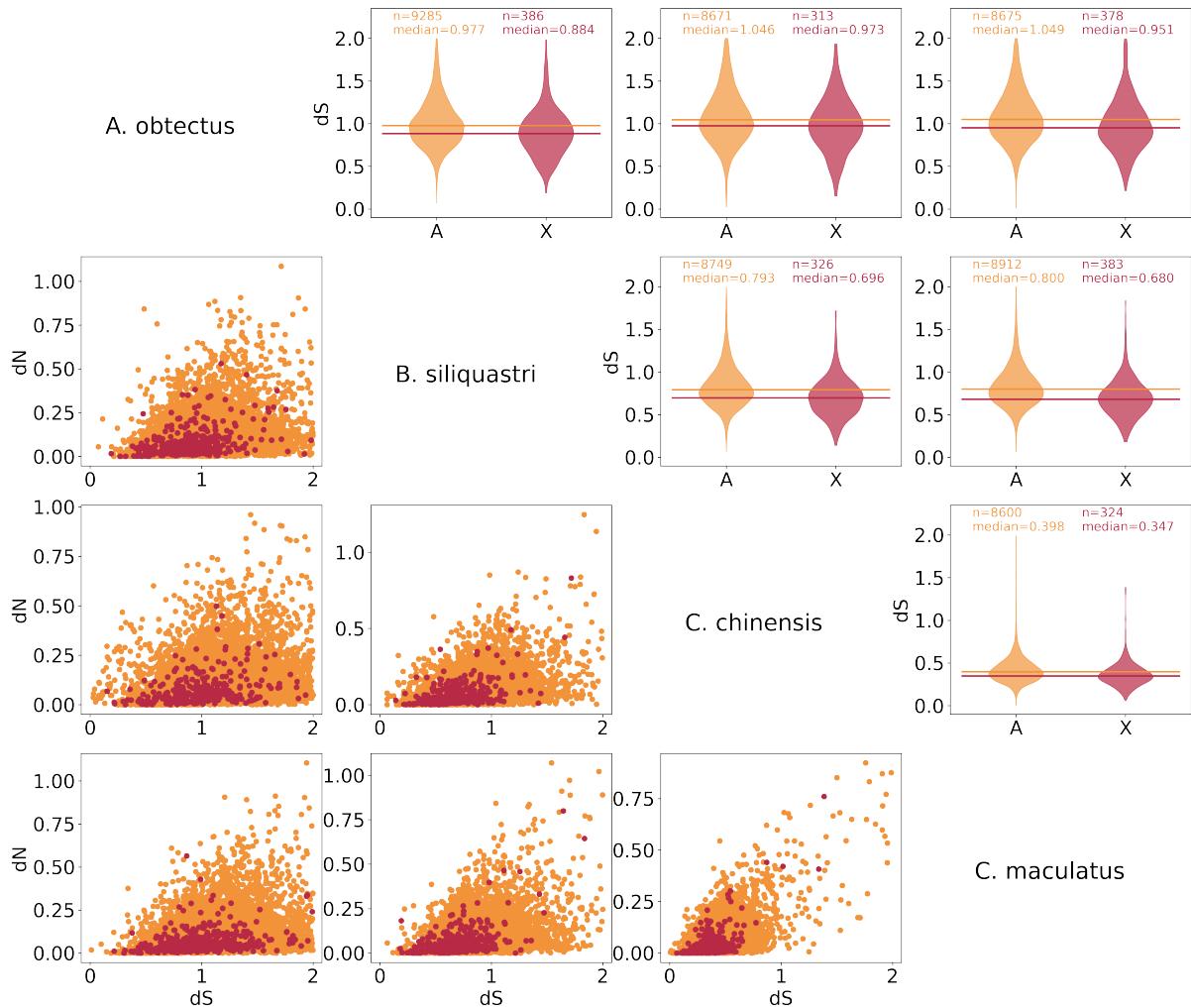
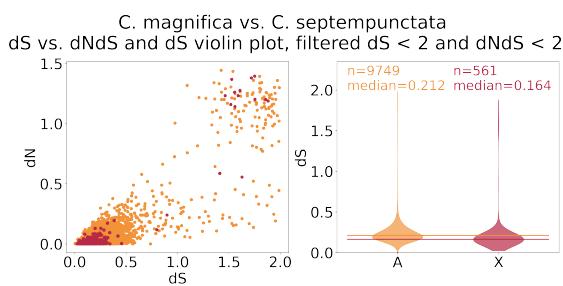


Bruchini: dS vs. dNdS and dS violin plot, filtered dS < 2 and dNdS < 2

**A**



**B**



**C**

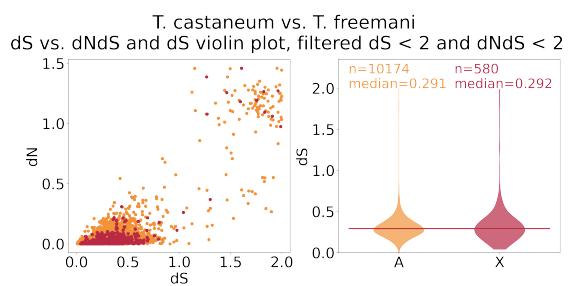
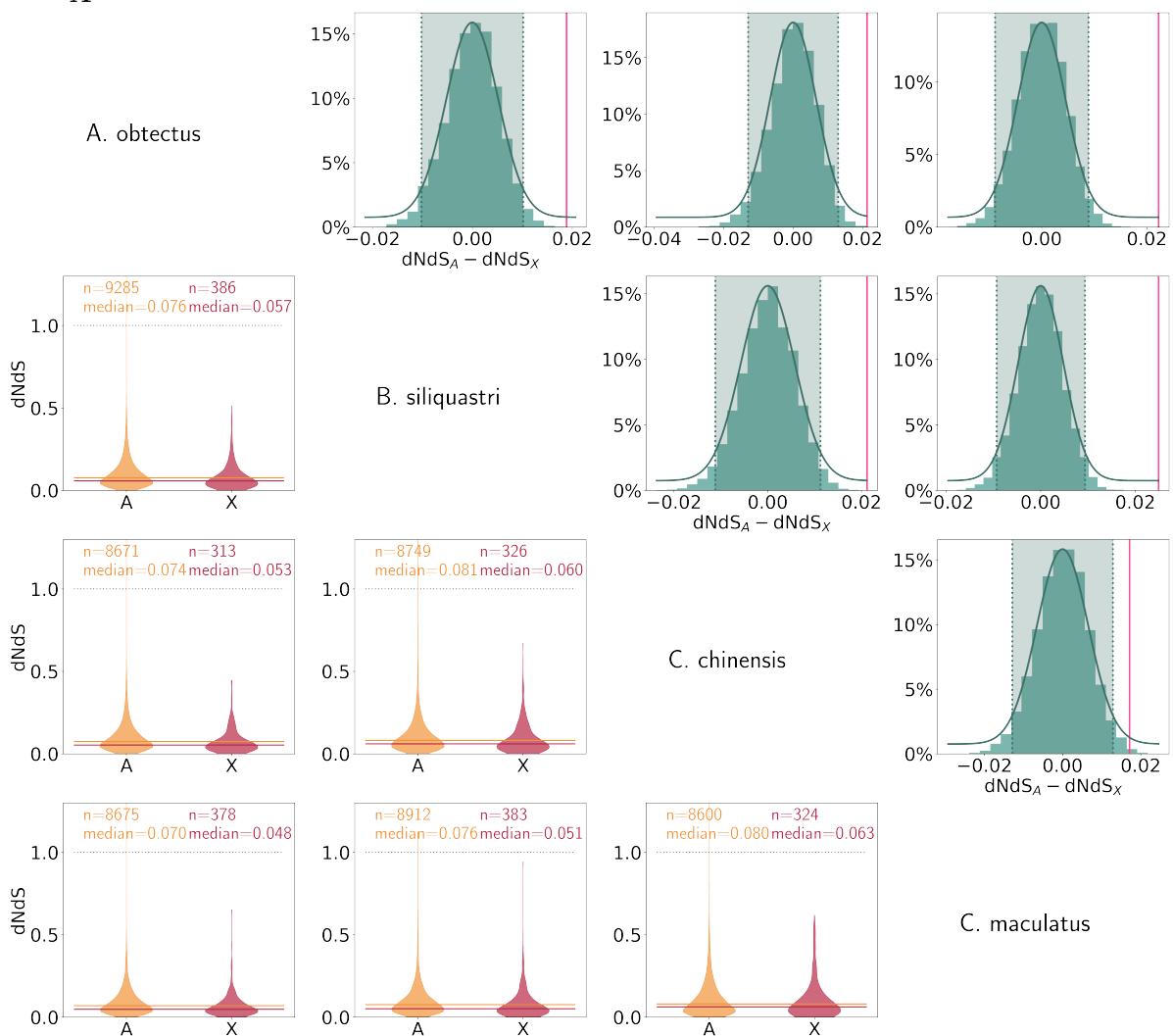
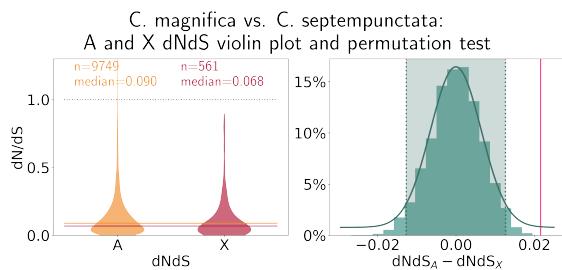
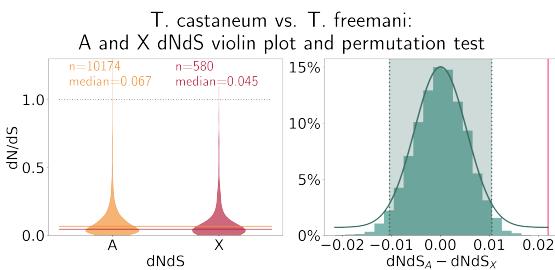


Figure 1: **d<sub>S</sub> vs. d<sub>N</sub> scatterplots and violin plots of d<sub>S</sub> values from X-linked and autosomal orthologs.** Pairwise comparisons between members of three species groups with violin plots of d<sub>S</sub> values for X-linked and autosomal 1-to-1 orthologs, and scatterplots of d<sub>S</sub> vs. d<sub>N</sub>. d<sub>S</sub> is lower for X-linked orthologs in all comparisons except *Tribolium*, where it is almost equal. The within-family comparisons are performed for three species groups: *Bruchini* (**A**), *Coccinella* (**B**) and *Tribolium* (**C**).

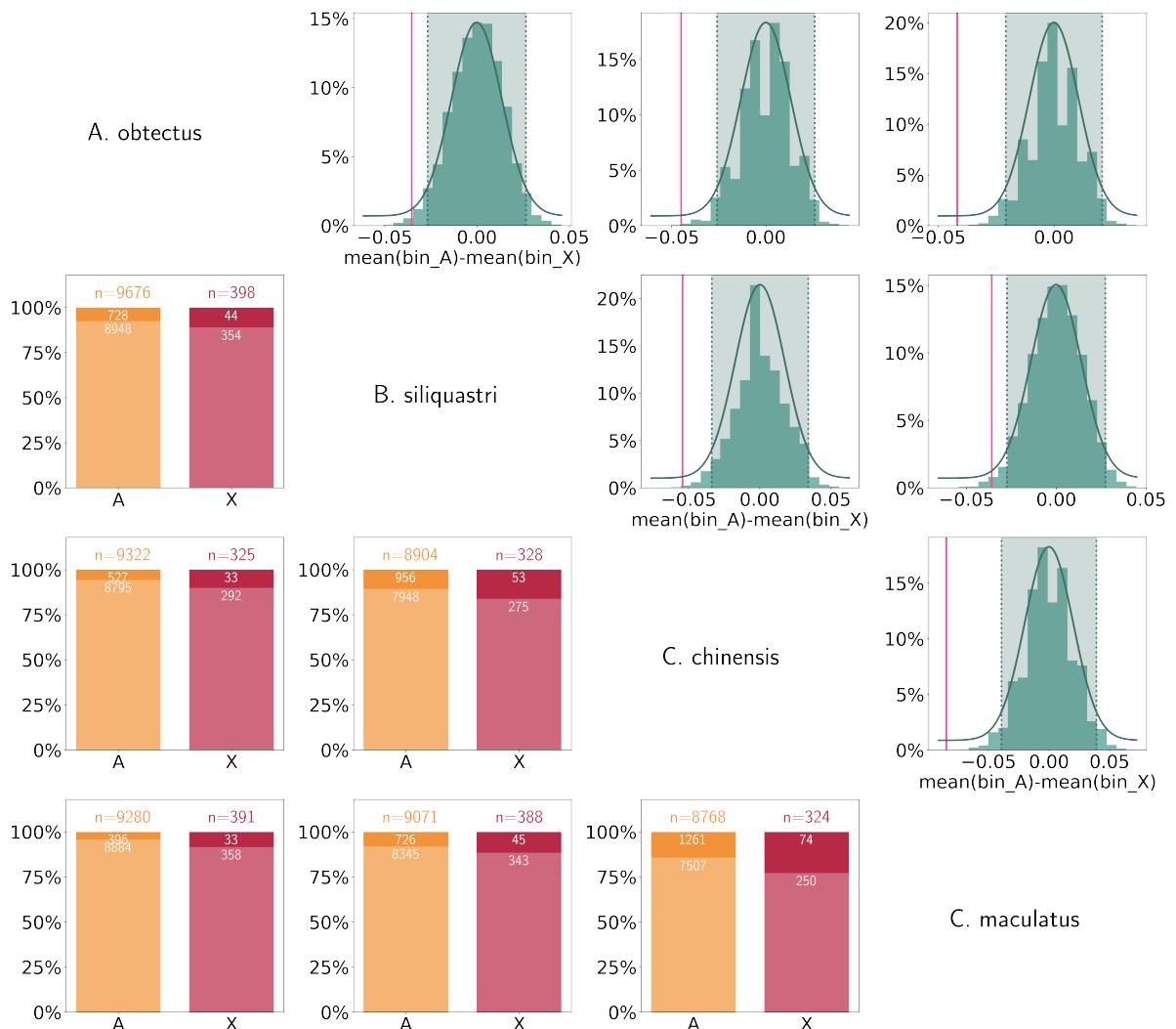
**A**

## Bruchini: A and X dNdS violin plot and permutation test

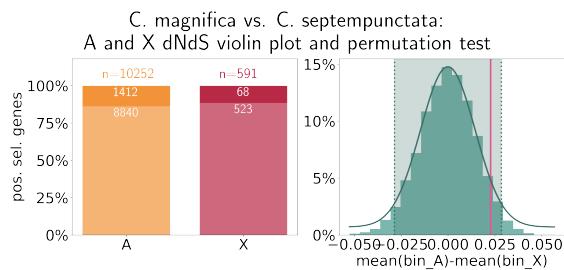
**B****C**

**Figure 2:  $d_N/d_S$  ratio and permutation tests for significance.** Pairwise comparisons between members of three species groups with violin plots of  $d_N/d_S$  values for X-linked and autosomal 1-to-1 orthologs, and permutation tests (10000 permutations) to assess significance. The permutation test show frequency histograms of median differences between autosomal and X-linked  $d_N/d_S$ , with the shaded area showing the 95% confidence interval according to a normal distribution. The vertical pink line shows the true observed difference in mean between autosomal and X-linked  $d_N/d_S$ . All pairwise comparisons show significantly lower  $d_N/d_S$  values on X-linked orthologs. The within-family comparisons are performed for three species groups: *Bruchini* (**A**), *Coccinella* (**B**) and *Tribolium* (**C**).

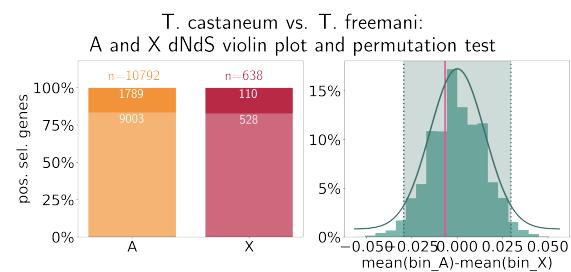
### Bruchini: A and X dNdS violin plot and permutation test



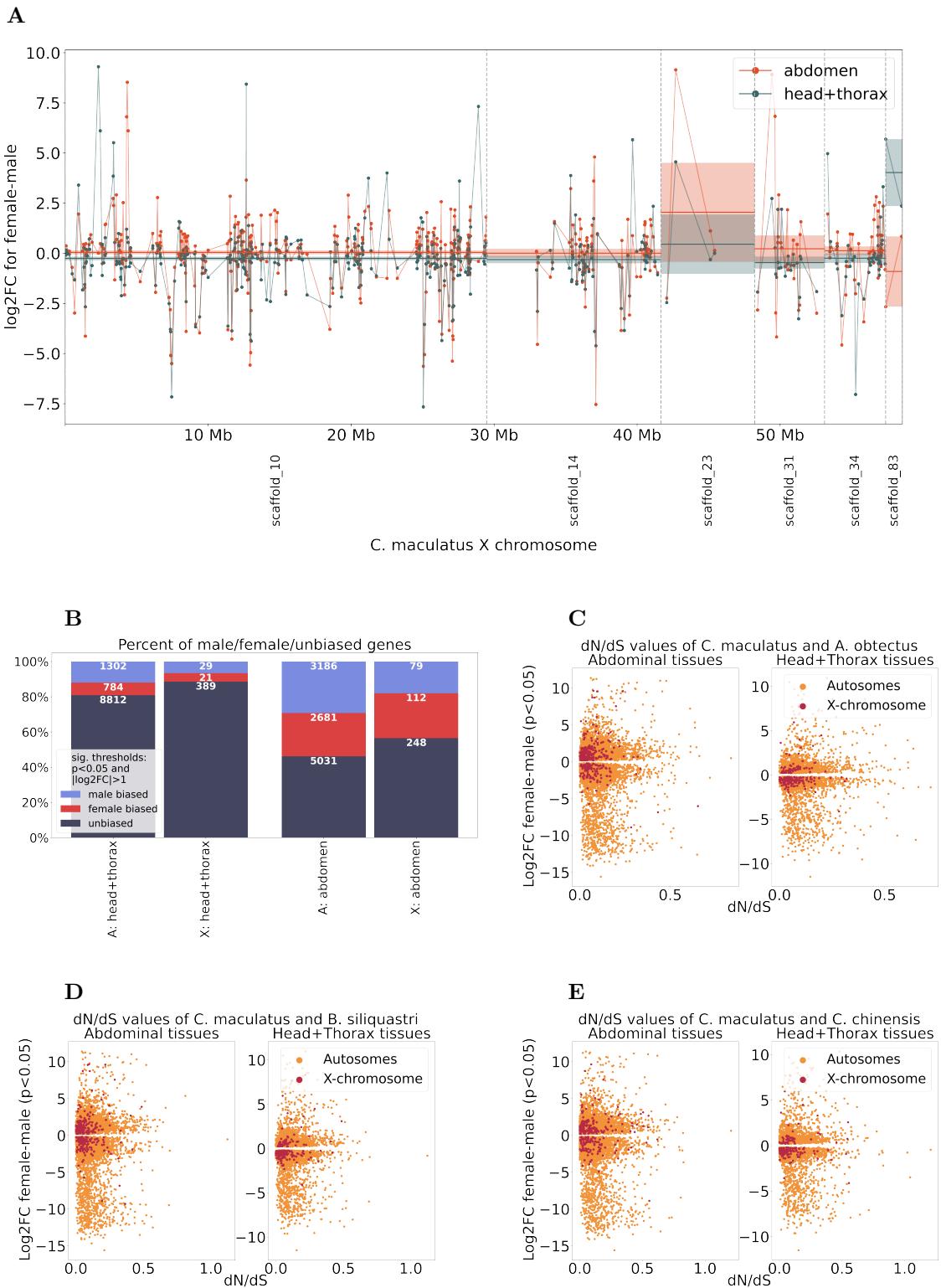
**B**



**C**



**Figure 3: positively selected orthologs split into autosomal or X-linked.** Positively selected genes are determined via likelihood ratio test comparison between paml site models M1a and M2a. The permutation test show frequency histograms of median differences between the percentage of orthologs with positively selected codons between autosomal and X-linked orthologs, with the shaded area showing the 95% confidence interval according to a normal distribution. The vertical pink line shows the true observed difference in percentage of orthologs with positively selected codons between autosomal and X-linked orthologs. *Bruchini* (**A**), show an enrichment of positively selected X-linked orthologs in all pairwise comparisons, while *Coccinella* (**B**) and *Tribolium* (**C**) show no significant difference.



**Figure 4: Differential expression analysis.** We are utilizing the log<sub>2</sub> fold change (og<sub>2</sub>LFC) information from genes in *C. maculatus* and combining it with d<sub>N</sub>/d<sub>S</sub> between these genes and their 1-to-1 orthologs in other bruchids. The log<sub>2</sub>FC contrast is always female-male, therefore positive values indicate female-bias and negative values indicate male-bias. **A:** log<sub>2</sub>FC of all genes on X-linked contigs in somatic (head+thorax) and reproductive (abdominal) tissues. **B:** number of sex biased genes in abdominal (reproductive) tissues and head and thorax (somatic) tissues. **C-E:** Scatterplot of significantly differentially expressed genes showing d<sub>N</sub>/d<sub>S</sub> of *A. obtectus* (**C**), *B. siliquastri* (**D**) and *C. chinensis* (**E**) respectively.