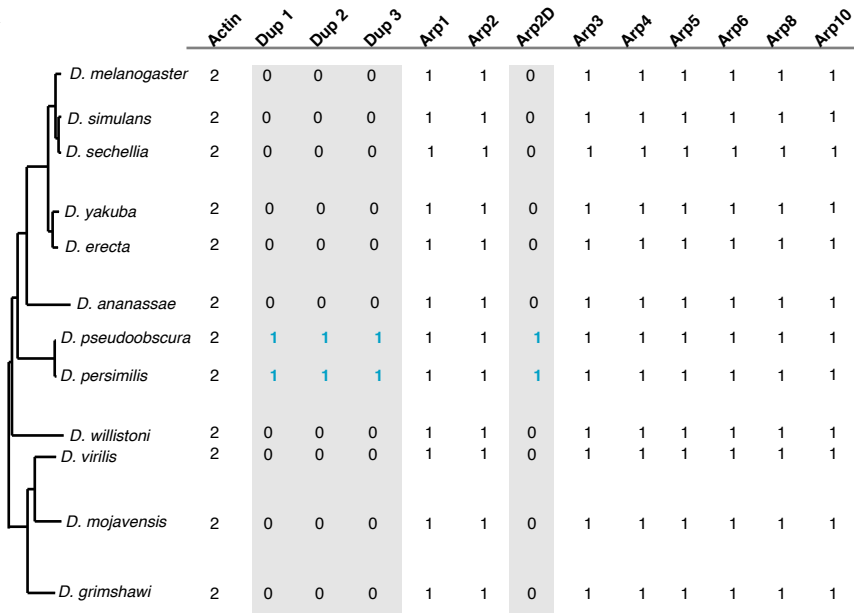


Supplemental Figure S1: Sequenced *Drosophila* species encode canonical Arps and lineage-specific Arps.

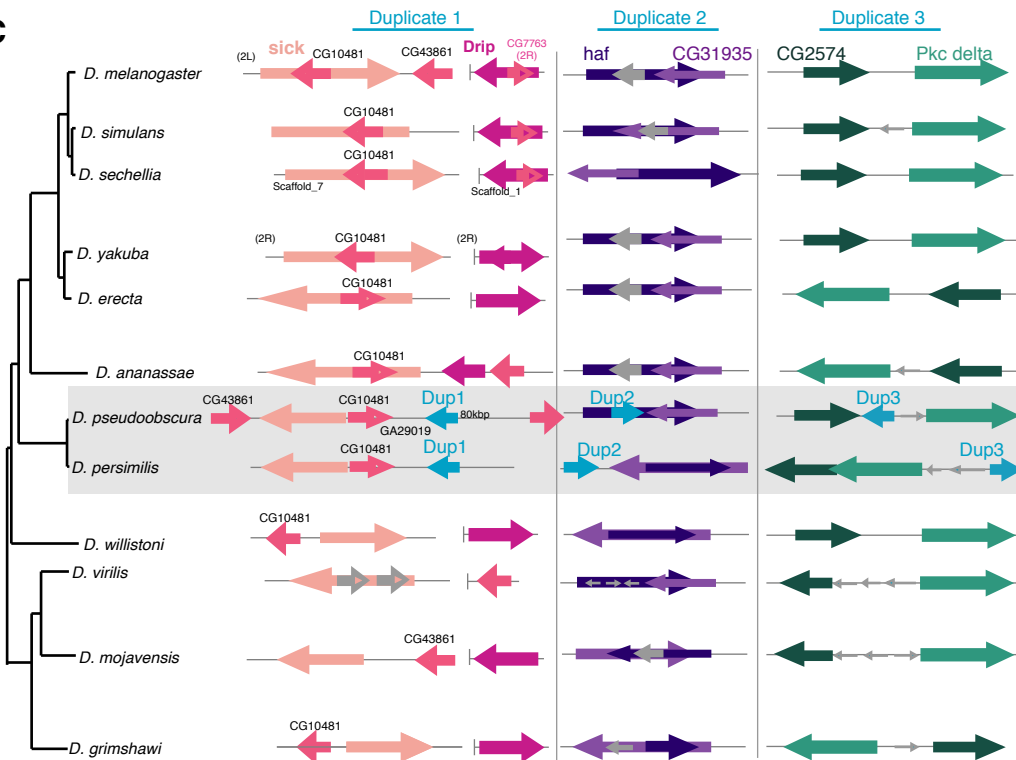
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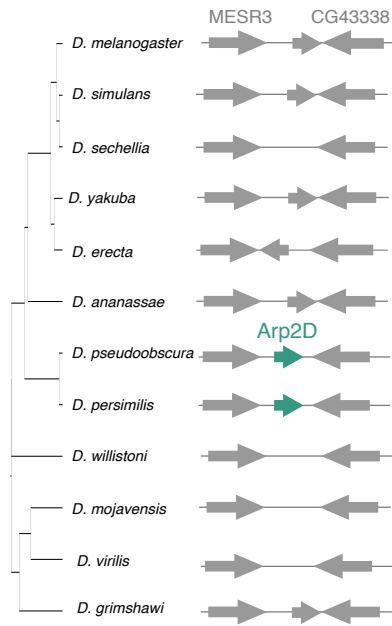
B

	Gene	Chromosomal location	
		<i>D. mel</i>	<i>D. pse</i>
Actin	Act5C	X	XL_group1e
	Act42A	2R	3
	Act57B	2R	3
Muscle Actin	Act87E	3R	2
	Act88F	3R	2
	Act79B	3L	XR_grp8
Duplicates	Arp2	X	XL_grp1e
	Dup 1	NA	4_grp4
	Dup 2	NA	4_grp3
	Dup 3	NA	XL_grp1a
	Arp2D	NA	4_grp3

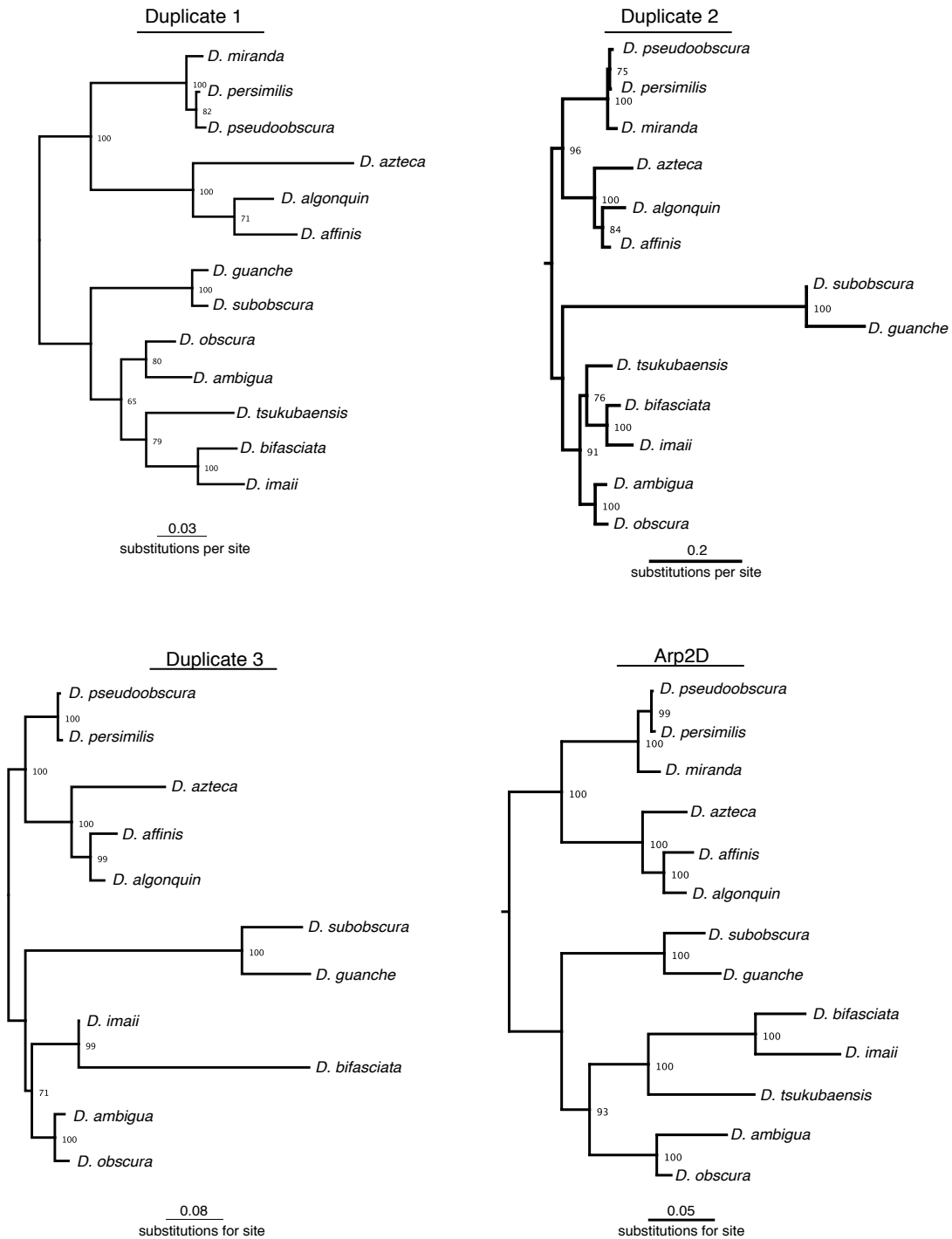
C



Supplemental Figure S2: Arp2D is present in only the obscure clade.



Supplemental Figure S3: Nucleotide trees including pseudogenized genes recapitulate overall species tree topology



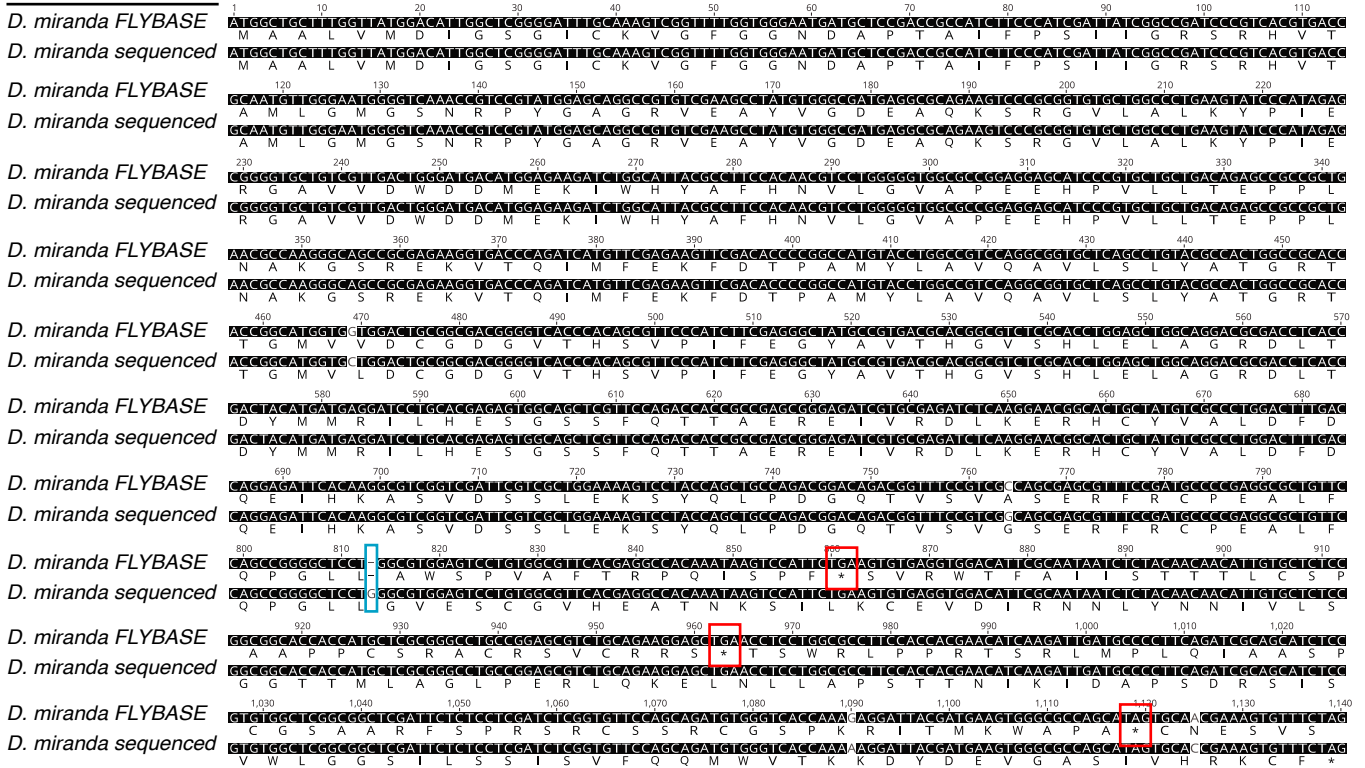
Supplemental Figure S4: A single base-pair deletion leads to pseudogenization of Arp2D in *D. subobscura*

D. pseu. Arp2D ATGGATACCAAGGGTCGGCACATAATTTGTGTGATAATGGCACAGGATCTGTAAGTGGGACTTCCGGCAGCAATTTGCCCGGCACATCTTCCCTCGATAGTGGGGCTCCTATTCTC
D. subobs. Arp2D M D T K G R H I I V C D N G T G S V K C G L A G S N L P A H I F P S I V G R P I L
 130 140 150 160 170 180 190 200 210 220 230 240
D. pseu. Arp2D CGGGCGATGAATCTTGCATGCGAATGGCGTGCAGATGGATGATGTGATGGTGGCGATGAGGCACCTCAAGCTGCGGCTATTGCTAGCGGTTCCCATCCCATGGAGAATGGTATAATCCGT
D. subobs. Arp2D R A M N T C D A N G V Q M D D V M V G D E A L K L R S L L A V S H P M E N G I I R
 250 260 270 280 290 300 310 320 330 340 350 360
D. pseu. Arp2D AACTGGGAGGACATGTGCCATGTGGACTATACATTTGGCCCAAGAAGATGAACATCGAACCGCAACTCGAAGATACTGCTGCCGAGCCGCCAATGATCCCCACGAGGTATCGGGAG
D. subobs. Arp2D AACTGGGAGGACATGTGCCAGTCCGGACTACATTTGGCCCAAGAAGTGGCCATTGAGCCGCAAAAATCAAAGATACTGCTGCCGAGGTGCCCAATGATGGTGGCAAAAGATCGGGAG
 370 380 390 400 410 420 430 440 450 460 470 480 490
D. pseu. Arp2D AAGATGATGGAGGTGATGTTGAGCATTACGGCTTTGATGCCACTGTTGGCCCTACAGGGCGGTGCTACGCTGTACGGCCAGGGTGTACTAGGGCGCGGTGATCGATCCCGGGATGGT
D. subobs. Arp2D X M W E V M F E H Y G F D A T Y L A S Q A V L T L Y G Q G L T T G A V I D A G D G
 500 510 520 530 540 550 560 570 580 590 600 610
D. pseu. Arp2D GTCAAGAACATTTGCTGCTACGAGGAGGCTGCCCTGCCACATTTGACGAAGCATTGAATGTTTGGCCCGTGTATACACGGCGCTGGTCAAGTTGCTTATGCAACGGCGGTACCGG
D. subobs. Arp2D ATGAGGCACATTTGCTCGTCTACGAGGAGGCTGCACCTGCCACATTTGACGAAGCATTGAATGTTTGGCCCGTGTATACACGGCGCTGGTCAAGTTGCTTATGCAACGGCGGTACCGG
 620 630 640 650 660 670 680 690 700 710 720 730
D. pseu. Arp2D TTGAATAATTCGGCGGACTTTGAAACGGTGCAGTGTGATGAGGAAAACCTGCTACGTTGGCTACGACATCGAGCAGGAGAAACGGCTGGCCGAGGACACACGACATGGTGGAGTCTAT
D. subobs. Arp2D L N S A D F E T V R L M K E K L C Y V G Y D I E Q E K R L A E D T T A L V E S Y
 740 750 760 770 780 790 800 810 820 830 840 850 860
D. pseu. Arp2D ACGTTGCCGATGGTGCAGTGTCAAGATCGGGCGGAGCGTTCGAGGCGCCGAGGCTTCTTCAACCAGCATTTGATCGATGTGAGGCGCGCGGCTTCTGAAATGGCCTTCAATGTG
D. subobs. Arp2D T L P D G R V I K I G G E R F E A P E A F F Q P H L I D V E A A G L S E M A F N V
 870 880 890 900 910 920 930 940 950 960 970 980
D. pseu. Arp2D ATTCAAGTCGGGACATCGACATACGTCGCGAGCTTCCGGCATACTGTTGTTGTTGGGGATCCACATGTTTCCGGGATTTCACGTCGACTGGAGAACGACTTGAAGAAATTTGTTCTTA
D. subobs. Arp2D I Q A A D I D I R P Q L F R H I V L S G G S T M F P G F P S R L E N D L K K L F L
 990 1,000 1,010 1,020 1,030 1,040 1,050 1,060 1,070 1,080 1,090 1,100
D. pseu. Arp2D GAGCGAGTGTCCAACCGGATCGCGAAAATATGCCAAATTTAAGATACGAATTAAGGATCCACCACACGCAAGAAATATGCTTCTCAATGGTGGCTTGTTCGGCAATTCACATAAGGAT
D. subobs. Arp2D E R V L Q R D A E N M P K F K I R I K D P P T R K N N V F N G G S V L A N V T K D
 1,110 1,120 1,130 1,140 1,150 1,160 1,170 1,180 1,190 1,200 1,210 1,221
D. pseu. Arp2D CGTGATGACTTTTGGATGCTCAAAGAGGAGTACGAGGAGCAGGGTCTTAAAGTGTGGACAGCTTAAAGCAGAAATCCAAAAGAGGAGAAATATGAAACAGATGAGGGGTAG
D. subobs. Arp2D R D D F W M S K E E Y E Q G L K V L D K L K Q K S K K K E Y E T D E G *
 CGGGAGAGTTTGGATGTCAAAGAGGAGTACGAGGAGCAGGGCTCAAAGTGTGGACAGCTTAAAGCAGAAATCCAAAAGAGGAGAACTAAGCTGGAT-----TGA
 G R S F G C P K R S T R S R A S K C W T S * K R V L P R R R K L S W I-----

Supplemental Figure S5: *D. miranda* encodes full-length Dup2 and Arp2D.

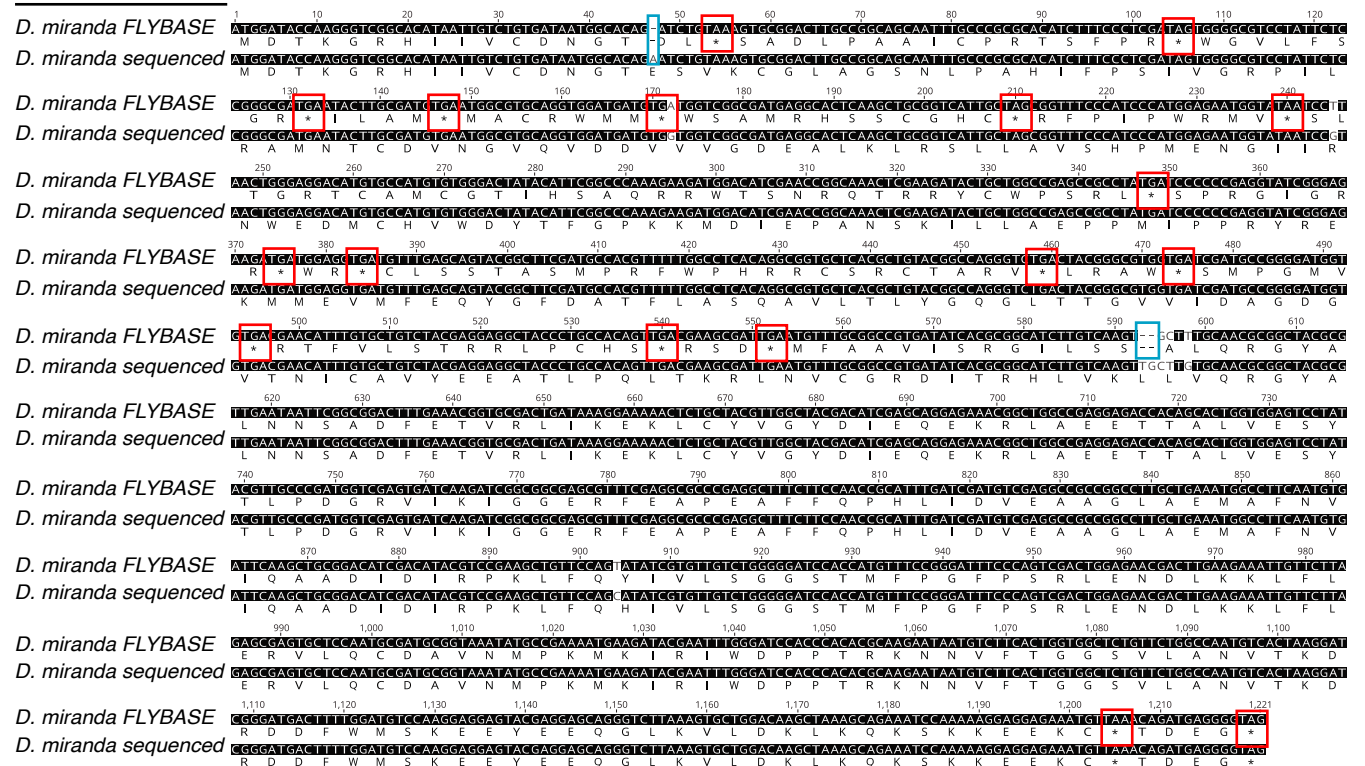
A

Dup2

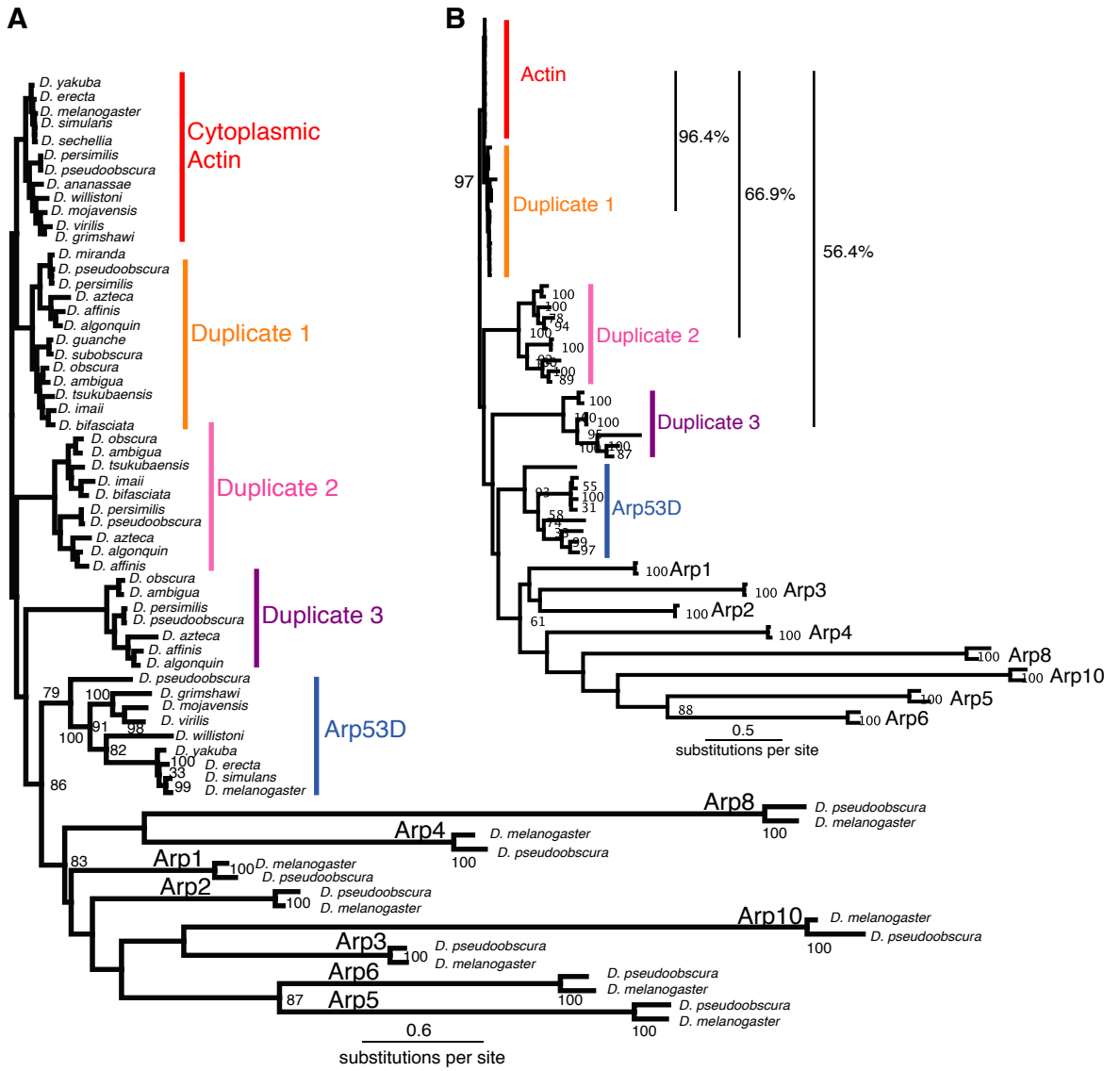


B

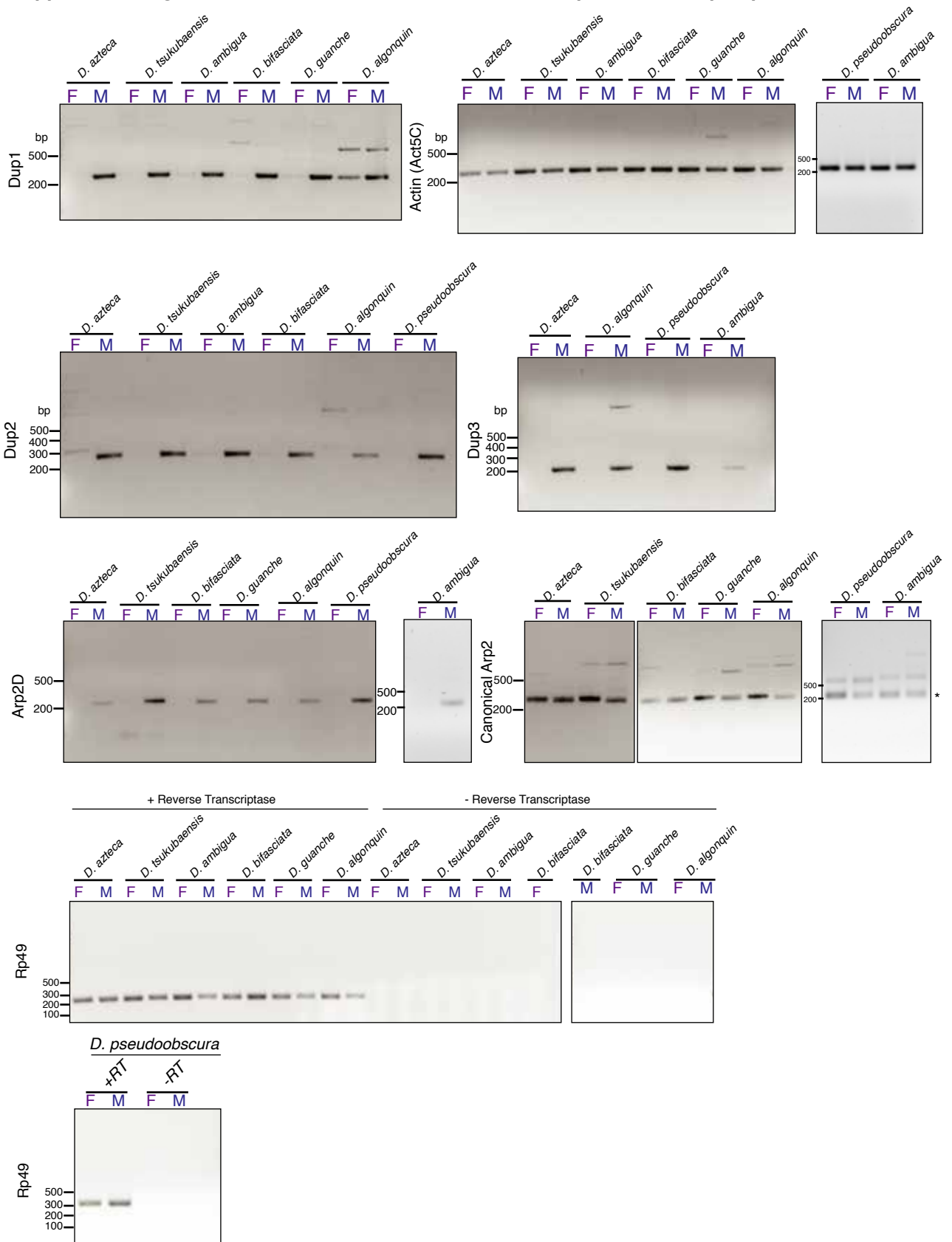
Arp2D



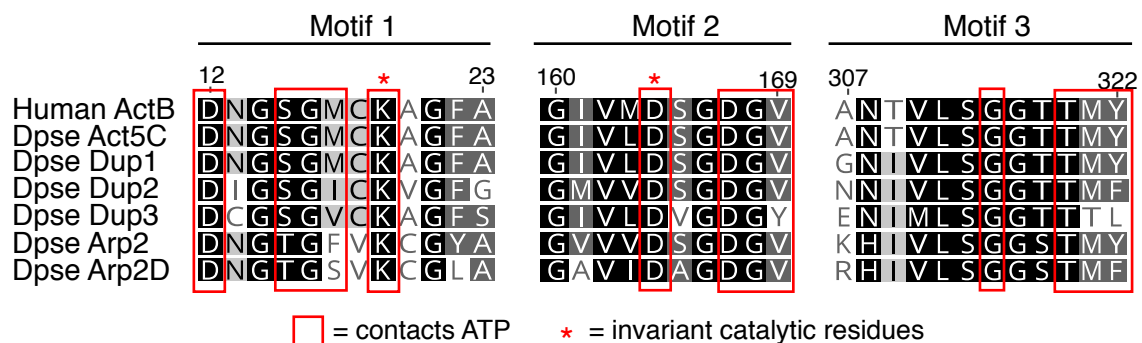
Supplementary Figure S6: Gene and protein trees of Duplicates 1-3 and canonical Arps



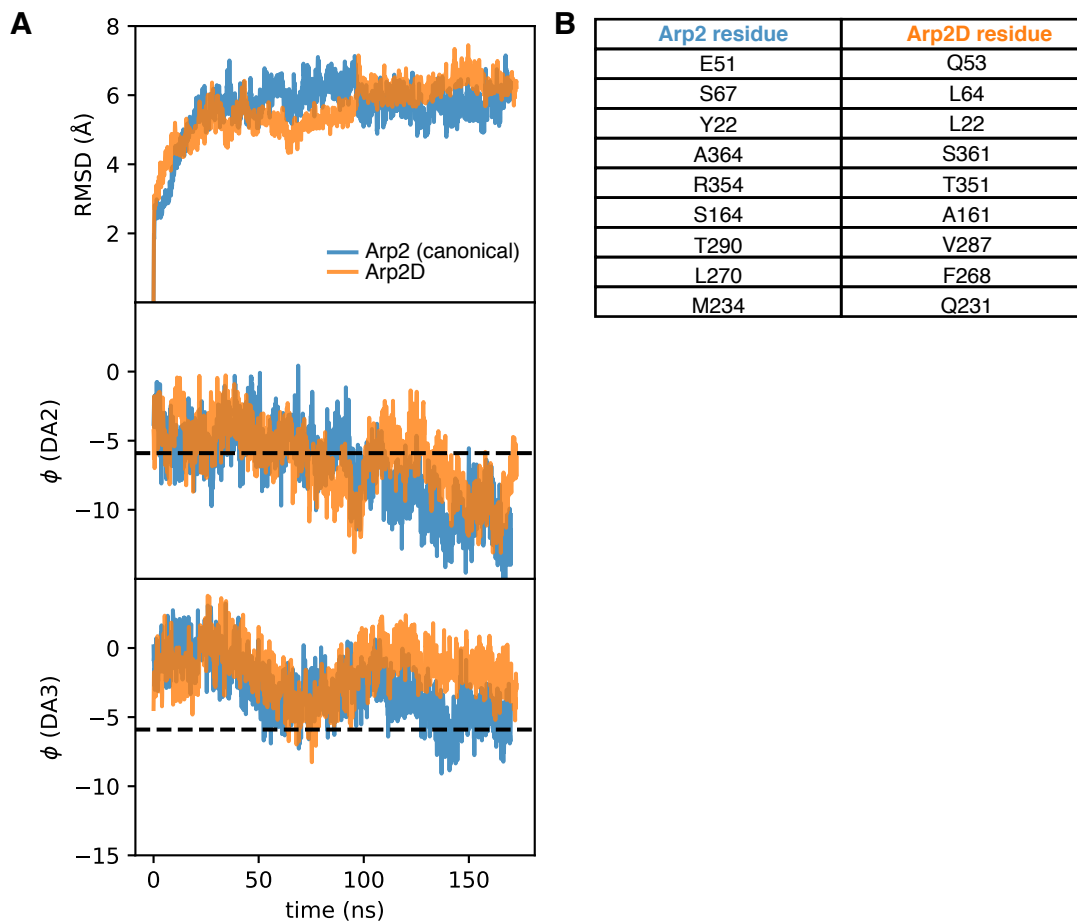
Supplemental Figure S7: RT-PCR indicates male-enriched expression of Arp duplicates



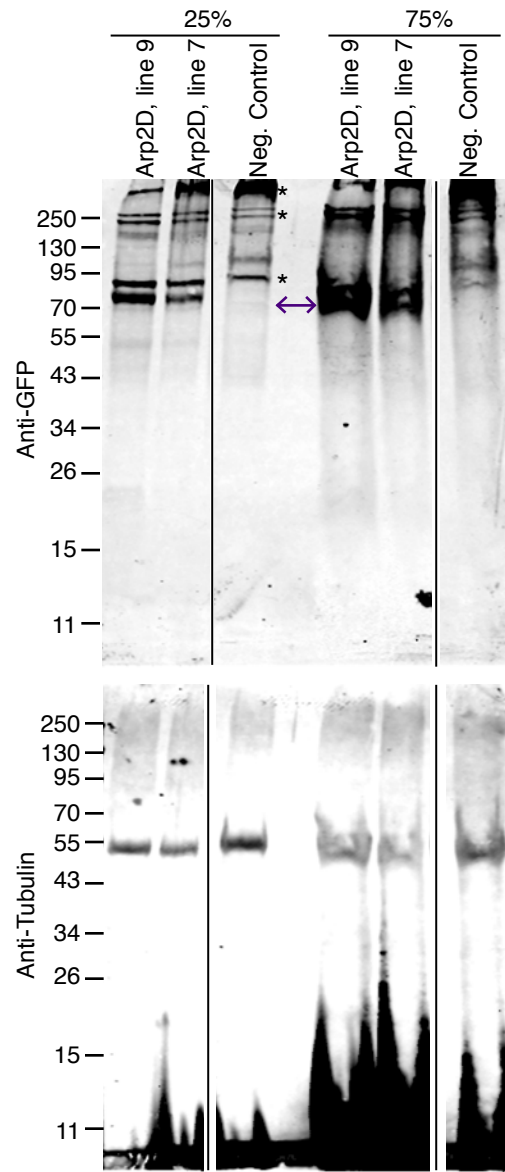
Supplemental Figure S8: The ATP-binding motifs are conserved in the *D. pseudoobscura* Arp duplicates



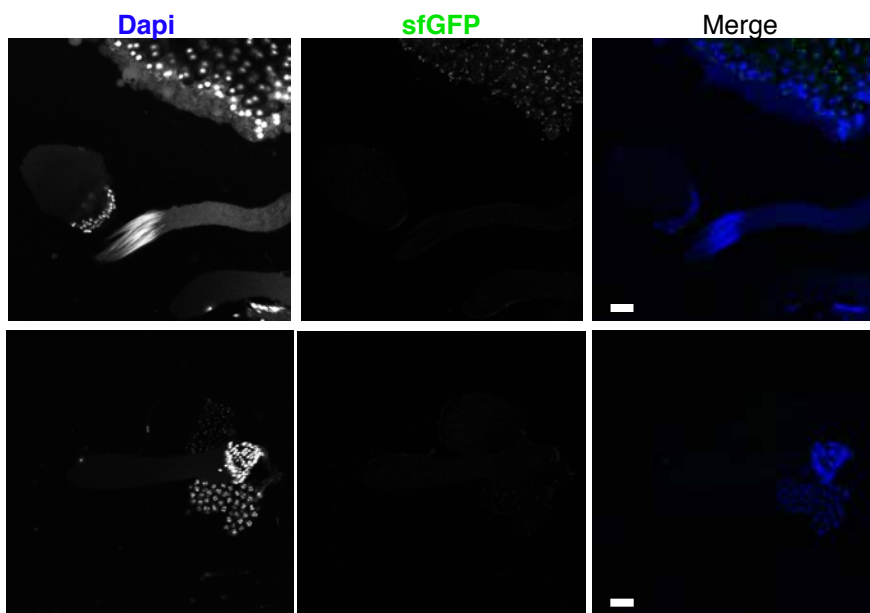
Supplemental Figure S9: Arp2D/3 exhibits a stable complex similar to Arp2/3 despite fixed residue changes



Supplemental Figure S10: Full-length Arp2D-sfGFP protein is expressed in the testis.



Supplemental Figure S11: *D. pseudoobscura* w- mature sperm exhibit autofluorescence but none in meiotic and post-meiotic cysts



Supplemental Figure S12: Arp2D localizes only to motile fan-like actin cones

