Supplementary Table 1: Description of the main features of the samples analyzed in this study.

	Clade	Number of RNA-seq samples	Sequencing depth (per-base read) <sup>a</sup>	Number of annotated introns	Number of analyzable introns <sup>b</sup>	Average number of introns per BUSCO gene	Fraction of major introns alternatively spliced <sup>c</sup>	Average AS rate among BUSCO introns	Fraction of rare SVs <sup>d</sup>
Vertebrates							*		
Callorhinchus milii	Chondrichthyes		1068	7700	7467	8.0	0.491	1.47 %	0.831
Gallus gallus	Aves	217	9657	8745	8625	8.4	0.854	1.59 %	0.958
Crocodylus porosus	Crocodylia	12	1819	7867	7668	8.5	0.817	3.02 %	0.908
Monodelphis domestica	Mammalia	269	11371	8538	8407	8.5	0.915	1.91 %	0.957
Heterocephalus glaber	Mammalia	54	2072	9409	9324	8.6	0.803	2.69 %	0.914
Macaca mulatta	Mammalia	177	5571	9328	9261	8.6	0.908	2.84 %	0.948
Oryctolagus cuniculus	Mammalia	338	15503	8036	7885	8.4	0.950	1.97 %	0.969
Rattus norvegicus	Mammalia	362	16611	8469	8196	8.5	0.953	1.89 %	0.965
Mus musculus	Mammalia	317	12245	9327	9080	8.4	0.937	1.87 %	0.958
Bos taurus	Mammalia	26	710	9046	8926	8.5	0.511	1.63 %	0.856
Loxodonta africana	Mammalia	23	3667	9001	8653	8.3	0.896	3.55 %	0.938
Sus scrofa	Mammalia	55	910	8982	8798	8.5	0.644	1.95 %	0.886
Canis lupus	Mammalia	5	348	9279	8628	8.2	0.436	2.18 %	0.764
Homo sapiens	Mammalia	313	10269	11122	10981	8.4	0.957	3.38 %	0.949
Equus caballus	Mammalia	19	998	9191	9073	8.5	0.658	2.16 %	0.884
Insects									
Bombyx mori	Lepidoptera	14	459	5001	4681	5.3	0.393	1.12 %	0.835
Athalia rosae	Hymenoptera	6	359	4772	4701	4.8	0.348	1.6 %	0.782
Cephus cinctus	Hymenoptera Hymenoptera	17	2566	5036	5017	4.8	0.348	2.4 %	0.782
•		2			5017 4664			2.4 %	
Orussus abietinus	Hymenoptera	114	197 4871	4801 4274		4.7	0.370	2.03 % 1.21 %	0.763
Nasonia vitripennis	Hymenoptera	114 4	4871 350		4159 3734	4.5	0.648 0.268	1.21 % 0.98 %	0.913
Trichogramma pretiosum	Hymenoptera			3794		4.4			0.782
Harpegnathos saltator	Hymenoptera	166	1888	4746	4712	4.7	0.565	2.02 %	0.886
Linepithema humile	Hymenoptera	23	1476	4726	4615	4.8	0.570	1.45 %	0.882
Camponotus floridanus	Hymenoptera	37	449	4596	4546	4.7	0.358	1.52 %	0.761
Pogonomyrmex barbatus	Hymenoptera	39	1388	4678	4440	4.5	0.579	1.91 %	0.866
Polistes canadensis	Hymenoptera	14	440	4665	4562	4.8	0.424	1.88 %	0.834
Polistes dominula	Hymenoptera	12	218	4698	4161	4.3	0.180	1.63 %	0.624
Solenopsis invicta	Hymenoptera	23	436	4516	4394	4.6	0.430	1.71 %	0.807
Acromyrmex echination	Hymenoptera	42	1470	4716	4638	4.7	0.529	2.15 %	0.835
Megachile rotundata	Hymenoptera	108	3400	5120	5086	4.8	0.898	3.81 %	0.927
Apis mellifera	Hymenoptera	40	1777	4940	4898	4.9	0.673	2.3 %	0.892
Apis florea	Hymenoptera	4	503	4881	4332	4.4	0.318	1.85 %	0.711
Apis cerana	Hymenoptera	12	1401	4508	4439	4.6	0.578	2.36 %	0.839
Bombus terrestris	Hymenoptera	33	2648	4857	4683	4.7	0.763	2.33 %	0.922
Acyrthosiphon pisum	Hemiptera	35	3163	4918	4844	6.0	0.709	1.09 %	0.933
Cimex lectularius	Hemiptera	10	462	5640	5588	6.3	0.431	1.61 %	0.838
Halyomorpha halys	Hemiptera	6	1460	5715	5676	6.5	0.591	1.73 %	0.885
Aedes aegypti	Diptera	27	2469	2369	2290	2.6	0.514	1.35 %	0.870
Drosophila grimshawi	Diptera	30	256	2190	2032	2.7	0.168	0.8 %	0.726
Drosophila pseudoobscura	Diptera	32	3628	2312	2244	2.6	0.433	1.32 %	0.871
Drosophila melanogaster	Diptera	129	4542	2415	2391	2.7	0.551	1.22 %	0.909
Drosophila suzukii	Diptera	23	1979	2187	2052	2.6	0.287	1.17 %	0.810
Ceratitis capitata	Diptera	29	1168	3067	3015	3.3	0.418	1.45 %	0.860
Lucilia cuprina	Diptera	23	2446	2566	2405	2.8	0.268	0.85 %	0.823
Musca domestica	Diptera	12	1056	2545	2401	2.9	0.254	0.98 %	0.795
Onthophagus taurus	Coleoptera	53	644	2836	2753	3.2	0.377	1.34 %	0.810
Tribolium castaneum	Coleoptera	14	2618	3333	3225	3.6	0.556	1.15 %	0.881
Dendroctonus ponderosae	Coleoptera	30	2262	4370	4269	4.9	0.505	1.26 %	0.882
Anoplophora glabripennis	Coleoptera	20	325	3764	3567	4.1	0.299	1.13 %	0.781
Leptinotarsa decemlineata	Coleoptera	21	2071	3373	3133	3.8	0.512	1.21 %	0.883
Blattella germanica	Blattodea	30	943	4911	4454	5.4	0.423	1.26 %	0.827
Cryptotermes secundus	Blattodea	11	481	6471	6391	6.4	0.573	2.32 %	0.832
Zootermopsis nevadensis	Blattodea	53	3944	6728	6614	6.4	0.802	2.36 %	0.927
Zootermopose nevadensis	Diamodoc				0011	V. 1	0.002	2.00 /0	

<sup>&</sup>lt;sup>a</sup> Median per-base read coverage computed on BUSCO gene exons <sup>b</sup> Number of analyzable introns (i.e. with Ns+Na  $\geq$  10) among BUSCO genes

 $<sup>^{\</sup>rm c}$  Proportion of major introns for which alternative splicing has been detected (i.e. with N2 > 0) among BUSCO genes

 $<sup>^{\</sup>rm d}$  Fraction of rare spliced variants in trons (i.e. with MIRA  $\leq 5\%$  ) among all protein-coding genes