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

| | Clade | Number of RNA-seq samples | Sequencing depth $(per	ext{-}base read)^a$ | Number of annotated introns | Number of analyzable introns ^b | Average number of introns per BUSCO gene | Fraction of major-isoform introns alternatively spliced $^{\rm c}$ | Average AS rate among BUSCO introns | Fraction of rare SVs ^d |
|---|--------------------|------------------------------|--|-----------------------------|--|--|--|-------------------------------------|--------------------------------------|
| Vertebrates | | | | | | | | | |
| Callorhinchus milii | Chondrichthyes | 11 | 1068 | 7700 | 7467 | 8.0 | 0.491 | 1.47 % | 0.831 |
| Gallus gallus | Aves | 217 | 9657 | 8741 | 8621 | 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 | 9000 | 8652 | 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 | 9190 | 9072 | 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 | 17 | 2566 | 5035 | 5016 | 4.7 | 0.744 | 2.4 % | 0.907 |
| Orussus abietinus | Hymenoptera | 2 | 197 | 4801 | 4664 | 4.7 | 0.370 | 2.03 % | 0.763 |
| Nasonia vitripennis | Hymenoptera | 114 | 4871 | 4273 | 4158 | 4.5 | 0.648 | 1.21 % | 0.913 |
| Trichogramma pretiosum | Hymenoptera | 4 | 350 | 3794 | 3734 | 4.4 | 0.268 | 0.98 % | 0.782 |
| Harpegnathos saltator | Hymenoptera | 166 | 1888 | 4745 | 4711 | 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 | 4939 | 4897 | 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 6 | 462 1460 | 5640 5715 | 5588 5676 | 6.3 6.5 | 0.431 0.591 | 1.61 % 1.73 % | 0.838 0.885 |
| Halyomorpha halys | Hemiptera | | | | | | | | |
| Aedes aegypti | Diptera | 27 | 2469 | 2369 | 2290 | 2.6 | 0.514 | 1.35 % | 0.870 |
| Drosophila grimshawi | Diptera | 30 | 256 | 2190 2312 | 2032 2244 | 2.7 2.6 | 0.168 | 0.8 % 1.32 % | 0.726 |
| Drosophila pseudoobscura Drosophila melanogaster | Diptera | 32 129 | 3628 4542 | 2312 | 2390 | 2.6 | 0.433 0.551 | 1.32 % | 0.871 0.909 |
| Drosophila suzukii | Diptera | | | 2187 | 2052 | 2.6 | | 1.17 % | |
| Ceratitis capitata | Diptera Diptera | 23 29 | 1979 1168 | 3067 | 3015 | 3.3 | 0.287 0.418 | 1.45 % | 0.810 0.860 |
| | | 29 | | 2566 | | 2.8 | | 0.85 % | |
| Lucilia cuprina | Diptera Diptera | 12 | 2446 1056 | 2545 | 2405 2401 | 2.9 | 0.268 0.254 | 0.98 % | 0.823 0.795 |
| Musca domestica Onthophagus taurus | Coleoptera | 53 | 644 | 2836 | 2753 | 3.2 | 0.254 0.377 | 0.98 % 1.34 % | 0.795 |
| Tribolium castaneum | Coleoptera | 53 14 | 2618 | 2830 3333 | 3225 | 3.2 | 0.556 | 1.34 % | 0.810 |
| Dendroctonus ponderosae | Coleoptera | 30 | 2262 | 4370 | 4269 | 4.9 | 0.505 | 1.15 % | 0.882 |
| Anoplophora glabripennis | Coleoptera | 20 | 325 | 4370 3764 | 4269 3567 | 4.9 | 0.505 | 1.26 % | 0.882 |
| Anopiopnora giabripennis Leptinotarsa decemlineata | Coleoptera | 20 | 325 2071 | 3372 | 3132 | 3.8 | 0.299 | 1.13 % | 0.781 |
| Blattella germanica | Blattodea | 30 | 943 | 4911 | 3132 4454 | 5.4 | 0.512 | 1.21 % | 0.883 |
| Cryptotermes secundus | Blattodea | 30 11 | 481 | 6471 | 6391 | 6.4 | 0.423 | 2.32 % | 0.827 |
| Zootermopsis nevadensis | Blattodea | 53 | 3944 | 6727 | 6613 | 6.4 | 0.802 | 2.36 % | 0.832 |
| Zootermopsis nevadensis | Diamoded | 99 | 0344 | 0121 | 0010 | 0.4 | 0.002 | 2.30 /0 | 0.321 |

 $^{^{\}rm a}$ Median $per\mbox{-base}$ read coverage computed on BUSCO gene exons

b Number of analyzable introns (i.e. with $N_s + N_a \ge 10$) among BUSCO genes c Proportion of major-isoform introns for which alternative splicing has been detected (i.e. with $N_a > 0$) among BUSCO genes d Fraction of rare spliced variants introns (i.e. with MIRA $\le 5\%$) among all protein-coding genes