

Exon number	222222222	222222222	223333333	333333333	334444444	444444444	444445555	555555555
	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
	5	15	25	35	45	55	65	75
A. lyrata t2	MFTRSNRKGG	GGNGE <b>E</b> KKNR	FTAE <b>E</b> KG <b>G</b> K	MPSKSSKEEL	<b>W</b> ELPPSWDWR	DYPGVIGPVM	<b>N</b> Q <b>K</b> L <b>Q</b> GFCTA	SQCTPNTRDN
Klet	MFTRSNRKGG	GGNGE <b>E</b> RKNR	FTAE <b>E</b> KG <b>E</b> K	MPSKSSKEES	<b>S</b> ELPPSWDWR	DYPGVIGPVM	<b>N</b> Q <b>K</b> L <b>Q</b> GFCTA	SQCTPNTRDN
Kowa	MFTRSNRKGG	GGNGE <b>E</b> NKNR	FTAE <b>E</b> KG <b>G</b> K	MPSKSSKEES	<b>A</b> ELPPSWDWR	DYPGVIGPVM	<b>N</b> Q <b>K</b> L <b>E</b> GFCTA	SQCTPNTRDN
Mias	MFTRSNRKGG	GGNGE <b>E</b> RKNR	FTAE <b>E</b> KG <b>E</b> K	MPSKSSKEES	<b>S</b> ELPPSWDWR	DYPGVIGPVM	<b>N</b> Q <b>K</b> L <b>Q</b> GFCTA	SQCTPNTRDN
Zapa	MFTRSNRKGG	GGNGE <b>E</b> NKNR	FTAE <b>E</b> KG <b>G</b> K	MPSKSSKEES	<b>A</b> ELPPSWDWR	DYPGVIGPVM	<b>N</b> Q <b>K</b> L <b>E</b> GFCTA	SQCTPNTRDN
Exon number	555555555	555666666	666666666	666666666	666666666	666666667	777777777	777777777
	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
	85	95	105	115	125	135	145	155
A. lyrata t2	NVFKKL <sup>U</sup> CRH	PD <b>N</b> I <b>H</b> <b>Y</b> -IKV	DEFEYLTNVN	DEELQAI <sup>V</sup> VQ	QPVIGILRNT	<b>N</b> DE <b>F</b> L <b>A</b> IGSG	IYRSPSGDVD	<b>V</b> N <b>F</b> H <b>Q</b> VLIIG
Klet	NVFKKL <sup>U</sup> CRH	PD <b>N</b> I <b>H</b> <b>Y</b> NIKV	DEFEYLTNVN	DEELQAI <sup>V</sup> VQ	QPVIGILRNT	<b>N</b> DD <b>F</b> L <b>G</b> IGSG	IYRSPSGDVD	<b>I</b> N <b>F</b> H <b>Q</b> VLIIG
Kowa	NVFKKL <sup>U</sup> CRH	PD <b>K</b> I <b>H</b> <b>T</b> -IKV	DEFEYLTNVN	DEELQAI <sup>V</sup> VQ	QPVIGILRNT	<b>N</b> DD <b>F</b> L <b>A</b> IGSG	IYRSPSGDVD	<b>V</b> N <b>F</b> H <b>Q</b> VLIIG
Mias	NVFKKL <sup>U</sup> CRH	PD <b>N</b> I <b>H</b> <b>Y</b> -IKV	DEFEYLTNVN	DEELQAI <sup>V</sup> VQ	QPVIGILRNT	<b>N</b> R <b>D</b> F <b>L</b> GIGSG	IYRSPSGDVD	<b>I</b> N <b>F</b> H <b>Q</b> VLIIG
Zapa	NVFKKL <sup>U</sup> CRH	PD <b>K</b> I <b>H</b> <b>T</b> -IKV	DEFEYLTNVN	DEELQAI <sup>V</sup> VQ	QPVIGILRNT	<b>N</b> DD <b>F</b> L <b>A</b> IGSG	IYRSPSGDVD	<b>V</b> N <b>F</b> H <b>Q</b> VLIIG
Exon number	777777777	777777777	777777777	777777777	777777777	77777777	77777777	
	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	
	165	175	185	195	205	215		
A. lyrata t2	YGYD <b>--</b> NGKP	YWIIQ <b>N</b> SYGE	GWGNGGFGYV	YRRI <b>K</b> SGQGS	<b>E</b> FFAVAYPKI	RG <b>F</b> PRK <b>P</b> R*		
Klet	YGYD <b>D</b> SNGKP	YWIIQ <b>N</b> SYGE	GWGNGGFGYV	YRRI <b>N</b> SGQGS	<b>E</b> FFAVAYPKI	RG <b>Y</b> PRK <b>P</b> R*		
Kowa	YGYD <b>--</b> NGKP	YWIIQ <b>N</b> SYGE	GWGNGGFGYV	YRRI <b>K</b> SGQGS	<b>V</b> FFAVAYPKI	RG <b>F</b> PRK <b>Q</b> R*		
Mias	YGYD <b>D</b> SNGKP	YWIIQ <b>N</b> SYGE	GWGNGGFGYV	YRRI <b>N</b> SGQGS	<b>E</b> FFAVAYPKI	RG <b>Y</b> PRK <b>P</b> R*		
Zapa	YGYD <b>--</b> NGKP	YWIIQ <b>N</b> SYGE	GWGNGGFGYV	YRRI <b>K</b> SGQGS	<b>E</b> FFAVAYPKI	RG <b>F</b> PRK <b>P</b> R*		

**Figure S6.** Alignment of the predicted variants of the *A. halleri* Cysteine Protease-Like 1 (CPL1) protein. Shown are the conceptually translated consensus coding sequences of AL8G32870 from two metallicolous (Klet and Mias) and two non-metallicolous (Kowa and Zapa) populations of *A. halleri* based on the *A. lyrata* genome annotation version 2.1 (transcript variant t2). Amino acids that are polymorphic among the five genotypes are printed in bold type, with those common to both metalliferous (M) sites shown in red fonts and those common to both non-metalliferous (NM) sites shown in blue fonts, and highlighted in yellow when M populations share derived alleles. A grey bar above the alignment marks the PF00112 papain family cysteine protease domain. Catalytic residues are underlined.