

Supplementary Table S1. Abbreviations and accession numbers used in Figure 4 and Figure 5. All accession numbers from NCBI.

Abbreviation	Full name	N-term accession	C-term accession	Repeat unit
Aa AcSp1 or Aar AcSp1	<i>Argiope argentata</i> aciniform spidroin 1	AHK09813.1		
Av AcSp1	<i>Araneus ventricosus</i> aciniform spidroin 1	AUH99620.1		
At AcSp1	<i>Argiope trifasciata</i> aciniform spidroin 1	AHK09776.1	AAR83925.1	ADN39425.1
Ad AcSp1	<i>Araneus diadematus</i> aciniform spidroin 1	AWK58687.1	AWK58698.1	
Sg AcSp1	<i>Steatoda grossa</i> aciniform spidroin 1	AWK58693.1		
Pt AcSp1	<i>Parasteatoda tepidariorum</i> aciniform spidroin 1	AWK58692.1		
Pb AcSp1	<i>Parawixia bistriata</i> aciniform spidroin 1		ADG57593.1	
Lh AcSp1	<i>Latrodectus hesperus</i> aciniform spidroin 1	AFX83557.1		
Lh TuSp1	<i>Latrodectus hesperus</i> tubuliform spidroin 1	ABD24296.1	AAZ28931.1	
Ab TuSp1	<i>Argiope bruennichi</i> tubuliform spidroin 1	BAE86855.1		
Av TuSp1	<i>Araneus ventricosus</i> tubuliform spidroin	ASO67373.1		

	1			
Cm TuSp1	<i>Cyrtophora moluccensis</i> tubuliform spidroin 1		AAV28944.1	
Ad TuSp1	<i>Araneus diadematus</i> tubuliform spidroin 1	AWK58641.1	AWK58742.1	
Nc TuSp1	<i>Nephila clavipes</i> tubuliform spidroin 1	PRD35275.1		
Nc Flag	<i>Nephila clavipes</i> flagelliform spidroin	AAC38846.1	AAC38847.1	
Ag TuSp1	<i>Araneus gemmoide</i> tubuliform spidroin 1		AAX45294.1	
Nim Flag	<i>Nephila inaurata madagascariensis</i> flagelliform spidroin	AAF36091.1	AAF36092.1	
Av MiSp	<i>Araneus ventricosus</i> minor ampullate spidroin	AFV31615.1		
Mg MiSp	<i>Metepeira grandiosa</i> minor ampullate spidroin	ADM14328.1	ADM14320.1	
St MiSp	<i>Steatoda grossa</i> minor ampullate spidroin	ARA91200.1	ARA91183.1	
LhMiSp	<i>Latrodecus hesperus</i> minor	ARA91152.1		

	ampullate spidroin			
Lh MaSp1	<i>Latrodecus hesperus</i> major ampullate spidroin 1	ABR68856.1		
Lh MaSp2	<i>Latrodecus hesperus</i> major ampullate spidroin 2	ABR68855.1		
Nc MaSp1	<i>Nephila clavipes</i> major ampullate spidroin 1	ACF19412.1	AAT75312.1	
Ab MaSp2	<i>Argiope bruennichi</i> major ampullate spidroin 2	AFN54363.1		
Pt PySp1	<i>Parasteatoda tepidariorum</i> pyriform spidroin 1	XP_015910086.1	XP_015910080.1	
Nc PySp1 or Ncl PySp1	<i>Nephila clavipes</i> pyriform spidroin 1	AWK58660.1	ADN39427.1	
Aa PySp1	<i>Argiope argentata</i> pyriform spidroin 1	AQR58363.1		
Av PySp1	<i>Araneus ventricosus</i> pyriform spidroin 1	MH376748		
Sg TuSp1	<i>Steatoda grossa</i> tubuliform spidroin 1	AWK58693.1		
Ag PySp1	<i>Araneus gemmoides</i> pyriform spidroin 1			AEP25627.1
Lh PySp1	<i>Latrodecus hesperus</i> pyriform spidroin 1	AWK58659.1	ACV41934.1	

Ncr PySp1	<i>Nephila cruentata</i> pyriform spidroin 1		ADK56477.1	
Bc fib1	<i>Bothriocyrtum californicum</i> fibroin 1	HM752562	EU117162	

Supplementary Table S2. The percent identity of PySp1 repetitive units among five spider species in Figure 5B.

Species	Percent of identity
<i>Araneus ventricosus</i> and <i>Argiope argentata</i>	64%
<i>Araneus ventricosus</i> and <i>Araneus gemmoides</i>	85%
<i>Araneus ventricosus</i> and <i>Nephila clavipes</i>	50%
<i>Araneus ventricosus</i> and <i>Argiope trifasciata</i>	53%
<i>Argiope argentata</i> and <i>Nephila clavipes</i>	55%
<i>Argiope argentata</i> and <i>Araneus gemmoides</i>	64%
<i>Argiope argentata</i> and <i>Argiope trifasciata</i>	80%
<i>Nephila clavipes</i> and <i>Araneus gemmoides</i>	55%
<i>Nephila clavipes</i> and <i>Argiope trifasciata</i>	47%
<i>Araneus gemmoides</i> and <i>Argiope trifasciata</i>	57%

Pt	FySp1	MY-WDFPALL-----LGALV-----ATTESAAISKALFOEGLAFYFIDRFVNYLRKCDVLDKREKFEESLNGIGRLYRK-----KAKPSRIKHMVTKIAEISEQFEAG-N	120
Nc	FySp1	MS-WLP-----VLSVFLVFL-----PQINALSASKSMFQDEGTTLYLMEYDNLKDCIVRFEQDAIINSLFESGLYRK-----NAKPSRIKHSMAKADIYIEGLEGGD	120
Aa	FySp1	MS-WL-----SLSVLYFLA-----PQINALSASKSMFQDEGTTLYLMEYDNLKDCIVRFEQDAIINSLFESGLYRK-----NAKPSRIKHSMAKADIYIEGLEGGD	120
Av	FySp1	MS-WLGLQFLFTFLVFA-----WRGALSDPSRWQDEATAVFLERTLVLGCGVLDKQERDAIDTDFQSSWFGQ-----NAKPSRIKHSMAKADIYIEGLEGGD	120
Lh	TuSp1	MY-WLSTLSTLVA-----LGLTFLA-----KSLSGVSAVNFVPSMAFSPFLNLSNLSFSPFQDEGTTLYLMEYDNLKDCIVRFEQDAIINSLFESGLYRK-----ASSAISTLSTALSSIAELVTEA--FE	120
Ab	TuSp1	MY-WLSTLSTLVA-----LGLTFLA-----KSLSGVSAVNFVPSMAFSPFLNLSNLSFSPFQDEGTTLYLMEYDNLKDCIVRFEQDAIINSLFESGLYRK-----ASSAISTLSTALSSIAELVTEA--FE	120
Nc	Frag	MACFTSAVITFLF--AQCAST--YG-----RGLVNSPFSNPNTAEAFARSPVSNVYSSGFGAGGEDFDIDTIGSLIQAG--SMGKGRHDTRAKAKAMVALASSIAELVTEA--GG	120
Nim	Flag	MACFTSAVITFLF--AQCAST--YG-----RGLVNSPFSNPNTAEAFARSPVSNVYSSGFGAGGEDFDIDTIGSLIQAG--SMGKGRHDTRAKAKAMVALASSIAELVTEA--GG	120
Be	F1B1	MACFTSAVITFLF--AQCAST--YG-----RGLVNSPFSNPNTAEAFARSPVSNVYSSGFGAGGEDFDIDTIGSLIQAG--SMGKGRHDTRAKAKAMVALASSIAELVTEA--GG	120
Lh	AcSp1	MN-WLSTLSTLFI--LAFVQVQVEGRGHHSSGSSSPANPAKANAFMQLIQK1STSPVFPQKEDDIEEIVTMSAFSSMSTGGSSNAKLAQMMAFASMAEIVAEADNDP	120
Av	AcSp1	MN-WLSTLSTLFI--LAFVQVQVEGRGHHSSGSSSPANPAKANAFMQLIQK1STSPVFPQKEDDIEEIVTMSAFSSMSTGGSSNAKLAQMMAFASMAEIVAEADNDP	120
Aar	AcSp1	MN-WLSTLSTLFI--LAFVQVQVEGRGHHSSGSSSPANPAKANAFMQLIQK1STSPVFPQKEDDIEEIVTMSAFSSMSTGGSSNAKLAQMMAFASMAEIVAEADNDP	120
At	AcSp1	????????????????VQDA-----VQAS-FISPSPIANPAKAGSLMVLNRLASSNVLVQKEREDESIMDTLMSLIGASAGKSSAAGLAQIMAVASSIAELVTEADNDP	120
Mg	MiSp	MQ-LFRLSFFL--VLFIQGL-----SQSPTITVNAAMTETNLVQASRSGTLTADQEDDMRQDVSNSQKMGFP--FKHRLRMYTAAHAEVAVT--SF--PQ	120
Mg	MiSp	?????RSLFPL--VLFIQGL-----AH-GHIGTPGAGKSVTGSIVQAGSGVSDQMDLGMADAVNRDLRPLN--APHRKGVITMMAIADAVN--SF--GQ	120
St	MiSp	MF-IPAGLSPLF--LIFSQ--FI-----FSDAASVWDTATAEAFISPSAMERGSVSSQVDEIAS1SDT11SALERP--NNSRKLQALMMAFASVSEIAPSE--GI	120
Lh	MiSp	MF-IPAGLSPLF--LIFSQ--FI-----FSDAASVWDTATAEAFISPSAMERGSVSSQVDEIAS1SDT11SALERP--NNSRKLQALMMAFASVSEIAPSE--GI	120
Lh	MaSp1	MT-WSTRALSLFLVLTQSLYA-----LAQANTPSSKANADAFINP1SAASNTGSPSQQEDDSLIGNTLMAAMDNGGR--ITPSKLAQDMFASSVAEIAASEG--D-	120
Lh	MaSp2	MN-WSTRALSLFLVLTQSLYA-----LAQANTPSSKANADAFINP1SAASNTGSPSQQEDDSLIGNTLMAAMDNGGR--ITPSKLAQDMFASSVAEIAASEG--D-	120
Nc	MaSp1	MT-WTRALSLFLVLTQSLYA-----QQ-NTFSSTELADAFINAFINEGRGTAFADQLDMIST1GDDT1KAMRQARSNSKGLQALMMAFASMAEIAAQQ--GL	120
Ab	MaSp2	MN-WSTRALSLFLVLTQSLYA-----VQAATPNSQLAEDFNSFLRF1AQGAFSPQLDDMS1GDTLTKATEKMGKSSKGLQALMMAFASMAEIAAQQ--GL	120
Pt	FySp1	DITFLDCAIQALAAFEECTGSDNDFIESVKEMTIVMNVNEVK-----QFVSAAKRRMAGMSNLSLSSIMGGKMV--FSAFNSLS--SVASQIRAGSSNLSKFEILVESLLETHS	240
Nc	FySp1	DITSYKLDVQAAMAAAMETAGVDNFIETIQELVVMNDYF-----QLTSAASQRISQLSNLSASAVNGRVN--HALNSLSL--SAASQIGG--SRLKTEVLETLETLA	240
Aa	FySp1	DITSYKLDVQAAMAAAMETAGVDNFIETIQELVVMNDYF-----QLTSAASQRISQLSNLSASAVNGRVN--HALNSLSL--SAASQIGG--SRLKTEVLETLETLA	240
Av	FySp1	DFSYLDIDVQAATAFAEATIGVDRDENFESVIELVLFNDEIEK-----QLMSPAANQR1AALSNSLRAAVGGQV--VGLNSLSL--SAASQIGG--SRLKTEVLETLETLA	240
Lh	TuSp1	DIDQDQVSLTSLSCQVETIGSPVPAFASKSLGLESQ-----LASTAATSRINDIAQSLSTLSSGGGLAFQVNFPLIQSS--IQSNPFLDQVFLIESLETYS	240
Ab	TuSp1	NYNSLSELIGLSDCFITGSDPAPVSRISQLSISVLSQADNIS-----LGSSAATRVSSLANSFASA1SSSGSGLS--VPTFLNLSVGAQVSSSSLSLEVNLEVLEIA	240
Nc	Frag	DIDQDQVSLTSLSCQVETIGSPVPAFASKSLGLESQ-----LASTAATSRINDIAQSLSTLSSGGGLAFQVNFPLIQSS--IQSNPFLDQVFLIESLETYS	240
Nim	Flag	DIDQDQVSLTSLSCQVETIGSPVPAFASKSLGLESQ-----LASTAATSRINDIAQSLSTLSSGGGLAFQVNFPLIQSS--IQSNPFLDQVFLIESLETYS	240
Be	F1B1	DIDQDQVSLTSLSCQVETIGSPVPAFASKSLGLESQ-----LASTAATSRINDIAQSLSTLSSGGGLAFQVNFPLIQSS--IQSNPFLDQVFLIESLETYS	240
Lh	AcSp1	STIEFQKVSALAAHATIGTGNVQFIREKRLTIFAREDETEN-----SRRVPMVNG1HSAMGGSGFN--YQFQGMLS--QYSQSGT--CNP--NNYVMDALL	240
Av	AcSp1	STIEFQKVSALAAHATIGTGNVQFIREKRLTIFAREDETEN-----SRRVPMVNG1HSAMGGSGFN--YQFQGMLS--QYSQSGT--CNP--NNYVMDALL	240
Aar	AcSp1	STIEFQKVSALAAHATIGTGNVQFIREKRLTIFAREDETEN-----SRRVPMVNG1HSAMGGSGFN--YQFQGMLS--QYSQSGT--CNP--NNYVMDALL	240
At	AcSp1	STIEFQKVSALAAHATIGTGNVQFIREKRLTIFAREDETEN-----SRRVPMVNG1HSAMGGSGFN--YQFQGMLS--QYSQSGT--CNP--NNYVMDALL	240
Mg	MiSp	S1AVYQMLGALGQCQVAVMGVDRFRNEINDLITMFAKAESENE-----2LRSASAAR1SQLTSSITNAVANGVD--ANLARSQ--SSFSALRS--SGMSSDAKIEVLETTIVG	240
St	MiSp	S1AVYQMLGALGQCQVAVMGVDRFRNEINDLITMFAKAESENE-----2LRSASAAR1SQLTSSITNAVANGVD--ANLARSQ--SSFSALRS--SGMSSDAKIEVLETTIVG	240
Lh	MiSp	SNDVNTISGCMQDAASVAVGVDQITREVAEIVNMLANENAVS-----LSSAAEKVSSVAALAS--GQV--AALAVYV--NTYGVAS--SGVSGEALVQALLEVSA	240
Lh	MaSp1	SNTVYQMLGALGQCQVAVMGVDRFRNEINDLITMFAKAESENE-----2LRSASAAR1SQLTSSITNAVANGVD--ANLARSQ--SSFSALRS--SGMSSDAKIEVLETTIVG	240
Lh	MaSp2	SNSKAIQ1DALRGALQITGVYDFLNEISLVMFQVSA-E-NAL-----LSSASSSR1SSAASLAT--GVLN--TALPSTVY--NMSQVASSPMSSEVYQALLEVSS	240
Ab	MaSp2	LGVTNLADALTSFVQITGVNSRFSERLSLIGMFAQASANDV-----4LAAPATSARISSHASALLS--NPTN--PASISVYV--NAVQ1SSSPGASCDVLVQALLEVTA	240
Pt	FySp1	ALMELLS-PG--AGNALLE-----TTL--SALA	280
Nc	FySp1	ALLESISIPGAGSGSPAQ-----AAL--AALA	280
Aa	FySp1	ALLDSVSGSS--AFQAQ-----AVV--QAFA	280
Av	FySp1	ALLSLLT-SGAST--ADFAQ-----VYL--AALG	280
Lh	TuSp1	ALQWAG--ITVMAFLPFAFTALVNYL-----	280
Ab	TuSp1	ALQWINGG--ITSYDLRVVPAQQLVYALS-----	280
Nc	Frag	ALQWINGG--ITSYDLRVVPAQQLVYALS-----	280
Nim	Flag	ALQWINGG--ITSYDLRVVPAQQLVYALS-----	280
Be	F1B1	ALQWINGG--ITSYDLRVVPAQQLVYALS-----	280
Lh	AcSp1	ALQVLSAQ--VNDVTSSTV-----VTSNLSKAL--	280
Av	AcSp1	LLQLSNTQ--IRQVNPATAS-----SVANSARSFELVA	280
Aar	AcSp1	LLQLSNTQ--IRQVNPATAS-----SVANSARSFELVA	280
At	AcSp1	LLQLSNTQ--IRQVNPATAS-----SVANSARSFELVA	280
Mg	MiSp	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
St	MiSp	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
Lh	MiSp	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
Lh	MaSp1	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
Lh	MaSp2	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
Nc	MaSp1	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280
Ab	MaSp2	LWHLSSAS--IGVYSSAGL-----NTMSLVQSTVGAAY	280

Figure S1. Alignment of concatenated N- and C-terminal regions from sequences with both terminal regions on NCBI. N-terminal regions end at amino acid position 170 (blue line). Missing data coded as "?". 120 amino acids long per line. Species abbreviations and accession numbers in Supplementary Table S1.

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Codon usage:
gca Ala (A) 312 # cag Gln (Q) 92 # uug Leu (L) 16 # uaa Ter (.) 0
gcc Ala (A) 107 # --- Gln (Q) 430 # --- Leu (L) 193 # uag Ter (.) 0
gcg Ala (A) 65 # gaa Glu (E) 16 # aaa Lys (K) 0 # uga Ter (.) 0
gcu Ala (A) 142 # gag Glu (E) 1 # aag Lys (K) 0 # --- Ter (.) 0
--- Ala (A) 626 # --- Glu (E) 17 # --- Lys (K) 0 # aca Thr (T) 6
aga Arg (R) 61 # gga Gly (G) 110 # aug Met (M) 0 # acc Thr (T) 0
agg Arg (R) 0 # ggc Gly (G) 2 # --- Met (M) 0 # acg Thr (T) 33
cga Arg (R) 3 # ggg Gly (G) 18 # uuc Phe (F) 15 # acu Thr (T) 90
cgc Arg (R) 0 # ggu Gly (G) 30 # uuu Phe (F) 18 # --- Thr (T) 129
cgg Arg (R) 14 # --- Gly (G) 160 # --- Phe (F) 33 # ugg Trp (W) 1
cgu Arg (R) 0 # cac His (H) 1 # cca Pro (P) 149 # --- Trp (W) 1
--- Arg (R) 78 # cau His (H) 0 # ccc Pro (P) 33 # uac Tyr (Y) 16
aac Asn (N) 47 # --- His (H) 1 # ccg Pro (P) 45 # uau Tyr (Y) 47
aau Asn (N) 63 # aua Ile (I) 67 # ccu Pro (P) 66 # --- Tyr (Y) 63
--- Asn (N) 110 # auc Ile (I) 0 # --- Pro (P) 293 # gua Val (V) 21
gac Asp (D) 0 # auu Ile (I) 76 # agc Ser (S) 99 # guc Val (V) 47
gau Asp (D) 16 # --- Ile (I) 143 # agu Ser (S) 123 # gug Val (V) 60
--- Asp (D) 16 # cua Leu (L) 18 # uca Ser (S) 172 # guu Val (V) 131
ugc Cys (C) 0 # cuc Leu (L) 32 # ucc Ser (S) 124 # --- Val (V) 259
ugu Cys (C) 0 # cug Leu (L) 54 # ucg Ser (S) 78 # nnn ??? (X) 0
--- Cys (C) 0 # cuu Leu (L) 44 # ucu Ser (S) 224 # TOTAL 3372
caa Gln (Q) 338 # uua Leu (L) 29 # --- Ser (S) 820 #

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Figure S2. The codon usage of PySp1 gene.