Identification of potential chemical signals in the European water vole *Arvicola terrestris*

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Supplementary Table S1. Identified and partially identified (*italics*) compounds in *A. terrestris* male and female (N=68) lateral scent glands by GC/MS (NIST = NIST library; Std = commercial compound) - * refers to compounds not present in the chromatogram of **Fig. 1** (Male 18 – October), but identified in other animals.

Name	Rt (min)	Compound	Identification	Name	Rt (min)	Compound	Identification	Name	Rt (min)	Compound	Identification
ATG1	03.75	3-ethyl, hexane	NIST + Std	ATG18	25.16	1-heptadecanol	NIST	ATG35	*36.47*	*Xdecyl ester, hexanoic acid*	Fragmentation
ATG2	05.96	3-methyl, butanoic acid	NIST	*ATG19*	*27.72*	*1-octadecanol*	*NIST*	*ATG36*	36.55	nonadecyl ester, octanoic acid	
ATG3	06.18	2-methyl, butanoic acid	NIST + Std	ATG20	30.52	pentadecyl ester, octanoic acid	Fragmentation	ATG37	37.06	nonadecyl ester, octanoic acid	Fragmentation
ATG4	08.23	6-methyl, 2-heptanone	NIST	*ATG21**	31.45	*Xdecyl ester (?), nonanoic acid*	Fragmentation	ATG38	37.38	nonadecyl ester, nonanoic acid	Fragmentation
ATG5	08.46	benzaldehyde	NIST + Std	*ATG22*	*31.50*	*3-methyl-, hexadecyl ester(?), butanoic acid*	*Fragmentation*	ATG39	37.92	decadecyl ester, octanoic acid,	Fragmentation
ATG6	*08.81*	*4-methyl-5,6-dihydropyran-2-one*	*See ATG8 spectrum*	ATG23	32.67	hexadecyl ester, octanoic acid	Fragmentation	ATG40	38.40	octadecyl ester, decanoic acid,	Fragmentation
ATG7	09.42	2-methyl-3-methylene, nonane	NIST	ATG24	32.75	unidentified compound 3		ATG41*	38.75	decadecyl ester, nonanoic acid	Fragmentation
ATG8	10.06	5,6-dihydro-2H-Pyran-2-one	NIST	ATG25*	33.01	hexadecyl ester, pentanoic acid	Fragmentation	ATG42	39.24	nonadecyl ester, decanoic acid	Fragmentation
ATG9	11.40	nonanal	NIST + Std	ATG26	33.60	heptadecyl ester, octanoic acid,	Fragmentation	ATG43	40.52	(-)-Cholesterol	NIST
ATG10	12.72	2-isoamylpyrazine	NIST	ATG27*	34.14	unidentified compound 4		ATG44	40.68	unidentified compound 5	
ATG11	13.90	2-isoamyl-6-methylpyrazine	NIST	*ATG28**	34.16	*1-heneicosyl formate with 2 double bonds*	NIST	ATG45	41.07	decadecyl ester, decanoic acid	Fragmentation
ATG12	14.07	2-Decenal, (Z)-	NIST + Std	ATG29	34.53	heptadecyl ester, pentanoic acid	NIST	ATG46	41.87	undecadecyl ester, decanoic acid	Fragmentation
ATG13	15.58	undecenal	NIST + Std	ATG30*	35.06	octadecyl ester, octanoic acid	Fragmentation	ATG47*	43.02	unidentified compound 6	
ATG14	16.41	caryophyllene	NIST + Std	*ATG31*	35.26	*Octadecenol (?)*	Fragmentation	ATG48	43.05	unidentified compound 7	

ATG15	19.74	codecalactone	NIST library	**ATG32***	35.51	hexadecyl ester, decanoic acid	Fragmentation	**ATG49**	43.72	unidentified compound 8	
ATG16	23.72	unidentified compound 1		**ATG33**	35.61	octadecyl ester, octanoic acid (isomer of ATG30?)	Fragmentation	**ATG50**	44.05	unidentified compound 9	
ATG17	24.65	unidentified compound 2		**ATG34**	36.13	heptadecenyl ester, nonanoic acid	Fragmentation				

Supplementary Table S2. Identified and partially identified (*italics*) compounds in *A. terrestris* male and female urine (N= 33) by SPME-GC/MS (NIST = NIST library; Std = commercial compound) - * refers to compounds not present in the chromatogram of **Fig. 3** (Male 1 – May), but identified in other animals

Name	Rt (min)	Compound	Identification	Name	Rt (min)	Compound	Identification
ATU1*	01.95	1-penten-3-one	NIST + Std	**ATU16***	14.29	4-methylphenol (p-Cresol)	NIST
ATU2*	02.04	3-pentanone	NIST	***ATU17****	*14.54*	*6-Propenyl bicyclo[3.1.0] hexan-2-one*	*NIST*
ATU3*	02.10	heptane	NIST +Std	**ATU18**	14.67	2-methyl-6-(1-propenyl)-, (Z)- pyrazine	NIST
ATU4*	02.53	3-penten-2-one	NIST + Std	**ATU19**	15.30	nitrile benzyl	NIST + Std
ATU5	06.24	2,3-dimethyl, bicyclo[2.2.1]hept-2-ene (santene)	NIST	***ATU20****	*16.05*	*2,6,6-Trimethyl-2-cyclohexene-1,4-dione*	*NIST*
ATU6*	06.69	styrene	NIST + Std	**ATU21***	17.88	octanoic acid	NIST + Std
ATU7	07.27	1-nitro-3-methylbutane	NIST	***ATU22***	*21.12*	*di(2-phenylethyl) ester, oxalic acid*	*NIST*
ATU8	07.81	2,5-dimethyl-pyrazine	NIST + Std	**ATU23**	24.15	2-nitroethenyl, benzene (beta-nitro-styrene)	NIST
ATU9*	08.23	dimethyl sulfone	NIST + Std	**ATU24***	24.55	Unidentified compound 2	
ATU10	09.61	benzaldehyde	NIST + Std	**ATU25***	24.96	2H-1-benzopyran-2-one (Coumarin)	
ATU11	11.15	trimethyl pyrazine	NIST + Std	**ATU26**	25.96	1-(1,5-dimethyl-4-hexenyl)-4-methyl, benzene (Alpha-curcumene)	NIST
ATU12*	11.54	Unidentified compound 1		**ATU27**	27.95	Unidentified compound 3	
ATU13*	12.30	2-hydroxy benzaldehyde	NIST	**ATU28***	27.56	Unidentified compound 4	
ATU14*	12.53	acetaldehyde benzene	NIST + Std	***ATU29***	*30.48*	*1,1'-oxybis, octane*	*NIST*
ATU15	13.87	3-ethyl-2,5-dimethyl, pyrazine	NIST				

Supplementary Table S3. Animals caught in the field used for identification of urinary volatiles and proteins, and RNA sequencing

▨ no urine collected
🟩 only glands were collected, not enough urine for SPME
🟨 proteins were analysed (see Fig. 1)
🟪 only RNA from liver was analysed

2016 October Marcenat

Animal #	Sex	Weight (g)	Urine	LSG	Testis weight (mg)	Uterus weight (mg)	Proteins	Volatiles	RNA	Vaginal swab
1	M	79.7	X		356.6			no signal		
3	M	55	X	X	106.1		🟨	X		
5	F	61.1	X					no signal		
6	M	94.7	X	X	203.2		🟨	X		
7	F	63.7	X	X		102.3				
8	F	62.8	▨	🟩 X		7.4				
9	F	74.3	X	X		49.9	🟨	no signal		
10	M	71	▨	X	248.9			no signal		
11	F	108.7	▨	🟩 X		172				
12	M	44.5	X	X	50.3					
13	F	75.4	▨	🟩 X		36.2				
15	F	96.4	X	X		72.4	🟨	no signal		
16	M	88.3	▨	🟩 X	225.9					
17	F	84.1	▨	🟩 X		164.1				
18	M	65.2	X	X	332.1		🟨	X		
19	F	78.2	▨	🟩 X		121.5				
20	M	70.5	X		145.4			no signal		

21	F	83.5	▨	X		129.6			
22	F	65.9	X	X		91.7	🟨	X	
23	F	97.6	▨	X		264.8			Start of pregnancy
2017 May		Laqueuille							
1	M	141	X	X	494.9		🟨	X	
2	F	116	▨	X		223.5			diestrus
3	F	122	▨	X					pregnant
4	F	94	X	X		220.5		X	estrus
5	M	56.1	▨	X	136.1				
6	F	122	X	X		258.9	🟨	X	
7	F	38.3	X	X		26.7			
8	M	121	X	X	556.1		🟨	X	
9	F	122.4	▨	X		212.1			metestrus
10	F	127	X	X		185.8	🟨	X	metestrus
11	F	58	▨	X		96.3			estrus
12	F	43.7	▨	X		31.9			
13	F	89.9	X	X		244	🟨	X	estrus
14	M	87	X	X	523				
15	M	64	X	X	173.4			X	
17	F	40	▨	X		38.5			
18	M		X	X	400.2				
19	F	114	▨	X					pregnant
20	F	53.5	▨	X		89.7			estrus
21	F	114	X	X			🟨	X	pregnant
22	F	122	X	X		147.8		X	metestrus
23	M	131.6	X	X	498.7		🟨	X	
24	M	119	X	X	612.1		🟨	X	

25	M	69.9	X	X	181.1			X	
26	M	126		X	589.1				
28	F	106	X	X		303.6		X	estrus
2017 July		Laqueuille							
2	F	44.8	X	X		59		X	
3	F	77.9		X		61.5			diestrus
4	M	41.8	X	X	62.3		🟨	no signal	
5	F	90.6	X	X		186.1		X	diestrus
6	M	25.9	X	X	41.1				
7	F	24.6		X		12			
8	M	27.8	X	X	57		🟨	X	
9	F	60.2		X		98.4			metestrus
10	F	24.3	X	X		11.5			
11	M	65.2		X	344				
12	F	89.6	X	X		164	🟨	X	metestrus
13	F	96		X					pregnant
14	M	25.2	X	X	38			X	
15	F	97.4	X	X		173.2	🟨	X	diestrus
16	F	108.5		X					pregnant
17	F	92.9	X	X			🟨	X	pregnant
18	M	138.9	X	X	442.7			X	
19	F	142		X					pregnant
20	F	85		X		92			
21	F	90.3		X		295.4			diestrus
22	M	61.4		X	230				
23	F	55.7		X		49.2			diestrus
24	F	76.6		X					pregnant

25	M	114.2	X	X	520			X		
26	F	104.8		X		211				diestrus
2018 April		Saint-Julien-Puy-Lavèze								
1	M	73			323.1					
2	F	130								End of pregnancy
3	M	132			493.4					
4	M	122			392.1					
7	F	108				304.6				Diestrus
8	F	84				144.4				Estrus

Supplementary Table S4. Peptides identified in bands from 1D-E gels of males and females *A. terrestris* (pattern in Figure 4), which are homologous to peptides of *M. glareolus* OBPs. Band # is indicated in Fig.4. Peptides are replaced (in red) on the *M. glareolus* sequences below.

Protein name	Protein accession number	Calculated molecular mass	Band #	Peptide (s)	% of sequence coverage
Glareosin	Not available. Sequence in Loxley *et al.* (2017) supp. data	16933	1 to 9	WVTVALAADNVNK-TQGTTNVLYVYGK	9-17
MglaOBP1	D3VW62_MYOGA	16646	1, 2, 4, 5, 9	DLLATDTCPE-WVTTAIAADNVDK	8-14
MglaOBP2	D3VW63_MYOGA	16840	1 to 9	GQPLTPEQYEK-GQPLTPEQYEKLEEFAK-LIFVVGKGQPLTPEQYEK	10-15
MglaOBP3	D3VW64_MYOGA	16752	1 to 9	EQNIPTENIR-GQPLTPEQYEK-GQPLTPEQYEKLEEFAK	10-16

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Glaerosin       HSELDGKWVTVALAADNVNKLEEGGSLRLYLRELTCTEACNRLEVTFYLKANGQCTKTKV    60
D3VW62_MYOGA    QAELEGKWVTTAIAADNVDKIERGGPLRLYIRKITCTEACSKMEVTFYVNENNQCSQTKI    60
D3VW63_MYOGA    QAELEGKWVTTAIAADNIDTIEEEGPMRIYVRELTCSEACSKMGVTFYVNANGQCSETKV    60
D3VW64_MYOGA    YAELEGTWYTTAIAADNVDTIEEEGPLRLYVRELTCSKGCNKLGVTFYVNANGQCSKTTV    60

Glaerosin       VGNRQEDGKYRAQFENGDNTFGPVYATPEFTLFANHNVDRTQGTTNVLYVYGKHGPLTPE    120
D3VW62_MYOGA    TGYRQEDGNYRAQFE-GDNVFKPVAATEDIIVFASENVDRAGRTTNLVLVAGKGQPLTPE    119
D3VW63_MYOGA    IGYRQEDGKYRTQFE-GDNRFEPVHATPENIVFTNKNVDRTGRTTKLIFVVGKGQPLTPE    119
D3VW64_MYOGA    TGYMQEDGKYRTQFE-GDDRFKPVHATPDNIVFISQNVDRAGRTTNLIFVVGKGQPLTPE    119

Glaerosin       QYEKLEEFAKLWNLPRENLQDVRAS-ECPK   149
D3VW62_MYOGA    QHEKLEAYAKEHNIPPENIRDLLATDTCPE   149
D3VW63_MYOGA    QYEKLEEFAKEQGIPTENIREVLPTDTCPE   149
D3VW64_MYOGA    QYEKLEEFAKEQNIPTENIRNVLATDTCPE    149
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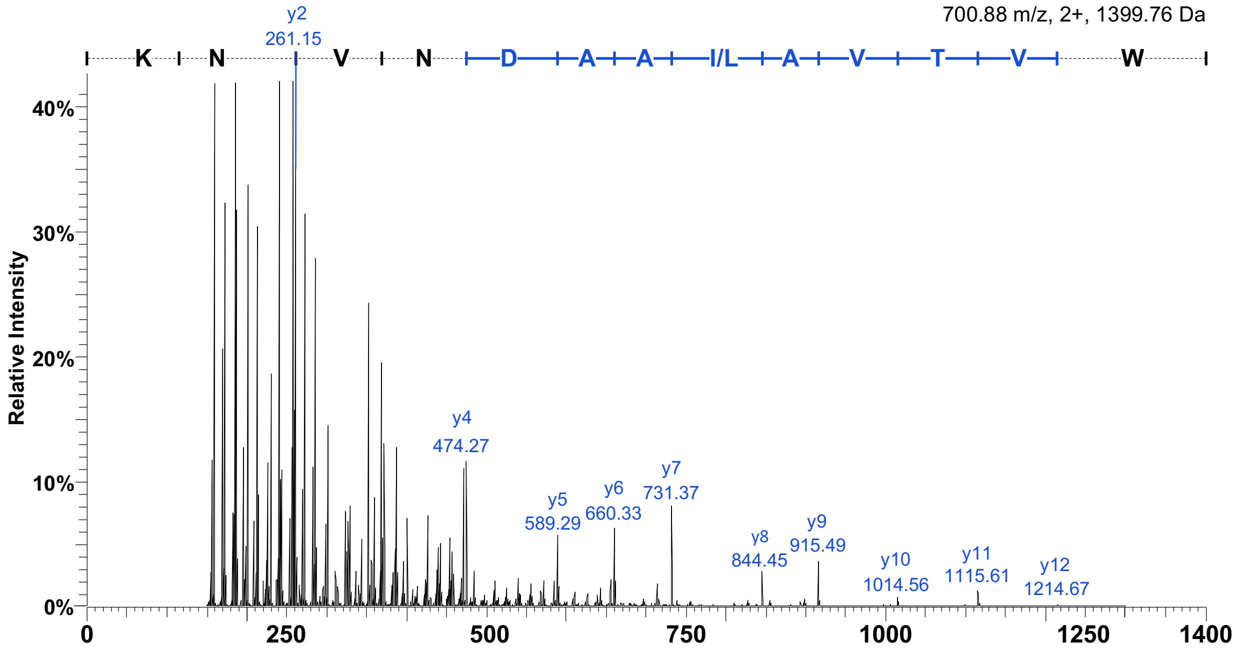
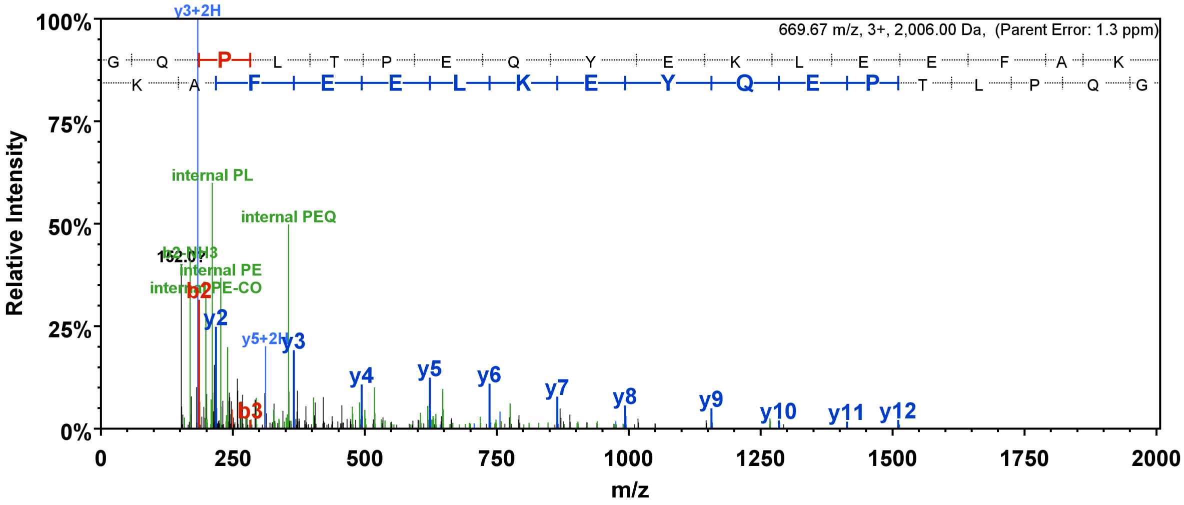
Supplementary Figure S1. MS/MS spectrum of the WVTVALAADNVNK peptide. *De novo* analysis allows identifying the tag sequence “T-ALAAD” in ion 700.88 m/z 2^+-charged. Manual annotation confirms the presence of the tag and completes sequencing with clear identification of the y2 fragment, corresponding to the dipeptide KN and allowing to deduce the dipeptide VN – the fragments y9 and y10 identifying Valine as the missing amino acid into the tag T-ALAAD – and the fragments y11 and y12 allowing to identify the WV dipeptide according to the measured mass of the peptide (1399.76 Da).



Supplementary Figure S2. MS/MS spectrum of the GQPLTPEQYEKLEEFAK peptide. Database searching allowed identifying the ion of m/z 669.67 (3+) as matching with GQPLTPEQYEKLEEFAK peptide mass with a precursor mass error of 1.3 ppm and an ion score of 70.9. Upper red and blue sequences indicate the amino acids validated by detection of respectively N-terminal and C-terminal peptide fragments (b and y ions series).



Supplementary Table S5. Primers used for amplification of the sequence encoding glareosin-like (arvicolin) from *A. terrestris* male liver

	5’ primer (forward)	3’ primer (reverse)
PCR with *de novo* MS information	Peptide WVTVALAADNV at N-ter position 5’-TGGGTTACCGTTGCCCTTGCTGCTGACAATGTG-3’	Peptide PLTPEQYEKLEEFA at C-ter position 5’-CCTTTGACTCCTGAACAATATGAAAAACTTGAGGAATTTGCC-3’
5’RACE PCR	GeneRacer™ 5’ primer 5’-CGACTGGAGCACGAGGACACTGA-3’	Gene Specific 5’ primer 5’-CCCGTCCTCTATCTTGTTCACATTGTCAGCAGC-3’
5’RACE nested PCR	GeneRacer™ 5’ Nested Primer 5’-GGACACTGACATGGACTGAAGGAGTA-3’	Gene Specific 5’ nested primer 5’-TCTATCACACGCCTCAGCACAAGTCAGTTCA-3’
3’RACE PCR	Gene Specific 3’ primer 5’-GTTACCGTTGCCCTTGCTGCTGACAATGTGAAC-3’	GeneRacer™ 3’ primer 5’-GCTGTCAACGATACGCTACGTAACG-3’
Full-length amplification from animal liver	5ARVICOLIN 5’-ATGGTCAAGTTTCTGCTGCTGGCTT-3’	3ARVICOLIN 5’-TTATTTAGGACAAATATCTGTAGCCAGAACATC-3’

Supplementary Table S6. Sequences used for the building of the phylogenetic tree (Fig. 6)

Accession	Description	Name in the tree
lcl|Query_9764	arvicolin	arvicolin
QEY02203	glareosin	glareosin
P09465	aphrodisin[Cricetulus griseus]	Aphrodisin_C. griseus
Q9Z1I7	Aphrodisin Mesocricetus auratus	Aphrodisin_M.auratus
NP_620258.1	odorant-binding protein precursor [Rattus norvegicus]	OBP_R.norvegicus
AIA65156.1	odorant binding protein 1 [Mus musculus musculus]	OBP1_M.musculus_musculus
AIA65157.1	odorant binding protein 2 [Mus musculus musculus]	OBP2_M.musculus_musculus
AIA65158.1	odorant binding protein 5 [Mus musculus musculus]	OBP5_M.musculus_musculus
AIA65159.1	odorant binding protein 6 [Mus musculus musculus]	OBP6_M.musculus_musculus
AIA65155.1	odorant binding protein 7 [Mus musculus domesticus]	OBP7_M.musculus
XP_005346787.2	odorant-binding protein 2b [Microtus ochrogaster]	OBP2b_M.ochrogaster
ADC97771.1	odorant binding protein 2 [Myodes glareolus]	OBP2_M.glareolus
ADC97770.1	odorant binding protein 1 [Myodes glareolus]	OBP1_M.glareolus
ADC97772.1	odorant binding protein 3 [Myodes glareolus]	OBP3_M.glareolus
XP_028719045.1	aphrodisin-like [Peromyscus leucopus]	Aphrodisin _P. leucopus
XP_028717128.1	odorant-binding protein 1a-like [Peromyscus leucopus]	OBP1a_P. leucopus
XP_028717126.1	odorant-binding protein 1b-like [Peromyscus leucopus]	OBP1b_P. leucopus
AGT28425.1	odorant-binding protein [Phodopus sungorus]	OBP_P. sungorus
XP_027278553.1	odorant-binding protein 2a isoform X2 [Cricetulus griseus]	OBP2a_C.griseus
EGW10044.1	Odorant-binding protein 2b [Cricetulus griseus]	OBP2b_C.griseus
XP_005406585.1	PREDICTED: male-specific submandibular salivary gland protein-like [Chinchilla lanigera]	SAL_C.lanigera
XP_028639070.1	odorant-binding protein 2a-like [Grammomys surdaster]	OBP2a_G.surdaster
XP_021044251.1	odorant-binding protein 1a-like [Mus pahari]	OBP1a_M.pahari
XP_021012716.1	odorant-binding protein 2a [Mus caroli]	OBP2a_M.caroli
XP_012876753.1	odorant-binding protein 2b [Dipodomys ordii]	OBP2b_D.ordii
KFO21503.1	Odorant-binding protein 2b [Fukomys damarensis]	OBP2b_F.damarensis
XP_010604442.1	PREDICTED: female-specific lacrimal gland protein [Fukomys damarensis]	LGP_F.damarensis
XP_004867396.1	female-specific lacrimal gland protein-like [Heterocephalus glaber]	LGP_H.glaber
VEV85354.1	allergen lipocalin Cav p 1 isoform 2 precusrsor [Cavia porcellus]	Lipo_C.porcellus

Supplementary Figure S3. Sequences used in Megablast search

1. *Microtus ochrogaster*

>ENSMOCT00000026459.1 Moch1
EGKWDTIAVAADNVAKIEEGGPLRLYARELTCSEECNKLEVTF
YVNANGQCSETKITGYRQEDGTYRTQYEGDGRFKPVHGISDNIVFICQNVDRAGQTTNLI
YVIGKGQPLTPEQYEKLEEFAKEQNMPTENIRNVLATDTCPQ

>ENSMOCT00000020380.1 Moch2
YTELEGAWFTTAIAADNVDTIEEEGPMRLYVRELTCSEACNEMD
VTFYVNANGQCSETTVTGYRQEDGKYRTQFEGDNRFEPVYATSENIVFTNKNVDRTGRTT
NQIFVVGKGQPLTPEQYEKLEEFAKQQNIPKENIRQVL

>ENSMOCT00000021279.1 Moch3
ELEGKWVTTAIAADNVDKIEEGGPMRFYMRELTCCEECSQME
ITFYVNVNDQCSQTKITAYRQEDGNYRTQFEGDHVFKPVFATEDIIVLAGDNVDRASRRT
KLIFVLEPLTPEQHEKLEAYVEERSIPPENIRDVLATDTFPK

>ENSMOCT00000011433.1 Moch4
YAELGGKWLTTAIAADNVDKIEKEGPLRLYVREITCSEACSQMG
VTFYVNANGQCSATKVIAYRQEDGKYRTQFEGDNRFEPVHATPENIVFTSKNVDRAGQTT
NLIFVVGKGKPLNPEQYEKLEEFAKEQNIPKENIREVLAT

>XP_005358711.1 aphrodisin-like [Microtus ochrogaster]
HAEIEGKWVTTAIAADNADIIEEGQPLRLYVRELACTEACNRLKFTFYTKVNGQ
CTKTKVIANRQADDQYRARFEGDNIIVPVYVTPEIIIFVVQNVNRTGRKTNLIYVLGKGQSLTSVQYEKLEEFAK
VQNIPRKNIQDILATDICPK

2. Hamsters

>CAA46342.1 aphrodisin precursor protein [Cricetus cricetus]
QDFAELQGKWYTIVIAADNLEKIEEGGPLRFYFRHIDCYKNCSEMEITFYVITN
NQCSKTTVIGYLKGNGTYQTQFEGNNIFQPLYITSDKIFFTNKNMDRAGQETNMIVVAGKGNALTPEENEILVQF
AHEKKIPVENILNILATDTCPE

>CAA12414.1 aphrodisin [Mesocricetus auratus]
QDFAELQGKWYTIVIAADNLEKIEEGGPLRFYFRHIDCYKNCSEMEITFYVITN
NQCSKTTVIGYLKGNGTYQTQFEGNNIFQPLYITSDKIFFTNKNMDRAGQETNMIVVAGKGNALTPEENEILVQF
AHEKKIPVENILNILATDTCPE

3. *Myodes glareolus*

>Glaerosin
HSELDGKWVT VALAADNVNK LEEGGSLRLY LRELTCTEAC NRLEVTFYLK ANGQCTKTKV
VGNRQEDGKY RAQFENGDNT FGPVYATPEF TLFANHNVDR TQGTTNVLYV YGKHGPLTPE
QYEKLEEFAK LWNLPRENLQ DVRASECPK

>ADC97770.1 odorant binding protein 1 [Myodes glareolus]
QAELEGKWVTTAIAADNVDKIERGGPLRLYIRKITCTEACSKMEVTFYVNENNQ
CSQTKITGYRQEDGNYRAQFEGDNVFKPVAATEDIIVFASENVDRAGRTTNLVLVAGKGQPLTPEQHEKL
EAYAKEHNIPPENIRDLLATDTCPE

>ADC97771.1 odorant binding protein 2 [Myodes glareolus]
QAELEGKWVTTAIAADNIDTIEEEGPMRIYVRELTCSEACSKMGVTFYVNANGQ
CSETKVIGYRQEDGKYRTQFEGDNRFEPVHATPENIVFTNKNVDRTGRTTKLIFVVGKGQPLTPEQYEKL
EEFAKEQGIPTENIREVLPTDTCPE

>ADC97772.1 odorant binding protein 3 [Myodes glareolus]

```
YAELEGTWYTTAIAADNVDTIEEEGPLRLYVRELTCSKGCNKLGVTFYVNANGQ
CSKTTVTGYMQEDGKYRTQFEGDDRFKPVHATPDNIVFISQNVDRAGRTTNLIFVVGKGQPLTPEQYEKL
EEFAKEQNIPTENIRNVLATDTCPE
```

4. *Peromyscus maniculatus bairdii*

```
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gene:ENSPEMG00000023943.2 transcript:ENSPEMT00000032820.2
gene_biotype:protein_coding transcript_biotype:protein_coding
description:aphrodisin-like [Source:NCBI gene;Acc:102925781]
TMVKFLLLALAFGLAHAYAELQGKWKTTAAAADNVEKIEKGGNLRLYVREVTCNHDCTEM
YITFYVNLNGQCSKTTVTGYKQEDGTYKTQFEGDNVFQPVYATSKNIVFTGKNTDRAGKE
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gene_biotype:protein_coding transcript_biotype:protein_coding
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SDECQTLTITFKVKVEGECQTHTVVGRKEKDGLYMTDYSGKNYFRVIEKADGIIIFHNVN
VDNSGKETNVILVAGKEESLSKAQKQELKKLANKLNIPNENIQHLEPADTCHQ

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gene_biotype:protein_coding transcript_biotype:protein_coding
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GVTFYVKESVFFKYFHFTDEGDNKYELVYLTEEHAVFTSKNVDRAGTETNLIFVLGKGGA
LNEEEHEKLVKFAEDKNIPTENIRNVLATGNINENFLAISYMCVCVLMYINKYMYAYI

>ENSPEMP00000029229.1 pep
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gene_biotype:protein_coding transcript_biotype:protein_coding
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FYVNRLFMFLIFKYFHFTDEGDNKYELVYLTEEHAVFTSKNVDRAGTETNLIFVLGKNGS
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gene_biotype:protein_coding transcript_biotype:protein_coding
gene_symbol:Pbsn description:probasin [Source:MGI Symbol;Acc:MGI:1860484]
TMRVFILLLMLDLLGVSSVMMNKNLRKKIQGNWRTVYLASSTVEKISVDSPLRTYFRRIE
CGKRCRQIHFYFYIKKGARCQQYEVIGKKRQEFYQAQYEGKITVMIKMVNDKILLFHYFN
KDVRGKVTRVAGVLARARKLTKDEMTQYMDLVEEIGIEDENVQRVLDTDTCP

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gene_biotype:protein_coding transcript_biotype:protein_coding
gene_symbol:Pbsn description:probasin [Source:MGI Symbol;Acc:MGI:1860484]
TMRVFILLLMLDLLGVSSVMMNKNLRKKIQGNWRTVYLASSTVEKISVDSPLRTYFRRIE
CGKRCRQIHFYFYIKKGARCQQYEVIGKKRQEFYQAQSRARKLTKDEMTQYMDLVEEIGI
EDENVQRVLDTDTCP

>ENSPEMP00000010512.1 pep
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gene_biotype:protein_coding transcript_biotype:protein_coding
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Symbol;Acc:MGI:1924954]
MVVLLVLGLVPSLAAAQFNWEIISQKNYNMAKISGIWYSIFMASDNMTRIEENGDLRVFM
RNINPLKNGSLKFDFFFMVHGECVAVTMVCEKTENYGEFTVAYEGENRVLLTETDYRMYI
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>ENSPEMP00000019617.1 pep
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gene:ENSPEMG00000017847.2 transcript:ENSPEMT00000023958.2
gene_biotype:protein_coding transcript_biotype:protein_coding
gene_symbol:Lcn11 description:lipocalin 11 [Source:MGI
Symbol;Acc:MGI:2684955]
IPGRHSRGMKLLLLLSVGLGLAWTLQDFSPEQVIGSWETLKLASNDRSVVEEEGAYECFM
TGIALLDNGNLNVSYFHRKDGKYVKEFYVAEKTDTPGRYTFEYHGKNYLTFVAVTEEFAI
IDLENQRDGEPLIVVELHGRTQEEGKLGMAHYRRQTARRGILPSNIVDVFLKRPCATQE

>ENSPEMP00000011685.1 pep
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gene:ENSPEMG00000012251.2 transcript:ENSPEMT00000015875.2
gene_biotype:protein_coding transcript_biotype:protein_coding
gene_symbol:Obp2b description:odorant-binding protein 2b-like [Source:NCBI
gene;Acc:102925381]
MKNLLIFLLLGLVAVLKAQEVPSDDQEELSGTWHIKALVCDKNHTEREGPKKVFPMTVTA
LEGGDLEVEITFWKKGQCHKKKIVMHKTDEPGKYTAFKGKKVIYIQELSVKDHYIFYCEG
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gene_biotype:protein_coding transcript_biotype:protein_coding
MKLLLMLLLLALELTLVCVHAEGETSARGKNFNPKKIEGKWYSVGLASDKREMIEEGGSM
RVFVEDIDVFENSSLAFKFHTKINGVCTGLYLVCDETEKDGEYFVEYDGRNKFTVLDTDY
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LQARDG

>ENSPEMP00000035169.1 pep
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gene_biotype:protein_coding transcript_biotype:protein_coding
LVCDETEKDGEYFVEYDGRNKFTVLDTDYDGYVIFHLRNVNKGETFQLMELYGRKQDLSS
KIKEKFAELCKKHGIVEENILDLTEADRCLQARDG

>ENSPEMP00000012546.2 pep
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gene:ENSPEMG00000012837.2 transcript:ENSPEMT00000016754.2
gene_biotype:protein_coding transcript_biotype:protein_coding
gene_symbol:Ambp description:alpha 1 microglobulin/bikunin precursor
[Source:MGI Symbol;Acc:MGI:88002]
MQNLKTQRPQSHAAPPEKAHPAETLPDIKGQHNGNTSRQTGIYGKWFNLAIGSTCPWLRK
IKDKISMSTLVLQEGETEAEISVSSTRWRRGVCEEISWTYEKTDIDGKFLSYNPKWNATL
ETYVVHTNYDEYAIFITKKFTRDHGPTITAKLYGREPQLRDSLLLEFREVALSVGIPENS
IIFMVDRGKCQQSVDGPHPWLWVIRRRPSTKCSKAQNTLFLRV
```
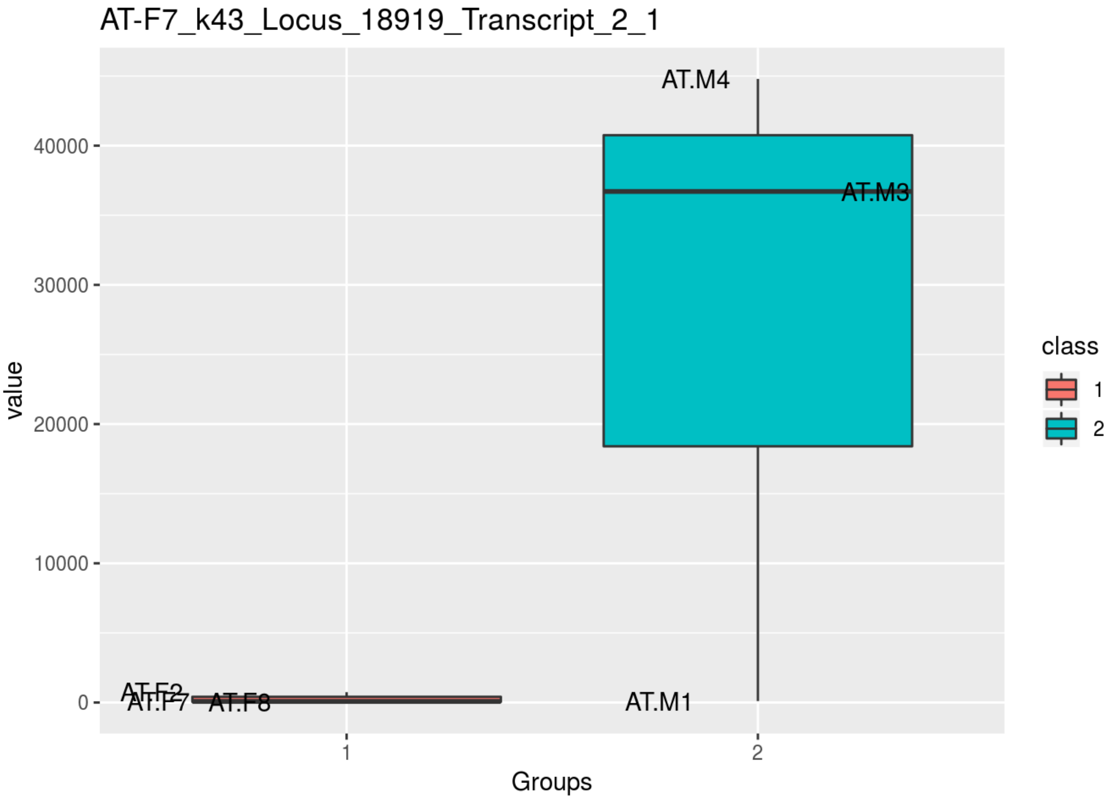
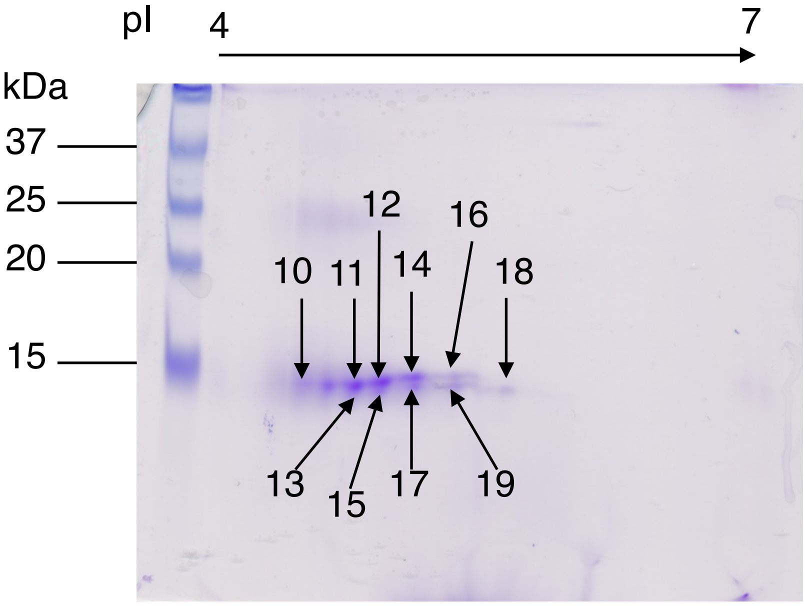

Supplementary Figure S4. Number of arvicolin reads counted in the liver transcriptome of *A. terrestris* males and females. Raw data coming from the sequencing by Illumina of the liver transcriptome of the six animals were deposited in the sequence Read Archive of NCBI under Bioproject #PRJNA545200.

Sex	Animal	reads by million of sequences
Female	AT-F2	745,7882
Female	AT-F8	15,82129
Female	AT-F7	79,36758
Male	AT-M1	101,3807
Male	AT-M3	36710,23
Male	AT-M4	44792,08

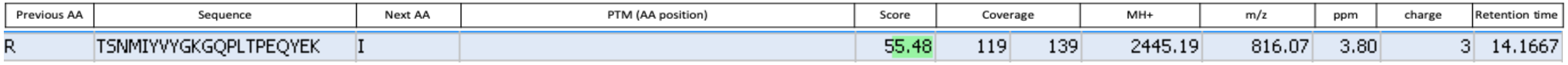


Supplementary Figure S5. Two-dimensional electrophoresis of male 6 (October) extract of urinary proteins. Spots 10-19 were cut out and analysed by nano-LC-MS/MS.

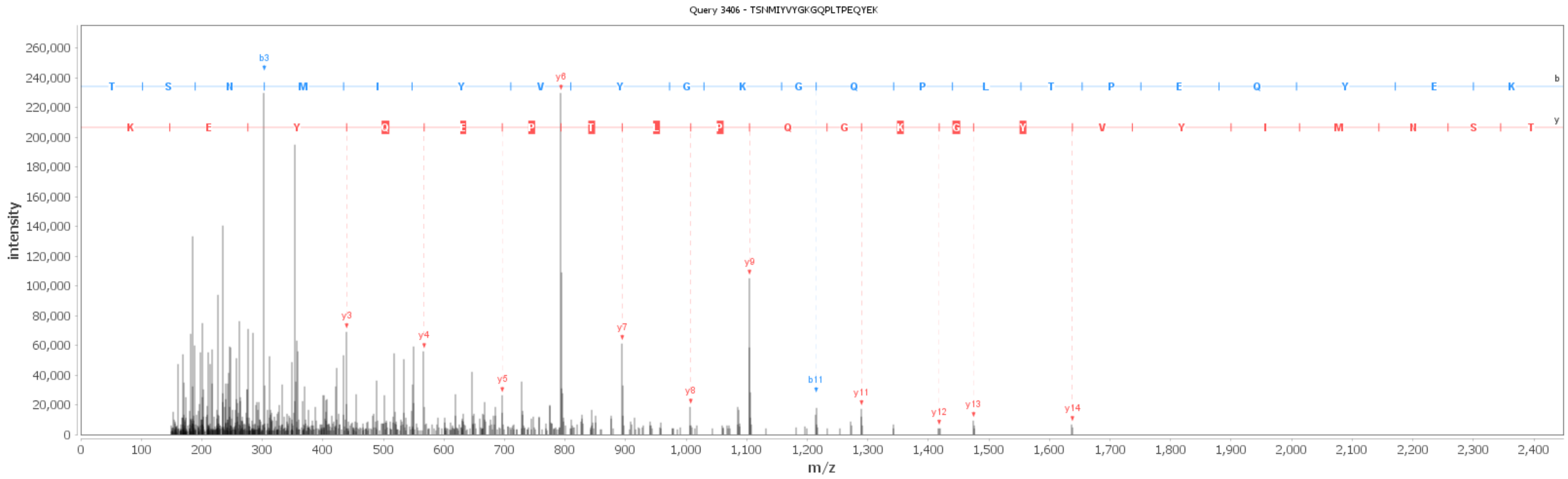


Supplementary Figure S6. MS/MS spectra of identified phosphopeptides from arvicolin. *m/z*, ion score, retention time and spectra of the naked peptides (A and C) and the corresponding phosphopeptides (B and D).

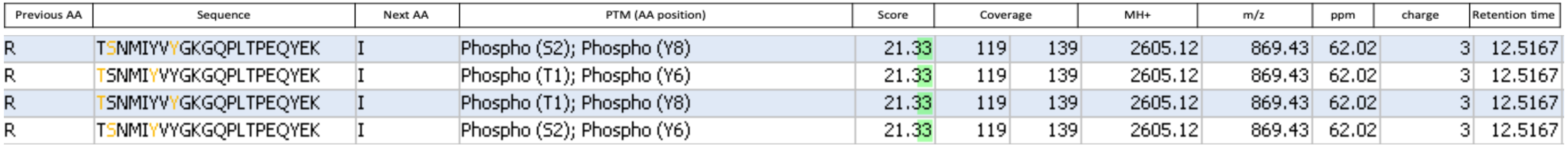
A: naked peptide (TSNMIYVYGKGQPLTPEQYEK)



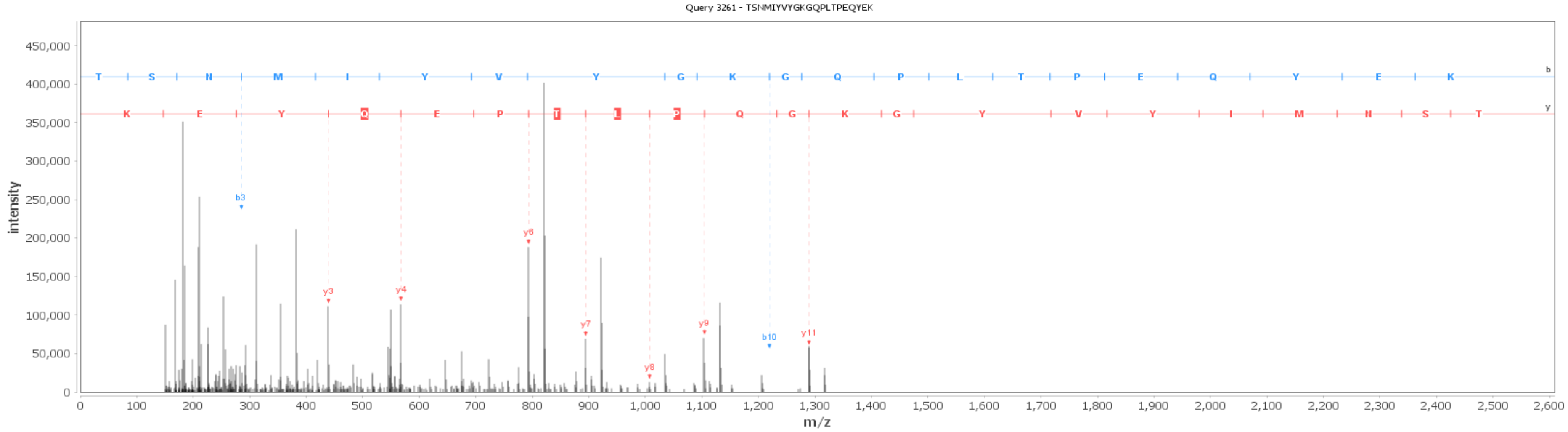
Previous AA	Sequence	Next AA	PTM (AA position)	Score	Coverage		MH+	m/z	ppm	charge	Retention time
R	TSNMIYVYGKGQPLTPEQYEK	I		55.48	119	139	2445.19	816.07	3.80	3	14.1667



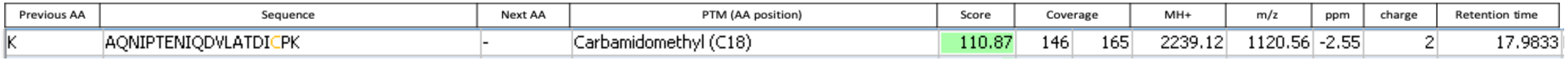
B: phosphorylated peptide (TSNMIYVYGKGQPLTPEQYEK)



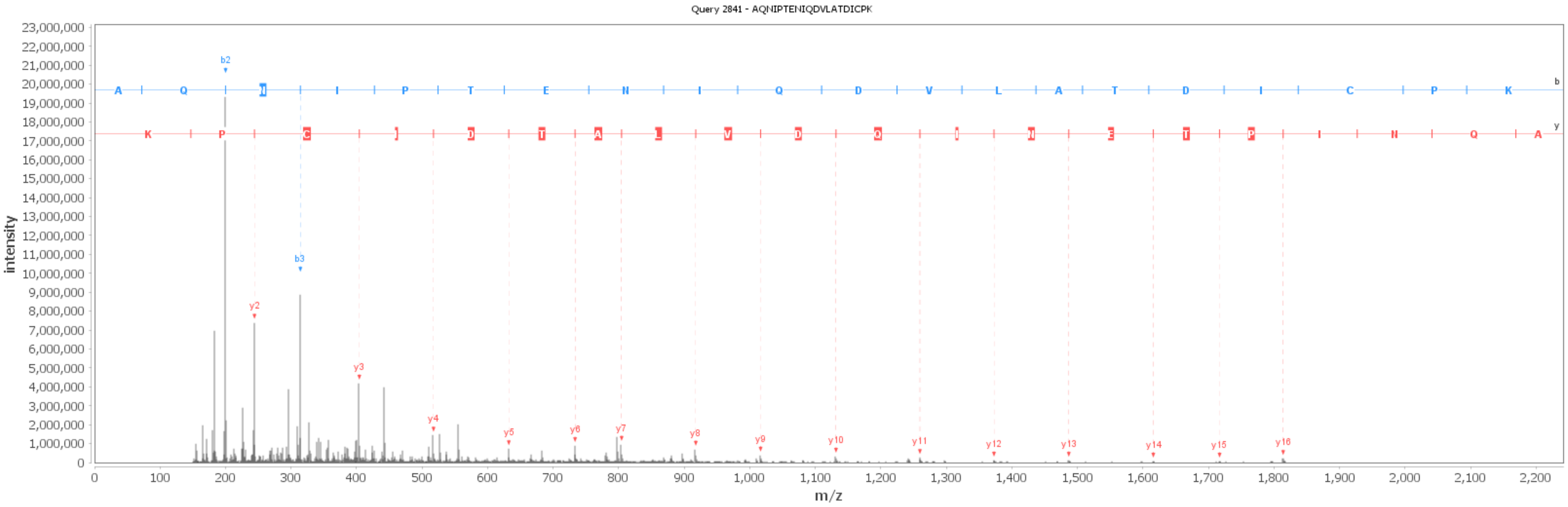
Previous AA	Sequence	Next AA	PTM (AA position)	Score	Coverage		MH+	m/z	ppm	charge	Retention time
R	TSNMIYVYGKGQPLTPEQYEK	I	Phospho (S2); Phospho (Y8)	21.33	119	139	2605.12	869.43	62.02	3	12.5167
R	TSNMIYVYGKGQPLTPEQYEK	I	Phospho (T1); Phospho (Y6)	21.33	119	139	2605.12	869.43	62.02	3	12.5167
R	TSNMIYVYGKGQPLTPEQYEK	I	Phospho (T1); Phospho (Y8)	21.33	119	139	2605.12	869.43	62.02	3	12.5167
R	TSNMIYVYGKGQPLTPEQYEK	I	Phospho (S2); Phospho (Y6)	21.33	119	139	2605.12	869.43	62.02	3	12.5167



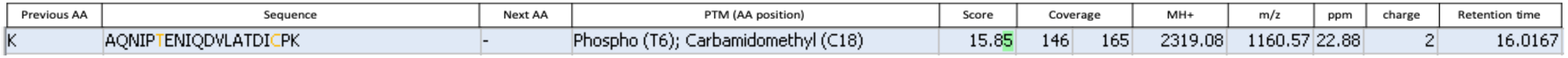
C: naked peptide (AQNIPTENIQDVLATDICPK)



Previous AA	Sequence	Next AA	PTM (AA position)	Score	Coverage		MH+	m/z	ppm	charge	Retention time
K	AQNIPTENIQDVLATDICPK	-	Carbamidomethyl (C18)	110.87	146	165	2239.12	1120.56	-2.55	2	17.9833



D: phosphorylated peptide (AQNIPTENIQDVLATDICPK)



Previous AA	Sequence	Next AA	PTM (AA position)	Score	Coverage		MH+	m/z	ppm	charge	Retention time
K	AQNIPTENIQDVLATDICPK	-	Phospho (T6); Carbamidomethyl (C18)	15.85	146	165	2319.08	1160.57	22.88	2	16.0167