-	KF358111	-	-
<i>Acrolophia ustulata</i>	KF318963	-	-	KF358121	-	-
<i>Brassia aurantiaca</i>	AF350518	-	-	AF350597	AF350676	FJ563573
<i>Capanemia superflua</i>	AF350549	-	-	FJ563840	AF350707	-
<i>Catasetum barbatum</i>	MF987482	MF987475	-	-	-	-
<i>Catasetum bertioguense</i>	KU295252	KU295217	-	-	-	-
<i>Catasetum bicolor</i>	MF987483	-	MF987494	-	-	MF987512
<i>Catasetum blackii</i>	KU295253	KU295253	-	-	-	KU350872
<i>Catasetum boyi</i>	MF987484	-	MF987495	-	-	MF987513
<i>Catasetum callosum</i>	KU295254	MF987476	MF987496	-	-	KU350873
<i>Catasetum cernuum</i>	KU295255	KU295219	-	-	-	KU350874
<i>Catasetum ciliatum</i>	KU295256	KU295219	-	-	-	KU350875
<i>Catasetum collare</i>	KT768384	KT768350	KT768454	-	-	KT768491
<i>Catasetum complanatum</i>	KU295257	KU295221	-	-	-	KU350876
<i>Catasetum denticulatum</i>	KU295258	KU295222	MF987497	-	-	KU350877
<i>Catasetum discolor</i>	KU295259	KU295223	MF987498	-	-	MF987514
<i>Catasetum expansum</i>	KU295260	KU295224	MF987499	KF660300	-	KU350878
<i>Catasetum fimbriatum</i>	KU295261	KU295225	MF987500	-	-	KU350879

<i>Catasetum galeritum</i>	KU295262	KU295226	-	-	-	KU350880
<i>Catasetum gladiatorium</i>	KU295263	KU295227	-	-	-	KU350881
<i>Catasetum globiflorum</i>	-	-	-	-	-	MF987515
<i>Catasetum gnomus</i>	KU295264	KU295228	-	-	-	KU350882
<i>Catasetum hopkinsonianum</i>	KU295265	-	-	-	-	-
<i>Catasetum integerrimum</i>	MF987485	MF987477	-	-	-	MF987516
<i>Catasetum juruenense</i>	KT768385	KT768351	KT768455	-	-	KT768492
<i>Catasetum laminatum</i>	LK054139	-	-	-	-	-
<i>Catasetum lanciferum</i>	KU295266	KU295229	MF987501	-	-	KU350884
<i>Catasetum luridum</i>	KU295267	KU295230	-	-	-	-
<i>Catasetum macrocarpum</i>	KT768386	KT768352	KT768456	-	-	KT768493
<i>Catasetum maculatum</i>	MF987486	MF987478	MF987502	-	-	-
<i>Catasetum meeae</i>	KT768387	KT768353	KT768457	-	-	-
<i>Catasetum ochraceum</i>	MF987487	MF987479	MF987503	-	-	MF987517
<i>Catasetum ornithoides</i>	MF987488	-	MF987504	-	-	MF987518
<i>Catasetum osakadianum</i>	KU295268	KU295231	-	-	-	KU350886
<i>Catasetum osculatum</i>	MF987489	-	MF987505	-	-	MF987519
<i>Catasetum pileatum</i>	KU295269	-	-	-	-	KU350887
<i>Catasetum purum</i>	KU295270	KU295232	-	-	-	KU350888
<i>Catasetum rodigasianum</i>	KU295271	-	-	-	-	-
<i>Catasetum rooseveltianum</i>	KU295272	KU295233	-	-	-	KU350890
<i>Catasetum x roseoalbum</i>	KT768388	KT768354	KT768458	-	-	KT768494
<i>Catasetum saccatum</i>	KU295273	KU295234	-	-	EU441214	KU350891
<i>Catasetum sanguineum</i>	KU295274	-	MF987507	-	-	KU350892
<i>Catasetum spitzii</i>	KU295275	KU295235	MF987508	-	-	-
<i>Catasetum stenoglossum</i>	KU295276	KU295235	-	-	-	KU350893
<i>Catasetum tabulare</i>	MF987491	MF987480	MF987509	-	-	MF987520
<i>Catasetum uncatum</i>	MF987492	-	MF987510	-	-	-
<i>Catasetum viridiflavum</i>	KU295277	KU295237	-	-	-	MF987521

<i>Caucaea cucullata</i>	AF432952	-	-	AF433005	AF433024	-
<i>Chytroglossa aurata</i>	-	-	-	FJ564753	-	FJ563245
<i>Chytroglossa marileoniae</i>	DQ210301	-	-	FJ565112	FJ562408	-
<i>Cischweinfia colombiana</i>	FJ565464	-	-	-	-	-
<i>Clowesia amazonica</i>	-	-	-	-	-	KU350895
<i>Clowesia dodsoniana</i>	MF987493	MF987481	MF987511	KF660299	-	KF660524
<i>Clowesia rosea</i>	U257701	KU257693	-	-	-	-
<i>Clowesia russelliana</i>	KT768389	-	-	-	-	KT768495
<i>Clowesia thylaciochila</i>	LK054140	-	-	-	-	-
<i>Comparettia langkastii</i>	FJ565460	-	-	FJ563962	FJ562383	-
<i>Comparettia macroplectron</i>	AF350541	-	-	AF350620	AF350699	-
<i>Comparettia schaeferi</i>	FJ565337	-	-	FJ563932	FJ562368	-
<i>Cuitlauzina candida</i>	EF079417	-	-	EF079217	FJ562416	-
<i>Cyanaeorchis arundinae</i>	KF771816	KF771816	-	KF771820	-	KU257725
<i>Cyanaeorchis minor</i>	KF771818	KF771818	-	KF771822	-	KU350898
<i>Cyanaeorchis praetermissa</i>	KF771819	-	-	KF771823	-	-
<i>Cycnoches aureum</i>	KT768390	KT768355	KT768459	-	-	KT768496
<i>Cycnoches barthiorum</i>	KT768391	KT768356	KT768460	-	-	KT768497
<i>Cycnoches chlorochilon</i>	KT768392	KT768357	KT768461	-	-	KT768498
<i>Cycnoches cooperi</i>	KT768393	KT768358	KT768462	KF660301	-	KT768499
<i>Cycnoches densiflorum</i>	KT768394	KT768359	KT768463	-	-	-
<i>Cycnoches diana</i>	KT768395	KT768360	KT768464	-	-	KT768501
<i>Cycnoches egertonianum</i>	KM598425	KT768360	KT768464	EU214159	EU214159	KT768501
<i>Cycnoches guttulatum</i>	KT768398	KT768363	KT768467	-	-	KT768504
<i>Cycnoches haagii</i>	KU257702	KT768364	KT768468	-	-	-
<i>Cycnoches herrenhusanum</i>	KU257703	KT768365	KT768469	-	-	KT768506
<i>Cycnoches lehmannii</i>	KT768401	KT768366	KT768470	KF660266	-	KT768507
<i>Cycnoches loddigesii</i>	KT768402	KT768367	KT768471	-	-	KT768508
<i>Cycnoches manoelae</i>	KT768403	KT768368	KT768472	KF660254	-	KT768509

<i>Cycnoches pachydactylon</i>	KT768404	KT768369	KT768473	KF660258	-	KT768510
<i>Cycnoches pentadactylon</i>	KU257704	KT768369	KT768473	-	-	KT768510
<i>Cycnoches peruvianum</i>	KT768405	KT768372	KT768475	-	-	KT768513
<i>Cycnoches quatuorcristis</i>	MF285500	MF285467	-	-	-	-
<i>Cycnoches suarezii</i>	KT768408	KT768374	KT768476	-	-	KT768515
<i>Cycnoches ventricosum</i>	KT768409	KT768375	KT768477	-	-	-
<i>Cycnoches warszewiczii</i>	KT768410	KT768376	KT768478	EU214322	-	KT768517
<i>Cymbidiella pardalina</i>	AF470489	-	-	AF470459	-	-
<i>Cymbidium devonianum</i>	AF470515	-	-	-	-	-
<i>Cymbidium eburneum</i>	AF284709	-	KT768479	-	-	-
<i>Cymbidium tracyanum</i>	KT768412	-	-	-	-	-
<i>Cymbidium wenshanense</i>	AF284708	-	-	-	-	-
<i>Cyrtochiloides cardiochila</i>	AF432947	-	-	AF433011	AF433030	-
<i>Cyrtochilum aurantiacum</i>	AF350872	-	-	AF433008	AF433027	-
<i>Cyrtochilum cimiciferum</i>	AF350560	-	-	AF350639	AF350718	-
<i>Cyrtochilum edwardii</i>	AF350557	-	-	FJ565148	AF350715	-
<i>Cyrtochilum graminoides</i>	AF350875	-	-	AF433013	AF433032	-
<i>Cyrtochilum meirax</i>	AF432959	-	-	AF433016	AF433035	-
<i>Cyrtopodium aliciae</i>	EU877156	-	-	-	-	KU350900
<i>Cyrtopodium andersonii</i>	AF470490	-	MF285528	KF660267	-	KF660329
<i>Cyrtopodium glutiniferum</i>	-	-	-	-	-	MF285554
<i>Cyrtopodium holstii</i>	-	-	-	-	-	KU350901
<i>Cyrtopodium longibulbosum</i>	-	-	-	KF660285	-	KF660453
<i>Cyrtopodium macrobulbon</i>	-	-	-	LN609766	-	-
<i>Cyrtopodium punctatum</i>	AF239412	-	-	AF239508	AF239604	KT750238
<i>Cyrtopodium virescens</i>	-	-	-	-	-	KU350902
<i>Dipodium paludosum</i>	KY988619	-	KT750227	KT750201	-	KT750239
<i>Dressleria dilecta</i>	AF239411	-	GU004391	EU214339	AF239603	KU257728
<i>Dressleria fragrans</i>	-	-	-	KF660265	-	KF660327

<i>Dressleria helleri</i>	-	-	-	KF660264	-	KF660326
<i>Dressleria severiniana</i>	MF285505	-	KU257721	-	-	KU257729
<i>Dressleria sp</i>	KT768413	KU25769	-	-	-	KU257730
<i>Erycina glossomystax</i>	FJ565586	-	-	FJ565073	FJ562398	-
<i>Eulophia acutilabra</i>	KF318954	-	-	LN831656	-	-
<i>Eulophia angolensis</i>	KF318932	KU257698	-	KF358075	-	KU257731
<i>Eulophia calanthoides</i>	KF318896	-	-	KF358107	-	-
<i>Eulophia callichroma</i>	KF318903	-	-	-	-	-
<i>Eulophia coeloglossa</i>	KF318961	-	-	-	-	-
<i>Eulophia cucullata</i>	KF318946	-	-	-	-	-
<i>Eulophia flava</i>	JN114508	-	-	-	-	-
<i>Eulophia flavopurpurea</i>	KF318930	-	-	-	-	-
<i>Eulophia graminea</i>	AF284727	-	-	KF358078	-	-
<i>Eulophia guineensis</i>	KF318960	-	-	LN831662	AF239605	EU490745
<i>Eulophia hereroensis</i>	KF318940	-	-	KF358066	-	-
<i>Eulophia hians</i>	KF318892	-	-	KF358060	-	-
<i>Eulophia horsfallii</i>	KF318922	-	-	LN831636	-	-
<i>Eulophia livingstoneana</i>	LN831402	-	-	LN849252	-	-
<i>Eulophia longisepala</i>	KF318918	-	-	KF358086	-	-
<i>Eulophia macowanii</i>	KF318904	-	-	KF358120	-	-
<i>Eulophia meleagris</i>	KF318936	-	-	KF358083	-	-
<i>Eulophia ovalis</i>	KF318905	-	-	KF358064	-	-
<i>Eulophia parviflora</i>	KF318893	-	-	KF358062	-	-
<i>Eulophia parvilabris</i>	KF318927	-	-	KF358130	-	-
<i>Eulophia ruwenzoriensis</i>	EU877159	-	-	-	-	-
<i>Eulophia schnelliae</i>	KF318891	-	-	KF358126	-	-
<i>Eulophia schweinfurthii</i>	KF318924	-	-	KF358133	-	-
<i>Eulophia speciosa</i>	KF318967	-	-	KF358123	-	-
<i>Eulophia stachyodes</i>	KF318953	-	-	-	-	-

<i>Eulophia streptopetala</i>	KF318934	-	-	EF079258	-	MF987522
<i>Eulophia tenella</i>	KF318900	-	-	KF358119	-	-
<i>Eulophia tuberculata</i>	KF318945	-	-	KF358076	-	-
<i>Eulophia zeyheriana</i>	-	-	-	KF358091	-	-
<i>Fernandezia ecuadorensis</i>	FJ565635	-	-	FJ565127	FJ562413	-
<i>Fernandezia ionanthera</i>	FJ565236	-	-	AF239486	AF239582	-
<i>Fernandezia tica</i>	FJ565453	-	-	FJ564944	DQ315917	-
<i>Galeandra batemanii</i>	-	EU877122	-	-	-	-
<i>Galeandra baueri</i>	EU877138	EU877121	-	-	-	-
<i>Galeandra beyrichii</i>	EU877151	EU877133	-	-	-	-
<i>Galeandra blanchetii</i>	EU877140	EU877123	-	-	-	-
<i>Galeandra cristata</i>	-	EU877124	-	-	-	-
<i>Galeandra devoniana</i>	EU877142	KT768378	-	EU877181	-	KF660330
<i>Galeandra greenwoodiana</i>	EU877144	EU877127	-	-	-	-
<i>Galeandra junceaoides</i>	EU877145	EU877128	-	-	-	-
<i>Galeandra leptoceras</i>	-	KU257699	KU257722	-	-	KX495755
<i>Galeandra macroplectra</i>	-	-	-	-	-	KX495756
<i>Galeandra magnicolumna</i>	EU877146	EU877129	-	-	-	-
<i>Galeandra minax</i>	EU877147	EU877130	-	-	-	KU350903
<i>Galeandra paraguayensis</i>	EU877153	EU877134	-	-	-	-
<i>Galeandra santarena</i>	EU877143	EU877126	-	-	-	-
<i>Galeandra stangeana</i>	EU877148	EU877132	-	-	-	-
<i>Galeandra stylomisantha</i>	EU877154	-	-	-	-	-
<i>Geodorum densiflorum</i>	LN831388	-	-	JN004440	-	-
<i>Geodorum recurvum</i>	KF560539	-	-	KF673833	KF650564	-
<i>Gomesa dasytyle</i>	AF350551	-	-	AF350630	AF350709	-
<i>Gomesa radicans</i>	FJ565624	-	-	FR832799	FJ562410	-
<i>Grammatophyllum papuanum</i>	-	-	-	EF079262	-	-
<i>Grammatophyllum speciosum</i>	AF470488	-	-	AF470458	-	-

<i>Grandiphyllum auricula</i>	FJ565663	-	-	FJ565155	FJ562382	-
<i>Grandiphyllum divaricatum</i>	FJ565455	-	-	FJ564946	FJ562381	-
<i>Grobya amherstiae</i>	EU877158	EU877137	-	-	-	-
<i>Grobya cipoensis</i>	-	-	KU295244	-	-	-
<i>Grobya fascifera</i>	-	-	KU295245	-	-	-
<i>Grobya galeata</i>	AF470487	-	KU295246	AF470457	-	-
<i>Hofmeisterella eumicroscopica</i>	AF350510	-	-	FJ565091	AF350668	-
<i>Ionopsis minutiflora</i>	AF350548	-	-	AF350627	AF350706	-
<i>Lockhartia micrantha</i>	FJ565172	-	-	FJ564691	FJ562347	-
<i>Macradenia rubescens</i>	FJ565345	-	-	FJ564839	FJ562370	-
<i>Macroclinium aurorae</i>	FJ565626	-	-	FJ565118	-	-
<i>Macroclinium dalstroemii</i>	FJ565585	-	-	FJ565072	-	-
<i>Miltonia phymatochila</i>	FJ565222	-	-	FJ563856	FJ562409	-
<i>Miltoniopsis roezlii</i>	FJ565671	-	-	FJ565165	FJ562417	FJ563833
<i>Mormodes andreetae</i>	LK054149	-	-	-	LK054186	-
<i>Mormodes aromatica</i>	KM598430	-	-	AY368417	LK054209	-
<i>Mormodes badia</i>	KT768415	KT768380	KT768484	-	LK054181	KT768525
<i>Mormodes buccinator</i>	LK054148	LK054148	-	-	-	KU350904
<i>Mormodes castroi</i>	LK054150	-	-	-	LK054187	-
<i>Mormodes colossus</i>	LK054157	-	-	-	LK054194	-
<i>Mormodes dasilvae</i>	LK054151	LK054151	-	-	LK054188	KU350905
<i>Mormodes elegans</i>	LK054152	LK054152	-	-	LK054189	-
<i>Mormodes escobarii</i>	LK054159	-	-	-	LK054196	-
<i>Mormodes fractiflexa</i>	LK054160	-	-	-	LK054197	-
<i>Mormodes hookeri</i>	LK054161	-	-	-	LK054198	-
<i>Mormodes horichii</i>	LK054162	-	-	-	LK054199	-
<i>Mormodes ignea</i>	LK054163	-	-	-	LK054200	-
<i>Mormodes lawrenceana</i>	LK054146	-	-	-	-	-
<i>Mormodes lineata</i>	LK054164	-	-	-	-	-

<i>Mormodes lobulata</i>	LK054165	-	-	-	LK054201	-
<i>Mormodes luxata</i>	KT768417	KT768382	KT768486	-	-	KT768527
<i>Mormodes maculata</i>	LK054170	-	-	-	LK054206	-
<i>Mormodes nagelii</i>	LK054172	-	-	-	LK054208	-
<i>Mormodes oestlundiana</i>	LK054144	-	-	-	LK054182	-
<i>Mormodes paraensis</i>	LK054153	LK054153	-	-	LK054190	-
<i>Mormodes pardalinata</i>	LK054175	-	-	-	LK054210	-
<i>Mormodes punctata</i>	KT768418	KT768383	KT750231	-	-	-
<i>Mormodes rolfeana</i>	LK054156	-	-	-	-	-
<i>Mormodes sanguineoclaustra</i>	LK054176	-	-	-	LK054211	-
<i>Mormodes skinneri</i>	LK054167	-	-	-	LK054203	-
<i>Mormodes sotoana</i>	LK054168	-	-	-	LK054204	-
<i>Mormodes tapoayensis</i>	LK054154	-	-	-	LK054191	KU350906
<i>Mormodes tezontle</i>	LK054145	-	-	-	LK054183	-
<i>Mormodes theiochlora</i>	LK054147	-	-	-	LK054185	-
<i>Mormodes tuxtensis</i>	LK054171	-	-	-	LK054207	-
<i>Mormodes uncia</i>	LK054177	-	-	-	LK054212	-
<i>Mormodes variabilis</i>	LK054169	-	-	-	-	-
<i>Mormodes warszewiczii</i>	LK054155	-	-	-	LK054192	-
<i>Mormodes williamsii</i>	LK054178	-	-	-	LK054213	-
<i>Nohawilliamsia orthostates</i>	FJ565399	-	-	FJ563950	FJ562375	-
<i>Notylia barkeri</i>	AF350545	-	-	AF350624	FJ564794	-
<i>Notylia ecuadorensis</i>	FJ565477	-	-	FJ564961	FJ562385	-
<i>Notyliopsis beatricis</i>	FJ565597	-	-	FJ565086	FJ562401	-
<i>Oeceoclades bernetii</i>	KF318937	-	-	KF358068	-	-
<i>Oeceoclades maculata</i>	KF318917	-	-	-	-	-
<i>Oeceoclades pulchra</i>	KF318911	-	-	KF358079	-	KT768523
<i>Oliveriana brevilabia</i>	FJ565321	-	-	EF079202	FJ562367	-
<i>Oncidium chrysomorphum</i>	AF350796	-	-	FJ564996	AF433039	-

<i>Oncidium epidendroides</i>	AF350777	-	-	FJ564802	FJ562387	-
<i>Oncidium harryanum</i>	EF079421	-	-	EF079216	AF350727	-
<i>Oncidium lehmannianum</i>	FJ565395	-	-	FJ564891	FJ562373	-
<i>Oncidium multistellare</i>	AF350729	-	-	EF079213	AF350729	-
<i>Oncidium noezlianum</i>	AF350728	-	-	EF079211	AF350728	-
<i>Oncidium peruvianoides</i>	FJ565512	-	-	FJ564995	FJ562392	-
<i>Oncidium sphacelatum</i>	AF350564	-	-	AF350643	AF350722	-
<i>Oncidium strictum</i>	AF350568	-	-	FJ564985	AF350726	-
<i>Ornithocephalus bicornis</i>	FJ565564	-	-	FJ565049	FJ562397	-
<i>Ornithocephalus suarezii</i>	FJ565560	-	-	FJ565048	FJ562396	-
<i>Orthochilus aculeatus</i>	KF318949	-	-	-	-	-
<i>Orthochilus chloranthus</i>	KF318939	-	-	-	-	-
<i>Orthochilus ensatus</i>	KF318956	-	-	-	-	-
<i>Orthochilus foliosus</i>	KF318920	-	-	KF358101	-	-
<i>Orthochilus leontoglossus</i>	KF318941	-	-	KF358132	-	-
<i>Orthochilus mechowii</i>	KF318914	-	-	-	-	-
<i>Orthochilus odontoglossus</i>	KF318951	-	-	-	-	-
<i>Orthochilus tabularis</i>	KF318907	-	-	-	-	-
<i>Otoglossum chiriquense</i>	AF350561	-	-	AF433017	AF350719	-
<i>Otoglossum coronarium</i>	EF079410	-	-	FJ565100	FJ562406	-
<i>Otoglossum harlingii</i>	AF350879	-	-	-	-	-
<i>Phymatidium falcifolium</i>	FJ565362	-	-	-	-	-
<i>Plectrophora cultrifolia</i>	FJ565495	-	-	-	-	-
<i>Plectrophora triquetra</i>	FJ565494	-	-	-	-	-
<i>Polyotidium huebneri</i>	FJ565458	-	-	FJ565458	-	-
<i>Polystachya cultriformis</i>	GU556643	-	-	-	-	KF660306
<i>Psychopsiella limminghei</i>	FJ565660	-	-	FJ565152	-	FJ563600
<i>Psychopsis papilio</i>	AF350582	-	-	-	AF350740	-
<i>Psychopsis sanderae</i>	FJ565198	-	-	-	-	-

<i>Pterostemma antioquiense</i>	FJ565396	-	-	FJ563948	FJ562374	-
<i>Rhynchostele londesboroughiana</i>	AF350530	-	-	EF065561	AF350688	-
<i>Rodriguezia arevaloi</i>	FJ565331	-	-	FJ564824	-	-
<i>Rodriguezia delcastilloi</i>	AF350543	-	-	AF350622	AF350701	-
<i>Rodriguezia satipoana</i>	AF350544	-	-	AF350544	AF350702	-
<i>Solenidium lunatum</i>	FJ565359	-	-	FJ563940	-	-
<i>Solenidium portillae</i>	FJ565472	-	-	FJ564956	FJ562384	-
<i>Systemoglossum acuminatum</i>	AF350528	-	-	AF350607	AF350686	-
<i>Systemoglossum bennettii</i>	FJ565214	-	-	FJ564728	-	-
<i>Telipogon bowmanii</i>	-	-	-	DQ315912	DQ315938	-
<i>Telipogon hystrix</i>	DQ315841	-	-	DQ315899	DQ315925	-
<i>Telipogon nervosus</i>	DQ315870	-	-	HQ219263	DQ315933	-
<i>Telipogon obovatus</i>	FJ565603	-	-	FJ565093	-	-
<i>Telipogon pogonostalix</i>	AF350511	-	-	AF239488	AF350669	-
<i>Telipogon pulcher</i>	DQ315875	-	-	DQ315910	DQ315936	-
<i>Telipogon venustus</i>	FJ565183	-	-	FJ564703	-	-
<i>Thysanoglossa jordanensis</i>	EF079389	-	-	EF079224	-	-
<i>Tolumnia variegata</i>	AF350533	-	-	AF350612	AF350691	-
<i>Trichoceros muralis</i>	FJ565468	-	-	FJ563966	DQ315939	FJ562901
<i>Trichopilia brevis</i>	AF350581	-	-	EF079229	AF350739	-
<i>Trichopilia fragrans</i>	FJ565568	-	-	FJ565053	-	-
<i>Trichopilia sanguinolenta</i>	AF350580	-	-	-	-	-
<i>Vitekorchis lucasiana</i>	FJ565573	-	-	-	-	-
<i>Warmingia eugenii</i>	FJ565196	-	-	FJ563841	-	-
<i>Zygostates alleniana</i>	EF079390	-	-	EF079225	DQ315941	-
<i>Zygostates grandiflora</i>	AF350508	-	-	AF350587	AF350666	-

Table S2. Primer and PCR settings used for amplifying plastid and nuclear DNA loci.

Loci	Primer	Sequence	Reference	Pre-melt	Amplification	Final extension	Number of amplification cycles
ITS	ITS 4	TCC-TCC-GCT-TAT-TGA-TAT-GC	Baldwin, 1992 ¹²	95°C (3 min)	95°C (30 secs) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
	ITS 5	GGA-AGT-AAA-AGT-CGT-AAC-AAG-G		95°C (3 min)	95°C (30 secs) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
ETS	EST-Orchid	CAT-ATG-AGT-TGT-TGC-GGA-CC (AT)-T	Monteiro et al. 2010 ¹³	95°C (3 min)	95°C (30 secs) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
	18-IGS	AGA-CAA-GCA-TAT-GAC-TAC-TGG-CAG-G	Baldwin and Markos, 1998 ¹⁴	95°C (3 min)	95°C (30 secs) + 52°C (1 min) + 68°C (1 min)	68°C (10 min)	39
<i>Xdh</i>	X502F	TGT-GAT-GTC-GAT-GTA-TGC	Górniak et al. 2010 ¹⁵	95°C (3 min)	95°C (30 secs) + 53°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39
	X1599R	G(AT)G-AGA-GAA-A(CT)TG-GAG-CAA-C		95°C (3 min)	95°C (30 secs) + 53°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39
<i>ycf1</i>	3720F	TAC-GTA-TGT-AAT-GAA-CGA-ATG-G	Neubig et al. 2009 ¹⁶	95°C (3 min)	95°C (30 secs) + 54°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39
	5500R	GCT-GTT-ATT-GGC-ATC-AAA-CCA-ATA-GCG		95°C (3 min)	95°C (30 secs) + 54°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39
<i>trnS-G</i>	trn-S(GCU)	GCC-GCT-TTA-GTC-CAC-TCA-GC	Hamilton, 1999 ¹⁷	95°C (3 min)	95°C (30 secs) + 51.5°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39
	trn-G(UCC)	GAA-CGA-ATC-ACA-CTT-TTA-CCA-C		95°C (3 min)	95°C (30 secs) + 51.5°C (1 min) + 68°C (1.5 min)	68°C (10 min)	39

Table S3. Alignment characterisation.

Partition	Model	Likelihood
ITS	GTR+G	-22881.15
ETS	TIM3+G	-4003.25
<i>Xdh</i>	TPM2uf+G	-3555.32
<i>matK</i>	TVM+G	-14706.06
<i>trnL-F</i>	TPM1uf+G	-10074.65
<i>ycf1</i>	TPM1uf+G	-7596.36

Table S4. Likelihood of biogeographical models (DEC, DIVA, BAYAREA) as implemented in BIOGEOBEARS.

Model H₁	Model H₀	logL¹ H₁	logL H₀	D-statistic	p-value	AIC H₁	AIC H₀
DEC+J	DEC	-875.5	-893.9	36.78	1.3e-09	1757	1792
DIVALIKE+J	DIVALIKE	-912.3	-926.6	28.62	8.8e-08	1831	1857
<i>BAYAREALIKE+J</i>	BAYAREALIKE	<i>-860.3</i>	-931	141.5	1.3e-32	<i>1727</i>	1866

¹Log Likelihood

Table S5. Model selection and parameter values of BiSSE analysis on lineages with ESD and alternative sexual systems.

Model	logL¹	AICc²	λ^3 (0)	λ (1)	μ^4 (0)	μ (1)	q_{0-1}⁵	q_{1-0}⁶
<i>M</i> ₁	-262.414	531.022	-	2.2724	-	2.1596	-	0.01347
<i>M</i> ₂	-248.854	506.035	1.2539	2.0244	-	1.1816	-	0.00586
<i>M</i> ₃	-250.101	508.528	-	1.5893	1.5308	0.6345	-	0.00366
<i>M</i> ₄	-260.683	529.691	-	2.2688	-	2.1554	0.02002	0.000001925
<i>M</i> ₅	-248.654	507.800	1.2327	2.0959	1.1590	1.2670	-	1.26707
<i>M</i> ₆	-248.273	507.039	1.2496	2.0113	-	1.1742	0.00689	0.000002158
<i>M</i> ₇	-249.814	510.120	-	1.5992	1.5393	0.6592	0.00403	0.000006212
<i>M</i> ₈	-247.921	508.538	1.1827	2.2634	1.1007	1.4974	0.00904	0.000005874

¹log Likelihood

²Akaike Informative Criterion corrected

³Speciation rates

⁴Extinction rates

⁵transition rates from state 0 to 1

⁶transition rates from state 1 to 0

Table S6. Model selection and parameter values of BiSSE analysis on lineages pollinated by male euglossine bees and alternative pollination syndromes.

Model	$\log L^1$	AICc ²	λ^3 (0)	λ (1)	μ^4 (0)	μ (1)	q_{0-1}^5	q_{1-0}^6
M_1	-245.514	497.222	-	2.2924	-	2.1767	-	0.0030665
M_2	-240.045	488.416	1.8276	2.1389	-	1.8172	-	0.0020853
M_3	-240.741	489.807	-	2.0960	2.0865	1.7851	-	0.0019566
M_4	-244.693	497.712	-	2.2919	-	2.1762	0.006970	0.000002017
M_5	-238.972	488.437	1.2969	2.4619	1.2625	2.1751	-	2.1751137
M_6	-239.715	489.922	1.8364	2.1388	-	1.8221	0.003017	0.000003762
M_7	-240.449	491.390	-	2.1084	2.0950	1.8048	0.002745	0.0000034742
M_8	-238.273	489.241	1.2101	2.5350	1.1618	2.2638	0.006002	0.0000026449

¹log Likelihood

²Akaike Informative Criterion corrected

³Speciation rates

⁴Extinction rates

⁵transition rates from state 0 to 1

⁶transition rates from state 1 to 0

Table S7. Model selection and parameter values of BiSSE analysis on epiphytic lineages and alternative plant habits.

Model	logL¹	AICc²	λ^3 (0)	λ (1)	μ^4 (0)	μ (1)	q_{0-1}⁵	q_{1-0}⁶
<i>M</i> ₁	-258.626	523.446	-	1.7808	-	1.6653	-	0.01611
<i>M</i> ₂	-256.090	520.505	1.2590	1.5762	-	1.2320	-	0.01462
<i>M</i> ₃	-256.221	520.768	-	1.5223	1.5019	1.1720	-	0.01326
<i>M</i> ₄	-257.123	522.572	-	1.7813	-	1.6656	0.04960	0.000579
<i>M</i> ₅	-255.714	521.919	1.1414	1.6908	1.1089	1.4030	-	1.40300
<i>M</i>₆	-253.720	517.931	1.2762	1.5697	-	1.2374	0.02558	0.00001974
<i>M</i> ₇	-254.303	519.098	-	1.5598	1.5259	1.2443	0.02234	0.00002881
<i>M</i> ₈	-253.018	518.731	0.8744	1.7400	0.8010	1.4450	0.043234	0.00016626

¹log Likelihood

²Akaike Informative Criterion corrected

³Speciation rates

⁴Extinction rates

⁵transition rates from state 0 to 1

⁶transition rates from state 1 to 0

Supplementary figures

Fig. S1. Conflicting nuclear-plastid associations (i.e. pair of nuclear / plastid taxa) obtained by PACo analysis, using Bayesian posterior probability trees. Taxa with normalised squared residual scores above the threshold value (red line) indicate potential conflicting associations.

Fig. S2. Phylogenetic relationships of Catasetinae and sister subtribes (Eulophiinae, Dipodiinae, Oncidiinae, Cyrtopodiinae, Cymbidiinae) independently derived from nuclear and plastid datasets. potential outlier, congruent and excluded terminals (i.e. taxa with sequences included on the plastid loci but missing on the nuclear dataset prior analysis) are shown in red, green and blue, respectively.

Fig. S3. Best scoring ML tree of Catasetinae and sister subtribes (Eulophiinae, Dipodiinae, Oncidiinae, Cyrtopodiinae, Cymbidiinae) obtained from non-conflicting concatenated nuclear and plastid loci. Node charts indicate Bootstrap Support (BS > 75), where fully black diagrams indicate BS of 100.

Fig. S4. Chronogram of Catasetinae and sister subtribes obtained under a relaxed clock model, applied to a non-conflicting, concatenated nuclear and plastid loci. Minimum and maximum age intervals are provided. MRCA of Catasetinae as well as multiple origins of ESD are shown.

Fig. S5. Probabilities of areas estimated as inferred under the BayArea+J model using a relaxed clock model dated phylogeny. Grey colours at pie diagrams indicate probabilities of shared ancestral areas (i.e. two or more biogeographical regions) estimated at nodes. The LCA of Catasetinae is indicated with a black star and gains of ESD are indicated with red circles. Coded biogeographical areas are colour-coded following inset map, and are shown in front of taxa names (grey colours indicate taxa

distributed in more than one biogeographical region). Sub-tribe members sampled in our phylogeny are color-coded. Right panels show selected representatives of (a) Cymbidiinae (*Grammatophyllum measuresianum*); (b) Cyrtopodiinae (*Cyrtopodium polyphyllum*); (c) Eulophiinae (*Eulophia streptopetala*); (d, e, f) Catasetinae (*Catasetum fimbriatum*, *Galeandra macroplectron*, and *Mormodes punctata*, respectively); (h, i) Oncidiinae (*Trichoceros* sp., *Rossioglossum grande*). Photos (except b by L. Varella): O. Pérez. [Inset: Codes areas used for biogeographical analysis. Geopolitical boundaries map generated by ArcMAP (<http://www.sri.com>) using political divisions and elevation data from DIVA-GIS (<http://www.diva-gis.org/data>)].

Fig. S6. Maximum Likelihood optimisation (1000 iterations) under an Asymmetrical Rate Model (ARD) of Environmental Sex Determination (ESD) of Catasetinae on a BEAST maximum credibility clade chronogram. Posterior probabilities of each state are shown at nodes. The LCA of Catasetinae is indicated with a black star.

Fig. S7. Bioclimatic variables and their corresponding contributions to Axis 1 (47.9% variance) of PCA analysis. Note that there is no evident discrimination between temperature and seasonality.

Fig. S8. Bioclimatic variables and their corresponding contributions to Axis 2 (23.6% variance) of PCA analysis. Note that there is no evident discrimination between temperature and seasonality.

Fig. S9. Presence and absence of Environmental Sex Determination overlaid on: (a) A Principal Component Analysis (PCA), and (b) a non-dimensional metric scaling analyses (NMDS) plot. Note that in both analyses, climatic niche spaces of lineages

with and without ESD overlap (normal confidence ellipses drawn at a confidence level of 0.95 largely intersect).

Fig. S10. Mean ancestral values and 95% confidence intervals (CI) of bioclimatic variables estimated at all nodes of the Catasetinae phylogeny by ACE analyses. (A) Altitude; (B) Annual mean temperature (Bio 1); (C) Mean diurnal range (Bio 2); (D) Isothermality (Bio 3); (E) Temperature seasonality (Bio 4); (F) Maximum temperature of warmest month (Bio 5); (G) Maximum temperature of coldest month (Bio 6); (H) Temperature annual range (Bio 7); (I) Mean temperature of wettest quarter (Bio 8); (J) Mean temperature of driest quarter (Bio 9); (K) Mean temperature of warmest quarter (Bio 10); (L) Mean temperature of coldest quarter (Bio 11); (M) Annual precipitation (Bio 12); (N) Precipitation of wettest month (Bio 13); (O) Precipitation of driest month (Bio 14); (P) Precipitation seasonality (Bio 15); (Q) Precipitation of wettest quarter (Bio 16); (R) Precipitation of driest quarter (Bio 17); (S) Precipitation of warmest quarter (Bio 18); (T) Precipitation of coldest quarter (Bio 19). Mean ancestral values are indicated by red bars. Blue circles represent mean ancestral values at ancestors where ESD originated independently. Blue bars indicate CI of ancestral values at nodes with ESD as inferred by character state estimation analyses, and orange bars indicate CI of mean ancestral values at nodes without ESD. Note the little differences between ancestral mean values of bioclimatic variables in nodes inferred to have ESD, and those inferred to have alternative sexual systems (i.e. absence of ESD).

Fig. S11. Density probability plots of speciation rates (A); differences in speciation and extinction rates (B); net diversification rates (C), and differences in net diversification rates (D) in lineages with Environmental Sex Determination and alternative sexual systems as inferred by BiSSE.

Fig. S12. Density probability plots of speciation rates (A); differences in speciation and extinction rates (B); net diversification rates (C), and differences in net diversification rates (D) in euglossine-bee pollinated lineages and alternative pollination syndromes as inferred by BiSSE.

Fig. S13. Density probability plots of speciation rates (A); differences in speciation and extinction rates (B); net diversification rates (C), and differences in net diversification rates (D) in epiphytic lineages and those with other habits as inferred by BiSSE.

Fig. S14. Null distribution of AIC of BiSSE simulations in A) ESD, and B) euglossine-bee pollination syndrome.

Figure S1

Association

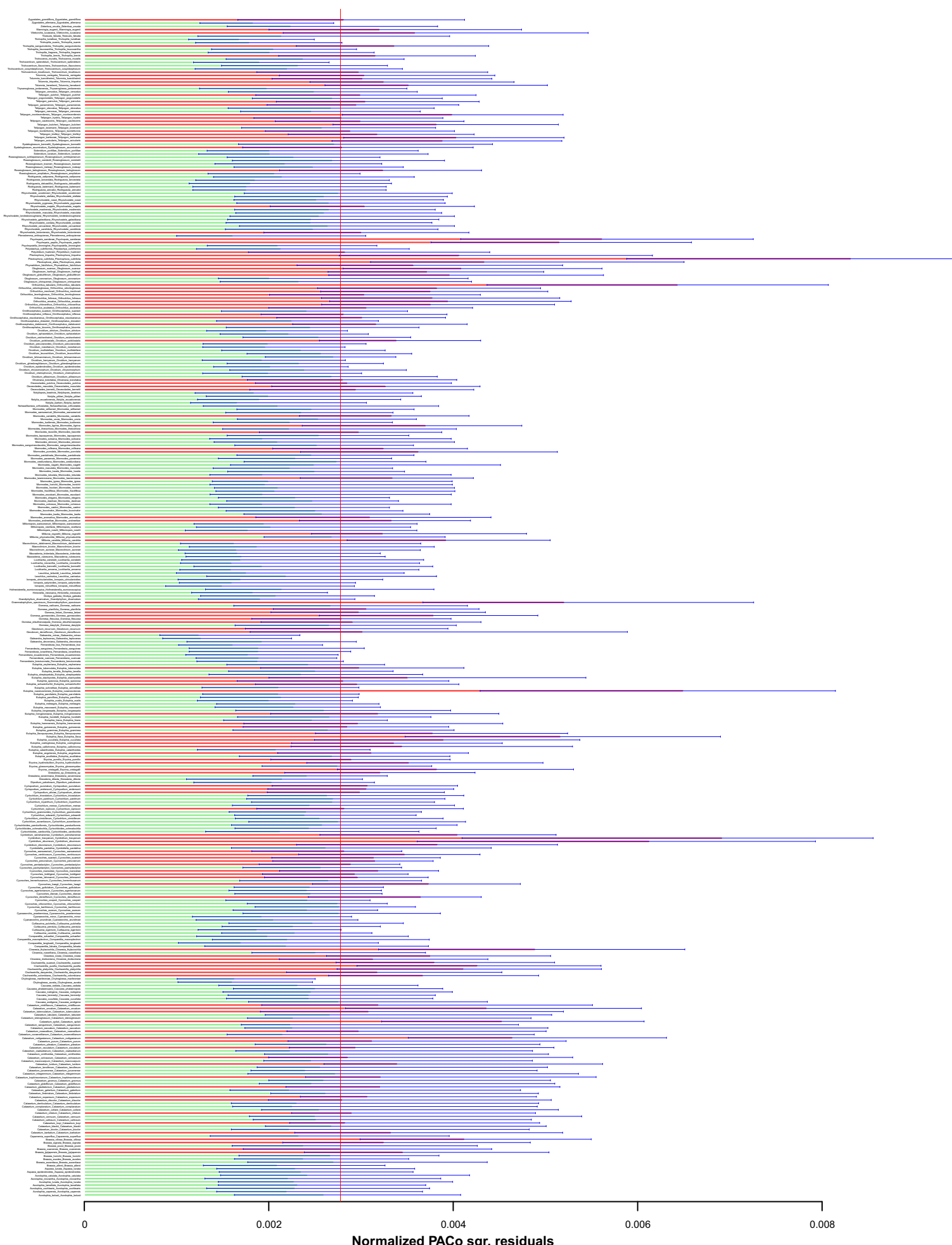
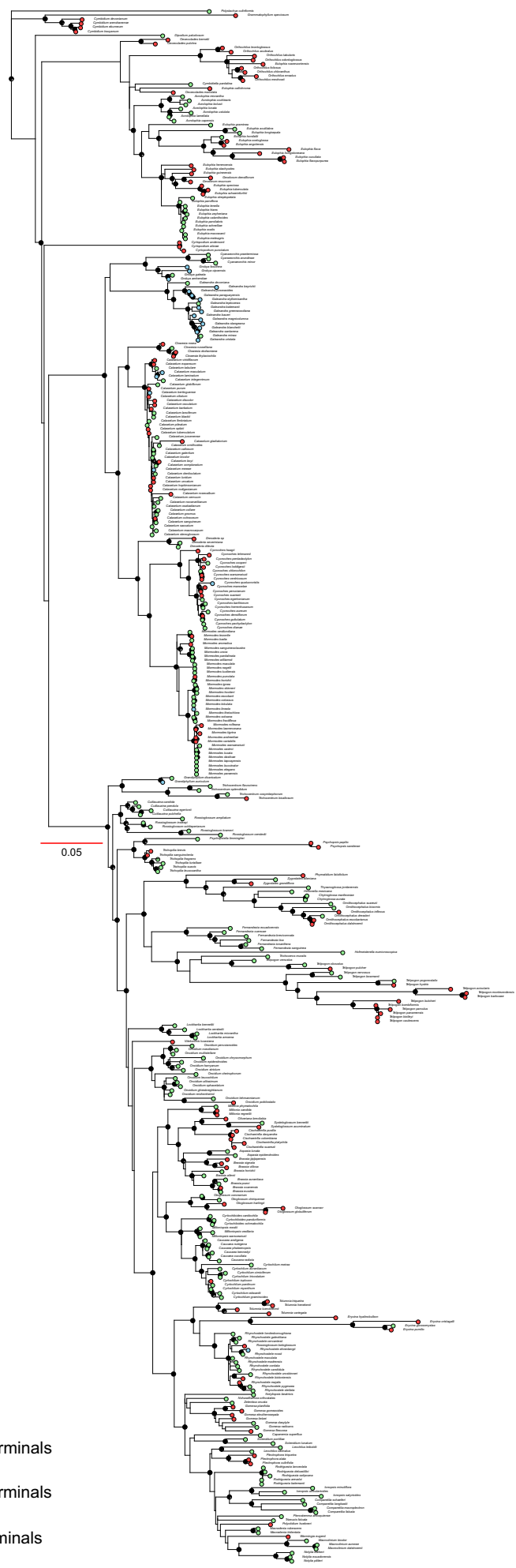
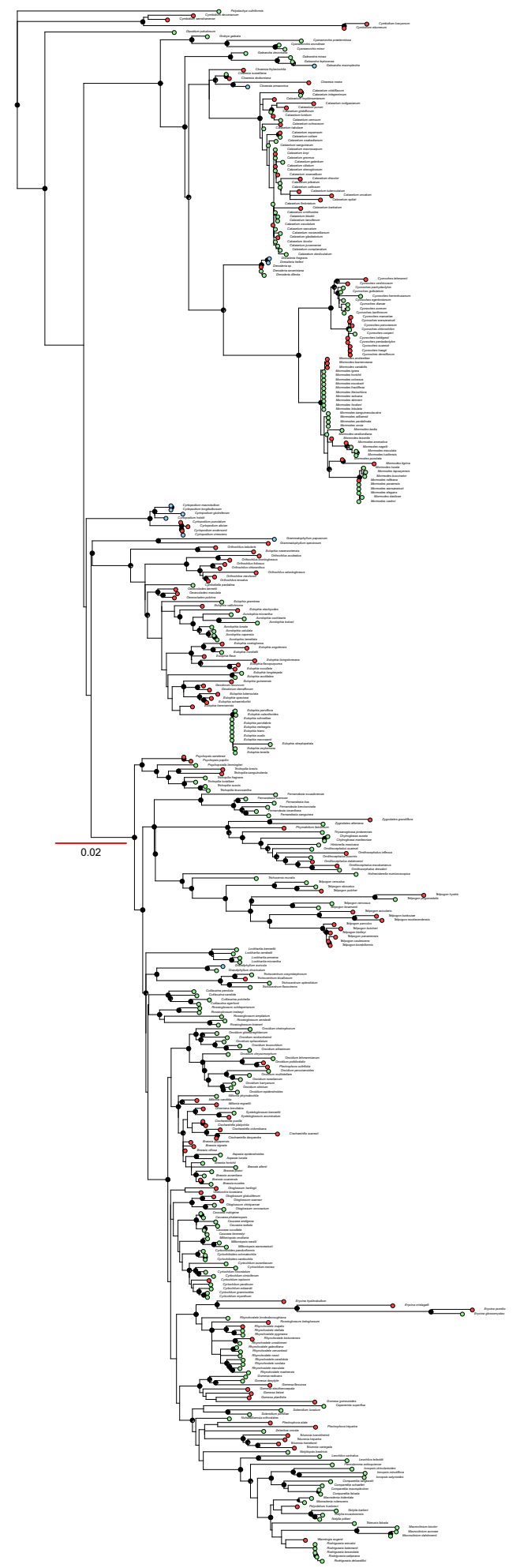


Figure S2

ITS - ETS - *Xdh* ML consensus ML tree



matK - *trnL-F* - *ycf1* ML consensus ML tree



- Congruent terminals
- Conflicting terminals
- Excluded terminals

Figure S3

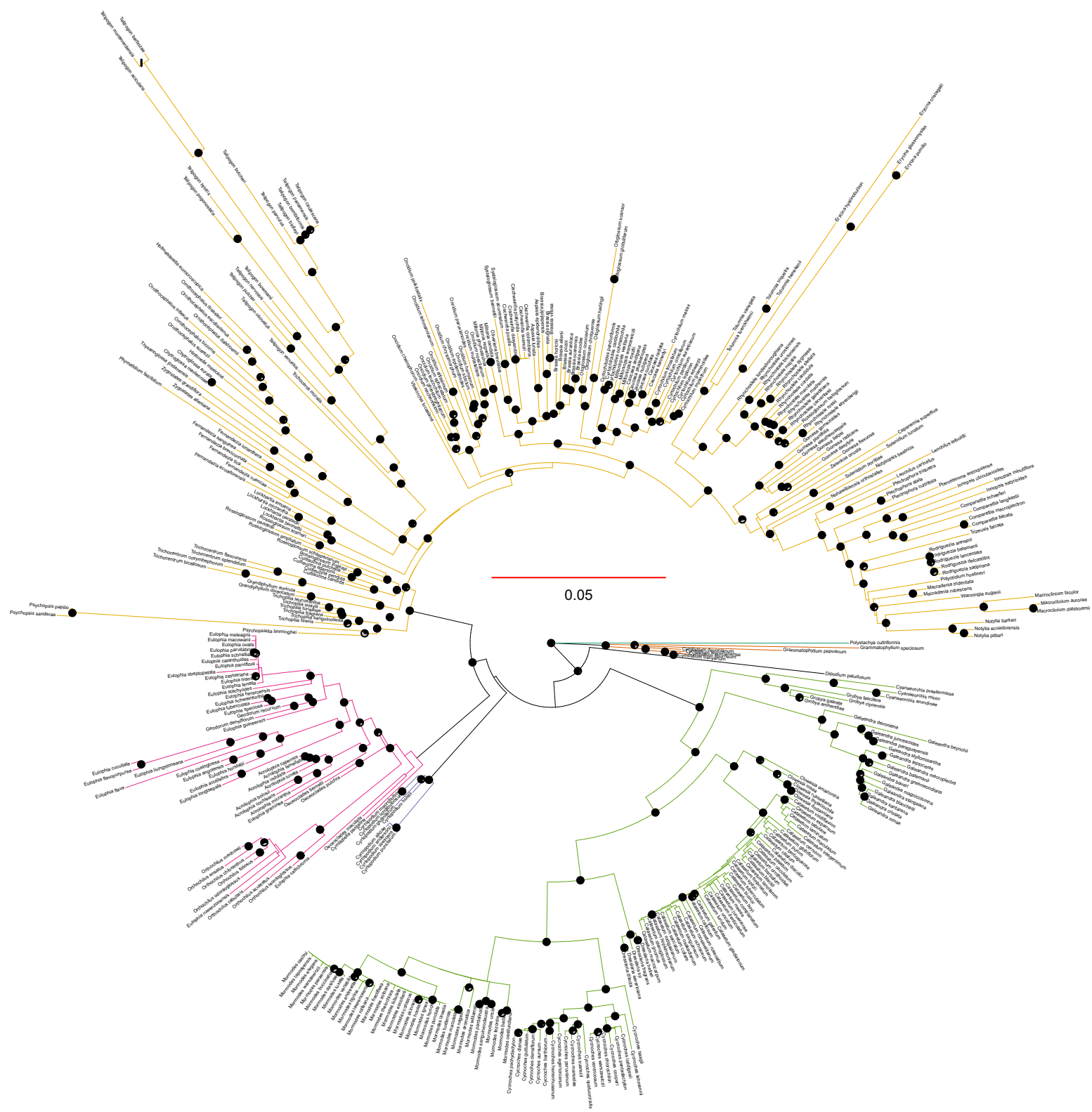
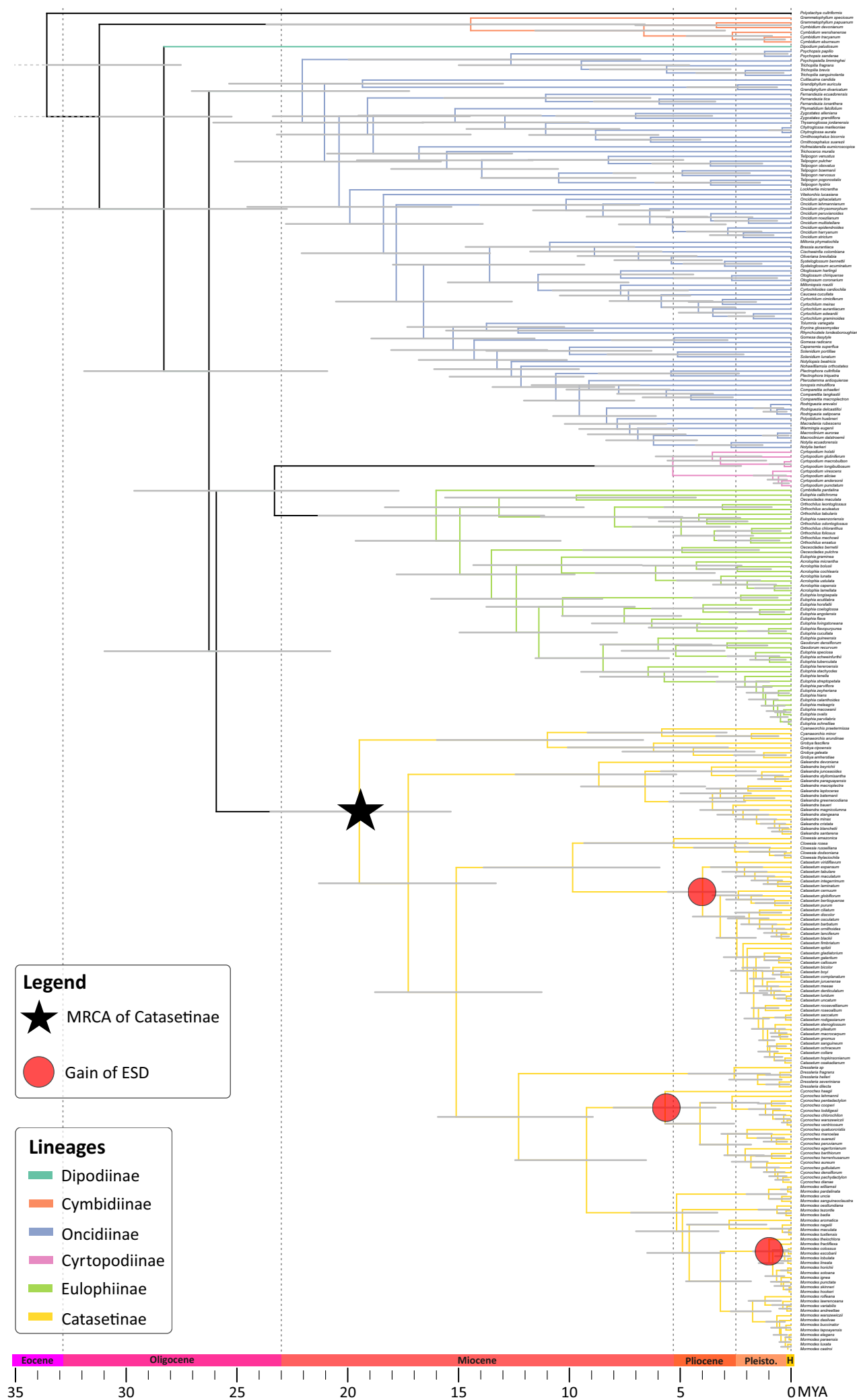


Figure S4



Legend

- ★ MRCA of Catasetinae
- Gain of ESD

Lineages

- Dipodiinae
- Cymbidiinae
- Oncidiinae
- Cyrtopodiinae
- Eulophiinae
- Catasetinae

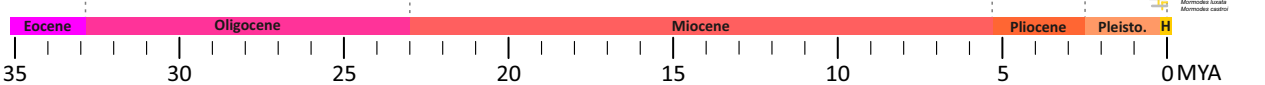


Figure S5

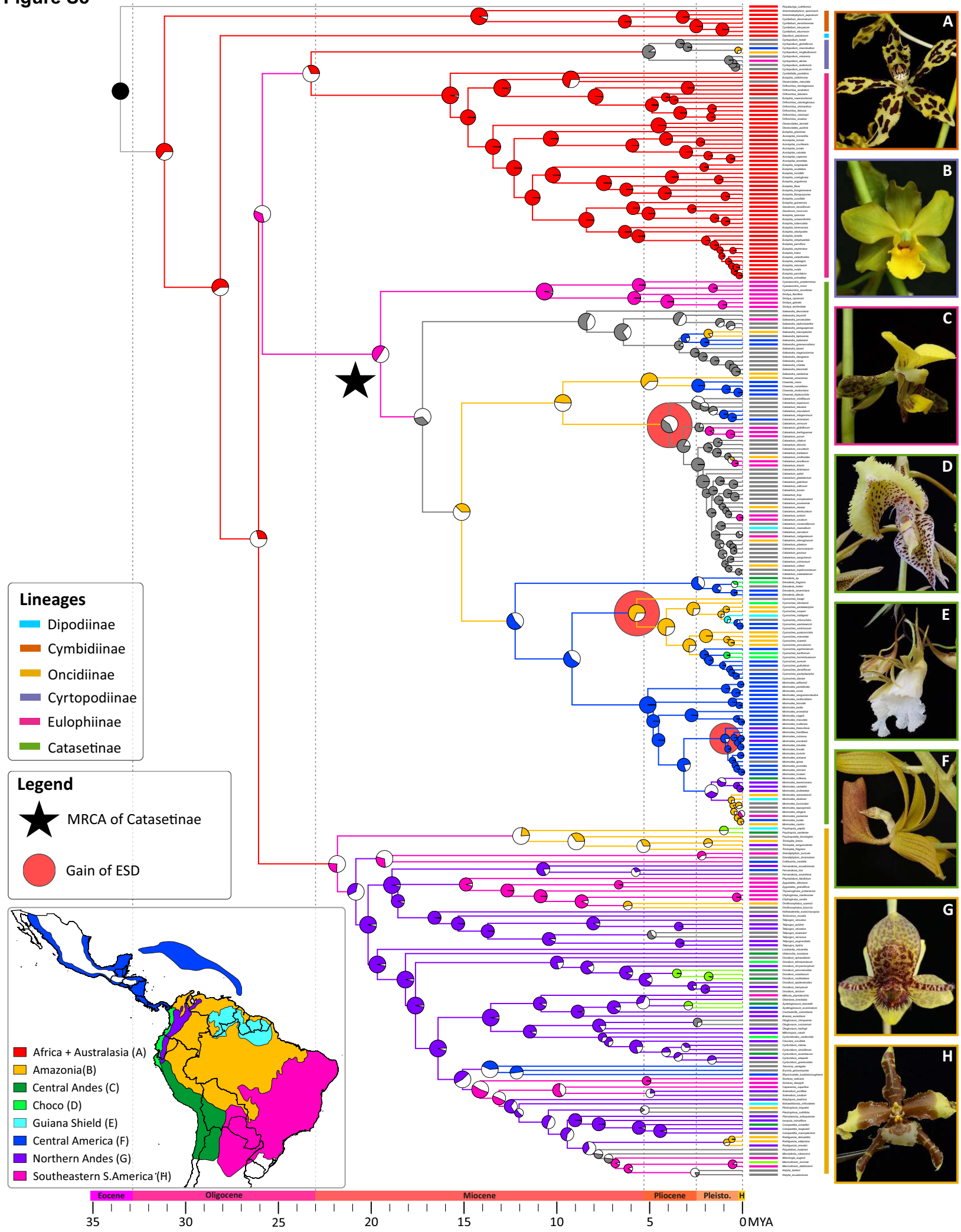


Figure S6

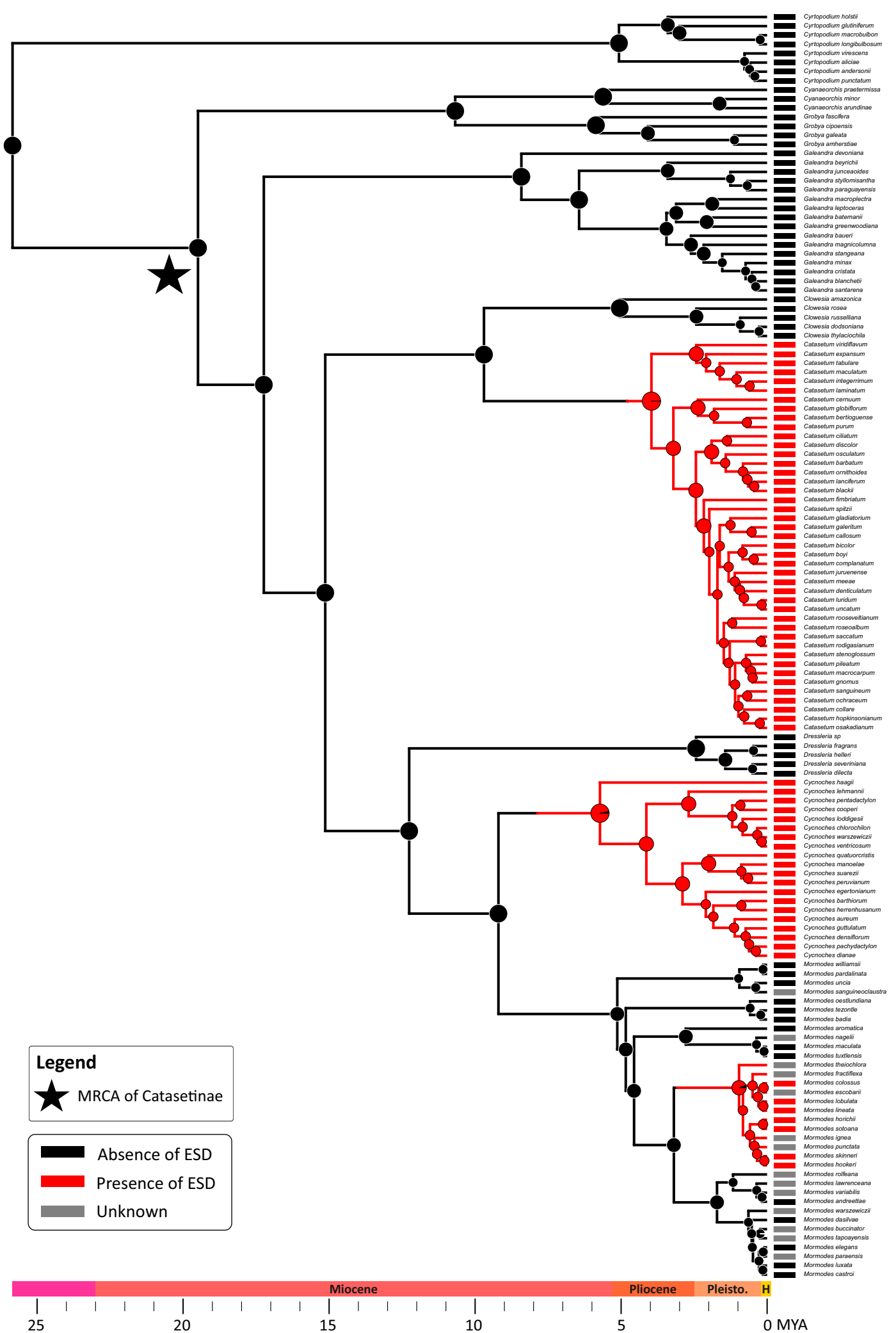


Figure S7

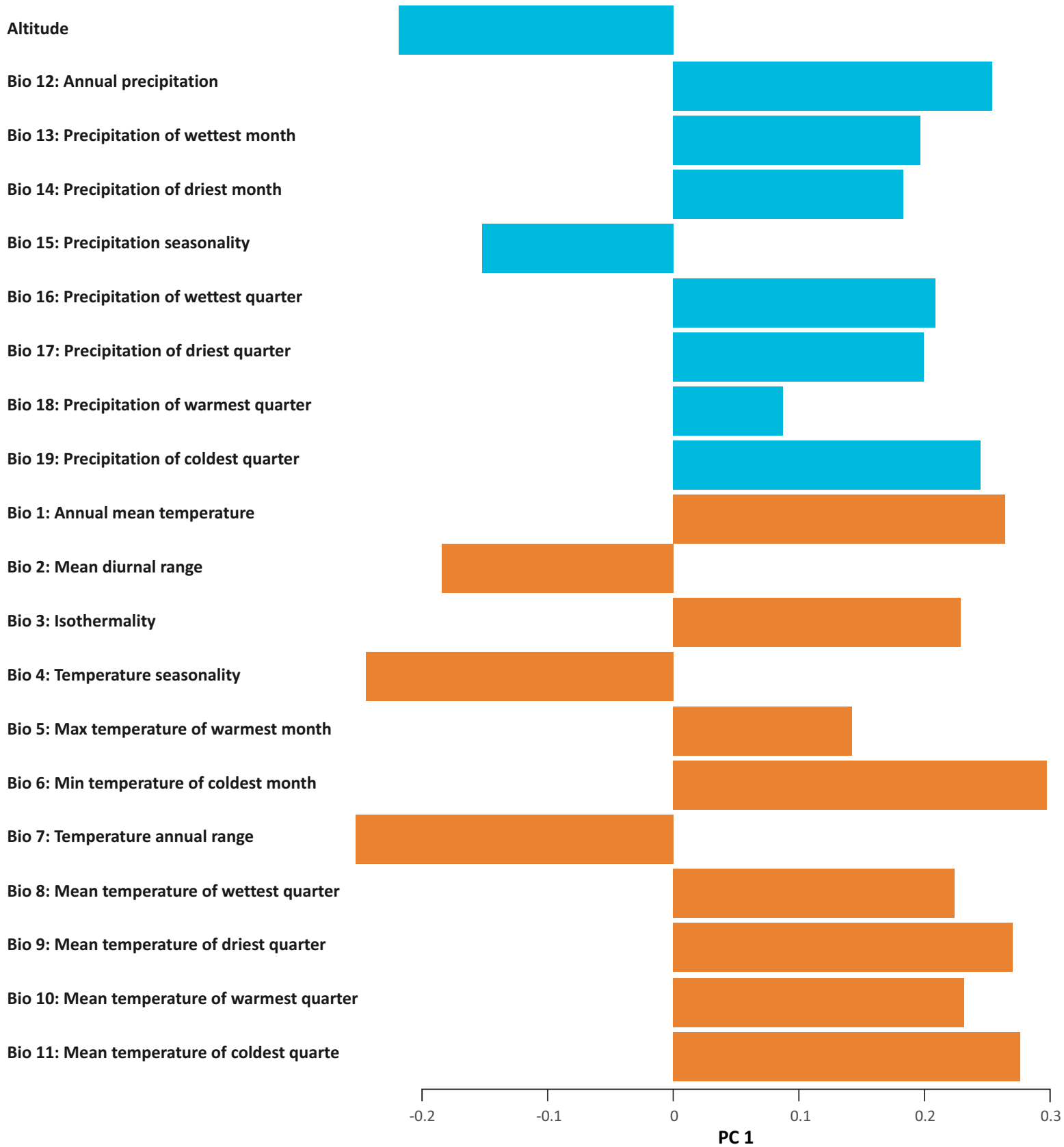
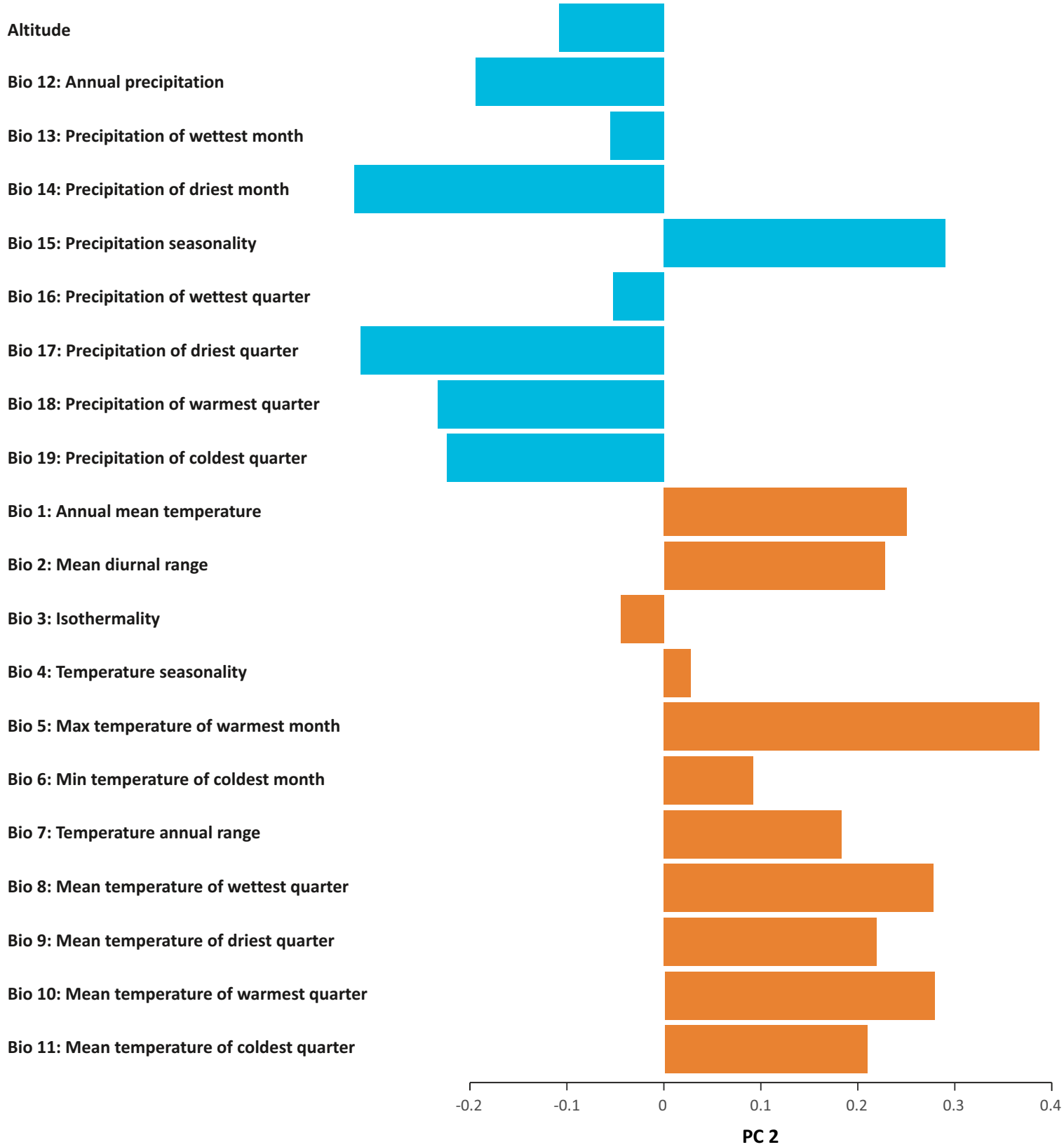
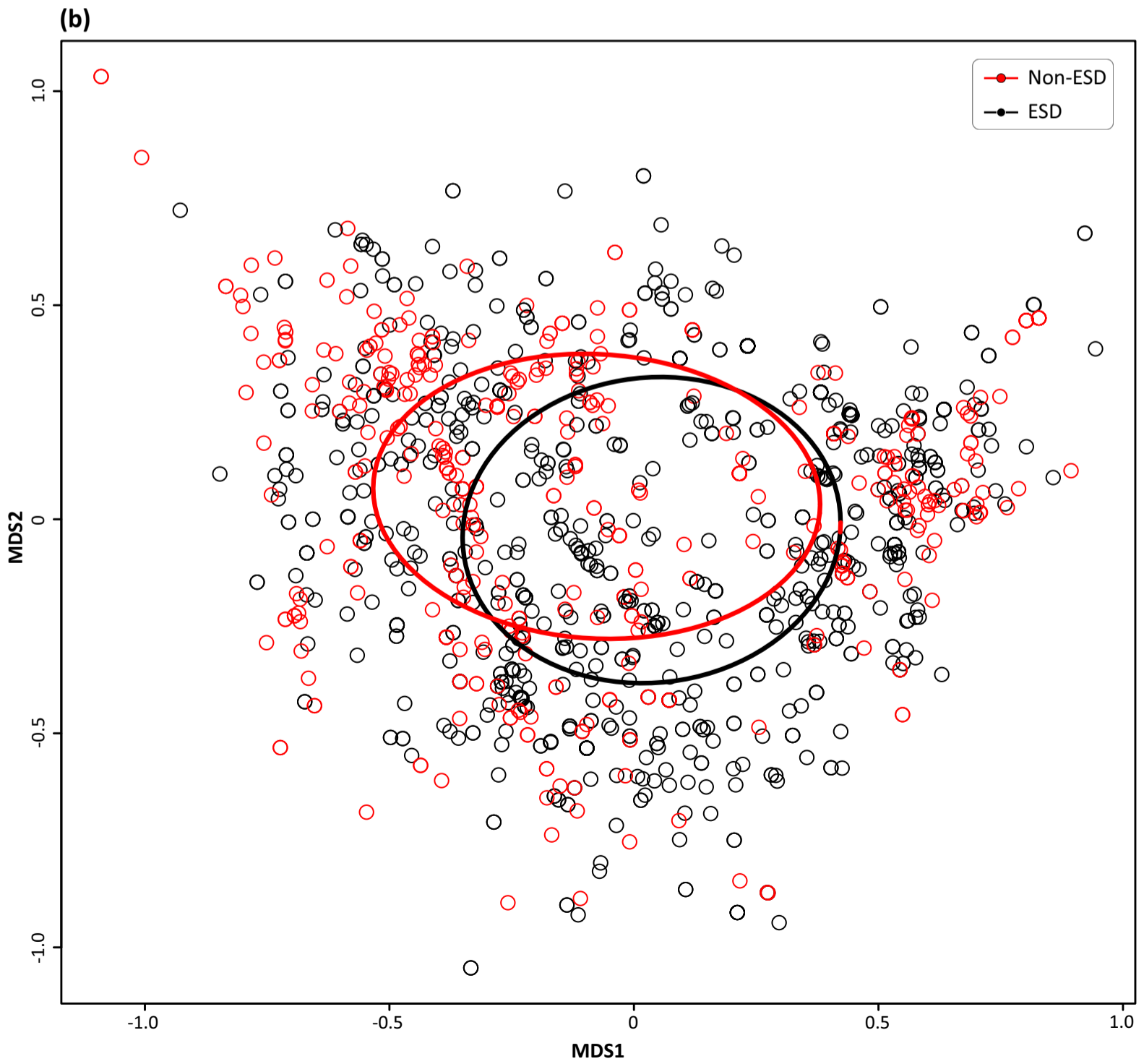
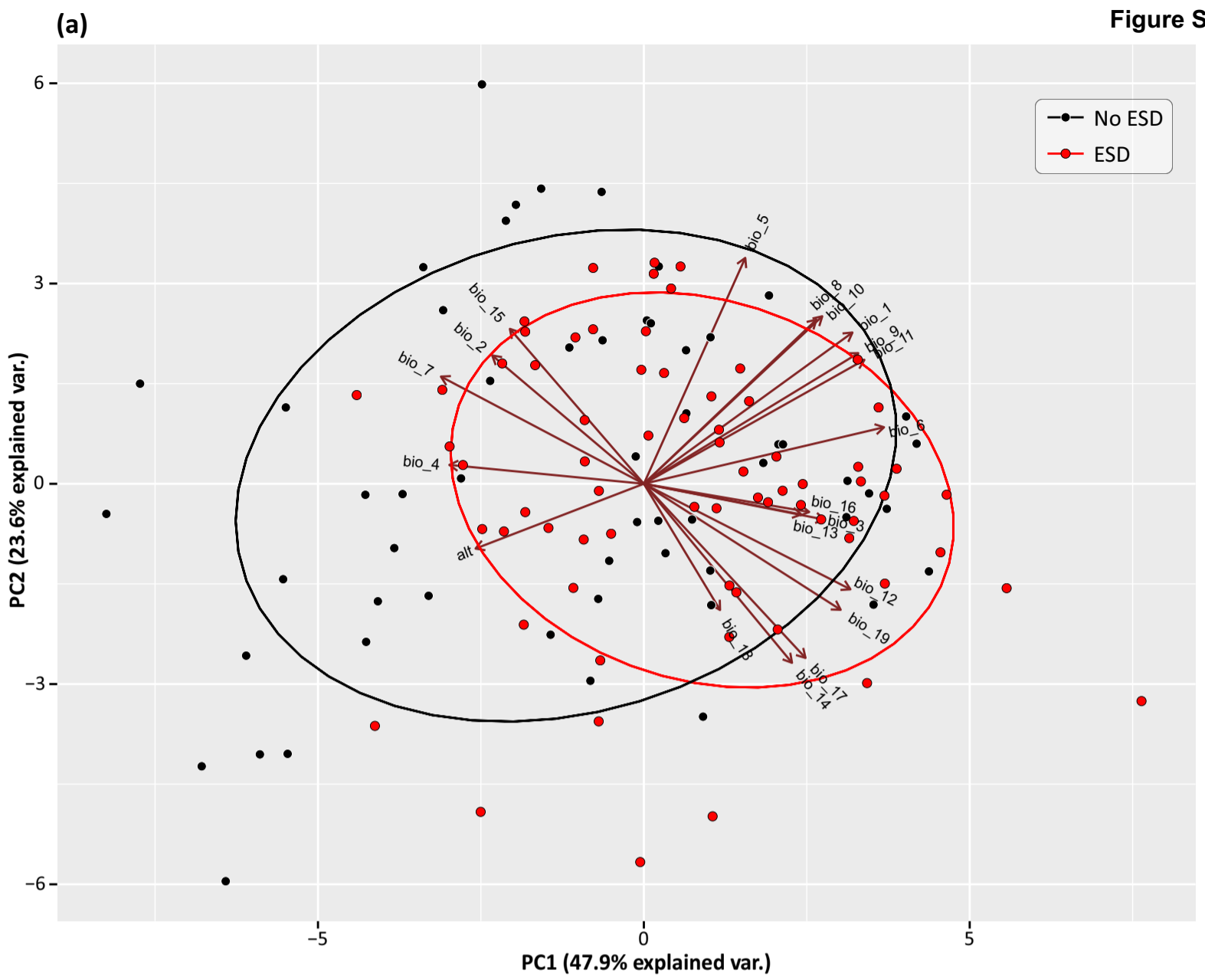
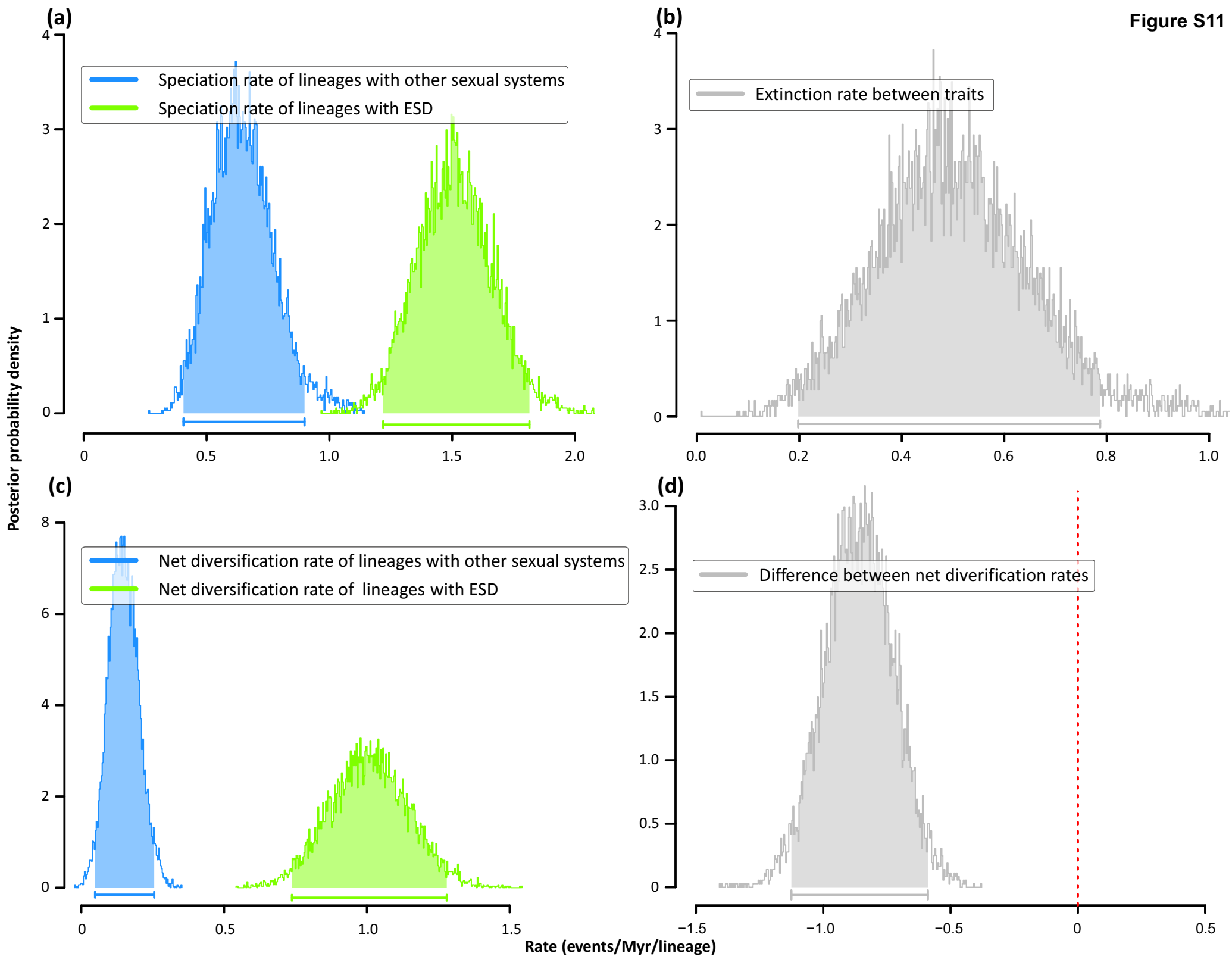
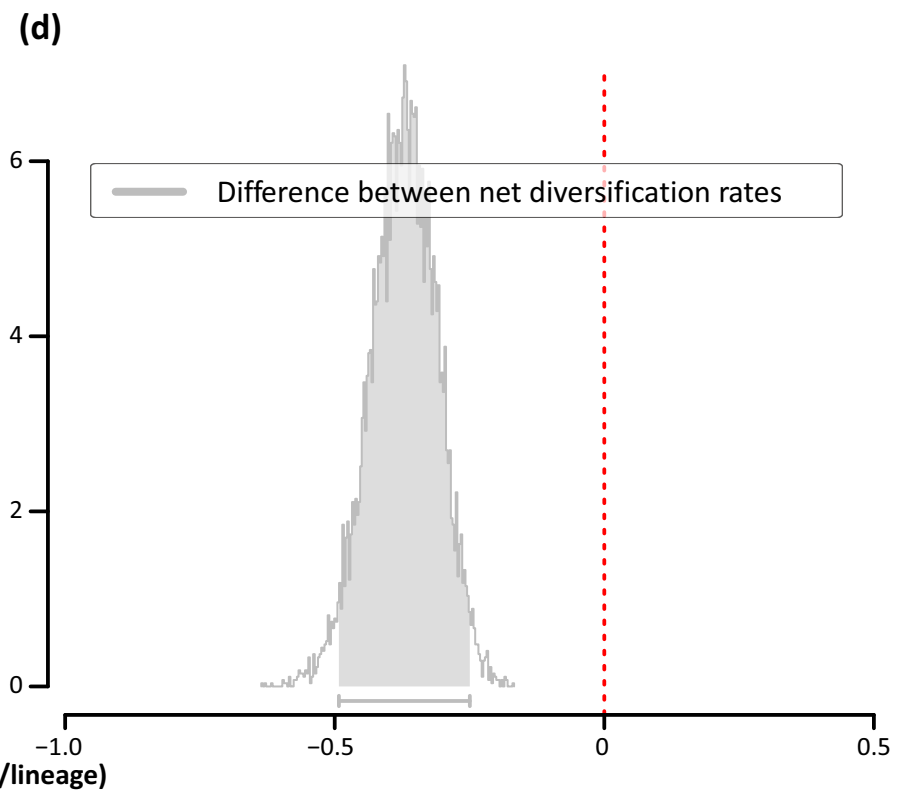
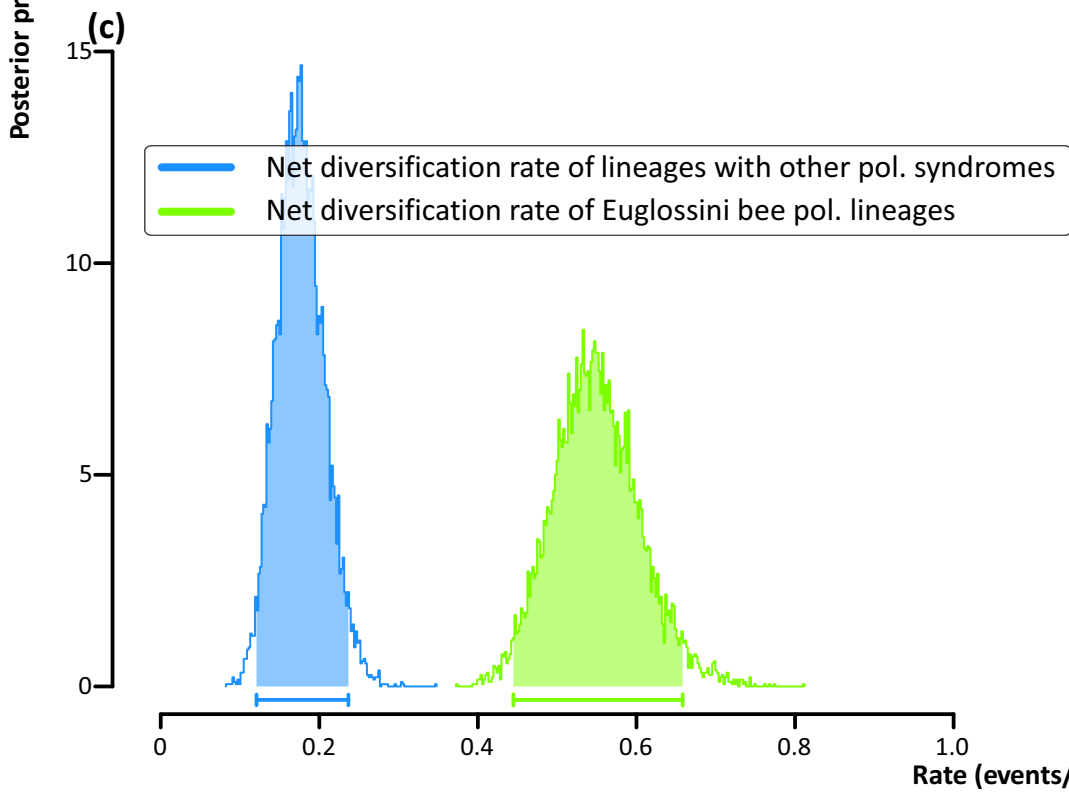
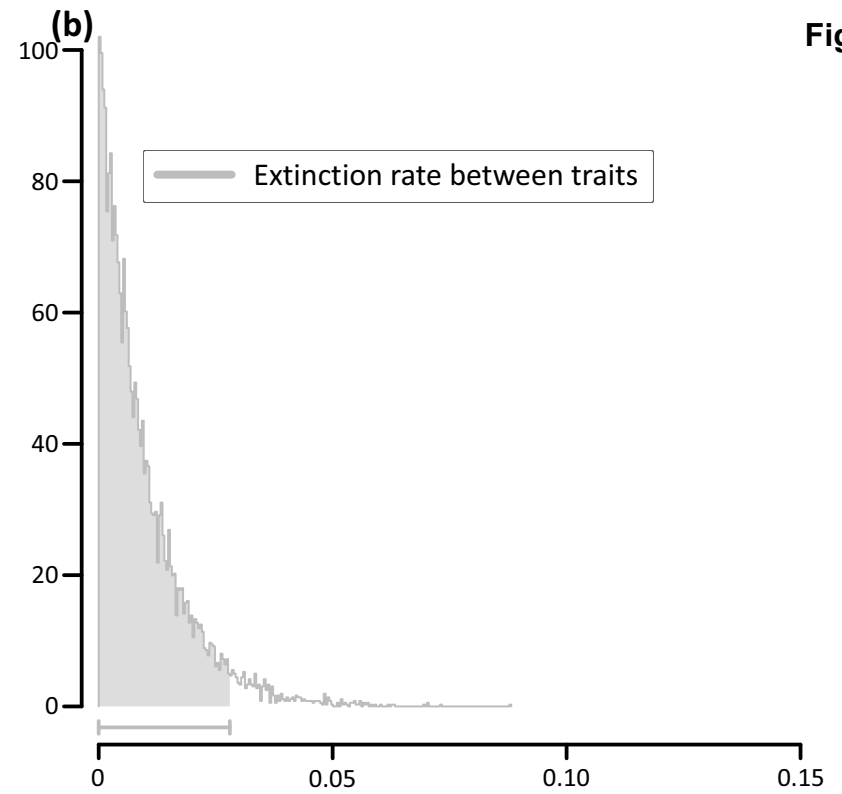
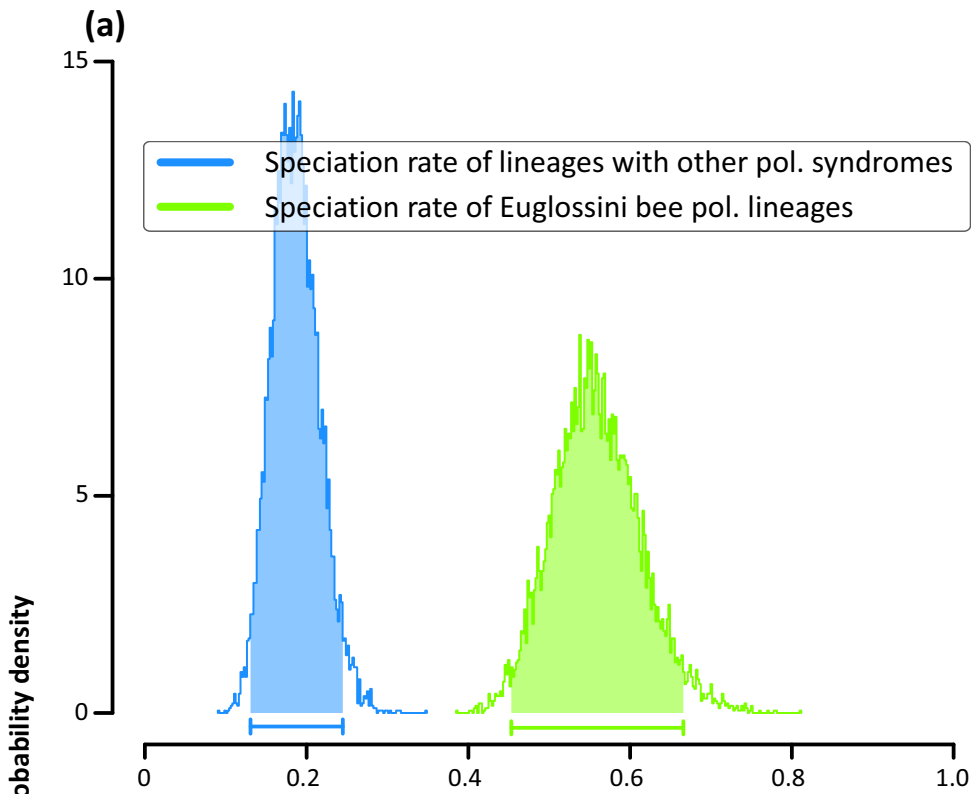


Figure S8









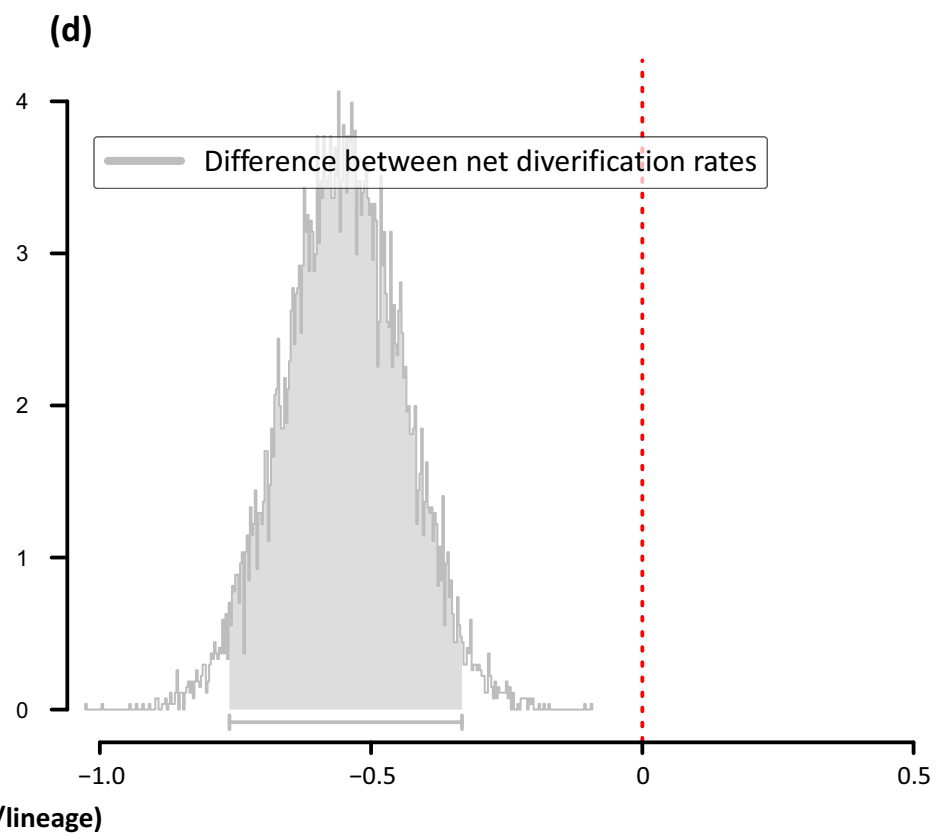
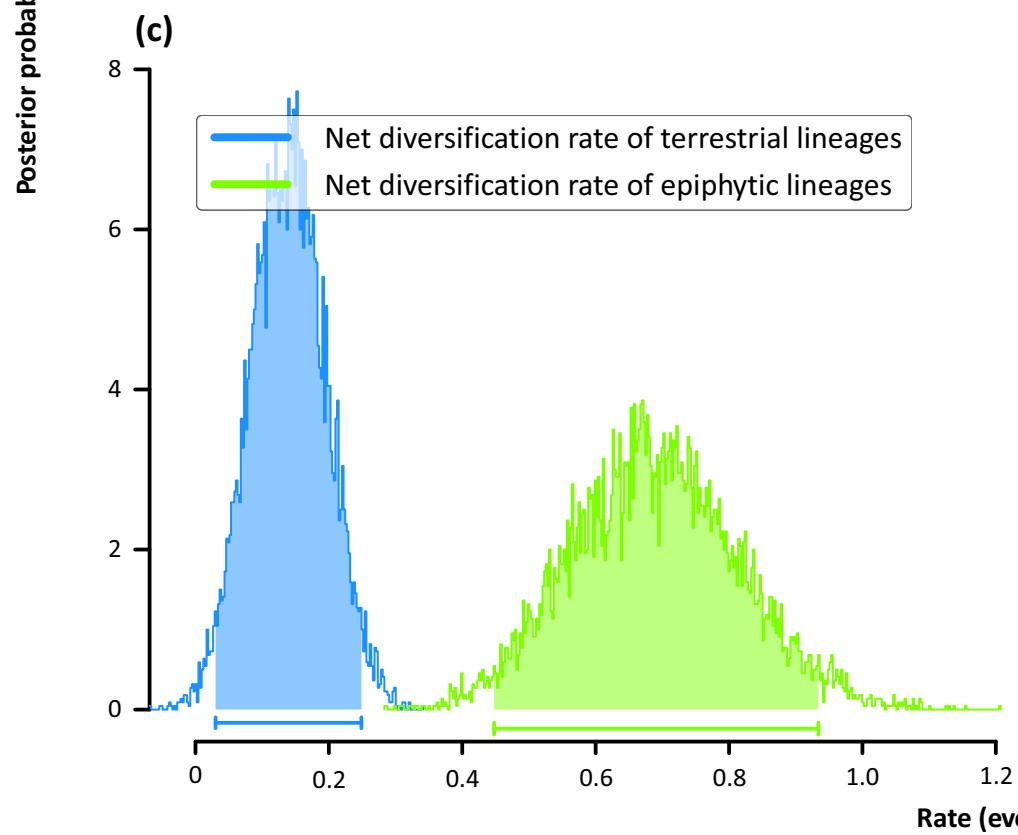
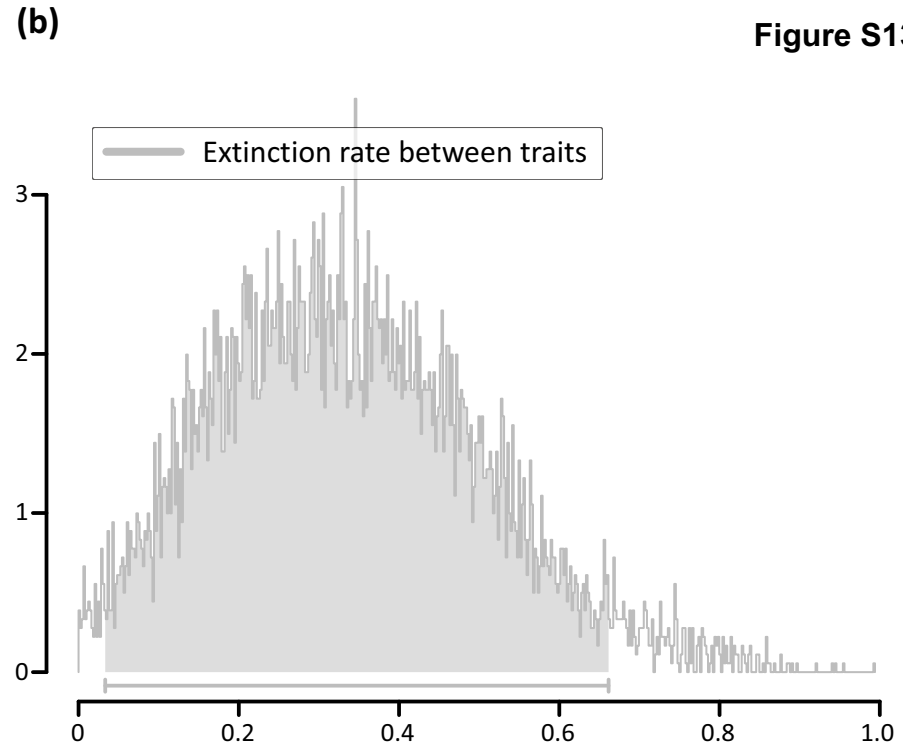
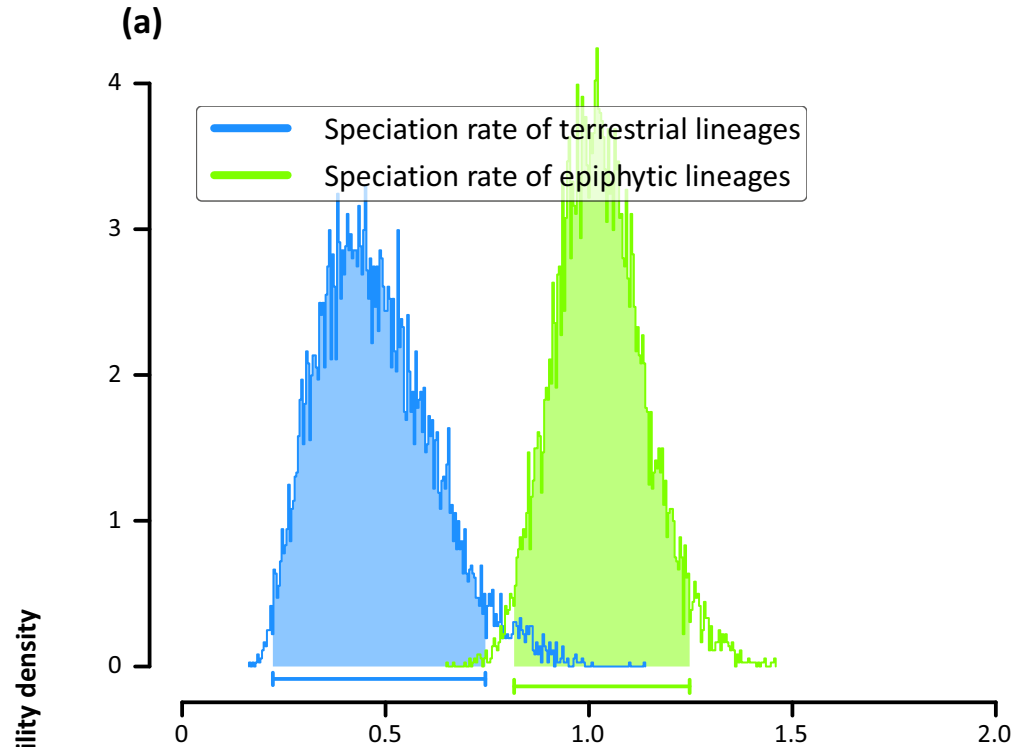


Figure S14

