

Fig. s1. The predicted 3D structure assessment of Bx-CPI-1. Laplace image (Ramachandran Plot) of 3D structure of Bx-CPI-1 (**A**). Residues in most favoured regions [A,B,L] are 88 (81.5%); Residues in additional allowed regions [a,b,l,p] are 14 (13.0%); Residues in generously allowed regions [$\sim a, \sim b, \sim l, \sim p$] are 4 (3.7%); Residues in disallowed regions are 2 (1.9%). Number of non-glycine and non-proline residues is 108; Number of end-residues (excl. Gly and Pro) is 2; Number of glycine residues (shown as triangles) is 6; Number of proline residues is 5. Total number of residues is 121. Verify 3D model of Bx-CPI-1 (**B**). The 66.99% of the residues have averaged 3D-1D score \geq 0.2.

Buraphelenchus xylophius (Bx-CFI-1) MKVULALLUSVPIMAGIM GUSTLELKDFVQCFILTVEKYQCEBEL, YHHAGIMS Actionates gp. (KR3021 RSK 000099) MEridagyne enterolobii (scaffoldi588 cov156.gl8152) Fabditophanes gp. (KR3021 RSK 00019990.1) MFYCKAFFIFELINSEGIFILLIFLANDERING SVCGUVEKUKLEKKLASSTAATTAESEN. FYCFFKELS 97 Meloidogyne enterolobii (scaffoldi588 cov156.gl8152) Frichuris trichura (TEE 000083001-mRNA-1) Trichuris trichura (TEE 000083001-mRNA-1) Elacobarde sp. (KR3021 RSK 0001001-mRNA-1) Fastiona kastu (SLC 000091001-mRNA-1) Elacobarde stercorali (SLF 000083001-mRNA-1) Fisheria hava (TO2 18141.1) MERICASSTANDESENTER MERICASSTANDESE			
Globodera pallida (GPLIN 000129900) Divjenchus destructor (Dd 00099) Heterodera glycines (Hecqly.GOGODO1419.2) MERCIASGEORVOMAGETYCONTEQUIVALENTSKENU GWITEQUIVALENTSKENU, FYGPTKEISS 67 Heterodera glycines (Hecqly.GOGODO1419.2) MERCIASGEORVOMAGETYCONTEQUIVALENTSKENU, FYGPTKEISSENU, FYGPTKEISS 67 Parastrongylcides trichoura (TFK 0001769700.1) MERCIASGEORVOMAGETYCONTEQUIVALENTSKENU GWITEQUIVALENTSKENU, FYGPTKEISS 67 Stongylcides steroralis (STF 0000414900.1) Plectus sambesi (FSAMS.scaffcld664size4241.g7865.1) Ceanchabdits elegans (ROBIO.1-mRNA-1) Trichuris trichura (TFK 000095301-mRNA-1) Strongylcides steroralis (STF 0000016301) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU (MERCIASSENU (MERCIASSENU) MERCIASGEORVOMAGETYCONTEGLASSENU (MERCIASSENU	Bursaphelenchus xylophilus (Bx-CPI-1)	NKVVLALLLVSVFIMAGQLVGENSKLDLKDFVVQEYLTKVEKQYNQESNEKYHHRALQAVS	61
Ditylenchus destructor (Dd 00099) Heteroders dyvines (Hetqly.GGOOOL419.2) Heteroders (Hetqly.GGOOOL419.2) Heteroders dyvines (Hetqly.GGOOL419.2) Heteroders (Hetqly.GGOOL419.2)	Globodera pallida (GPLIN 000129900)	MAMADQSQHNSVVCCWTDQDVADDSVKALASRSVAKIMAESNDRFQLFFVQIIS	54
<pre>Heteroders qlycines (Hetqly.GOOOD1419.2) Heteroders qlycines (Hetqly.GOOO</pre>	Ditylenchus destructor (Dd 00099)	MISNLYCSIAIFLAVIINISKSEMLVGEWVEHDVNKDEIKNLSKKVIHKINAESNDMFYOFPKEILS	67
Meloidogume entercolobii (scaffoldi585 cov156.gl8152)	Heterodera glycines (Hetgly.G000001419.2)	MRFQKAKFAIFRINSHQIFILLILIKLAMTDQQNSIVGEWIEQNVDEKIKSLASRSVAKINSESNDRFSLFPLEVLS	79
Rabditophanes sp. (KR001 RSKR 000109900.1) MRTILAVATIAFTMITHERPVARTIGEVARTIFUELDITENHVGENTEQLITENTVERGEATHEUXENED BREASTORQUIGES STEROTABLE VARUATURE CONTROLOGUE AND	Meloidogyne enterolobii (scaffold15838 cov156.g18152)		58
Parastrongyloides trichosusi (FTER 0001760700.1)	Rhabditophanes sp. (KR3021 RSKR 0001099900.1)	MRFIILAVATIAIFTCMTLATEPVAETTIHRAERIKLNPCITEHHMVGEWTECLVISPTIEYARRATKTFNLESNDRFYYSFIAVIS	88
Strongyloides sterozalis (STF 000041490.1)	Parastrongyloides trichosuri (PTRK 0001760700.1)	MRFVLLVAIVLSTLFAVMSPIMTGEWTECDATHFDIVSLGCHAVTKYNCCSNDLHYHGFVEVLS	64
Plectus sambesii (PSAMS.scaffold664ize4441.g7865.tl) Caenothabdits elegans (ROBBO.1s.2) Meator americanus (NECAME 1133) Trichurs trichurs (TRE 000083501-BRNA-1) Trichurs trichurs (TRE 000093501-BRNA-1) Trichurs trichurs (TRE 000093501-BRNA-1) MESTUCZELNALUCTWASHAGUNG FIXQBESERNEYDENSES.NUMLERIVE 66 Trichurs trichurs (TRE 000093501-BRNA-1) MESTUCZELNALUCTUASHAGUNG FIXQBESERNEYDENSES.NUMLERIVE 66 Trichurs trichurs (TRE 000093601-BRNA-1) MESTUCZELNALUCTUASHAGUNG FIXQBESERNEYDENSES.NUMLERIVE 66 Trichurs trichurs (TRE 000093601-BRNA-1) MESTUCZELNALUCTUASHAGUNG FIX BASELSEND Trichurs (TRE 0000093601-BRNA-1) ACGON SMMKIKYETEKTRELGSVCASDAFV.TEKHNVERNISSE 113 Trichurs (TRE 0000093601-18) Resolution (TRE 000019900.1) Resolution (TRE 00019900.1) Resolution (TRE 000019900.1) Resolution (TRE 000019900.1) Resolution (TRE 000019900.1) Resolution (TRE 000019900.1) Resolution (TRE 000019900.1) Resolution (TRE 00001990.1) Resolution (TRE 000019900.1) Resolution (TRE 00001990.1) Resolution (RESOLUTI	Strongyloides stercoralis (SSTP 0000414900.1)	MSRCGMTGENTECSVNDTDIICLACKSVDRFNCCSNDLVYHGFVKVLS	48
Caenochabdicis elegans (ROBBIO.1s.2) Meator americanus (ROCHZE 1133) Trichuris trichura (TER 000089501-mENA-1) Trichuris trichura (TER 000089501-mENA-1) Trichuris trichura (TER 000089501-mENA-1) Trichuris trichura (TER 000089501-mENA-1) Strong (TDF 000098501-mENA-1) Strong (TDF 000098501-mENA-1) Stron	Plectus sambesii (PSAMB.scaffold664size44241.g7865.tl)		78
Nector smericanus (NECAME 1113)	Caenorhabditis elegans (R01B10.1a.2)		66
Trichuris trichura (TTRE 0000983501-mRNA-1)	Necator americanus (NECAME 11133)		66
Trichinella mativa (T02 14141.1)	Trichuris trichiura (TTRE 0000893501-mRNA-1)	MLRVIAFLFAFIFAVSSGTLEGEWFFLOVKSEEARRVAHRSLADBUTKSNSAYHDMLIRIVE	62
Elscophors elsphi (EEL 000011001-mENA-1) Brugis timori (STM 7000961601-mENA-1) Brugis timori (STM 7000961601-mENA-1) Brugis timori (STM 7000961601-mENA-1) Brugis timori (STM 7000961601-mENA-1) Bursephelenchus xylophius (Bx-CPI-1) Bursephelenchus xylophius (Bx-CPI-1) Bursephelenchus xylophius (Bx-CPI-1) Brecovers glycines (Herdy: GOCO001415-2) AkSgrid ClyEikLivMsStR FKSALTABEAKCAGGS.ARKITYEAGINSKE.S. Birsephelenchus xylophius (Bx-CPI-1) Bursephelenchus setturotor (Dd 00099) AkSgrid ClyEikLivMsSterkers, StateABACHEGG, ARKITYEAGINSKE, S. Halditophanes sp. (RS031 BSRR 000199900.1) AkSgrid ClyEikLivMsSterkers, StateABACHEGG, ARKITYEAGINSKE, S. Bursephelenchus stercoralis (SIT 000019990.1) AkSgrid ClyEikLivMsSterkers, StateABACHEGG, ARKITYEN, RESCITIKER, S. Bursephelenchus stercoralis (SIT 000019900.1) Parastrongyloides trichosuri (FTR 000093501-mENA-1) ASGRID CLYEILINGENGARKUG, RENESCIRE, SARLESANGCURG, GRAIESSANGCURG, SARLESANGCURVERNES, BEGVITIKENSKER, SCITWALESSANGCURVER, SCINALINGER, SCINALINGEN, SCINALINGER, SCINALINGEN, SCINALINGER, SC	Trichinella nativa (T02 14141.1)		73
Brugis timori (BTMF 0000961601-BRNA-1) MISIEDOSLAVILLIFALALVELQASSIEESSTDIGUEQUE DEW DESSEDDENT CELLESVITT WEICOSMEC. VELMENTELLESSED CONSENSES BS Consensus MISIEDOSLAVILLIFALALVELQASSIEESSTDIGUEQUE DEW DESSEDDENT CELLESVITT WEICOSMEC. VELMENTELLESSED CONSENSES BS Surasphelenchus xylophilus (Bx-CPI-1) BLCCW SMMKIKVTEHTHELG	Elaeophora elaphi (EEL 0000011001-mRNA-1)	MIFSIKDGLLVILLLLFGVVALLFLCGLTSMESKGHAPLGCOTLIGENCERSPEDSEICELLPSILMKVNCCSNDEVELMPIKLLK	86
Fasciola hepatica (AAV68752.1)	Brugia timori (BTMF 0000961601-mRNA-1)	MISIRDGSLAVILLIFIAIALVELCRASDIESKTDICIGCOVLIGENCECSPDDNEICELLPSVLTKVNCCSNDEYHIMPIKLLK	85
Consensus 99 Burasphelenchus xylophilus (Bx-CPI-1) BECGW SCHWARLEYVESHTREEGSVCASDAFV.TDEHNVEAKIYSGFTK.TEEITIHPG	Fasciola hepatica (AAV68752.1)	MIRILGICILHEMSCRVFGENLWGEYTE PRSVTFEERSVFCPMILSKLITAGSV. ESSCELELLO	65
Bursaphelenchus xylophilus (Bx-CPI-1) ECONOMINERYFERTNELGSVCASDAFV.TDEHVEXENJSCETK.TEEITLHPQ	Consensus	qq	
Burasphelenchus xylophils (Bx-CPI-1) BCCDN GHNMKIKYETGINIEGG			
Globodera pallida (GPLN 00012990) a GGGUNGHERVEREVEREVES. ALTADERACAEGOS.ARKITYZAGINNSKE.S. (31) Bitylenchus destructor (dd 0099) akgGUNGTUREVES.ALTADERACAEGOS.ARKITYZAGINNSKE.S.(31) Hetarodera glycines (Hetgly.G00001419.2) akgGUNGTUREVESSKENKUSSKENE	Bursaphelenchus xylophilus (Bx-CPI-1)	ACCUNSCENNYKIKYEFERINCIEGSVCASDAFV.TDKHHVEAKIYSCENTK.TEEITLHPC	121
Datylenchus destructor (Dd 00099) AKGUNG CQUELKLIVNESS ENNENASVEFERTKOCECCE. NARVYTAKINIKEEN. FERITIKENSARNE	Globodera pallida (GPLIN 000129900)	ACSCWARMXYXLRIVVCKSNCFKSALTADFANCAEGDQS.ARKITYEAEGINNSRKE.SE	113
Heteroders qlycines (Hetqly.G000001419.2) Argenvert@vd.Hivenseshsulathancatenco_TARKTFLYIPEKTEC_EGITYKEE	Ditylenchus destructor (Dd 00099)	AKSOVASEIQYELKILVGKSSCKKNEVASVEFDETKCQEQDENARKVYTAKIWIKENEN.FEEITITKENSAENE	141
Meloidogume enterolobii (scaffoldi5838 covi56.d18152) affordiscilititevertelitte	Heterodera glycines (Hetgly.G000001419.2)	AKSCYNAEICYCLRIKYGRSNCSENSNLADHANCATEDDQ.TARKTFLVTIWEKPWEQ.FECITYKLE	145
Rhabditophanes sp. (KR3021 BSKR 000109900.1) #KSTSGSLHRUGFWGETMWENVESFGNWICTQLEFTWGKGQLVFINWEFKESNEERITIGFGI	Meloidogyne enterolobii (scaffold15838 cov156.g18152)	AESCYNSELLYILTERVERTOCIETCYNSENLSECNRYTGEL. ERICARR.	107
ParastrongyLoides trichosuri (FTR 0001460700.1) #XSGNWartNYLETVINGETTIKNEYNHEDLIAREHTNYRDGRKHIVTNNESSFRED.FEVILTEVENKOACA	Rhabditophanes sp. (KR3021 RSKR 0001099900.1)	AKSKINSESLHNICFRVEETNVMENKVSFCNVNCTCLKPIVNGKCCLVFIKVWIKPWESNFEKITIIGFKCI	160
Strongyloides steroralis (STP 0000414900.1) #KSGYWACHXELQLIARTLENKWSHELCECREVKSDGPQQIIY01895HED.FEEIFHGIRQA 119 Plects sambesi (PSAMB.scaffold664size44241.g7865.1) ATSGYWCYSTELEVLOESDDANTYSHEQVSATESIKETSTAPKKYCKTYWCKSMEN.FEEIKHGIRQA	Parastrongyloides trichosuri (PTRE 0001760700.1)	ARSOWAETNYELEVINGETGTIENCVKHEDLTAEHTKVKDD., GKKKIVTVNIWSKPNED. FEEYTIKGVKCA	135
Plectis sambesii (PSAMS.scaffold664sire4241.g7865.tl) ATECUNVCVSLUPUVCVSLUPUVCVSLUPUVCVSLUPUSCVSLGSLEFISTLAPKVCVTVVQCSKEN.FEZIAVTOCCNFVVENSN. 156 Caenorhabditis elegans (ROLBIO.ls.2) ASTOUNGLISTKLEVIVCESNCRGELGAMEITSSNCGIKGG.GSRALVQVTUREKPEN.FCQTVEKIRCVTADEQF 143 Necator americanus (NKCAME 11133) ASTOUNGLISTKLEVIVCESNCRGELGAMEITSSNCGIKGG.GSRALVQVTUREKPEN.FCQTVEKIRCVTADEQF 143 Trichuris trichurs (TTRE 0000893501-mRNA-1) ASGUVACHARKIUNYELSKARKEVG.RKRDS.GLEKGG.GSRALVQVTUREKPEN.FCQTVEKINVELSKARKEVG.ARTINSCLILKF.NQIFCS.HLEVC. 116 Elector advantik (EL 000001001-mRNA-1) ASGUVACHARKEVGARSEKRG.ISSEVNIKTOKIEG.HEQVTITEKPENER, LQVISIGTWUSSV 155 Brugias timori (BTHF 0000861601-mRNA-1) VSGUVACHARKEVGARSEKRG.ISSEVNIKTOKIEG.HEDQVTIEWERFRED.FLQVISIGTWUSSV 157 Paugias timori (BTHF 0000861601-mRNA-1) VSGUVACHARKEVGARSEKRG.ISSEVNIKTOKIEG.HEDQVTIEWERFRED.FLQVISIGTWUSSV 157 Fasciala hepatica (AAV68752.1) VSGUVACHARKEVGARSEKRG.ISSEVNIKTOKIEG.HEDQVTIEWERFRED.FLQVISIGTWUSSV 157 Fasciala hepatica (AAV68752.1) VSGUVACHARKEVGARSEKRG.ISSEVNIKTOKIEG.HEDQVTIEVERFRED.FLGVINIELSV 157	Strongyloides stercoralis (SSTP 0000414900.1)	AKSCYNACHNYELCILIGHTETLENKYSHGELTEECKHVKSD., GPCCIITVGIWSHFWED, FEEITFKGIKCA	119
Caenorhabditis elegans (R01B10.1a.2) ASTUNA EISTKLEVLVGESNCRGELQAMEITSSNCCIKDG.GSRALYQVTIMEKPEN.FECFTVEKIRDVTADECF 143 Necator americanus (NKCAME 11133) ASTUNA EISTKLEVLVGESNCRGELQAMEITSSNCCIKDG.GSRALYQVTIMEKPEN.FECFTVEKIRDVTADECF 143 Trichuris trichuris (TRR 000093501-mENA-1) ASTUNA EISTKLEVLVGESNCRGELQAMEITSSNCCIKEG.GSRALYQVTIMEKPEN.FECFTVEKIRDVTADECF 116 Trichuris trichuris (TRR 000093501-mENA-1) ASTUNA EISTKLEVLVGESNCRGELSKARKOVARSTELSKARKUVGESNERVSG.SNERVSARDVCKVSG.DSALCVVTIMEKPELDEVCTSVERAELQ 151 Elecohora elaphi (EEL 000001001-mENA-1) VSQUMAVVKMEVQVARSECKG.ISEVUNKTKKEG.HEDCVTILEVMEKPED.FLQVSIGITVKJSV 158 Fugia timori (BTMF 0000961601-mENA-1) VSQUMAVVKMEVQVARSECKG.ISEVUNKTKEG.HEDCVTILEVMEKPED.FLQVSIGITVLSSV 157 Fesciala hepatica (AAV68752.1) VSQUMAVVKMEVQVARSECKG.SATGPGCWEVVVFVLYSSKSAFSVGFFRVSCT	Plectus sambesii (PSAMB.scaffold664size44241.g7865.tl)	ATSCHARVSYELEVINGESDEARNTWSHECVSATRCSLKETSTAPKKVCKFTWCKSNEN.FEEIRMIGCENFVENSN.	156
Necator americanus (NECAME 1133) ALGOVACINE INFORMATING	Caenorhabditis elegans (R01B10.1a.2)	ASTOVACISTRIEVINGESNCKRGELCAHEITSSNCCIKDG., GSRALYCVTIWEKPNEN, FECFTVERIRCVTADECF	143
Trichuris trichiura (TTRE 0000893501-mRNA-1) ASSCHWEANYKLDYJGLSNCAKKDVG.RKENRSLCLIKF.MQIFCS.HLCEVC. 116 Trichinella nativa (TO2 14341.1) ATSCHWEINYKLDYJGLSNCAKKDVG.RKENRSLCLIKF.MQIFCS.HLCEVC. 116 Lacophore Lapht (EL 000001001-mRNA-1) VSSCHWEINYKREVCVASSCHWEINKELG.HEDCYTIEVERFELD.FLCYWINESV 155 Brugia timori (BTMF 0000961601-mRNA-1) VSSCHWEINYKREVCVASSCHWEINKEKSK	Necator americanus (NECAME 11133)	AESOWAAFIKYIFEVIFUESTCKKGEMSATELSAANCCLKEG., GNRALYRVELWEKEMEN, FECFNVEKIRNVCASEHI	143
Trichinella nativa (T02 14141.1) ATBCANG EINEKLTIYVEESECSENTYSAQDAHQNRCKLVSG. LDARLCKVTHEKEHLUVEENADFDCTSVFRAEALQ 151 Elacobora elaphi (EEL 0000011001-mRNA-1) VSGCUAUCVTKNEUCVARSECKNG. LSEEVNLTCKRLEG. HEQVITLEVNEKHELD, LQVSILGTNVLSSV 155 Brugia timori (BTNF 000064601-mRNA-1) VSGCUAUCVTKNEUCVARSECKNG. LSEEVNLTCKRLEG. HEQVITLEVNEKHELD, LQVSILGTNVLSSV 157 Pasciala hepatica (AAV68752.1) VSGCUAUCVTKNEUCVARSECKNG. SACCUSCT	Trichuria trichiura (TTRE 0000893501-mRNA-1)	ASSOURATION VIGLENCAREDVG. BRENBSLCIILKE. MOIDECS. BLCEVC.	116
Elacophora elaphi (EEL 0000011001-mRNA-1) VSSGVAGVKYKNEVQVARSECKKG.LSEEVNIKTCKRIEG.HPEQVFILEVMEKFHED.FLQVSILGIKVLSSV 155 Brugia timori (BTMF 0000961601-mRNA-1) VSSGVAGVYKKMEVQVARSECKKG.ASEQVNIKTCKRIEG.HPEQVMILEVMEKFHED.FLQVNILEIKVLSSV 157 Fasciala hepatica (AAV68752.1) VSTGVAGENKKRVSG.ATGFGCWEVVVVVPLYSSKSATSVGFFRVSCI	Trichinella nativa (T02 14141.1)	ATSCHAFTNYKLTTYVGESECSENTVSACDAHCNBCKLVSG., CDARLCKVTTHEKENINVEEVADFDCTSVPRAEALO	151
Brugia timori (BTMF 0000961601-mRNA-1) VSCOVORVEXENEVCVARSCERS.ASECVNEKTERLEG.HFDCVMTLEVMEREFED.SLCVNILETKVLSSV 157 Fasciala hepatica (AAV68752.1) VSCOVAETNEREFEVSCE.ATCFGCWEVVVFVFLYSSKSATSVGFFFRVSCT	Elaeophora elaphi (EEL 0000011001-mRNA-1)	VSSCHARVKYKMEUCUBSSCKKG, ISEEVNIKTCHBLEG, HPECVETLEUWENEWER, FLOVSILGTKVISSU	158
Fasciola hepatica (AAV66752.1) VSTOVACINIKERVSGC.ATCFGCWEVVVFVPLYSSKSATSVGTPTRVSCT	Brugia timori (BTME 0000961601-mBNA-1)	USSOUNDEVENEUCUDESSCRES, ASSOUNT KTORKING, HEDOUMTIEUWERENED, FLOUNTLETKULSSU	157
Consensus y d	Fasciola hepatica (BAV68752.1)	VSTOUVAL INVERTISCE, ATCRCWEVVVVVVVLSSKSATSVGTPTRVSCT	116
	Consensus	V a	

Fig. S2. Multiple alignments of predicted **Bx-CPI-1** protein sequence of *B. xylophilus* with other 15 cystatins. Letters in black boxes, red boxes, and blue boxes indicate invariant amino acid residues, highly conserved amino acid residues, and moderately conserved amino acid residues, respectively, among the CPI proteins.



Fig. S3. Symptoms in *P. massoniana* after inoculation with *B. xylophilus*. Each seedling was inoculated with about 10,000 nematodes. The early stage of PWD (early); the initial stage of PWD (initial); the middle stage of PWD (middle); the later stage of PWD (later); the nematodes cultured on *B. cinerea* (control).



Fig. S4. Morphology of *B. xylophlilus* after soaking in dsRNA (non-dsRNA and *Bx-cpi-1* dsRNA) for 48 h. Scale bars = $100 \ \mu m$.



Fig. S5. Symptoms in *P. massoniana* after inoculation with *B. xylophilus*. The 1-year-old pines inoculated with 500 nematodes, and 2-year-old pines inoculated with 1,500 nematodes. Photographs shows the symptoms at 16d, 22d and 28d. Within each photograph, pot 1 and b indicates pines inoculated with ddH₂O, pot 2 and a indicates pines inoculated with *B. xylophilus* soaked in non-dsRNA, and pot 3 and c indicates pines inoculated with *B. xylophilus* soaked in *Bx-cpi-1* dsRNA.



Fig. S6. The standard curve and melt curve of *actin* and *Bx-cpi-1*. The standard curve of *actin* and *Bx-cpi-1* (**A**). Cq value is proportional to template cDNA concentration. The slope of *actin* and *Bx-cpi-1* was -3.058 and -3.071, the correlation coefficient (R²) of *actin* and *Bx-cpi-1* was 0.998 and 0.999, the amplification efficiency of *actin* and *Bx-cpi-1* was 112.356% and 111.659%. The melt curve of *actin* and *Bx-cpi-1* (**B**).