Supplementary Results

Distribution of _D_ statistic.
First, we compared the distributions of _D_-statistic values between (Supplementary Fig. S4a available on Dryad) different taxa computed from ABBA-BABA (Patterson, et al. 2012) site patterns (Fig. 3a) by applying HyDe to various groups of four species (quartets). Although quartets with older divergence times tend to have only slightly decreased values of _D_ (Martin, et al. 2015) (Wilcoxon rank-sum test [WRST], $P = 3.116 \times 10^{-8}$), this effect can be more pronounced for extremely old divergences. Indeed, quartets that were formed within the most recently diverged clades in our phylogeny, including Calopterygoidea (Late Cretaceous, ~ 67 Ma), Coenagrionoidea (Early Cretaceous, ~ 116 Ma) and Libelluloidea (Late Cretaceous, ~ 87 Ma) have significantly greater _D_ values (WRST, $P = 2.276 \times 10^{-9}$) if compared with quartets that were formed from distantly related species of Zygoptera and Anisoptera (Late Triassic ~237 Ma). Quartets involving Zygoptera lineages (i.e. both intra- and inter-Zygoptera comparisons) exhibit significantly higher average _D_ statistic values (WRST, all $P < 0.05$, Fig. 3a, Supplementary Table S5 available on Dryad) when compared to the distribution of _D_ values computed across the entire order. Conversely, Epiprocta, Anisozygoptera, Cordulegastroidea (intra- and inter-superfamilial comparisons) and Libelluloidea (only inter-superfamilial comparisons) showed reduced _D_ values (WRST, all $P < 10^{-7}$, Fig. 3a, Supplementary Table S5 available on Dryad). We compared taxon-specific distributions of significant _D_ statistic values to those of the entire order to identify possible extreme skewness in site-pattern frequencies. Overall, we found several mostly minor but significant upward and downward departures of _D_ statistic averages from the entire order (Supplementary Fig. S4a available on Dryad). This result may imply that the ILS rate heterogeneity across different odonate linages can differentially affect the value of _D_ statistic.

Distribution of _γ_ statistic.
Overall, comparisons of _γ_ distributions (Fig. 3b) show several lineages with elevated average _γ_ values (WRST, all $P < 0.05$, Fig. 3b, Supplementary Table S5 available on Dryad) to the total distribution of _γ_. In contrast to the comparisons of _D_ distributions, Epiprocta and inter-superfamilial comparisons of its two groups, Anisozygoptera and Aeshnoidea (Aeshnidae), show significantly higher _γ_ (WRST, all $P < 0.05$, Fig. 3a, Supplementary Table S5 available on Dryad), whereas all inter-superfamilial comparisons within Zygoptera (except Calopterygoidea), and comparisons within Aeshnoidea (Aeshnidae) and Calopterygoidea show significantly lower _γ_ (WRST, all $P < 0.05$, Fig. 3a, Supplementary Table S5 available on Dryad).

Signatures of introgression identified by D_{FOIL}
Overall, the distributions of significant D_{FOIL} statistics (Fig. 4a) except for Anisoptera, introgression scenario 2, Aeshnoidea (Aeshnidae) and Libelluloidea were different from the D_{FOIL} distribution within the entire order across all taxonomic levels (WRST, all $P < 0.05$, Fig. 4b, Supplementary Table S6 available on Dryad). This observation is indicative of differences in the amount of ancestral introgression as well as its polarization, which is determined by significance of D_{FOIL} statistics. Further we note that average D_{FOIL} (Fig. 4a) was significantly different from 0 (one sample _t_-test [OSTT], all $P < 0.0223$, Fig. 4b, Supplementary Table S6 available on Dryad) for all tested cases except Aeshnoidea (Aeshnidae) and Libelluloidea. Together these significant deviations from 0 of D_{FOIL} statistics further support the hypothesis of

introgression for the tested taxonomic levels (Pease and Hahn 2015). Tests of individual quintets as implemented in D_{FOIL} identified significant cases of introgression for all tested introgression scenarios and within all clades except and Aeshnoidea (Gomphidae+Petaluridae) and Lestoidea (Fig. 4c).

The analysis of quintet fraction with significant introgression revealed that only introgression scenario 4 and Coenagrionoidea exhibit an excess of significant quintets (FET, all $P < 0.05$, Fig. 4c, Supplementary Table S6 available on Dryad), whereas introgression scenario 4 and Calopterygoidea show decrease of significant quintets (FET, all $P < 0.05$, Supplementary Fig. S6c and Table S6 available on Dryad) in comparison with the entire order. Despite the notion that D_{FOIL} approach exhibits low false positive rate, it requires tree symmetry (Fig. 2, see Materials and Methods) (Pease and Hahn 2015), thus not all the quintet combinations of taxa can be evaluated for introgression with this method.

Supplementary Figures
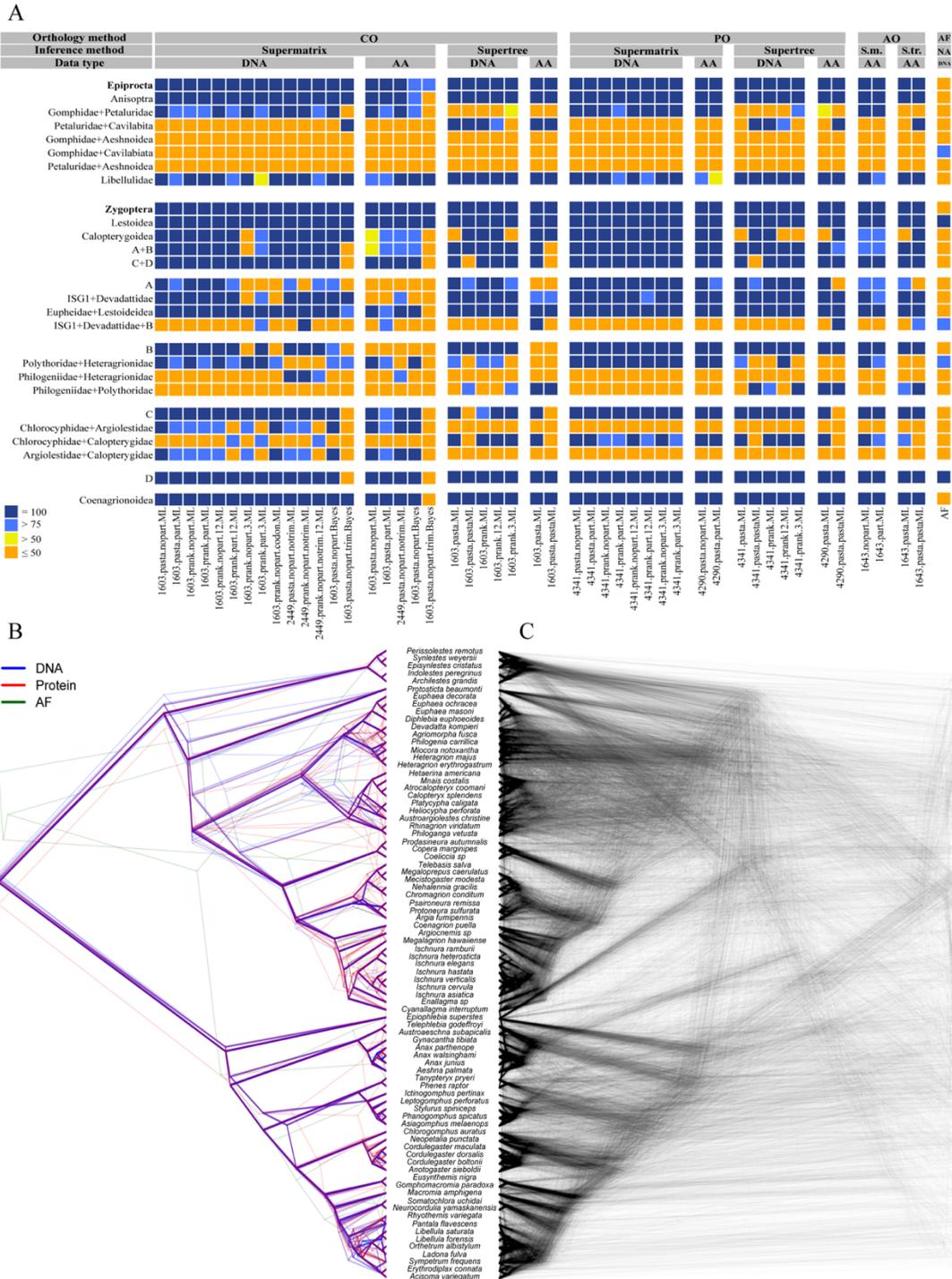
Figure S1. Comparison of Orthology Detection and Phylogenetic Tree Reconstruction Pipelines. (A) Comparison of support for major Odonata group relationship hypotheses. Supports present bootstraps scores for maximum likelihood (ML) and Alignment free (AF) inference, posterior probabilities for Bayesian inference (Bayes) and local branch support from quartet frequencies for Supertree approach using ASTRAL. The text below shows parameters of supermatrix and its analysis types (number_of_loci.alignment_method.partition.inference_method) within Supermatrix framework and parameters for gene alignments and its analysis types (number_of_loci.alignment_method.inference_method) within Supertree framework. The support value ≤ 50 indicates that either insignificant support or these relationships were not observed on a particular phylogeny. CO = single-copy orthologs; AO = all single-copy orthologs; PO = paralogy-parsed orthologs (B) Comparison of phylogenetic tree topologies estimated from different data types. (C) Comparison of BUSCO 1603 gene tree ML topologies.
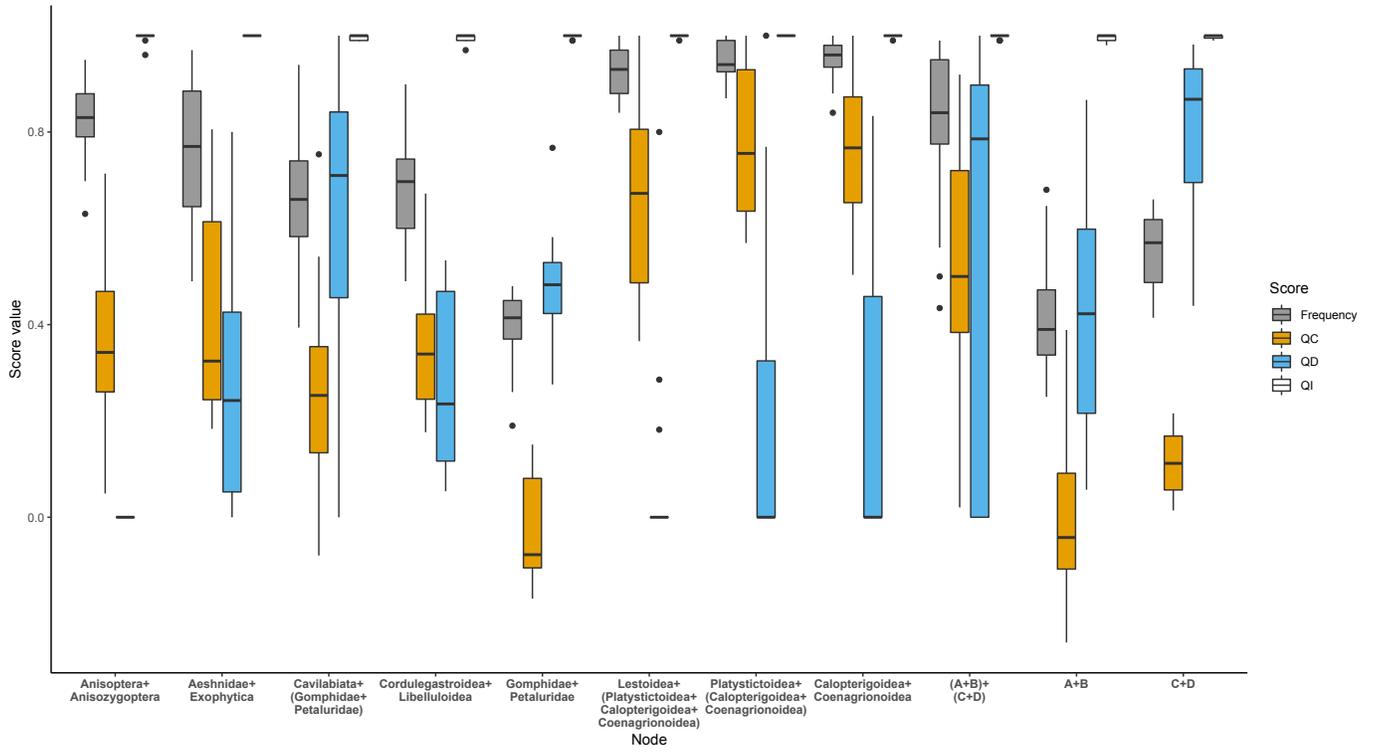
Figure S2. Quartet Sampling Analysis of Major Odonate Divergence Points. The boxplots represent distribution of Frequency, Quartet Concordance (QC), Quartet Discordance (QD) and Quartet Informativeness (QI) scores. Frequency indicates proportion of inferred quartets which coincide with the tree topology. QC indicates how often concordant quartets are inferred over the discordant ones. QD shows bias toward any particular discordant quartet. QI indicates whether the quartets are informative or not. All of the scores were derived for 15 supermatrices using tree topology as in Figure 1.
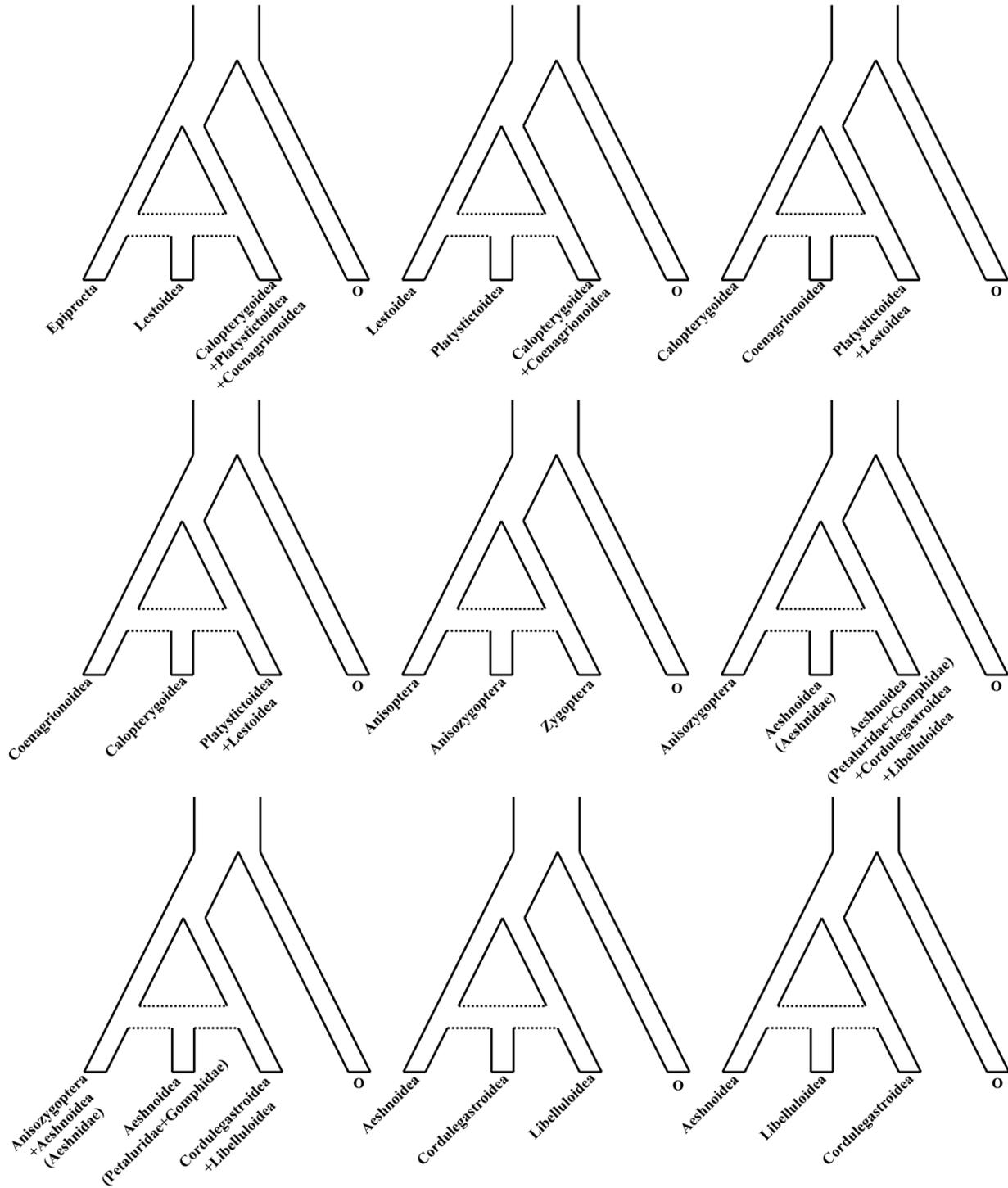
Figure S3. Hypotheses of Introgression/Hybridization scenarios between Odonata Superfamilies Tested in HyDe using D and γ Statistics.
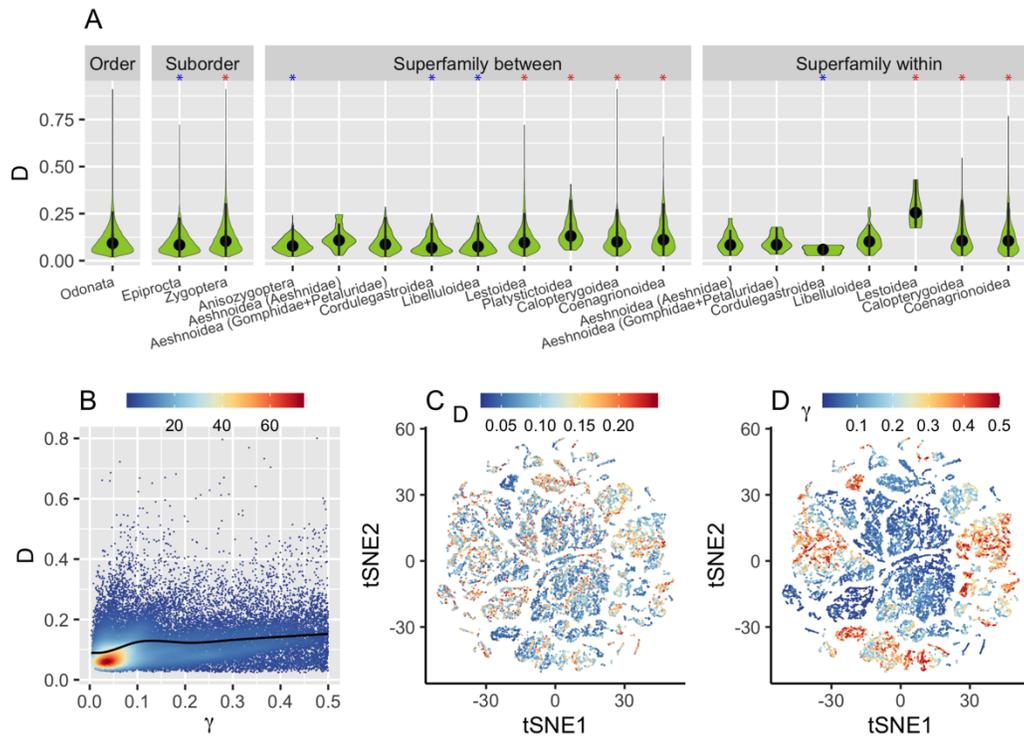
Figure S4. Distributions of the Patterson's *D* Statistic and Relations between HyDe γ and *D*.

(A) Estimated distributions of significant (Bonferroni corrected *P* < 0.05) *D* statistic from ABBA-BABA site pattern counts for each quartet using HyDe output. Black dots mark medians of violin plots. Asterisks indicate significantly greater (red) or lower (blue) *D* averages of various tested cases compared to *D* average of the entire order.

(B) Non-linear relationships between absolute values of significant *D* statistics and γ. The black line denotes a GAM fit. The color legend shows density of quartets across γ-*D* plane.

(C)-(D) tSNE projections of the 15 site pattern counts derived for each of the 32620 significant quartets from HyDe output (each dot on a tSNE map represents a quartet). Color schemes reflect significant values of *D* statistic (C) and significant values of of γ (D).
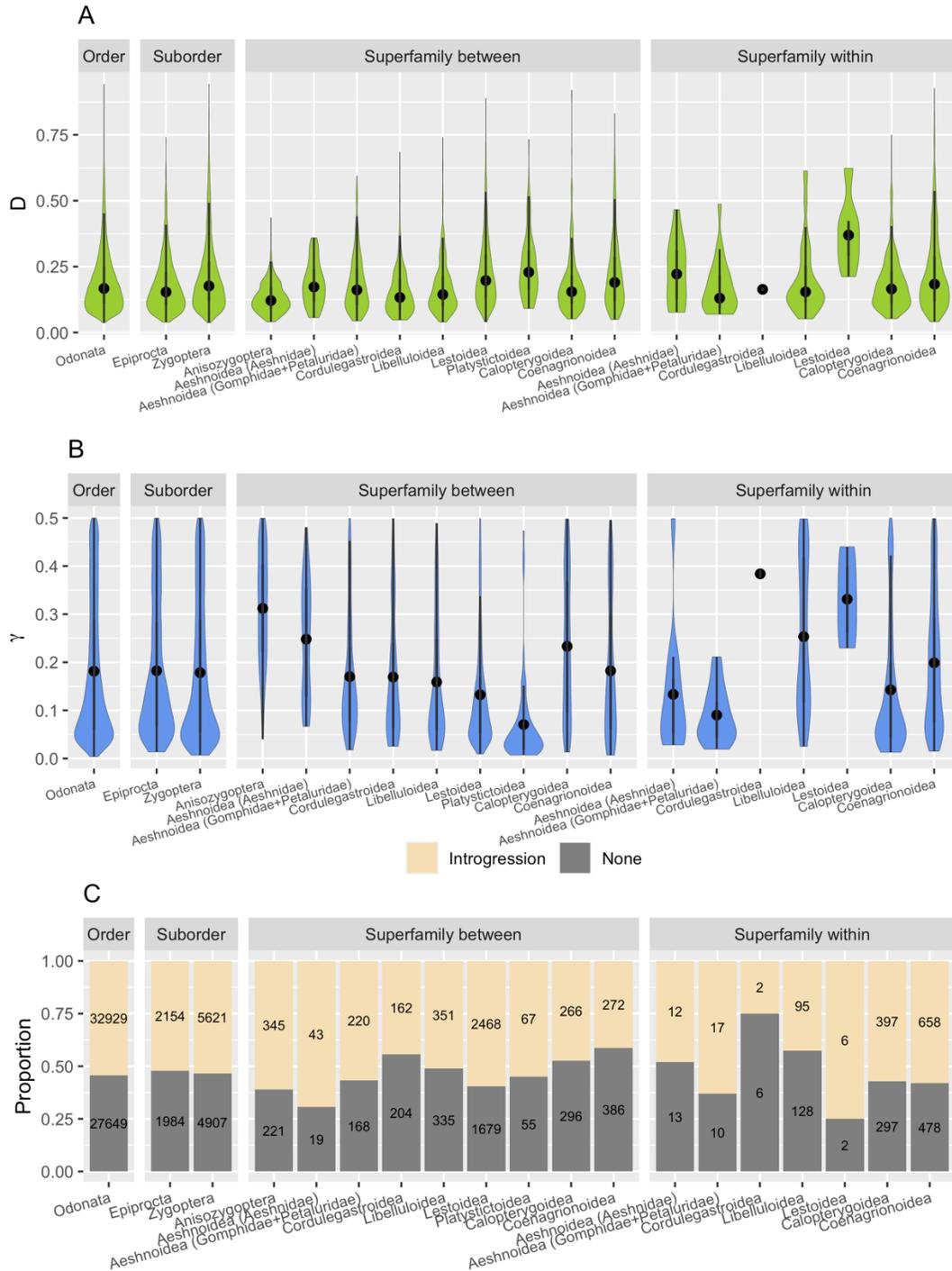
Figure S5. Distributions of the Patterson's *D* Statistic, HyDe γ and Their Relations Across Odonate Taxonomic Levels using the 1st and 2nd codon positions.

(A) Estimated distributions of significant (Bonferroni corrected *P* < 0.05) *D* statistic from ABBA-BABA site pattern counts for each quartet using HyDe output. Black dots mark medians of violin plots.

(B) Distribution of significant (Bonferroni corrected *P* < 10^{-6}) γ values for each quartet estimated by HyDe. In general, γ values that are not significantly different from 0 denote no relation of a putative hybrid species to either of the parental species P_1 (1-γ) or P_2 (γ) in a quartet.

(C) Proportions of quartets that support or reject introgression based on simultaneous significance of *D* statistics and
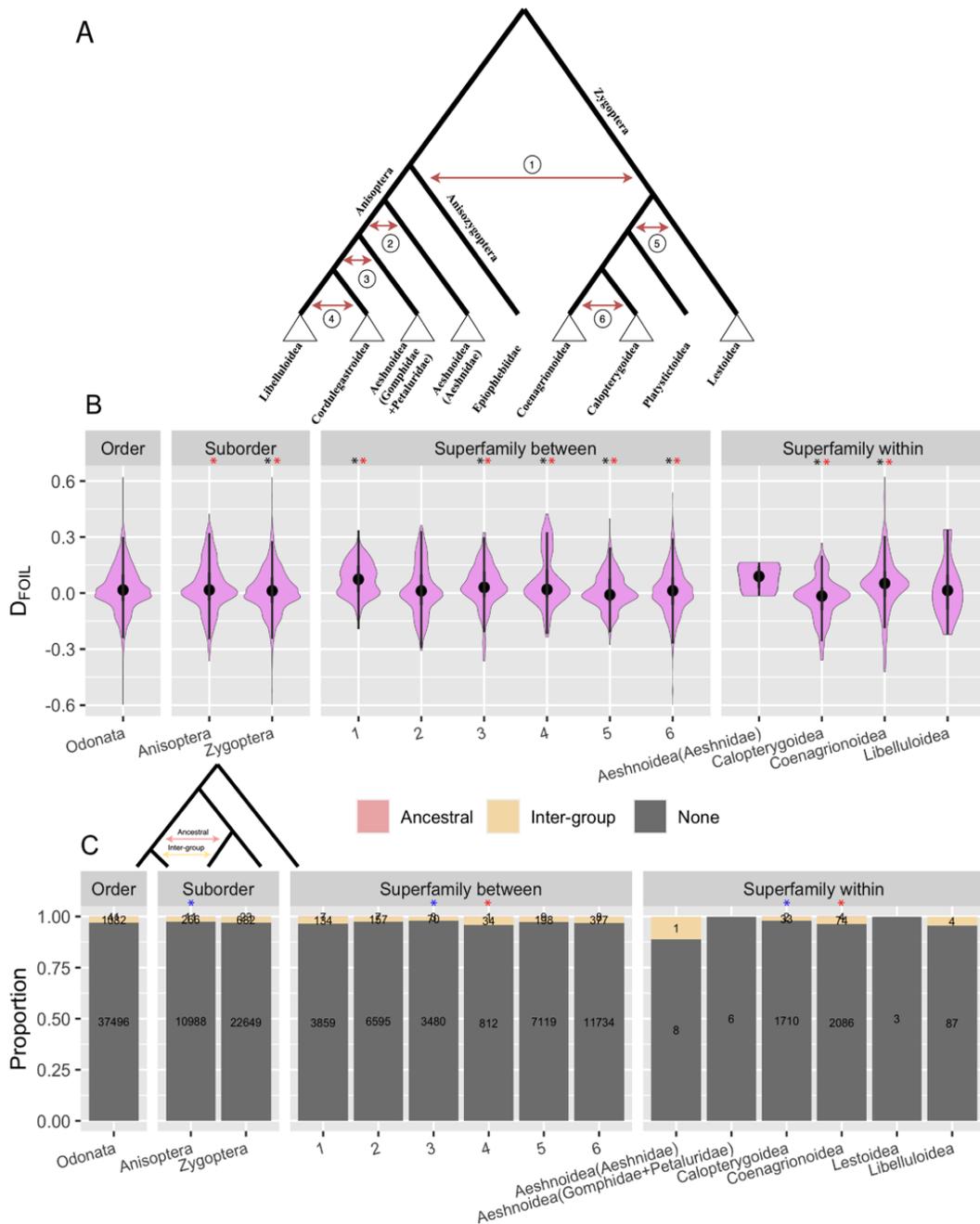
Figure S6. Distributions of the D_{FOIL} Statistics and Their Relations Across Odonate Taxonomic Levels.

(A) Tested scenarios of deep (numbered red arrows) and intra-superfamilial (white triangles) introgression for Anisoptera, Anisozygoptera and Zygoptera using D_{FOIL}.

(B) Estimated distributions of D_{FOIL} statistics from different site pattern counts for each symmetric quintet. D_{FOIL} allows to determine introgression and its polarization between a donor and recipient taxa by comparison of a sign (+/-/0) for all D_{FOIL} statistics. Black dots mark medians of violin plots. Lestoidea and Aeshnoidea (Gomphidae+Petaluridae) had no significant cases. Black asterisks indicate significant deviation of D_{FOIL} averages of various tested cases compared to D_{FOIL} average of the entire order. Red asterisks indicate whether the D_{FOIL} averages significantly different from 0.

(C) Counts of quintets that support ancestral, inter-group or no introgression scenarios based on significance of D_{FOIL} statistics (FDR corrected $P < 0.05$) and their directionality. The 5-taxon tree shows the difference between ancestral and inter-group introgression. Asterisks indicate significantly greater (red) and smaller (blue) fraction of quartets that support introgression compared to the entire order.

Chi-square test

Discordant topology T_1 Discordant topology T2   Concordant topology

S1   S3   S2   S2   S3   S1   S1   S2   S3
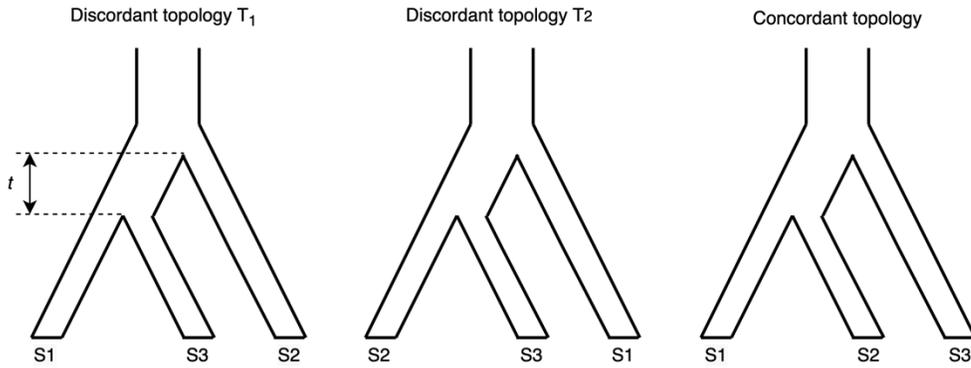
Assuming that a true species tree has ((S1,S2),S3) topology (concordant topology), the probabilities of a specific gene tree topology are:

$$p_{T_1} = \frac{e^{-t}}{3}$$

$$p_{T_2} = \frac{e^{-t}}{3}$$

$$p_{concordant} = 1 - \frac{2}{3}e^{-t}$$

Under Introgression scenario
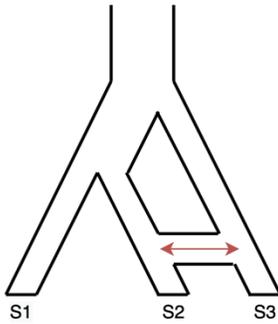
In the presence of introgression, the probability and hence, the proportion of the discordant tree topologies will be biased toward T1 or T2 depending on the introgressing species. If the difference between discordant gene tree topologies T1 and T2 is significant, this will be indicative of introgression, otherwise discordant gene tree topologies occur due to incomplete lineage sorting (ILS) alone.

S1   S2   S3

$$p_{T_2} > p_{T_1}$$

BLT

Concordant topology   Discordant topology T_1 (ILS)   Discordant topology T_1 (Introgression)   Discordant topology T2 (ILS)

S1   S2   S3   S1   S2   S3   S1   S2   S3   S2   S1   S3
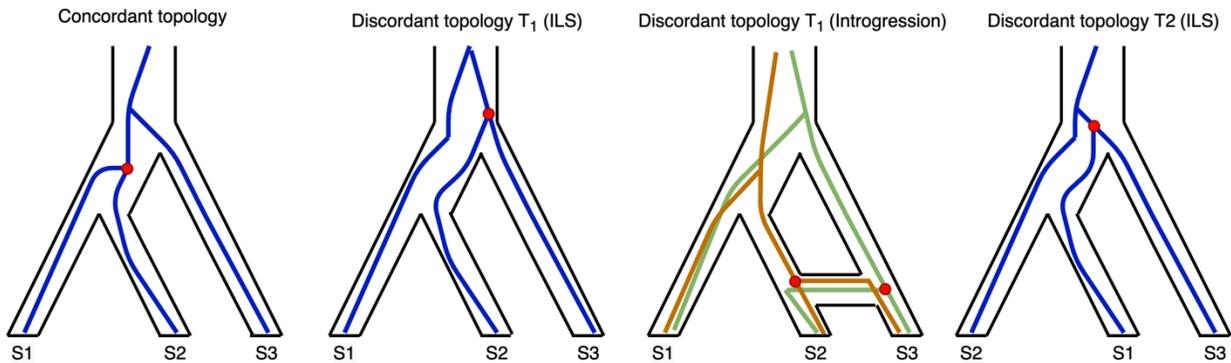
● First coalescent event

In the presence of introgression between species S2 and S3, the average genetic distance between these species for discordant gene trees T1 (introgression+ILS) will be smaller than the average genetic distance between S1 and S3 for the discordant topologies T2 generated purely by ILS.

Figure S7. The Rationale of Chi-square and Branch Length Test (BLT) procedures
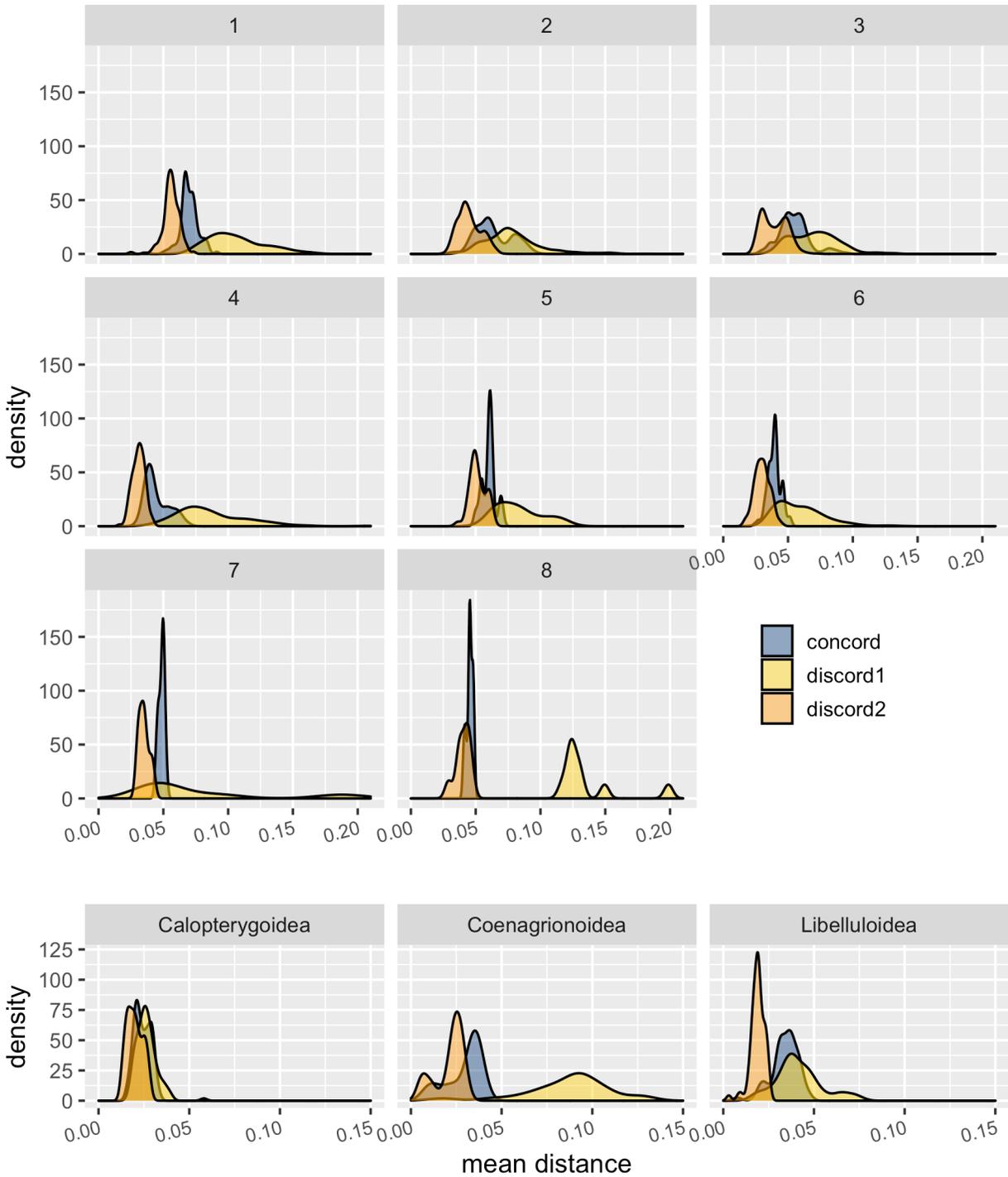
Figure S8. Normalized genetic mean distance between sister taxa across BUSCO gene trees for concordant and discordant topologies.
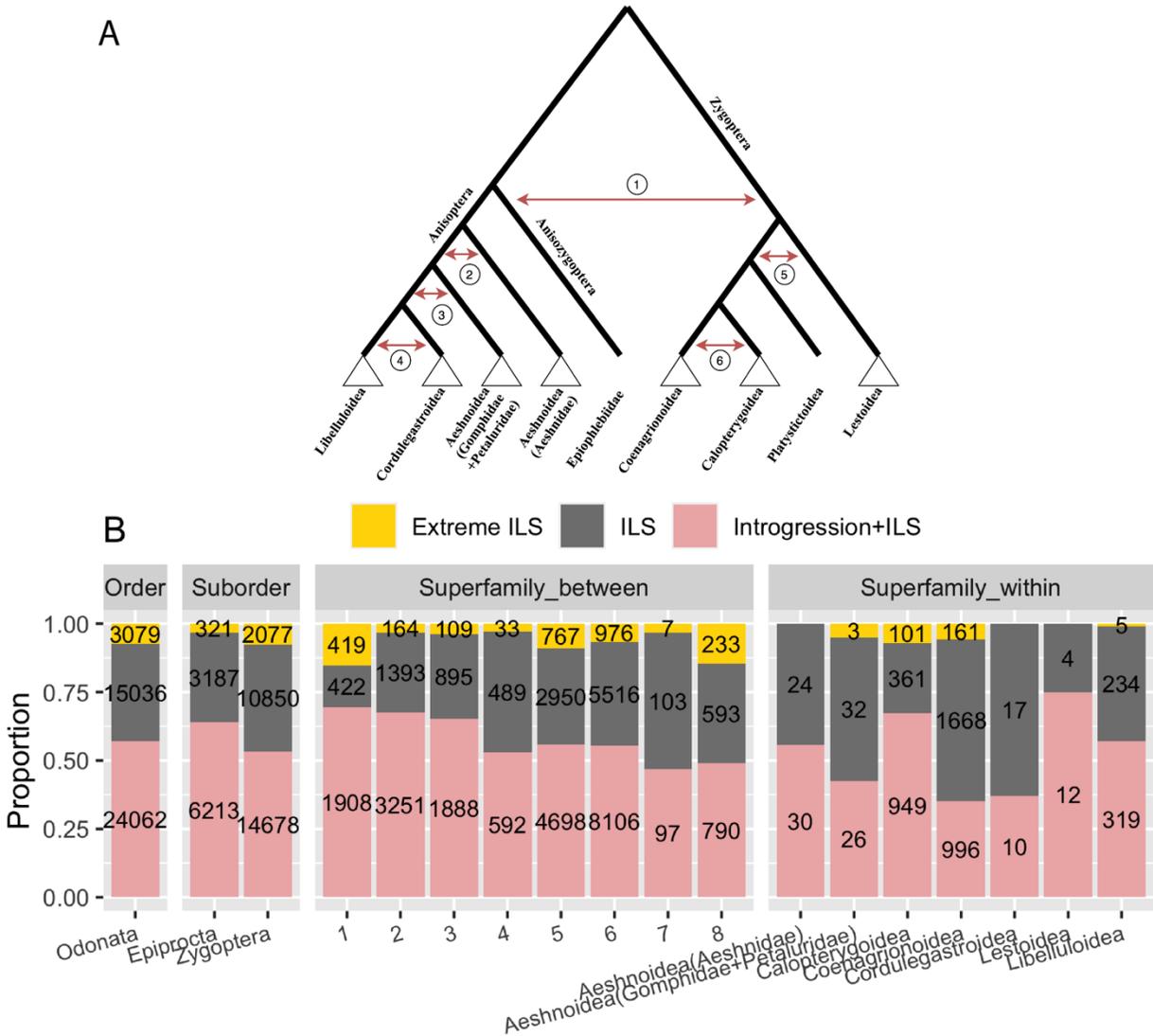
Figure S9. Overview of QuIBL for Odonate Taxonomic Levels.
(A) Tested scenarios of deep (numbered red arrows) and intra-superfamilial (white triangles) introgression for Anisoptera, Anisozygoptera and Zygoptera.
(B) Classification of triplets based on BIC criterion. Counts of triplets that exhibit extreme ILS (ΔBIC > -30 for the concordant, i.e. the common triplet topology), ILS (ΔBIC > -30 for a discordant triplet topology) and introgression+ILS (ΔBIC < -30 for a discordant triplet topology).
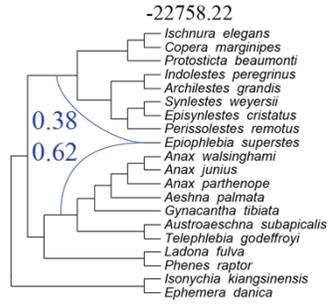
Figure S10. PhyloNet network inference using pseudo-likelihood approach for Anisozygoptera.
Phylogenetic network estimated from a set of ML gene trees using pseudo-maximum likelihood approach. *Epiophlebia superstes* was specified as a putative hybrid for Anisozygoptera clade. Blue lines indicate a reticulation event with the value of PhyloNet's estimated γ. Number above the network indicates the log-likelihood score.
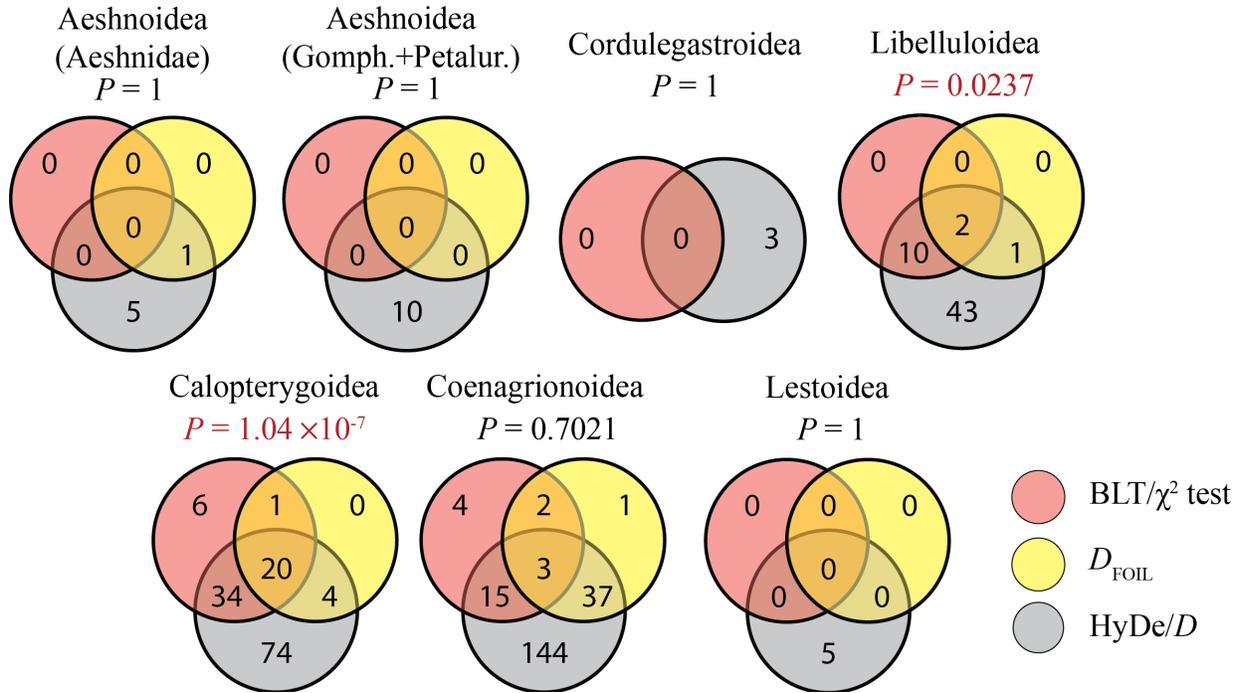
Figure S11. Overlap between Putatively Introgressed Species Pairs Inferred by Hyde/D, D_{FOIL} and BLT/χ^2 test
The numbers within sets represent the number of unique introgressing species pairs identified by a corresponding method. Significance of an overlap between all methods (intersection of all sets) for each scenario was determined by the exact multi-set interactions test. Significant P values are indicated in red. Note that due to the limitations of D_{FOIL}, introgression could not be tested within Cordulegastroidea using this method.

A   **Subordinal relationships**

Zygoptera
Zygoptera
Epiprocta
(Trueman 1996)

Zygoptera
Zygoptera (Lestes)
Anisoptera
(Saux et al. 2003,
Hasegawa & Kasuya 2006)

Zygoptera
Epiprocta
(Carle et al. 2008, Bybee et al. 2008,
Dumont et al. 2010, Suvorov et al. 2016)
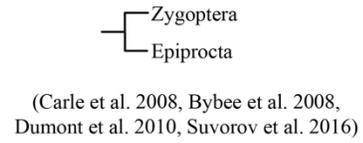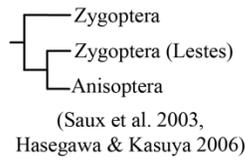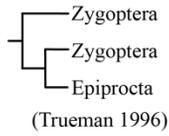
B   **Superfamilial relationships within Zygoptera**

Calopterygoidea
Lestoidea
Coenagrionoidea
(Bechly 1996, Rehn 2003)

Coenagrionoidea
Calopterygoidea
Lestoidea
(Hasegawa & Kasuya 2006)

Lestoidea
Calopterygoidea
Coenagrionoidea
(Carle et al. 2008, Bybee et al. 2008)

Lestoidea
Platystictoidea
Calopterygoidea (paraphyletic)
Coenagrionoidea
(Dijkstra et al. 2014)
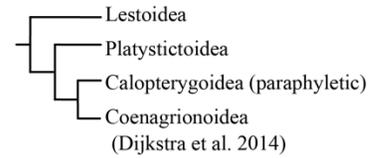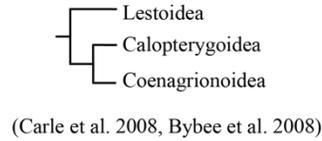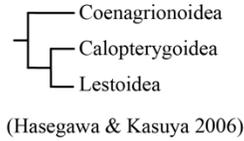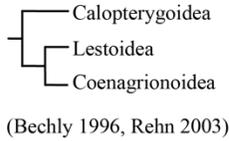
C   **The problem of Gomphidae and Petaluridae**

Anisozygoptera
Petaluridae
Remaining Anisoptera
(Bechly 1996, Trueman 1996,
Rehn 2003, Hasegawa & Kasuya 2006)

Anisozygoptera
Remaining Anisoptera
Petaluridae
Gomphidae
(Misof et al. 2001)

Anisoptera
Petaluridae
Gomphidae
Cavilabiata
(Bybee et al. 2008)

Anisoptera
Gomphidae
Petaluridae
Cavilabiata
(Carle et al. 2008)

Anisozygoptera
Gomphidae
Remaining Anisoptera
(Blanke et al. 2013)

Anisoptera
Cavilabiata
Petaluridae
Gomphidae
(Carle et al. 2015)

Figure S12. Phylogenetic Hypotheses of Odonata.