

Figure S1. cDNA Sequences of Piriform Silk Proteins.

A. A. trifasciata Piriform:

	<u>A P L P A P A P R P R P A P A P R P A P</u>	
3	gctccattgctgctccggctccgagaccagaccagcaccgctccgcgaccagcgcca	62
	<u>V Y A P A P V V S Q I Q A F T S N R G S</u>	
63	gtttatgctccagctccagttgtttcgcaaattcaggcatttacatcgaatcgaggttcc	122
	T Q Q N S F A Q Q S S V A Q Q S S V A Q	
123	actcaacagaatttcctttgcacagcaatcatctgttgacagcagtcacatctgttgacag	182
	Q S S V A Q Q S S V A Q Q S S T A Q Q S	
183	caatcatctgttgacagcaatcatctgttgacagcaatcatctactgcacagcaatca	242
	S V A Q Q S A V A Q S Q Q S S Y A A A S	
243	tccgttgctcaacagtcagcagttgcccaatcgcaacaaagttcctacgccgctgcgtct	302
	S G G S S A S Q S Q A T V S S A P V Y F	
303	tctggtggttcgagtgctgcagtcacaagctactgtctcaagcgccccagtatatttc	362
	N S Q I L T N N L A S S L Q S L S G L N	
363	aactcgcaaatgttgacaaataacctggcttcatctctgcaatcactgagtgccctaat	422
	Y V S S G Q L S S S D V A S I V A E A V	
423	tacgtatcaagtggtcagttgagttcctcggacgtcgcttccattgtcgcggaagctgta	482
	S Q S L G V S Q G S V Q N I I S Q R L N	
483	tcccagtcacctcgagtggtcacaaggtcagttcaaaatattataagccaaagattgaac	542
	G I G P G A S P S S V S A A I A N A V S	
543	ggcatagggccctggagcttccccatcatcagttcagcagcgatagcaaatgcagtatct	602
	S A V Q G S A S A A P G Q E Q S I A Q S	
603	tcggcagttcaaggatcagcctctgcagctcccgacaggaacaatcgattgcgcaaagt	662
	M S S A I S S A F Q Q I I S Q R T A V A	
663	atgtcttcagctatttccctcagctttccaacaaattatttcgcagagaacagcagtggt	722
	<u>P A P S T L P S P L P A P R P R P A P A</u>	
723	cccgtcctagcaccctaccatctccttctcctgctccgagaccgagaccagcgccagct	782
	<u>L Q P G P V Y A P A L A Y A P A P V Y A</u>	
783	ctacaaccagggccagtttatgcaccagcacttgcgtagtcaccagcgccagtttatgcc	842
	P A P V V S Q F H S S A S T E A T A E Q	
843	ccagctccagttgtttcgcagtttcatctgcttctactgaagccacagctgaacag	902
	N S F V Q T S L A Q S Q P G S Y S S Q S	
903	aattcctttgtacagacgtcacttgcccaatcgcaaccaggctcctattcgtcacaatca	962
	N A L S S N S G Q Q A S V A R S Q S S G	
963	aatgctctgtcttcaaattcaggtcaacaggcaagtgtagctcgttctcagcttagcgga	1022
	S S F S S G S S S D F G S S S S S G A F	
1023	tcttccttttctagcggatcgtcttcagatttccgggtcttctcgtcttctggtgcgttt	1082
	S T A S S P V S Y A P A T I S S S S S V	
1083	tcaactgccagcagccccgtgtcctatgctccagcaacaatcagcagctcttcgtctgtt	1142
	A A S S F S G S S S G F S S S S G S	
1143	gcggcctcatcggttttcgagtggtatcttccctcaggttttagctcttctgcttctggatca	1202
	A L A A S A A Q Q L L S P A A N Q R I A	
1203	gcattggccgcttccgctgccagcagctttgtcacctgcagcgaatcagaggattgca	1262
	A L S N T L K S A V S G G R V N Y G A L	
1263	gcactgtccaatactttgaagtctgctgtgtcaggaggacgcgtcaattacggggctctt	1322
	S N S L A S A A S Q I Q S S S R L S K N	
1323	tcgaattctcttgcgagcgctgcaagtcaaatacaaagttcctccagattatcaaaaaac	1382
	E V L V E V L L E T L A A L L D S L S I	
1383	gaagtcctagtcgaggttctcttggaaactttggccgctttgttgattccctttcaatt	1442
	S G S S S S Q F A Q A V L Q A F A *	
1443	tctggatcatccagctctcaattcgcacaagcagtgctacaagcatttgcctagatttctg	1502
1503	ctcctttactctggaaaaccacagcaaaaaataatatgaaattttaattgtatctttattt	1562
1563	gttatatatattgtatctgattttgggtaatggtaataaaaaatttaataagacataaaaaa	1622
1623	aaaaaaa 1629	

B. *N. cruentata* Piriform:

A P R P L P A P A P R P L P A P A P R P
 2 gtcctcgtcctctaccagctcctgctcctcgtcctctaccagctcctgctcctcgtccc 61
L P A P L P A P L P R P R P A P I V S Q
 62 ctaccagcacctttgccagctcctcttccaagaccagcccagcacccatagtttctcaa 121
V Q Q A S A L Q A Q S Q Q S A F A Q S Q
 122 gtgcaacaggcatccgctctacaggcacagtcacaacagtctgcttttgctcaatcccaa 181
Q S S I A Q S Q Q A S V A Q S Q R A S V
 182 caatcgtccattgcacaatctcagcaagcctctgctcgcccaatcccaacgagcctccgtc 241
S Q S Q Q S S N A F S S A A S F G A S S
 242 tctcaatcccagcaatctagtaatgcgttttcttctgcagcatcatttgagcttctagc 301
V A S S A S T Y F N S G I V Q S S I A S
 302 gtagcatccagtgccttcgacttactttaattcggaatagtacaaagtagcatcgctcg 361
S L Q S S S A L S S I A Y G Q T T A S I
 362 tcgttgcagtcttccagtgcctcagttccattgcctacggccagacaaccgcttctatc 421
N D I A S A V A G S I A N S I G L S Q Q
 422 aacgatatagcatcggcagtcgctggcagcattgcaaattcaatcggaactctcgcaaaa 481
T V Q S I I S Q Q L A S A G S G A S A Q
 482 accgttcaaagtattattagtcacaactagccagtgaggatccggagcatctgctcaa 541
T L A S L I S S A V S S L V Q Q S G S V
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S A G Q E Q S I S Q A L S S S I S S S L
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N Q L V A A R P L P A P A P R P L P A P
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 722 ttgcctgctcctcttccaagaccagcccagcacccatagtttctcaagtgcagcaggca 781
S A L Q A Q S Q Q S A F A Q S Q Q S S I
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A Q S Q Q A S V A Q S Q Q S S I S Q S Q
 842 gcacagtctcagcaagcctctgctcgcccaatcccaacaatcctccatctcccaatcccaa 901
Q A S V S Q S Q Q S S N A F S S A A S S
 902 caagcctccgtctctcaatcccagcaatctagtaatgcgttttcttctgcagcatcttct 961
G A S S V A S S A S T Y F N S G I V Q S
 962 ggagcttctagcgtagcatccagtgccttcgacttacttcaattcgggcatagtacaaagc 1021
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 1022 agcatcgctcgtcgttgcagtccttccagtgctctcagttccattgcctacggccagaca 1081
T A S I S D I A S A V A G S I A N S I G
 1082 accgcttctatcagcgatataagcatcggcagtcgctggcagcattgcaaattcaatcgga 1141
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 1142 ctctcgcaaaaaaccgttcaaagtgttattagtcacaactagccagtgaggatccgga 1201
A S A Q T L A S L I S S A V S S L V Q Q
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 1322 tcgagttctttgaatcagttggtagccgcaagacctctaccagcccctgctcctcgtccc 1381
L P A P L P A P L S R P R P V P V Q R P
 1382 ctaccagcacctttaccagctcctcttccaagacctcgaccagttccagtcctcaagacct 1441
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 1442 caaccgcgtattttcacccagtcctcgtcctatgctcctgccccattcactcagcag 1501
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 1502 tcgacttttgcctcagtcctcaacaagcttctcttgcctcaatctcaacaacaagcatctatc 1561
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Q V A T A Q S Q Q S S G G F S T S S T G
 1622 caagtagctacagcacaatctcagcaatcttctggtggttctccacatcatctactgga 1681

A S Q I S S S A I S T S S G S A L A N S
 1682 gcttctcaaatacagttcttcagccataagtaacttcttcgggatctgcattggctaattcc 1741
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 1742 gcacaacaactcacatcacctgcagcctctcaaagaatatctcagttatccaattcccta 1801
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 1802 gcattctgcagtttctggtggacaggtcaactatgcagccttatctaattctattgctagt 1861
 A A S Q I G G G S G L S K T E V L I E T
 1862 gctgcaagtcaaattggaggtggatctggattatccaaaacggaagttctaattgaaact 1921
 L L E T L A A L L E S L S L P G S A S G
 1922 ctcttagaaaccctggctgctttattggaatctctttctcttcctggatcagctagtgggt 1981
 G S Q F A Q A M L A A L A *
 1982 ggaagtcaattcgctcaagcaatgcttgcagctcttgcataaaatgtgggtaataacaaa 2041
 2042 taattttgttggaatgcttatgaatatatttttaggggaatatatgatatacactttaaatg 2101
 2102 aataatattgtataactttttgttaatgggaaataaaatttatttagcaagcatcaaaaaa 2161
 2162 aaaaaaaaaa 2172

C. N. clavipes piriform:

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61 gtcctcgtccaatcccagctcctcttccccgccagtagcccatagtttctcaagtacag 120
Q A S I Q Q A Q S S S A Q S R Q S S V A
121 caggcatccattcaacaggcacagtcgtcttctgctcagtcctcgacaatcggtcggtgct 180
Q Q A S I S Q S Q Q A S V S Q S Q Q A S
181 cagcaagcctccatctctcaatcccagcaggcttcggtatcccaatcccagcaggcttcg 240
V S Q S Q Q A S V S Q S Q Q S S N A Y S
241 gtatcccaatcccagcaggcttcagtagtcccaatctcagcagtccttctaattgcattct 300
A A S N A A S S V S Q A S S D S S Y F N
301 gctgcttctaattgccgcatccagcgtatctcaagcatcaagcgattcaagttactttaac 360
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361 tcgcaagtagtacagagcgactctcatcgtccttgcaatcttctagtgctctcagttca 420
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421 attgcttacggtcagacctcagccaatataaatgatgtagcagctgcggtcgctagaagc 480
V S Q S L G V S Q Q A A Q S V I S Q Q L
481 gtttcacaatcccttggagtttcccaacaagccgcacaaagtgtcatcagccaacaacta 540
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541 gcaagtgcaggatcgaggagcatcggtcaaacattggcgcaattgatatccagcgagtg 600
S S L V Q Q S G T V S A G Q E Q S I S Q
601 tcttccttgggttcagcaatctggaacgggtgtctgcccggacaagaacagagtatttcaca 660
S L S S I L S S L S Q V V A Q R P L P
661 tccctttctagttcaatcttgtcttctctcagtcagtagttgcccagaggccactacca 720
V P A P R P L P A P L P A P R P I P A P
721 gtacctgctcctcgtccactcccagcacctttgcccgctcctcgtccaatcccagctcct 780
L P R P V P I V S Q V Q Q A S I Q Q A Q
781 ctccccgccagtagcccatagtttctcaagtacaacaagcatccattcaacaggcacag 840
S S S A Q S R Q S S V A Q Q A S V S Q S
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Q Q A S V S Q S Q Q A S V S Q S Q Q S S
901 cagcaggcttctgtttcccaatcccagcaggcttcggtctcccaatctcagcagtcctct 960
N A Y S A A S N A A S S V S Q V S S A S
961 aatgcataattctgctgcttctaattgccgcatccagcgtatctcaagtatcaagcgcttca 1020
S Y F N S Q V V Q Q A L S S L Q S S
1021 agttacttttaactcgcaagtagtacagagcgactctcatcgtccttgcaatcttctagt 1080
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1261 tccagcgagtgctcttccttgggttcagcaatctggaacgggtgtctgcccggacaagaacag 1320
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1381 aggcctctaccagcaccagcaccctgctcctcgtccactcccagaacctctgccc 1440
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1561 cagcaagcctccgtctctcaatcccagcaggcttcggtctcccaatcccagcaggcttcg 1620
V S Q S Q Q A S L S Q T Q Q A S V S Q S
1621 gtatcccaatcccagcaggcttcgctatcccaaaccagcaggcttcggtatcccaatct 1680

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1681 cagcagtccttctaataatgcataattctgctgcgtcctaatagccgcatccagcgtatctcaagca 1740
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1741 tcaagcgttcaagttactttaactcgcaagtagtacagagtacactctcatcatccttg 1800
Q S S S A L S S I A Y G Q T S A N I N D
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V A A A V A R S V S Q S L G V S Q Q A A
1861 gtagcagcagcggtcgctagaagcggtttcacaaatcccttgaggtttcccaacaagccgca 1920
Q S V I S Q Q L A S A G A G A S A Q T L
1921 caaagtgtcatcagccaacaactagcaagtgcaggagcaggagcatcggctcaaacattg 1980
A Q L I S S A V S S L V Q Q S G T V S A
1981 gcacaattgatatccagcgcagtgctcttccttggttcaacaatctggaacgggtatctgcc 2040
G Q E Q S I S Q A L S S S I L S S L S Q
2041 ggacaagaacagagtagttttcacaaagcactttctagttcaatcttgcttctctcagtc aa 2100
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2101 gtagttgccagaggccactaccagtagctcctcgtccactcccagcacctttgcc 2160
A P R P I P
2161 gctcctcgtccaatcccag 2179
V I L S S L S Q V V A Q R P L P A P R P
3001 gtcattctgtcttctcttagtcagtagttgccagagacctctaccagctcctcgtcca 3060
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R P Q P A P I Q R P Q P V F T P S P S P
3121 agaccccaacctgctcctatccaaagacctcaaccgctatttactccaagtccttctccg 3180
V F A P S A F T **Q Q S T I A Q S Q Q V S**
3181 gtatttgctccttcagcattcactcaacaatcgactattgcgcaatcccaacaagtctct 3240
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3241 caagcacaaatcctcttttagcgcagtcctcaacaatcctcttttagcgcagtcctcaacaatcc 3300
S L A Q S Q Q S S L A Q S Q Q S S L A Q
3301 tccttagcgcagtcctcaacaatcctcttttagcgcagtcctcaacaatcctcttttagcgcag 3360
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3661 tcccttgcatctgctgtttcaaattggacgcgttaactatgctggccttatcgaaactctatt 3720
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3721 gccagtgctgcaagtcagattggaagtggatctagattgtccaaaactgaagttctaatt 3780
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3901 taacaattatttgttaaaatcgtgggtgaaagttttttggaattatataacagaatatcc 3960
3961 aattaactcttta 3974

Figure S2. Alignment of Spidroin C-terminal amino acid sequences.

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A_trifas_Flag -GGGQSSG-----GVLPSTSYAPTTSGYERLPNLINGIKSSM--QGGGFNYQNFGNILS
N_cruent_Flag -GAGSGTP-----SYSGSRVPDLVNGIMRSM--QGSGFNYQMFGNMMLS
N_clavps_Flag GPYGPSPG-----GAYGPSPGGAYYPSRVPDMVNGIMSAM--QGSGFNYQMFGNMMLS
A_trifas_AcSp -GVSVI-----SSLNSPVGLRSASAASRLSOLTSSITNAV--GANGVDANSLARSLO
N_cruent_TuSp -----PVLSSSGLASSSATSRVGSLSAQSLASALQSSGGTLDVSTFLNLLS
N_clavps_TuSp -GLQTTL-----APVFSSSGLSSASANARVSSLSAQSFASALSASRGTLVSTFTLLS
N_cruent_MiSp -GVGVGS-----TVASTTSLRSSAQASSRISAAAATLI-----SGGYLNTSALPSVIS
N_clavps_MiSp2 -----SRLSSAEACSRISAAAATLV-----SGSLNTAALPSVIS
N_clavps_MiSp1 -GDKEIACWSRCRYTVASTTSLRSSAEASSRISAAAATLV-----SGGYLNTAALPSVIS
N_clavps_MaSp2 -SAGAGSAGYGPQSASAAAASRLASPDSGARVASAVSNLV-----SSGPTNSAALSSTVIS
A_trifas_MaSp1 GGAASVA-----TASAAAASRLSSPGAASRVSSAVTSLVS-----SGGPTNSAALSNTIS
A_trifas_MaSp2 -GPQSQAP-----VASAAAASRLSSPQASSRVSSAVSTLV-----SSGPTNPASLSNAIS
N_cruent_MaSp  GGAGSGAA-----AASAAAASRLSSPEASSRVSSAVSNLV-----SSGPTNSAALSNTIS
N_clavps_MaSp1 -GVGSGAS-----AASAAAASRLSSPQASSRVSSAVSNLV-----ASGPTNSAALSSTIS
E_chisos_Fbrn -----SGLLSPAANQRIASLIPLILSAI--SPNGVNFVIGSNIA
L_hesper_PySp ----SNA-----ALTSSAHQLVSAAAKRRIASLSQAMSSVI--SGGRVNYAALSSTLS
A_trifas_PiSp ----SGSA-----LAASAAQQLLSPAANQRIASLSNTLKSASV--SGGRVNYGALSNSLA
N_cruent_PiSp ----SGS-----ALANSAQQLTSPAASQRIISQLSNSLASAV--SGGQVNYAALSNSIA
N_clavps_PiSp ----SGS-----ALASSAQQLTSAASQRIISQLSNSLASAV--SNGRVNYAALSNSIA
consensus -g--s-----gs-lss--a-sRlssl--slms-m--sgg-vn-aal-n-is

A_trifas_Flag QYATGSGTCN-----YYDINLMDALLAALHTLNYQGASYVPSY--PSPSEMLSYTENV
N_cruent_Flag KYASGSGACNSN-----DVNVLMDALLAALHCLSSHGSPSFGSS--PTPSAMNAYSNSV
N_clavps_Flag QYSSGSGTCNPN-----NVNVLMDALLAALHCLSNHGSSSFAPS--PTPAAMSAYSNSV
A_trifas_AcSp SSFSAL--RSSGMSSSDAKIEVLLLETIVGLLQLLSNTQVRGVNPA--TASSVANSAARSF
N_cruent_TuSp PISTQI-QANTSLNASQAIQVVLLEAVALLOIINGAQITSVNFG--SVSS----VNTAL
N_clavps_TuSp PISSQI-RANTSLDGTQATVQVLLLEALALLOVINAAQITEVNVS-----NVSSANAAL
N_cruent_MiSp DLFAQVSASSPGVSDSEVLIQVLLLEIVSSLIHILSSSSVGGQVDFN--SVGSSAAAVGQSM
N_clavps_MiSp2 DLFAQVSASSPGVSGNEVLIQVLLLEIVSSLIHILSSSSVGGQVDFS--SVGSSAAAVGQSM
N_clavps_MaSp2 DLFAQVGASSPGVSDSEVLIQVLLLEIVSSLIHILSSSSVGGQVDFS--SVGSSAAAVGQSM
N_clavps_MaSp1 NAVSQIGASNPGLSGCDVLVQALLEIVSACVTILSSSSIGQVNYG--AASQFAQVVGQSV
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A_trifas_MaSp2 SVVSQVSSSNPGLSGCDVLVQALLEIVSALVHILGSSSIGQINYA--ASSQYQVLVGQSL
N_cruent_MaSp SVVSQISASNPGLSGCDVLVQALLEVVSALIHILGSSSIGPVNYG--SASQSTQIVGQSV
N_clavps_MaSp1 NVVSQIGASNPGLSGCDVLVQALLEVVSALIQILGSSSIGQVNYG--SAGQATQIVGQSV
E_chisos_Fbrn SLASQISQSGGGIAASQAFQALLELVAAFIQVLSSAQIGAVSSSSASAGATANAFQSL
L_hesper_PySp GLASEI-QNESNLSKTEVLVEALLETLAALLDSI-----TPI--KSPGSEENFVESV
A_trifas_PiSp SAASQI-QSSRLSKNEVLVEVLLLETLAALLDSLS-----ISGSSSSQFAQAV
N_cruent_PiSp SAASQI-GGSGLSKTEVLIETLLETLAALLLESLSL-----GSAGGSQFAQAM
N_clavps_PiSp SAASQI-GSGRLSKTEVLIETLLETLAALLESISIP-----GSAGGSQFAQAM
consensus -i-sqi--sn-gls--dvlqlLle-v-all-lss--v--v-----s-a---aqsv

A_trifas_Flag RRYF--
N_cruent_Flag RRMFQF
N_clavps_Flag GRMFAY
A_trifas_AcSp ELVLA-
N_cruent_TuSp ATALAG
N_clavps_TuSp VSALAG
N_cruent_MiSp QVVMG-
N_clavps_MiSp2 QVVMG-
N_clavps_MiSp1 QVVMG-
N_clavps_MaSp2 LSAF--
A_trifas_MaSp1 NQAFS-
A_trifas_MaSp2 TQALG-
N_cruent_MaSp YQALG-
N_clavps_MaSp1 YQALG-
E_chisos_Fbrn SSAFAG
L_hesper_PySp LQAFP-
A_trifas_PiSp LQAF-
N_cruent_PiSp LAALA-
N_clavps_PiSp LAALA-
consensus --ala-

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