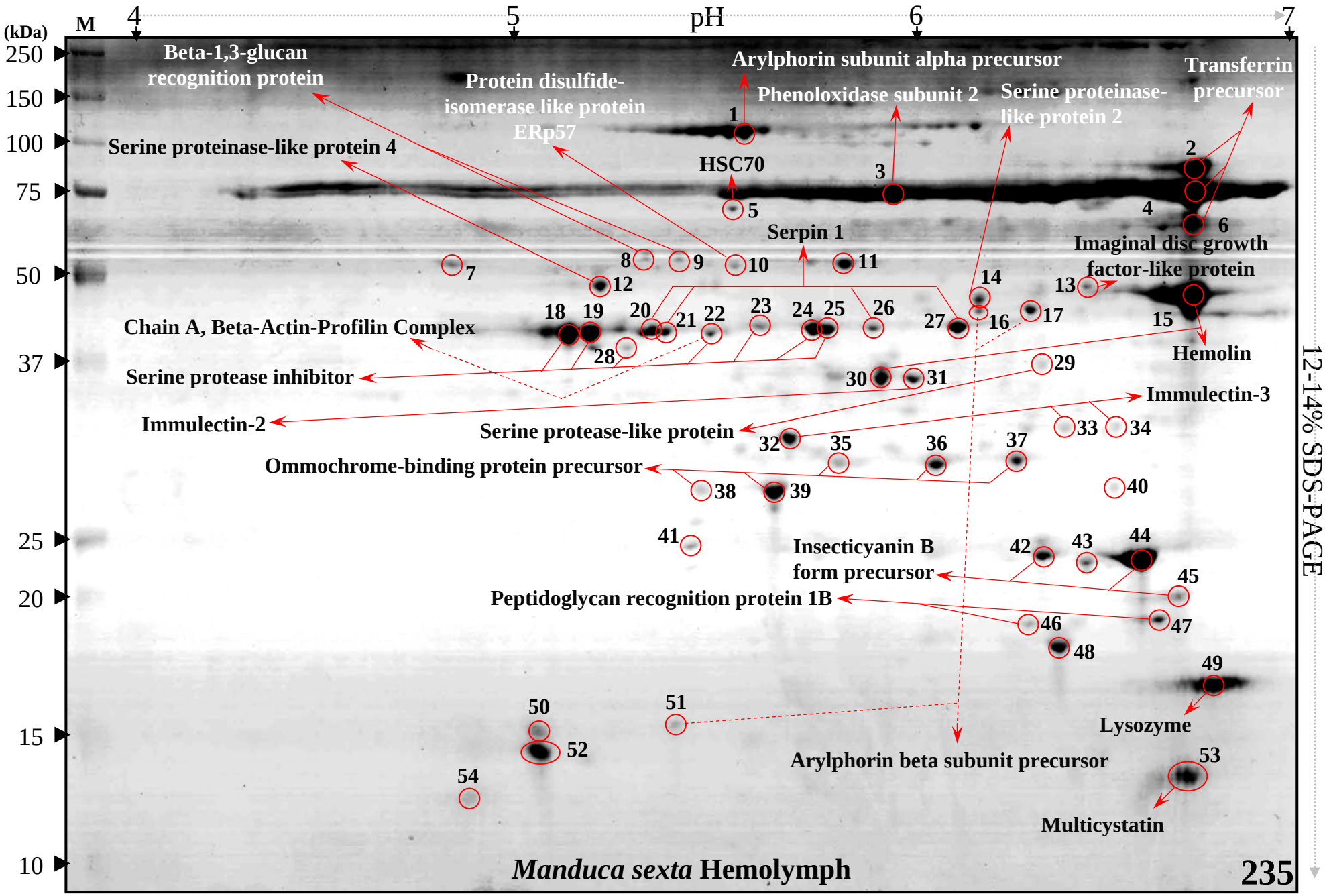
(kDa)
M
4
5
pH
6
7
250
150
100
75
50
37
25
20
15
10
Beta-1,3-glucan recognition protein
Protein disulfide-isomerase like protein ERp57
Arylphorin subunit alpha precursor
Phenoloxidase subunit 2
Serine proteinase-like protein 2
Transferrin precursor
Serine proteinase-like protein 4
HSC70
Serpin 1
Imaginal disc growth factor-like protein
Chain A, Beta-Actin-Profilin Complex
Hemolin
Serine protease inhibitor
Immulectin-3
Immulectin-2
Serine protease-like protein
Ommochrome-binding protein precursor
Insecticyanin B form precursor
Peptidoglycan recognition protein 1B
Lysozyme
Arylphorin beta subunit precursor
Multicystatin
12-14% SDS-PAGE
Manduca sexta Hemolymph
235

Development of 2-D gel map of the fifth instar hemolymph of *M. sexta*. The proteins were extracted by TCA/acetone protocol and the pellet was solubilized in LB-TT. The total soluble proteins were separated on pre-cast IPG strips (18 cm, pH 4-7) in the first dimension followed by pre-cast gradient (12-14%) ExcelGelTM SDS-PAGE in the second dimension. Proteins were visualized by colloidal CBB G-250 staining. Molecular masses were determined by running standard protein markers (2.5 ml/gel; Bio-Rad). Total spot numbers (ImageMaster 2D platinum software 5.0) detected on gel is given at the bottom right-hand corner. Marked protein spots were analyzed; spots numbers 1, 2, 3, and 4 were analyzed by nLC-IT-TOF-MS/MS and the rest of the proteins by nESI-LC-MS/MS. Proteins were identified using MASCOT search engine and SwissProt/NCBI non-redundant protein databases. The identified proteins are shown in Table III.

Table III

M. sexta hemolymph proteins separated by 2-DGE and their identification with nLC-IT-TOF-MS/MS and nESI-LC-MS/MS.

Spot No	Protein Name	*pI* / MW (Da) Theoretical	Accession	Queries Matched	Score	Sequence Coverage (%)	Matched Amino Acid Sequence
			Defense (27)				
			Serine Protease Inhibitor (11)				
18	**Serine protease inhibitor***	6.15 / 35117.36	gi|431335	11	401	30	DVFADLNR, GLELNDDFAAVSR, DVFGSEVQNVDFVK, SVEAAGAINK, NLVDPDALDETTR, SVLVNAIYFK, VPTMIGKK, YADVPELDAK, KLFTPGAAR
19	**Serine protease inhibitor***	5.56 / 43683.28	gi|431347	20	234	35	ESNDQFTAQMFSEVVK, GVDLK, GLELNDDFAAVSR, DVFGSEVQNVDFVK, SVEAAGAINK, IKNLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, TIKVPTMIGK, YADVPELDAK, LYNTEVEIYLPK, KLFTPGAAR
20	**Serpin 1***	5.19 / 43941.50	gi|1378127	21	632	38	ESNDQFTAQMFSEVVK, DVFADLNR, GVDLK, DVFGSEVQNVDFVK, SVEAAGAINK, NLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, TIKVPTMIGK, YADVPELDAK, IETTTDLK, EVLSNMNIK, KLFTPGAAR, SNGQHLFNGICFQP
21	**Serpin 1***	5.25 / 43457.91	gi|1378128	23	209	37	ESNDQFTAQMFSEVVK, DVFADLNR, GVDLK, SVEAAGAINK, IKNLVDPDALDETTR, NLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, VPTMIGK, YADVPELDAK, LYNTEVEIYLPK, IETTTDLK, EVLSNMNIK, KLFTPGAAR, QDKTTLFSGVFQS, TTLFSGVFQS
22	**Serine protease inhibitor**	5.56 / 43683.28	gi|431347	6	124	17	YADVPELDAK, NLVDPDALDETTR, LYNTEVEIYLPK, DVFGSEVQNVDFVK, ESNDQFTAQMFSEVVK
23	**Serine protease inhibitor**	5.56 / 43683.28	gi|431347	18	170	31	ESNDQFTAQMFSEVVK, GLELNDDFAAVSR, DVFGSEVQNVDFVK, NLVDPDALDETTR, SVLVNAIYFK, TIKVPTMIGK, YADVPELDAK, LYNTEVEIYLPK, TKESLYVDAAIQK, ITTYSFHFVPK
24	**Serine protease inhibitor**	5.56 / 43683.28	gi|431347	26	349	46	DVFADLNR, GVDLK, DVFGSEVQNVDFVK, IKNLVDPDALDETTR, NLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, YADVPELDAK, LYNTEVEIYLPK, IETTTDLK, IETTTDLKEVLSNMNIK, EVLSNMNIK, KLFTPGAAR, TKESLYVDAAIQK, AFIEVNEEGAEAAAANAFK, ITTYSFHFVPK, VEINKPFFFSLK, NSMFSGVCVQP

25	**Serine protease inhibitor**	5.56 / 43683.28	gi|431347	32	293	50	ESNDQFTAQMFSEVVK, DVFADLNR, GVDLK, DVFGSEVQNVDFVK, SVEAAGAINK, IKNLVDPDALDETTR, NLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, TIKVPTMIGK, VPTMIGK, YADVPELDAK, LYNTEVEIYLPK, FKIETTTDLK, IETTTDLKEVLSNMNIK, EVLSNMNIK, EVLSNMNIKK, KLFTPGAAR, LFTPGAAR, TKESLYVDAAIQK, AFIEVNEEGAEAAAANAFK, ITTYSFHFVPK
26	**Serpin 1***	5.88 / 44032.88	gi|1378125	26	144	45	ESNDQFTAQMFSEVVK, DVFADLNR, AVKGVDLK, GVDLK, SVEAAGAINK, WVEDQTNNR, IKNLVDPDALDETTR, SVLVNAIYFK, TMDRDFHVSK, TIKVPTMIGK, YADVPELDAK, LYNTEVEIYLPK, FKIETTTDLK, EVLSNMNIK, KLFTPGAAR, TKESLYVDAAIQK, VIPPVLK, ANDQSLFNGICLQP
27	**Serpin 1**	5.88 / 44032.88	gi|1378125	35	217	44	DVFADLNR, GVDLK, DVFGSEVQNVDFVK, SVEAAGAINK, IKNLVDPDALDETTR, NLVDPDALDETTR, SVLVNAIYFK, DKFVK, TMDRDFHVSK, TIKVPTMIGK, VPTMIGK, YADVPELDAK, LYNTEVEIYLPK, FKIETTTDLK, IETTTDLK, IETTTDLKEVLSNMNIK, KLFTPGAAR, LFTPGAAR, LENLLK, TKESLYVDAAIQK, ESLYVDAAIQK, VIPPVLK, ANDQSLFNGICLQP
28	**serine protease inhibitor**	5.56 / 43683.28	gi|431347	21	235	42	ESNDQFTAQMFSEVVK, DVFADLNR, GLELNDDFAAVSR, DVFGSEVQNVDFVK, IKNLVDPDALDETTR, NLVDPDALDETTR, SVLVNAIYFK, TMDRDFHVSK, YADVPELDAK, LYNTEVEIYLPK, FKIETTTDLK, IETTTDLKEVLSNMNIK, LFTPGAAR, TKESLYVDAAIQK, ESLYVDAAIQK, AFIEVNEEGAEAAAANAFK
			Pattern Recognition (10)				
8	**Beta-1,3-glucan recognition protein [*Manduca sexta*]***	5.27 / 54567.17	gi|7262927	13	112	18	LEAIYPK, CTGLLGTAQCK, DPSDAIIVPPIVTAK, TFAFK, VEISAK, NYGIR, NYVSGLLR, TLVGGPIMSEAEPYR, VTAPAGGFYK, ALLVDYVR
9	**Beta-1,3-glucan recognition protein [*M. sexta*]**	5.27 / 54567.17	gi|7262927	16	178	25	LEAIYPK, VSIPDDGYTLFAFHGK, GQMLFEDNFNKPLADGR, RDPSDAIIVPPIVTAK, DPSDAIIVPPIVTAK, KTFAFK, TFAFK, VEISAK, NYGIR, VACVKGNTEYIK, GNTEYIK., TLVGGPIMSEAEPYR, VTAPAGGFYK, VTAPAGGFYKEANEQNVEAAAR, EANEQNVEAAAR
15	**Hemolin***	7.91 / 45665.21	gi|511297	10	174	23	DQPAEVLFR, KDEGSLVFLKPEAK, DEGSLVFLKPEAK, KPVEGSWLK, ITQSPEGDLYFTSVEK, YVCAAK, SPAIDGDVPLVGYTIK, NGELVPMYVSNDMIAK, VVASPSGLTIK
30	**Hemolin**	7.91 / 45665.21	gi|511297	3	79	8	ESQATVLECVTENGDKDVK, QLGEDESIADSILAR

31	**Immulectin-2 [*M. sexta*]***	6.10 / 37423.55	gi|9651929	2	88	9	CHLEGAVLASPLNSNLK, GDFFSVEGIPLKK
32	**Immulectin-3 [*M. sexta*]***	5.86 / 33867.46	gi|55139125	12	130	34	TRDMFTEEYSSGPHCAR, DMFTEEYSSGPHCAR, LIPQEGLVAGSCSDALPYICYK, TAELSMTECGTVDK, TAELSMTECGTVDKGYQLSAK, TGHCYK, ELLAR, YPTGLIK, SGQLDDIGCAK, VPFICEKHPNNIMPVPNNV, HPNNIMPVPNNV
33	**Immulectin-3 [*M. sexta*]**	5.86 / 33867.46	gi|55139125	9	110	24	TAELSMTECGTVDK, CIAEGGQLAVINSAVEANVLK, ELLAR, YPTGLIK, SGQLDDIGCAK, VPFICEK, HPNNIMPVPNNV
34	**Immulectin-3 [*M. sexta*]**	5.86 / 33867.46	gi|55139125	12	191	30	TRDMFTEEYSSGPHCAR, DMFTEEYSSGPHCAR, TAELSMTECGTVDKGYQLSAK, CIAEGGQLAVINSAVEANVLK, ELLAR, SGQLDDIGCAK, VPFICEKHPNNIMPVPNNV
46	**Peptidoglycan recognition protein 1B [*M. sexta*]***	6.29 / 21530.35	gi|27733409	1	64	10	LVIIQHTDTPGCDTDDACAAR
47	**Peptidoglycan recognition protein 1B [*M. sexta*]**	6.29 / 21530.35	gi|27733409	14	129	37	LVIIQHTDTPGCDTDDACAAR, ITFIGSYNSK, ITFIGSYNSKEPNSQQLNAIK, EPNSQQLNAIK, CGVDNGHLSSDYK, CGVDNGHLSSDYKVVGHR, QLLDTDSPGR, QLLDTDSPGRK
			Pro-phenol oxidase (4)				
3	**Phenoloxidase subunit 2 (proPO-p2)*** **	5.68 / 80060.70	gi|75038472	66	2212	71	IFIAPK, WQDLK, LAYWR, FSDWR, IPEFPK, EAAAVIPK, GLDFSDR, TPIIIPR, IEQPDGR, DPFFYR, LDSLTSAR, LCNSLKR, MFIEMDR, FGDEEEVSR, KMFIEMDR, LSDVTEPNPR, FGDEEEVSRK, NLPWALSDQR, NLDKIPEFPK, EPIPEAYYPK, DLSIQGSDPRR, VQNYAEIFPAK, FLDSQVFTQAR, FTHLNHRPFR, EAPHNVRPYSR, NLPWALSDQRK, GLDFSDRGPVYAR,**DYTATDLEEEHR**, FVVPLSAGENTITR, VHAWVDDIFQSFK, ELSCVEASMFCGLK, NIEEAIATGNVILPDK, RPVDGLNVTIDDMER, YLESFGVIADEATTMR, QSTESSLTIPFEQTFR, ELSCVEASMFCGLKDK, FSDWREPIPEAYYPK, NIEEAIATGNVILPDKSTK, KLDIDMLGNMMEASVLSPNR, INLNPQLFNYCYTVAIMHR, MFIEMDRFVVPLSAGENTITR, SELAAFNYCGCGWPQHMLVPK, YLESFGVIADEATTMRDPFFYR, GEDKVLFELTEQFLTPEYANNGLELNNR
12	**Serine proteinase-like protein 4 [*M. sexta*]***	6.10 / 43149.26	gi|56418466	12	56	11	CFASGWGK, YQVILKK, KIEVPVVDR, IEVPVVDR, DTCKGDGGSPLVCPSEYEK, GDGGSPLVCPSEYEK, GDGGSPLVCPSEYEKDR,

14	**Serine proteinase-like protein 2 [*M. sexta*]***	5.83 / 43488.52	gi|21630233	25	300	47	HGEYCQCVPYYLCK, DGLTINDPTLDGNGLLDIR, FGEDENNDRLCQESVER, LCQESVER, VLKDPSEVVKPKPDPSK, AGEWDTQTIKEELEHQER, DVQEILIHK, DFKPLSLK, NDIALLR, NCVANGWGK, NVFGVQGLYAVILK, KVEQDMVPHSR, VEQDMVPHSR, CNTQLQK, DTCQGDGGAPLACPIGNNR, HWVDENMNK, WGYGSSTYSV
29	**Serine protease-like protein [*M. sexta*]***	6.30 / 33065.59	gi|27733421	11	135	25	DNYAVALGK, SDVKEIHIPPQFK, LQLKDGIMGK, DGIMGK, ETLKLSK, VVENPYIDAATCISESPASFR, GDGGAGVAFPSQEQGVQR
Lysozyme (1)							
49	**Lysozyme [*M. sexta* , Peptide Partial, 120 aa]***	8.94 / 13983.80	gi|233964	16	123	71	KHFSR, CELVHELR, CELVHELRR, QGFPENLMR, DWVCLVENESSR, YTDKVGR, NGSRDYGLFQINDK, CSDLLIDDITK, CSDLLIDDITKASTCAK, NHCQGSLPDISSC
Cystatin Protease Inhibitor (1)							
53	**Multicystatin [*M. sexta*]***	8.81 / 70612.02	gi|110225182	3	54	3	YLQSIGSTKPHK, EITVDCK, EITVDCKINNQK
Transport and Metabolism (8)							
Protein (Amino acid) Transport and Metabolism (5)							
35	**Ommochrome-binding protein precursor (OBP) (YCP)***	5.89 / 30614.72	gi|400673	12	205	24	MGYLNLATK, SFGEISGVK, DGMATAVDTTNHIVYLGGK, DGIYTYDYATK, LYFSSPVGFYAVNEADR, LYFSSPVGFYAVNEADRK
36	**Ommochrome-binding protein precursor (OBP) (YCP)**	5.89 / 30614.72	gi|400673	15	178	31	TVLKMGYLNLATK, MGYLNLATK, SFGEISGVK, DGMATAVDTTNHIVYLGGK, DGIYTYDYATK, GKLYFSSPVGFYAVNEADR, LYFSSPVGFYAVNEADR, DDNIVYSNEDNIVK
37	**Ommochrome-binding protein precursor (OBP) (YCP)**	5.89 / 30614.72	gi|400673	22	221	22	MGYLNLATK, SFGEISGVK, DGIYTYDYATK, VIDLGDYNVNAFTK, GKLYFSSPVGFYAVNEADR, LYFSSPVGFYAVNEADR, LYFSSPVGFYAVNEADRK
38	**Ommochrome-binding protein precursor (OBP) (YCP)**	5.89 / 30614.72	gi|400673	6	133	18	DGMATAVDTTNHIVYLGGK, VIDLGDYNVNAFTK, LYFSSPVGFYAVNEADR, LYFSSPVGFYAVNEADRK
39	**Ommochrome-binding protein precursor (OBP) (YCP)**	5.89 / 30614.72	gi|400673	2	107	12	DGMATAVDTTNHIVYLGGK, VIDLGDYNVNAFTK
Iron Transport and Metabolism (3)							

2	**Transferrin precursor* ** **	6.98 / 75222.87	gi|136206	46	1773	60	TWVAAK, WSPDPK, VLGLSEK, ALSTFFAK, YLCVDGSK, IPLTMLMK, IPLTMLMK, VALECVPAR, AMSVFAFSR, MNDHSISPK, GHGAPELVVR, LCVPAAYMK, YEAVIVVHK, TPNYAVAVVK, ANYTEVIER, QCGSDSSAWK, TDEEPDAPFR, LCVTSNVALSK, NNNVIFNNAAK, TIHDVISSCGLA, DLPINNLDQLK, DCEQMLEVPTK, GLATTEKLDFEK, CLAHNNGEVAFTK, DRVECLSFVQQR, IPNQDFVVFQEYR, SVQDNGSDLASVDDMR, QLADSGAADKPEWFTK, CDYPDNYSGYEGALR, DVLSSFATCNVAMAPSR, QADFVPVDPEDMYVASK, DIRPILDCVQENSEDACLK, FFGLPVGTTPASPSNENPEEFR, KFFGLPVGTTPASPSNENPEEFR, NLGEFFSGGSCLPGVDKPENNPSGDDVSK
4	**Transferrin precursor****	6.98 / 75222.87	gi|136206	98	3656	74	IDDLR, LDFEK, TWVAAK, RAVFPK, WSPDPK, VLGLSEK, LAPLREK, ALSTFFAK, IPLTMLMK, VALECVPAR, AMSVFAFSR, MNDHSISPK, GHGAPELVVR, LCVPAAYMK, AIQSDHCVK, YEAVIVVHK, TPNYAVAVVK, QCGSDSSAWK, TDEEPDAPFR, TPNYAVAVVKK, LCVTSNVALSK, NNNVIFNNAAK, SKVALECVPAR, KQCGSDSSAWK, VECLSFVQQR, GTAYNKIDDLR, DLPINNLDQLK, VALECVPARDR, DCEQMLEVPTK, GLATTEKLDFEK, CLAHNNGEVAFTK, YLCVDGSKAPITGK, DRVECLSFVQQR, FKTIHDVISSCGLA, MNDHSISPKENELK, IPNQDFVVFQEYR, SVQDNGSDLASVDDMR, QLADSGAADKPEWFTK, CDYPDNYSGYEGALR, DVLSSFATCNVAMAPSR, YNLHPVFHEVYGELK, QADFVPVDPEDMYVASK, SQYSHLCSMCEHPER, VKQLADSGAADKPEWFTK, TDEEPDAPFRYEAVIVVHK, SVQDNGSDLASVDDMRVAAAAK, DIRPILDCVQENSEDACLK, DAGGRDVLSSFATCNVAMAPSR, ACSWAARPWQGLIGHNDVLAK, FFGLPVGTTPASPSNENPEEFR, LCVPAAYMKDCEQMLEVPTK, KFFGLPVGTTPASPSNENPEEFR, SCHSSYSTFSGLHAPLFYLINK, IPNQDFVVFQEYRTDEEPDAPFR, NLGEFFSGGSCLPGVDKPENNPSGDDVSK
6	**Transferrin precursor**	6.98 / 75222.87	gi|136206	8	225	8	ALSTFFAK, CLAHNNGEVAFTK, YLCVDGSK, VLGLSEK, TPNYAVAVVK, AIQSDHCVK, TWVAAK
			Lipid Transport and Metabolism (3)				
42	**Insecticyanin B form precursor (Blue biliprotein) (INS-b)***	6.29 / 23182.27	gi|9718	16	123	45	LPLENENQGK, CTIAEYK, CTIAEYKYDGK, EYMEGDLEIAPDAK, YVMTFK, NYAINYNCNYHPDK, NYAINYNCNYHPDKK, AHSIHAWILSK, SKVLEGNTK, VLEGNTKEVVDNVLK, EVVDNVLK, TFSHLIDASK
44	**Insecticyanin B form precursor (Blue biliprotein) (INS-b)**	6.29 / 23182.27	gi|9718	41	264	64	LPLENENQGK, CTIAEYK, CTIAEYKYDGK, ASVYNSFVVNGVK, EYMEGDLEIAPDAK, YVMTFK, VVNLVPWVLATDYK, NYAINYNCNYHPDK, NYAINYNCNYHPDKK, VLEGNTK, EVVDNVLK, TFSHLIDASK, FISNDFSEAACQYSTTYSLTGPDR, FISNDFSEAACQYSTTYSLTGPDRH

45	**Insecticyanin B form precursor (Blue biliprotein) (INS-b)**	6.29 / 23182.27	gi|9718	11	87	33	LPLENENQGK, CTIAEYK, EYMEGDLEIAPDAK, YVMTFK, NYAINYNCNYHPDK, EVVDNVLK, TFSHLIDASK
				Storage (4)			
1	**Arylphorin subunit alpha precursor*** **	6.10 / 83866.31	gi|114240	4	65	4	IFLGPK, TGFMPK, SYEINAR, DFETFYK
16	**Arylphorin beta subunit precursor***	5.95 / 83830.79	gi|159491	11	343	12	AFDQKIDFHDFK, SYEIIAR, QVLGAAPKPFDK, YIYEYK, QYLQPYSSEK, VVDVVVDK, SSYPHDFK, IFMAPK, FVAGDNK, NSNDFLIFK
17	**Arylphorin beta subunit precursor**	5.95 / 83830.79	gi|159491	19	537	19	SYEIIAR, QVLGAAPKPFDK, QYLQPYSSEK, VVDVVVDK, SSYPHDFK, SEAAVDAVVK, IFMAPK, YDDNGFPLK, FVAGDNK, FVAGDNKIVR, NSNDFLIFK, LLEQNKVPHDMSEDYGYLPK, LMLPR, QHNMFFK, FNVPSHVMHSNVVPK
51	**Arylphorin beta subunit precursor**	5.95 / 83830.79	gi|159491	5	95	5	DVDAAFVER, EYNIEANIDNYSNK, EYNIEANIDNYSNKK, AVEEFLQLYR, TGFLPK
				Chaperone (1)			
5	**HSC70***	5.50 / 71863.38	gi|1495233	18	349	26	NTTIPTK, VAYKGEDK, STMEPVEK, MVNHFVQEFK, NALESYCFNMK, SQIHDIVLVGGSTR, TTPSYVAFTDTER, TFFPEEVSSMVLTK, QKELEGICNPIITK, IINEPTAAAIAYGLDK, NQVAMNPNNTIFDAK, IINEPTAAAIAYGLDKK, NQVAMNPNNTIFDAKR, TVQNAVITVPAYFNDSQR, GEDKTFFPEEVSSMVLTK, QTQTFTTYSDNQPGVLIQVFEGER
				Cell structure (1)			
22	**Chain A, Beta-Actin-Profilin Complex***	5.29 / 41828.20	gi|576368	22	129	30	GDDDIAALVVDNGSGMCK, AGFAGDDAPR, AVFPSIVGRPR, HQGVMVGMGQK, DSYVGDEAQSKR, LDLAGR, DLTDYLMK, GYSFTTTAER, MQKEITALAPSTMK, EITALAPSTMK, QEYDESGPSIVHR
				Cell fate (1)			
10	**protein disulfide-isomerase like protein ERp57 [*Bombyx mori*]***	5.25 / 55103.26	gi|62241290	4	77	9	STCEQFSVSGYPTLK, KGELSSEYNGPR, ESDLKGEFLK, ENYHGLVGVR
				Metamorphosis (1)			

13	**Imaginal disc growth factor-like protein [*Mamestra brassicae*]***	7.08 / 47827.53	gi|85726208	5	43	11	MVPLNENLDVDR, AITNFK, YPGLK, VLLSVGGDVDTEDADK, GLCTGDKYPILR
Unclassified (9)							
7, 11, 40, 41, 43, 48, 50, 52, 54	**N.D.**						

N.D. ; Not Determined , *; Non-redundant, ** ; nanoLC/Linear-Trap-TOF-MS