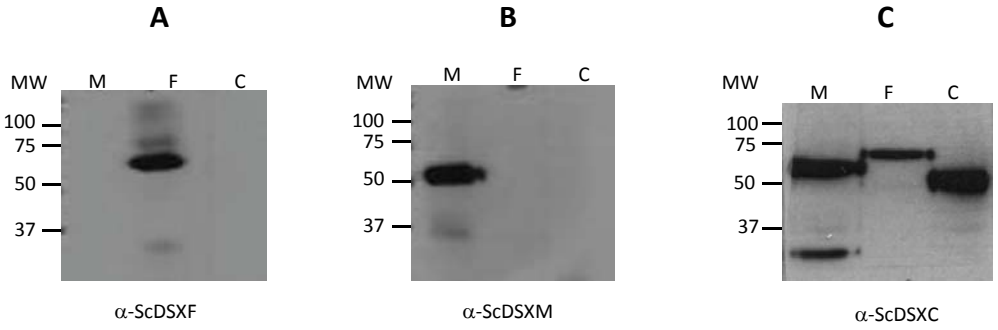

Figure S3 Specificity of the anti-sera against the *Sciara* male and female DSX proteins. Western - blots of GST-ScDSXM (lane M), GST-ScDSXF (lane F) and GST-DSXC (lane C) probed with α-ScDSXF serum **(A)**, with α-ScDSXM serum **(B)** and with α-ScDSXC serum **(C)**. To construct the GST-DSX fusion proteins, the procedure of Smith and Johnson [Smith DB, Johnson KS (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with gluthanione S-transferase. Gene 67:31–40] was followed with minor changes. To generate the GST-DSXF fusion, the whole ORF of *S. coprophila dsxF* was amplified from cDNA with primers p*GexDxsC* and p*GexdsxF* containing a restriction site for EcoRI and HindIII, respectively. To generate the GST-DSXM fusion, the whole ORF of *S. coprophila dsxM* was amplified from cDNA with primers p*GexDxsC* and p*GexdsxM* containing a restriction site for EcoRI and HindIII, respectively. To generate the GST-DSXC fusion, the fragment corresponding to the common N-terminal region of *S. coprophila dsx* was amplified from cDNA with primers p*GexDxsC* and p*GexdsxC2* containing a restriction site for EcoRI and HindIII, respectively. The amplicons were cloned in p*GEMT-easy* (Promega) and sequenced. The DNA of the p*GEMT-easy* vectors was digested with EcoRI and HindIII and the fragments were ligated in frame into the p*Gex-B* vector using the T4 DNA ligase (Roche). All the positive clones were sequenced to ascertain correct orientation.