## Evolutionary conservation of *bs* and *bsAS*

[ALTERNATIVE TEXT]

Results so far suggest that the expression of bsAS, through the regulation of isoform usage in bs, is essential for the proper development of *D. melanogaster* wings. To investigate to what extend the antisense regulation of bs has been conserved through evolution, and whether it may have conserved its functional role, we investigated the bs locus through metazoans. The bs gene is strongly conserved: it shares 37% identity and 49% similarity at protein level with the SRF, the human orthologue. Remarkably, in spite of dramatic changes in the exon structure of the gene through evolution, we have found that the MADS-box is systematically interrupted by an intron (often, but not always, the first one, Figure 5E). By analyzing the existing annotation, which we have manually extended using RNASeq and computational evidence, we found that the isoform structure of bs in *D. melanogaster* has actually been overall conserved within Arthropoda, but it is not present outside from this phylum (Figure 5E) Tracking the evolution of bsAS, however, is far more complicated, since lncRNA show generally poor sequence conservation, even at close phylogenetic distances (REF). We have therefore used RNASeq data available across the Metazoan kingdom to characterize antisense transcription within the long intron of the bs locus. Our analyses (Supplementary Information), show evidence of antisense transcription through all insects, including those in wingless taxa. RNASeq data available from adults heads in *D. mojavensis* *and D pseudobscura*, as well as from worker bees shows that both bs and bsAS are less expressed in heads than in whole animals (Figure 5C), mirroring our findings in *D. melanogaster*. Antisense transcription is also present in other hexapoda, outside from insects, as an antisense lncRNAs is annotated within the longest intron of the bs gene in *Folsomia candida*, a springtail from the *Collembola* class (Figure 5D). All these observations suggest that *bsAS* has been conserved all through hexapoda both transcriptionally and functionally. Since non-insect hexapods are wingless animals, bsAS regulation of isoform usage precedes emergence of wings in insects. While the long-short isoform structure is conserved in arthropods outside from hexapoda (chelicerates, myriapods and crustaceans), we have not been able to find evidence of antisense regulation in species from this taxa.