

QPGSQPIQVKPAQPGTTPGVVTGPDQKPSQVIVPPGGGSTPGTLPGGGKPVQVEPAKPGTTPGAIITGDRQVSKIIILPTGPGNAPQKPLGPGQTTQMIP
QPGSQPIQVKPAQPGTTPGVVTGPDQKPSQVIVPPGGGSTPGTLPGGGKPVQVEPAKPGTTPGAIITGDRQVSKIIILPTGPGNAPQKPLGPGQTTQMIP
QPGSQPIQVKPAQPGTTPGVVTGPDQKPSQVIVPPGGGSTPGTLPGGGKPVQVEPAKPGTTPGAIITGPDREVSKIIILPSSPGNAPQPMEPGVPQQPGQL

CTR TTPGASQEPGQPTGPGGAPQQPGQPMGGGSPQQPGQPLGPGNAPMKPGQTPKPVAPVSPQVVPQGGGLPIEILPAMPGTTPGAIITGPDNKISQIVLPPG
GGSTPGSVPGGRRPIYIEPGKPGNTPGAVTGDHQTIVLDPQATANAPLPGGSSLVGPPGGTTAGYFSPQTVTPAPNAAAYNQNPYNIGLGFGL
PNNQNLFQYLPNFETIFRQIVNRGNTRIPSLIQVMQSKGQSPDFTDLREFIRNLSAVFGVMRYENSNTYIEMYMELMMETLIGALEIIRVSDPSCLKP
TTLGGIPKYVNAFYDILI

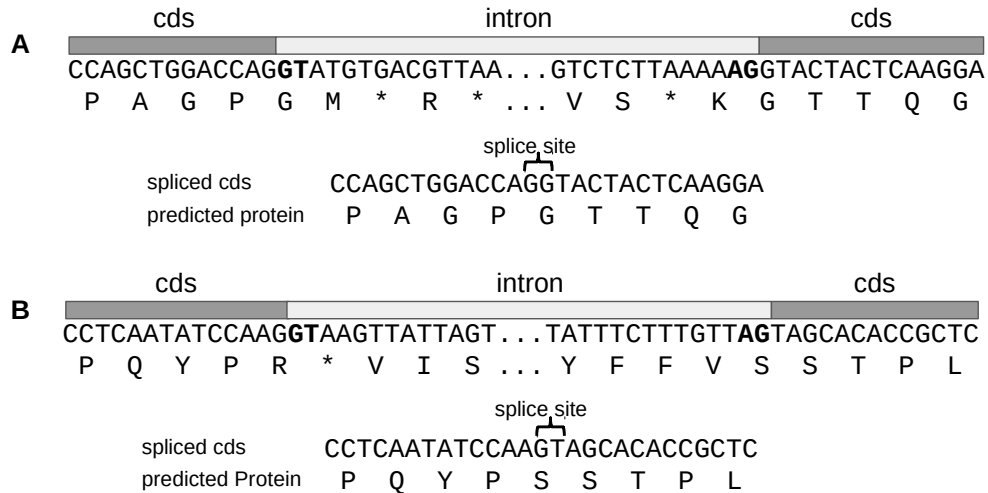


Figure S3: Putative splice sites for *A. trifasciata* AgSp1 (A) and AgSp2 (B) exons. Introns are capped with standard GT and AG dinucleotides (bold).

Ncl Motif 2 GPGGKPINIVPAGPTTPTGTVT GPDGKPSKFVVPDGAFTTPGSIP GPDGKPLVPEPAGPTTTPGLQT GPDGKPSKLVVP TTTKGPGPMGPGGPMGPGGPMGPGGPMGPGGQTTTTTPAPVK
Ncl Motif 3 GPDGKPLQIVPAGPTTPTGTVT GPDGKPSKFVVPKGAFTTPGSIP GPDGKPLVPEPAGPTTPGLET GPDGKPSKLVVP TTPKRPRGPGGSPGPGTQFQTGTTPTPVP
Atr Motif 1 GPDGKPLIEPAGPTTPTGTVT GPDGKPKFVLPKGAFTTPGSIP GPDGKPIHVQAPAGPTTPGAQT GPDGKINKLVVP TTTTPKGVPVGGMPLSPYSPQGGQPMYFPGGSPYGPGEQTTTTPIIP
Aau Motif 1 GPDGKPLQIEPAGPTTPTGTVT GPDGKPKFVVPKGAFTTPGSIP GPDGKPIHVQAPAGPTTPGAET GPDGKINKIVVP TTTTPKGPVGGGIPLSPIYPQGGQPMYFPGGSPYGPGEQTTTTPIIP
Pte Motif 2 GPGGRPIQLIPSRPGTTPGTVT GPDGYPIIILPSGSGTTPGSFE GPDGQPIKVVVPGTTPGAET RPDGSIKIIIFIP QSGTSPSGSTPSQVT
Pte Motif 3 GPGGNPIQLIPQGPPTPGAVT GPDGTIIYIILPKGSGTTPGSVE GPGGKPIKILPAKPTTPGAET GPDGTIEIIYIP QYPTTRRDGSTPSQIT
Sgr Motif 2 GPGGKPIQLVPSGPGSTPGVVT GPDGRPIKIFILPSGSGTTPGSIE GPNGKPIKIMPAGPTTPGAKT GPDGVSIIYVP SGPSGQTPGSRQPPGNTPSQIT
Lhe Motif 2 GPSGKPIQLVPSGPGSTPGVVT GPDGSPIKFILPKGSGTTPGSFE GPNQPIKVMVPGSTPGAKT GPDGVSIIYVP SQPSGQTPGSGGGSTPSQIT
Lge Motif 2 GPSGKPIQLVPSGPGSTPGVVT GPDGSPIRFILPKGSGTTPGSFE GPNQPIKVVVPGSTPGAKT GPDGVSIIYVP QQPSGQTPGSEGGSTPSQIT

Figure S4: Additional AgSp1 repeat motifs from orb weaving and cobweb weaving species.

A MGCSAVAILALCLISINVFQFAQEDTDPDFNETNEEFGRIFVNLLIESGVFGQTDDKDFNAVTESELLNAIQMLSKGQNPVSTKKTFFMMFASSLAELIVQESDNALTL
VEKTRAVTDAMRKAYMOTSGNPNALIQKVDVFLVGIIFLDVQQNAPEYDYEEYDTVIFEDEMLPLPEIIEETLPNIDPLPGHPEMPEVTSVDVIIGPGNDMDEPETFIGEVD
KMIKLEQDAFPNAPPEEQIQQIMLPGMSQPGEDDQKIVIPDSFPLESTLISEEEFPIPSVPLISLPKPFDPSPFMPRIEPIDEAPEIMDPFPTAISPKLEEPIREETT
IIQETPSTEPEPLDEQVILNAEPQISPTKDLIGPFMGPQLDMLNQPQIYPTYYPMTQNNMPEKAGNQDPYSNPALQNLMKKFNPSYLFGSPYQNLNEIEQTFNVVLRN
GETYIPAIMSAIIESRKTAPQTLDSLILVKKLSALFSDMTVENFYLSRTEVFIELEIETLISSLEIIRMADESCLNKVSAITSPYKYVNAFNDILF

B MGCSAVAILALCLISINVFQFAQEDTDPDFNETNEEFKLVNLLIESGVFGQTDDKDFNAVTESELLNAIKMLTKGQNPASTKKTFFMMFASSLAELIVQESDNALSVE
KTRAVTDAMRKAYMOTSGNPNALIQKVDVFLVGIIFLDVQQSAEDYEEYDTVIFEDEMLPLEIVGETLPNMDPSGHPDIVMESPEVTPGIVMGPNDIKEIVLPQSIESHM
DEPETYIGEVDKMIKFEQDAPSGNAPPEEQIQQIMLPGMSQPGEDNDEGDAQIIPDSSPLEEIPISAPVPIPSLNPFNVAFPPIPNLEPIDAALTPILDPFMPLITPAI
SPPKLEEPPKSDSDENMQIQQIMMDTGEPGEPSEETKIVQEGSNSEPIPEEMMPLLDLIPEMNEPIEGDTIIEQKGTDEGSPGMQIQVMPKVPSPSSGEQVINLPEPQ
ISPTKDLIGPFMGPQVDILNQPQISPFGGFGYYPIMPSPMPVENVDQPFNSPFLQNLMKKFNPTYLFGSPYQIGLNALIEQTFNVVLRNGETYIPAIMSAIIESKKTAP
QTLDLRVLVNLKLSPLFSDMTVENFYLSRTEIFIELILEALISSLEIIRMADESCINKVSAITSPYKYVNAFNDILF

Figure S5: *L. geometricus* (A) and *L. hesperus* (B) AgSp2 predicted amino acid sequences.

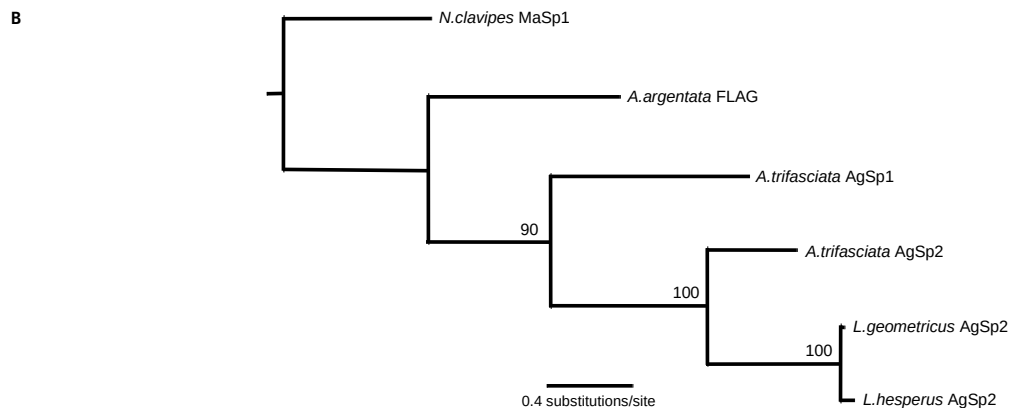
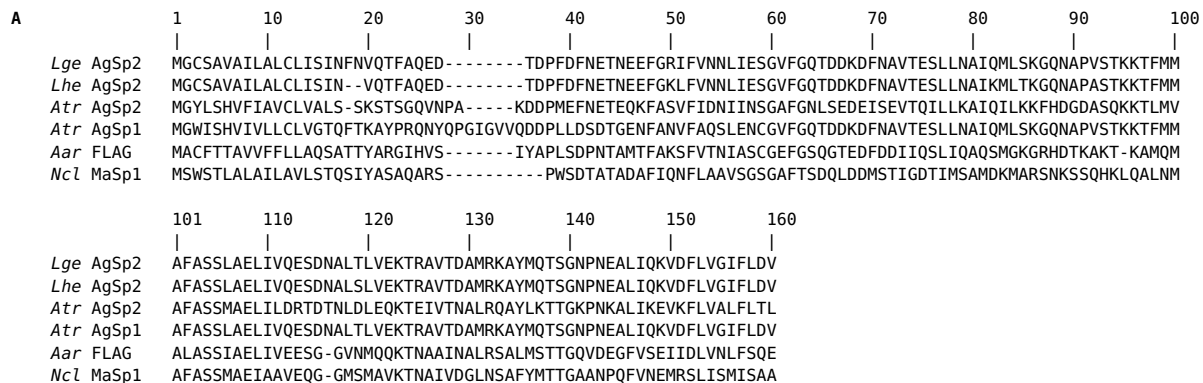


Figure S6: (A) Alignment of N-terminal encoding sequences from *L. geometricus* AgSp2, *L. hesperus* AgSp2, *A. trifasciata* AgSp2, *A. trifasciata* AgSp1, *A. argentata* FLAG, and *N. clavipes* MaSp1. (B) Maximum likelihood tree with bootstrap support (values >50 shown), rooted using N-terminal encoding *N. clavipes* MaSp1.

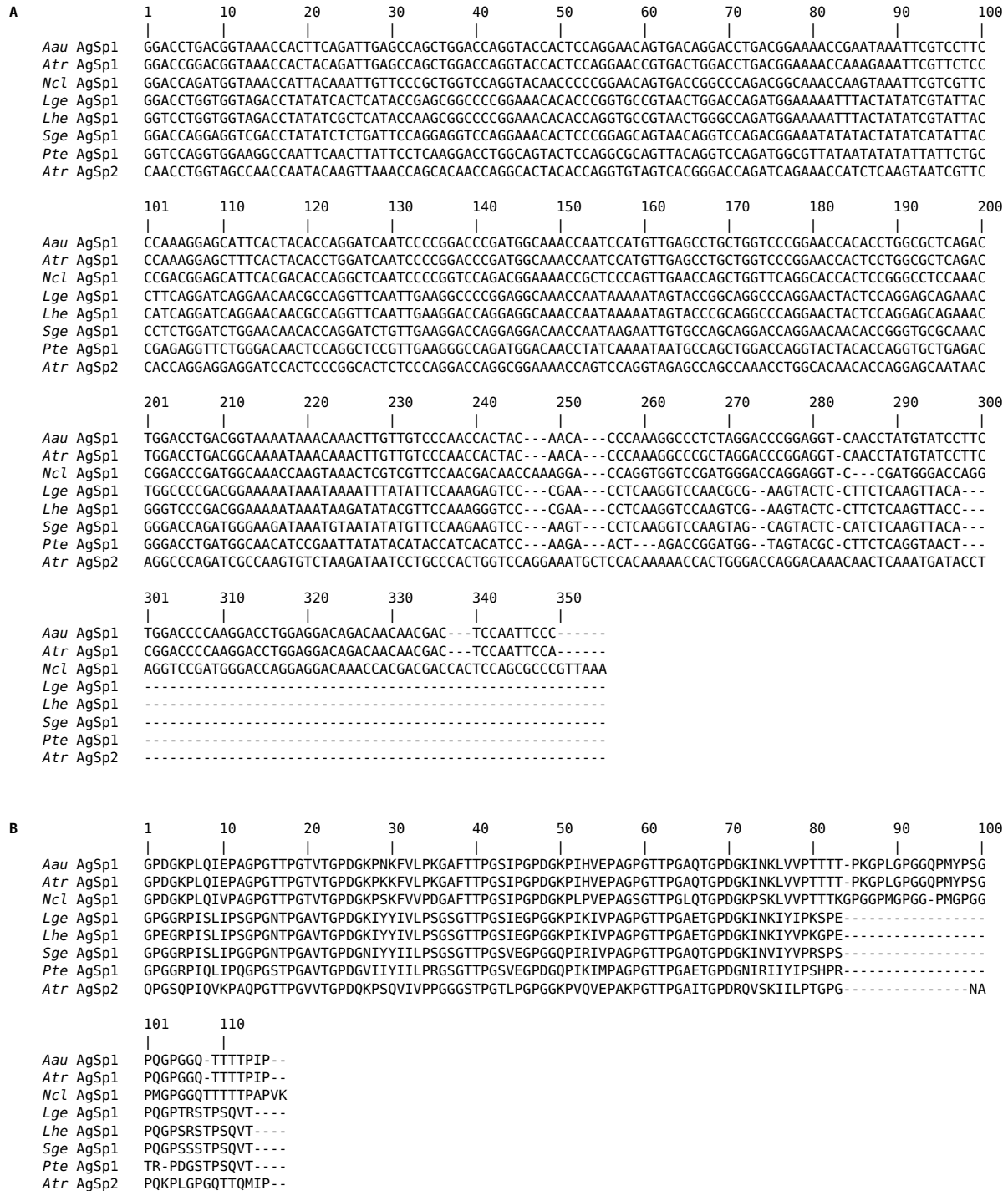


Figure S7: AgSp1 repeat motif nucleotide (A) and amino acid (B) alignments from three orb weaving and four cobweb weaving spider species and the AgSp2 repeat motif from *A. trifasciata*.

Table S1: Sequencing runs used to analyze AgSp sequences. *A. aurantia* and *A. trifasciata* data were generated for this study; other species data were accessed via NCBI. All samples were sequenced using the Illumina platform except AtriAg7-13, which were sequenced using the Oxford Nanopore MinION. Ag=aggregate, Ma=Major ampullate, Fb=Fat body, A=Anterior, P=Posterior.

Argiope trifasciata (Atr)

SRR7310653 (AtriAg1)
 SRR7310658 (AtriMa1)
 SRR7310659 (AtriFb1)
 SRR7310656 (AtriAg2)
 SRR7310657 (AtriMa2)
 SRR7310652 (AtriFb2)
 SRR7310647 (AtriAg3)
 SRR7310646 (AtriMa3)
 SRR7310649 (AtriFb3)
 SRR7310648 (AtriAg4)
 SRR7310655 (AtriAg5)
 SRR7310654 (AtriAg6)
 SRR7346453 (AtriAg7-9)
 SRR8167878(Atri_gDNA_10)
 SRR8167879(Atri_gDNA_11)
 SRR8167876(Atri_gDNA_12)
 SRR8167877(Atri_gDNA_13)

Argiope aurantia (Aau)

SRR7310651 (AaurAg1)
 SRR7310650 (AaurMa1)
 SRR7310667 (AaurFb1)
 SRR7310666 (AaurAg2)
 SRR7310665 (AaurMa2)
 SRR7310664 (AaurFb2)
 SRR7310663 (AaurAg3)
 SRR7310662 (AaurMa3)
 SRR7310661 (AaurFb3)
 SRR7310660 (AaurAg4)
 SRR7310669 (AaurAg5)
 SRR7310668 (AaurAg6)

Nephila clavipes (Ncl)

SRR5139355 (silk gland pair 03)
 SRR5139343 (silk gland pair 04)
 SRR5139327 (silk gland pair 05)

Latrodectus hesperus (Lhe)

SRR5285106 (AgA1)
 SRR5285107 (AgA2)
 SRR5285102 (AgP1)
 SRR5285103 (AgP2)

Latrodectus geometricus (Lge)

SRR5285086 (AgA1)
 SRR5285087 (AgA2)
 SRR5285082 (AgP1)
 SRR5285083 (AgP2)

Steatoda grossa (Sgr)

SRR5285128 (AgA1)
 SRR5285125 (AgP1)