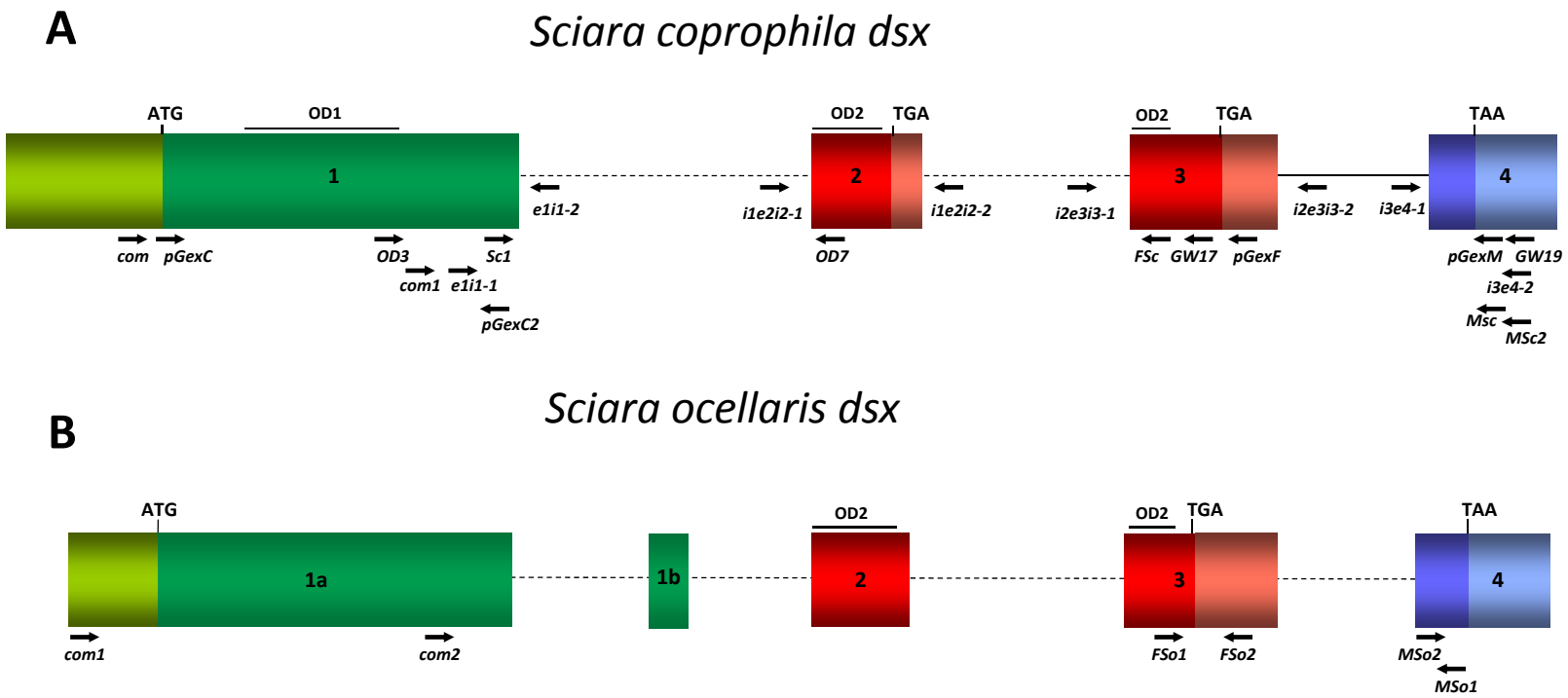

Figure S1 Location of the primers used in this work. The prefix-*dsx* has been omitted in the name of the primers.

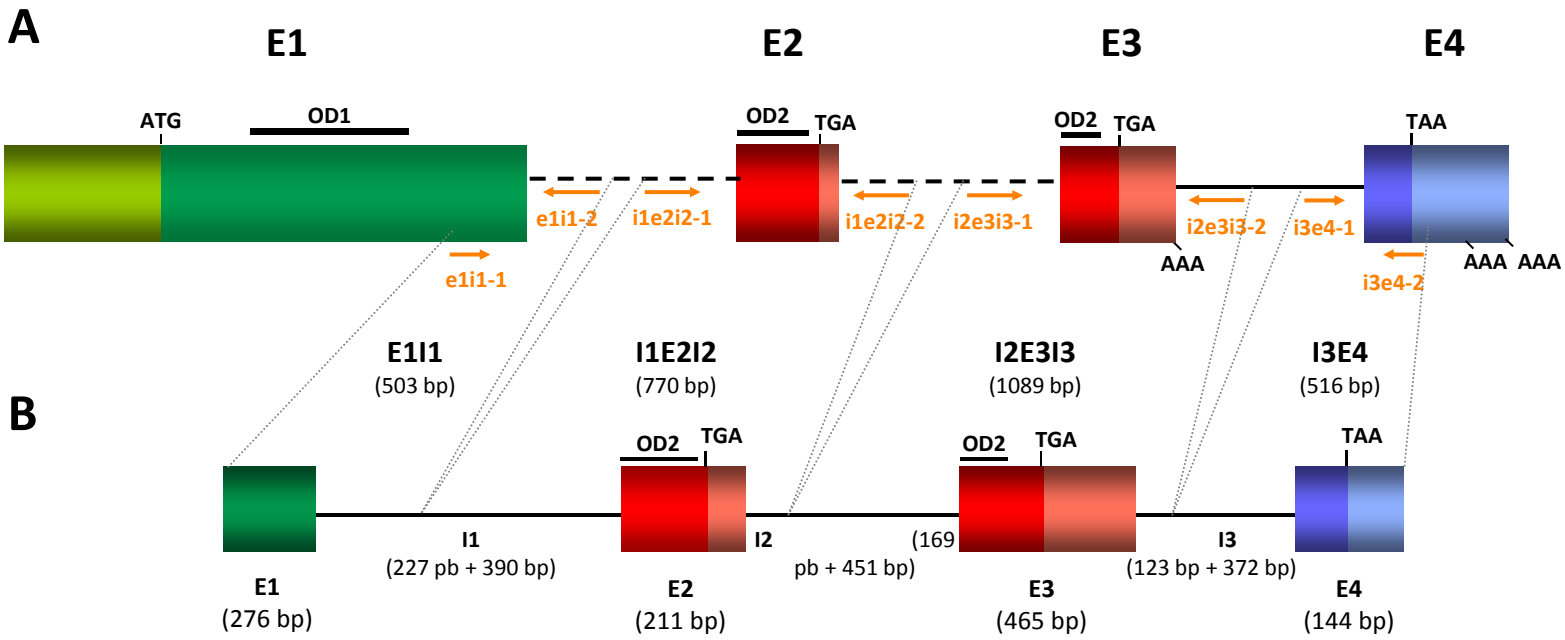

Figure S2 Construction of the *DScmg* mini-gene. For details, see Materials and methods. **(A)** Exons (boxes, E) and introns (lines) are not drawn to scale. The doted lines for introns 1 and 2 indicate that their lengths remain unknown. The beginning and the end of the ORF are indicated by ATG and TGA or TAA, respectively. AAA stands for poly-A(+). Orange arrows indicate the location of the primers used. **(B)** The *Dscmg* construct. The dotted lines from A to B indicate the regions of the whole primary transcript that is represented in *DScmg*, where the solid lines indicate now the intron sequences.

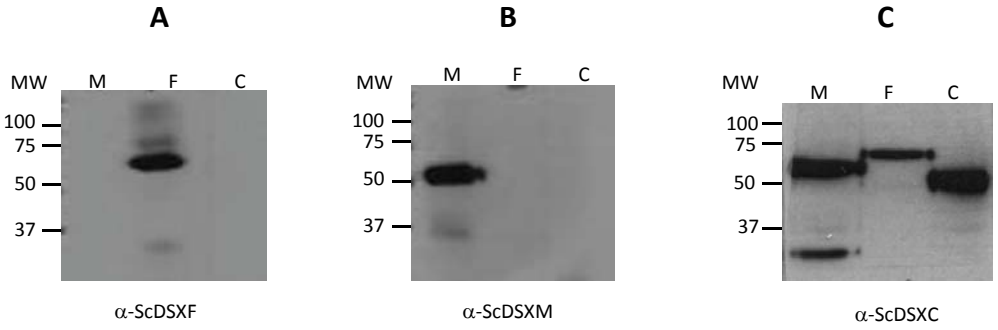

Figure S3 Specificity of the anti-sera against the *Sciara* male and female DSX proteins. Western - blots of GST-ScDSXM (lane M), GST-ScDSXF (lane F) and GST-DSXC (lane C) probed with α-ScDSXF serum **(A)**, with α-ScDSXM serum **(B)** and with α-ScDSXC serum **(C)**. To construct the GST-DSX fusion 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.

Table S1 Sequence of primers.

Primer	Sequence
dsxOD3	5′ TGGARAARTGYCGMYTRACNGCSGAYCG 3′
dsxOD7	5′ GTATCACATACATNARNGGC 3′
dsxSccom	5′ GGTCAGAACATAAGCGAAGTG 3′
dsxSc1	5′ ATCGTCGGGATTCCCTTACG 3′
dsxMSc	5′ TGTACTTCTAGGTAAAACGCAG 3′
dsxM2Sc	5′ CACTCATGGCTTTTATCATAAT 3′
dsxFSc	5′ GTCCGTTCCACAATTGACATG 3′
GW17	5′TCGTGTTATCATCCCCGTTTGGTATCG3′
GW19	5′TCAATCCATCGCATTCGCTTAATTAC3′
dsxcom1	5′CGTCACCGTCATTGACACCTGATCAG3′
e1i1-1	5′ ATAAGAAT**GCGGCCGC**CGTCATTGACACCTGATCAG 3′
e1i1-2	5′ GC**TCTAGA**TGCTAATCACGACACAGACG 3′
i1e2i2-1	5′ GC**TCTAGA**TGAAAGAAGGAAATCCTAAGTC 3′
i1e2i2-2	5′ TGCA**CTGCAG**GTTTTAGGACCAGGATGAGC 3′
i2e3i3-1	5′ TGCA**CTGCAG**TTCTGACTGTACTTTTGTTAGC 3′
i2e3i3-2	5′ CC**CTCGAG**GTCTCGATCAAGGATGTCTG 3′
i3e4-1	5′ CC**CTCGAG**AATCAATACGATACTGAGCATC 3′
i3e4-2	5′ GG**GGTACC**TAAATTCAATCCATCGCATTCG 3′
pGexdsxC	5′ G**GAATTC**GAATGGTCTCTAATGATATATCTG 3′
PGexdsxC2	5′CC**AAGCTT**GTCAGCCAGGCTTATAATGAGTATCC3′
pGexdsxF	5′CC**AAGCTT**GGTCCGTTCCACAATTGACATG 3′
pGexdsxM	5′ CC**AAGCTT**GTGTACTTCTAGGTAAAACGCAG 3′
DsxSo1	5′ TATGCTCATCATCTCACAATAG 3′
dsxMSo	5′ TGTAACTATAGTTGTAGAGTGC3′
dsxFSo	5′ CAACAACCTCAACATATACGAC 3′
rp49.1	5’ ATCCGCCACCAGTCGGATC 3’
rp49.2	5’ TGGCGCGCTCGACAATCTC 3’
rpl10.1	5’ GAGTGCATCCATTCCACGT 3’
rpl10.2	5’ GTATTTGACGTTGCAACCGT 3’
yp2Dm1	5’ GTCGTTGAGGCCACCATGC 3’
yp2Dm2	5’ GGAGTGGTTCGCTCGCATG 3’
M13 Forward	5’ GTAAAACGACGGCCAG 3’
M13 Reverse	5’ CAGGAAACAGCTATGAC 3’
Oligo d(T)	5’ TGC CAC GCT CGA CTA GTA CGT_{N22} 3’
T30	5’ T_{N30} 3’
AnchorT	5’ GCCACGCGTCGACTAGTACGT_{N22} 3’
Anchor	5’ GCCACGCGTCGACTAGTACG 3’

The highlighted black bases indicate the restriction sites used in the construction of the *DScmg* mini-gen and GST fusion proteins of *S. coprophila*.

Table S2 Insect species and GenBank accession numbers for the DSX sequences analyzed in the present work.

DSX	Taxa	Protein Acc#	
		Female-specific	***Male-specific***
DIPTERA			
Calliphoridae			
1.	*Lucilia cuprina*	ADG37649	ADG37648
Culicidae			
2.	*Aedes aegypti*	ABD96571	ABD96573
3.	*Anopheles gambiae*	AAX48939	AAX48940
Drosophilidae			
4.	*Drosophila erecta*	XP_001979242	XP_001979242
5.	*Drosophila melanogaster*	NP_731198	NP_731197
6.	*Drosophila persimilis*	XP_002013146	XP_002013146
7.	*Drosophila pseudoobscura*	XP_003736648	XP_001359020
8.	*Drosophila sechellia*	XP_002038750	XP_002038750
9.	*Drosophila simulans*	XP_002102542	XP_002102542
10.	*Drosophila yakuba*	XP_002086778	XP_002086778
Muscidae			
11.	*Musca domestica*	AAR23812	AAR23813
Phoridae			
12.	*Megaselia scalaris*	AF283695_1	AF283696_1
Sciaridae			
13.	*Sciara coprophila*	HG934386	HG934387
Tephritidae			
14.	*Anastrepha obliqua*	AAY25166	AAY25167
15.	*Bactrocera oleae*	CAD67986	CAD67987
16.	*Ceratitis capitata*	AAN63598	AAN63597
HYMENOPTERA			
Apidae			
17.	*Apis mellifera*	ABV55180	ABV55178
Pteromalidae			
18.	*Nasonia vitripennis*	ACJ65508	ACJ65511
LEPIDOPTERA			
Bombycidae			
19.	*Bombyx mori*	BAB13471	BAB13472
Saturniidae			
20.	*Antheraea assama*	ADL40848	ADL40846
COLEOPTERA			
Tenebrionidae			
21.	*Tribolium castaneum*	AFQ62106	AFQ62105
DIPLOSTRACA			
Daphniidae			
22.	*Daphnia magna*	BAJ78307	BAJ78307

Table S3 Comparison of DSX proteins in insects: nº of amino acids (degree of similarity = identical plus conserved amino acids)

Species	DSXF	DSXM	N-terminal region	OD1 domain	OD1-OD2 Intermediate region	OD2 domain	C-terminal region DSXF	C-terminal region DSXM
Sciara	273	221	41	46	111	61	14	23
Anophelex	241 (0,43)	283 (0,29)	37 (0,21)	46 (0,78)	82 (0,23)	61 (0,50)	15 (0,42)	72 (0,16)
Aedex	278 (0,38)	548 (0,33)	34 (0,17)	46 (0,78)	123 (0,24)	60 (0,50)	15 (0,42)	300 (0,12)
Ceratitis	315 (0,44)	394 (0,38)	40 (0,42)	46 (0,80)	152 (0,22)	62 (0,54)	15 (0,57)	109 (0,12)
Bactrocera	321 (0,45)	400 (0,38)	40 (0,42)	46 (0,80)	158 (0,23)	62 (0,54)	15 (0,64)	109 (0,12)
Anastrepha	317 (0,45)	396 (0,38)	40 (0,42)	46 (0,80)	154 (0,22)	62 (0,54)	15 (0,64)	109 (0,08)
Drosophila	427 (0,43)	549 (0,38)	40 (0,40)	46 (0,80)	264 (0,24)	62 (0,50)	15 (0,64)	152 (0,12)
Musca	397 (0,46)	527 (0,39)	42 (0,41)	46 (0,82)	232 (0,28)	62 (0,50)	15 (0,57)	160 (0,08)
Lucilia	396 (0,44)	532 (0,38)	42 (0,41)	46 (0,80)	231 (0,25)	62 (0,50)	15 (0,57)	166 (0,08)
Megaselia	310 (0,41)	573 (0,40)	37 (0,37)	46 (0,80)	151 (0,25)	61 (0,47)	15 (0,50)	293 (0,20)
Bombyx	264 (0,39)	266 (0,33)	31 (0,16)	46 (0,78)	91 (0,35)	62 (0,49)	34 (0,42)	51 (0,04)
Antheraea	265 (0,43)	279 (0,33)	31 (0,06)	46 (0,80)	92 (0,35)	62 (0,54)	34 (0,42)	63 (0,16)
Apis	277 (0,24)	336 (0,23)	59 (0,14)	45 (0,60)	101 (0,14)	49 (0,32)	23 (0,07)	87 (0,12)
Nasonia	235 (0,23)	224 (0,18)	30 (0,16)	45 (0,53)	100 (0,17)	47 (0,23)	13 (0,07)	5 (-)
Tribolium	227(0,44)	322(0,30)	25(0,24)	46(0,76)	84(0,29)	61(0,52)	11(0,36)	125(0,27)