SUPPLEMENTARY INFORMATION - SUMMARY

The Supplementary information contains:

- 7 Supplementary figures (Supplementary file 1)
- 18 Supplementary tables (Supplementary file 2)
- Supplementary references (Supplementary file 1; for Supplementary figures and Supplementary tables)

FIGURE S1

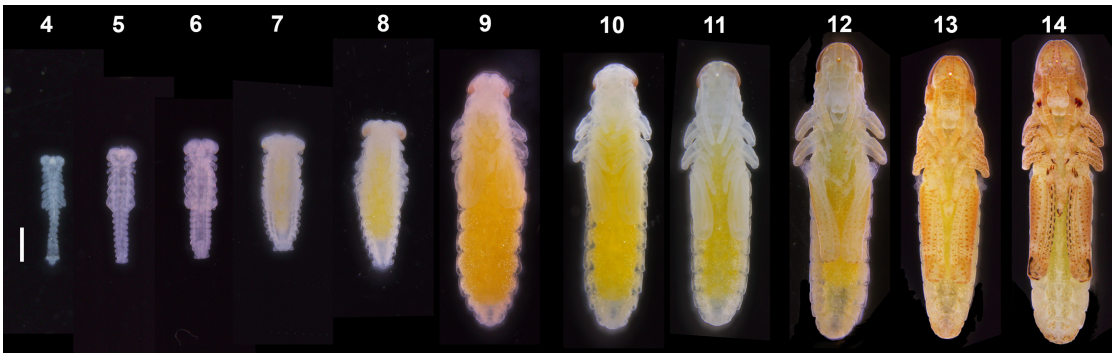


Figure S1. *Schistocerca* embryonic stages used in this study. Images of live embryos dissected out of the eggs; imaged under a stereomicroscope. Eggs and embryos of *Schistocerca* typically slightly vary in size. Numbers indicate age in days. Scale bar: 1 mm. Background in photos was cleaned (see Materials and Methods).

FIGURE S2

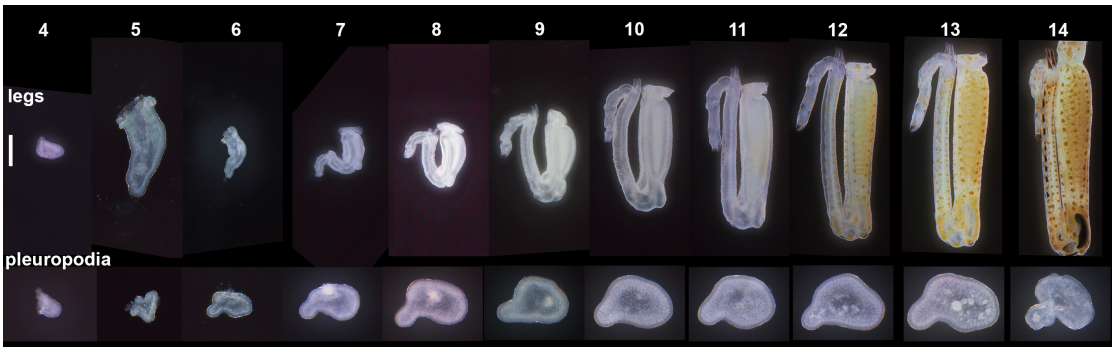


Figure S2. External features of developing hind legs and pleuropodia. Compare the sizes of the appendages; imaged under a stereomicroscope. Numbers indicate age in days. Scale bar: 0.2 mm for all pleuropodia and for legs at days 4 and 5; 0.5 mm for legs at days 6-14.

FIGURE S3

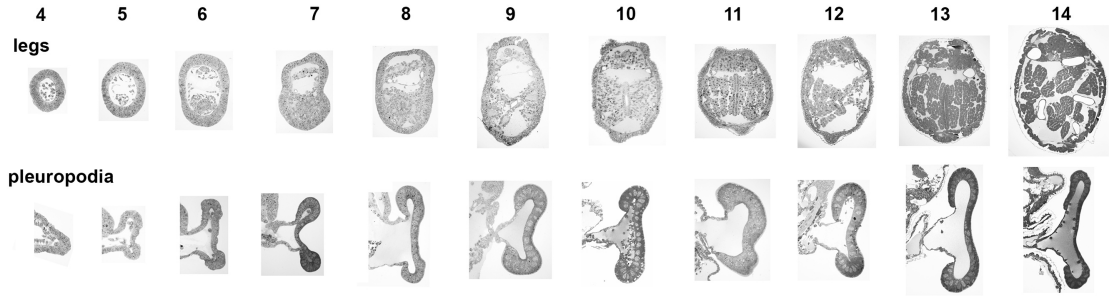


Figure S3. Cross-sections through developing hind legs and pleuropodia. Toluidine blue stained semithin sections of appendages embedded in epoxy resin. Numbers indicate age in days.

FIGURE S4

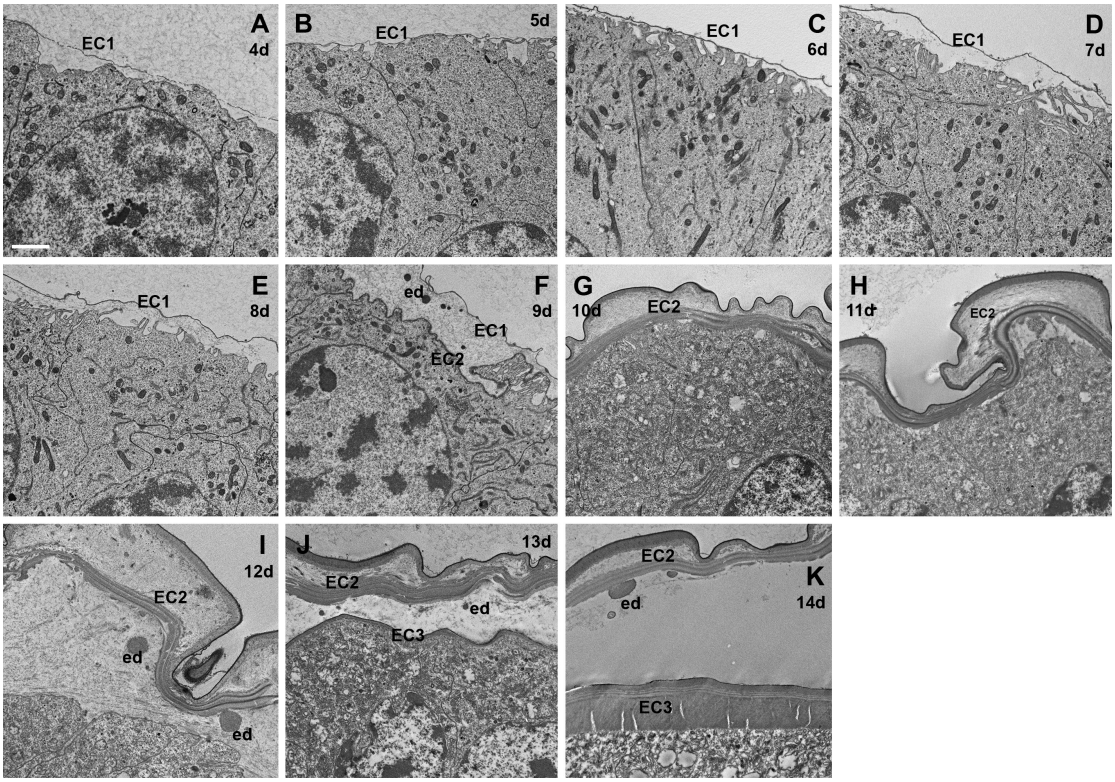


Figure S4. Ultrastructure of epidermal cells in developing hind legs. TEM micrographs. Compare with pleuropodia in Figure 3. Note the three different cuticles and appearance of ecdysial droplets (ed) during embryonic moulting.

EC1, EC2, EC3, the first, the second and the third embryonic cuticle, respectively (EC3 becomes the cuticle of the first instar larva). Scale bar: 2 μm.

FIGURE S5

(A)

Sg-nag1

MSVISTTVLVFALYGIFSCFATQAEEERPVWTWECRESRCEKVAAGEGEAQSLGACRLSCDPWATLWPRPRGGLQRTPGRLLA
LNPYSVSVEAAGRDLQPGVRQLLQEAGRIFHRKVERKARTGAKLRSAGERRSLFVTLTVSDGQTRSFHTDTSEAYSLSISEVTAG
RVNAAVTADTFFGARHALETLYQLIVYDDINKQLLLLSEINLSDSPAFPHRAIALDTARSYFSVASIKRTIDAMAANKLNTFHW
HITDSHSFPFVSETFPKLSQYGAYSPEKVYTPDEIKSVVEYARVRGVRIIPEFDAPAHVGEGWQWVGDNATVCFKADPWSQYCV
EPPCGQLNPTSEKMYQVLAGIYKDMLNVFDSDVFHMGGDEVNMNCWNTSEVITDWMDANGIPRTEEGLHELWDRFQSRAY
SLLAEANGKKELPVILWTSTLTDVAHVDKYLDNKRYIIQIWTRGTDLVIPELIRKGFRVIFSNYDALYFDCGFGAWIGSGNNWCS
PYIGWQKVYDNNVWDLLSAFGIDVGEGSEARKLVLGSEAALWSEQADEFALDGRLWPRAAALAERLWTDPVEGWMSAEHRF
LIQRQRLVDEGIAADTIEPEWCLQNQGHCYA*

Sg-nag2

MAPAPPAPHLLALTLLLTLLPSPPVVWANSPRWQWTCDSGLCVRSEAPPEPRLDAELEETVVQRSVHRLRPPWPSHELCRLT
CGPYGALWPRPTGHTLIADALVPFNPATARFDLSAVAGEQGRELVDAASRRWVRDLQHALAASGGHGGGGEVAGAAAGAGT
DVLVTVLTRDSPQALSWETDETYTLDVASSGHEVRVTVSAQTVWGALHGLTSLRQLVGCCSEDGAALMVAEARIVDGPVYAH
RGLLLDTARNFLPVETMMATMDAMAASKLNVLHWHATDSQSFPLLLPRVPQLARWGAFSARETYSSQQVSALLGYAHARGI
RLLLELDAPAHSGQGWQWGEAEGLGALALCVGQQPWRRLCIQPPCGQLNPANPRLVGVLADVYRDVVDLWPPGQPLHMGG
DEVSYSCWNSSAEVLEYMSKRRWDRSQDGFLRLWAEFQQAALEALDAARGSSDVPAILWSSHLTRPGNIERFLNSSRYVIETW
VEGGDPLPQQLLALGYRLVVATKDAWYLDHGFWGSTRYHDWKAVYSNRLPGSMAQGVLGGEVASWGELVDDQSLDARLWP
RAAALAERLWSNPGASAREAEPRLHAHRARLVAAGVRPEALAPRYCVLNEGACQ*

Sg-fdl

MSRQRLLWRLLGAALALTVAGLAAPPLFRLLVSPHSAANSVAGRRVYSSDPGPWTWSCESGRCVRALWQGGTQVSLDTCQW
TCAGWEAPLWPRPTGALRLANSTAALPEDLDVRLRLSGPQHEDTRGLLAAATERLARHLQLVRPAWAGRVACDAARGATVA
RLTVFVKLDADGSRPTGQLTLDTDESYRLQVRRESQDLQAEIDARSFFGARHALETLSQLAWWDPVSGCVHILDSAIVKDAPKF
RHRGLMVDTARNFIPLEALQRTVDAMASNKLNTLHWHLTDSTSFPYLSRALPTMARYGAYSPEQVYSMEDVSRLAEFARERG
VRLVVELDVPAHAAAGWPTEQVSCSEQRGSAANAPLVQQQQHRQNEDNGLQYRQEERRERRAQHGGEQQPAWWELCGQPP
CGQLPPADEAAFGTLRTLYQELRQASGASDVAHLGGDEVSAECWGGVRGERLWSLWGGFMRRAHRELVAASQGNPPTAVLV
WSSELTAPHNLRRYFDPSTHVVQVWGGSKWNETLPVLLAGFRAVVSHVDAWYLDCGWGDFRSGGPGPCGPVATWQTVYSH
RPWAAFPPGARSRLLGGEACLWSEKVDDQTLDVRLWPRAAALAERLWSDPPAGVHPDLPPPGSPQRDEPTLRRAYQRLSHH
RERLVARGVRAEAMWPRYCHLNPGACF*

Sg-hex

MGKKVEVVLCACVCVGLLLTVTAAEPLPRYITEPGPTVKATQGAVWPKPQNEQRFGGSVLIVPGNFTFQVEGPECDILSEAVSR
YEAILKEEAAIKGPRNASEASTQLSALLVRLDGECGDRPVFGMDESYELRINSPDLPGAMLLTSASVWGILRGLETFSQVATRVK
TADALILDNLAIADIPRFSHRGLLLDTSRHFIPVSYIKKTLDAMAYNKMNVFHWHIVDDQSFPYQSAAFPLLSEKGSYDPERFVY
SPADVAEVIEYARVRGIRVVPEFDTPGHTRSWGEAYPDLLTPCYNATGSPDGTYGPIDPTKNFTYEFLQTLFEEIVNVFPDEYFH
LGGDEVGFECWESNQDILDFMSEHNITESKDLESYYIQKIVDIASNLNSKSIVWQEVFDNEVRLSADTVVHIWTGDRNEELDSV
TAAGHYTLLSQCYYLDRFRYFGGDWHKFYNCEPLDFSADNVYQYDLVIGGEAAMWSEFVDESNVESRVWPRASAVAERLWSP
MNVTDIDEAATRIEEHYCRLRRRGINAQPPNGPGYCV*

(B)

Sg-cht5-1

MRTSAAWFLAVAGLCVVFCPPLVSGNVGDRGRVVCYFSNWAIYRPGIGRYGIDDVPASMCTHLVYSFIGVSNVTWGVLVIDPE
NDVENHGFANFTALKSKYPGLKTQLAIGGWAEGGRKYSAMAAVPARRRSLIASVVEYMKRYGFDGFDLDWEYPGAADRGGSF
SDKNHFKCFVQELREAFDAEGQGWEITMAVPLAKFRLQEGYHVPELCELVDAIHVMSYDLRGNWAGFADTHSPLYKRPHDQ
WAYEKLNVHDGLKLWQDMGCPAHKLVVGVPFYGRSFTLSAGNKDYKLGTYINKEAGGGKPGNYTQAKGFLAYYEICLEIQEVG
GWTEKWDEAGKVPYAYKGTQWVGFENPKSVQIKMDFIKAKGYGGAMTWAIDMDDFRGVCGPKDALISVMYNNMKDYIVPD
IQYSTTKRPDWDRPPPCDGKKPGAAPASTTTRRPTAAPTQSTTRRPAPTTTAAPSSSSSTTTTRRTTTASRPSTQPPPPPAAPD
DNELPPAAIDCSDGDFVPHHDCSKYYRCVYGKPVEFSCYEGTVWNPQLRVCDRPNDVHRTDCSMAKLHS*

Sg-cht5-2

MRAATQVGLLLAVALALAAASDEDTTPLDSSTGSPTNSVDEESSSSENAAVLSGGQRRGRVTCYFESWAVYRKRLRYGIEDIPG
DMCTHIIYSFVGLNNVTWELQVLDEKLDVQDGGFENFTALRQEFPGVRLQVALGGWAEGGHNYSAMVGDPARRASLVRSAVA
FLHRYGFDGFDVDWEYPGNAPRGGVPEDKDDFLCFMQELRVAFDAEGLGWELTMAVPLTEDKLRDGFHVPQLCSIVDAVHV
MAYDLRGEWDHFADVHSPLYRRPHDTGAYAKINTHDGLLLWEQLGGSSWGCHSTATPTNCVPTSPTTLPVLASFRAPEMTSA
E*

(based on alignment with homologous sequences this transcript might be misassembled and the amino acid sequenced prematurely terminated by introduction of a stop codon)

Sg-cht10-1

MWRPVALSLWLLLATSRGLHVPPADEPSFVRDAVEAPPGQSLALRRSATASRPRLPAFGTRQLPLRQAVESPPMAARLRSSER
LPLRDAVEHVPYEALPGAPTASEAFSLWRGFGDWLPENLPSTRQFNHSFAWWHDAIIAKLSLGGPRTKPPSLQAPSTHTSGIR
QFKVVCFVEGWAGYRRDPMRFTTADIDPFACTHIIYAFAVMDPHDLHIKPQDEQYDIIQGGYRSIVGLKRQNPQLKVMISVGG
WPEERRKFAEMTASASTRREFIRSVLHFIDEYGFDGIDLDWEYPGAADMGGSAREKEHFSLLVEELAEAFAPRGSVLSASVSPS
RFRVEDGYDVPRLARRLDFLNLMAFDLLTEQDAAADHHAPLTQRKHDYGLAVFYNVDYAVRYWLRKGARRDQLVVGIPFHG
HSFTLQDEAKNSPGAPVKGLGKEGPYTQEKGFLAYFEILQLLEEGHWMKATDDVGSPYMVKGNQWIGYEDQRSIATKVMYIK

KNLLGGAMVWALDLDDFEGAYGQKWPLLSVVKKGLLETTPQSDQQQASQEPTHVTPPIAGVPVSVDSSQYNCSGRGYVRDSA
SCQIYHRCEWGMKHTYICPEGLHYDSRTQLCDWPQIANCPMDNSSQRIEQENQSEVACNEEGLMEDPKDCNRYYMCHKGVA
QHYSCMLGQYFNVQKGICEYGSCMPKAPQDNIPSSQTRNLVGEDHYKVVCYYASWAWYRKEGGKFVPEHIDPTLCTHIVYAYA
SLDPNTLTMKYFDERADKENNFYERLTELPKKSGHHQQHASDVTVMIGLGGWTDSAGDKYSRLVSEGSARRRFVSKAVEFLH
RHQFGGLHLDWDYPRCWQSNCGRGPTSDKPNFTKLVQELRQAFKKQNPPLALAISISGYHEVIDEAYDLAELGRNTDFMSVM
TYDYHGSWEKSTGHVSPLYHRNGDIFPMYNTNDTMEYLVNKGAPRDKLLVGIPFYGQSYTLENPSNHDIGAPATGPGLAGEFT
MQPGMLAYYEICDRVRNNFWKIGRDRFGATGPFAYAGNQWVSFEDTKSVKEKAKYIKNMGYGGAMTFTLDLDDFENRCCRG
AFPLLRSINRVFGRIPDSAEPSGDDCTRPPPPVTPPPPTYTTGVDSGDHRPTTPISTTHQHPTSPKPSTTEYPWW

Sg-cht10-2
PSTTTSTTTSTTTTTTTTTTSTTTTPRPTTRPTTMSTTEYPWWTPSTTSTTRKPPTTRPTTTSTTEEYPWWTPPSTTKKPTTS
STTEQPWWTPSTTSTTSTAAPTTTMTTTEKPWWSTTPQKPLPPDSGPCEAGVYYPDPTNCNAYYRCVLGELRKEFCAGGLH
WNPDKKVCDWPSESKCDTKEPSETTVGSTTSSTTENPWWTPSKPSETQATTTTTEVPWWSTTRPPRPPTTEGNSEWVTTSR
PTTTQQPSEEVSECMNGQYYPVAGSCKSFYICVNGRLIKQTCAPGLVWNQDQTMCDWGFNVKCADDSEREAVHKAQPDDPC
NQGALNPYPGDCTRYLYCQWGRYHEADCAAGLHWNEMEKICDWPENAKCTDMESGSEAPAASSQKPVTEMSTSWTTAAPT
TKPPWTWATTTTVKPVTTTSTRAPPAQGPPISGYFKVVCYFTNWAWYRRGLGKYVPEDIDANLCTHIVYGFAVLDYENLIIKA
HDSWADFDNKFYERVVAYKKKGLKVSLAIGGWNDSAGDKYSRLVNSPSARRRFIKHVLEFLEKYGFDGLDLDWEYPVCWQVD
CAKGPASDKSSFAALVKELRQAFEPKGLLLSSAVSPSKTVIDAGYDVKTLAENLDWIAVMTYDFHGQWDKKTGHVAPLYFHPD
DDFYFFNANFSINYWISEGAPRRKIVMGMPLYGQSFQLEKASTNGLNARSTGPGQAGEFTRAAGFLAYYEICDRIKNKGWTVV
QDPERRMGPYAFKGNQWVSFDDVAMIQQKSEYIRKMGLGGGMIWALDLDDFRNRCGGGTHPLLNTIRTVLAAPPGGDGATE
MPPSWSTPGGGQPTMSTEEWMSSTSISSTEITDSGHHSTQDSGGEVTSVSPAITTTNRPAHPGTSSSPPPPPSQGEFKVVCYFT
NWAWYRQGVGKYLPNEIDPDLCTHIVYGFAVLNGDRLTIKPHDTWADYDNKFYEKVTEYKKKGIKVLVAIGGWNDSAGDKYS
RLVNSPGARRRFIEDVIDFIEQNNFDGLDLDWEYPKCWQVDCKKGPDSDKEAFAAFVRELRAAFNPKGLLLTAAVSPSKAVVD
AGYDVPTLSQNLDWIAVMTYDFHGQWDKITGHVAPMYTHPEDVDVTFNANFSIHYWIQKGASPKKIVMGMPMYGQSFSLAD
NSDHGLNAPTYGGGEAGESTRARGFLSYYEICTNIQKKGWRVVKDPEGRMGPYAYLRDQWVSFDDTSMIRYKSNFIRRMGLG
GGMIWALDLDDFRNVCSCEKYPLLKTINRVLRGYPGPGPNCDIEATEKPGSEETDNRIHPTIPPTSSTNNWNVISGGGGIVPKDP
TCGNRLFAPHDKDCNKYYLCQYGDFMEQSCPQGLYWNKDHCDWPSNTDCSKEDSSVINPAPIASTQEPEMSSTTENIHMSES
TVTTSIRPSEPGTSTVMTPSGDYMVVCYFTNWAWYRQGLGKYLPSDIDTSLCTHIAYGFAVLDGNSLTIKPHDSWADLDNEFY
TKVSGLKKKGIKVLLAIGGWNDSLGDKYSRLANNPSARRKFVEHVVKFIEKYGFEGLDLDWEYPKCWQVDCNAGPDSDKQGF
ADLVKELSMAFKPRGLLLSSAVSPSKVVIDSGYDVPVLSQYFDYISVMTYDFHGHWDKQTGHVAPLYYYPGDTYDYFNANFTM
HYWIEKGADRKKLIMGMPMYGQSFSLADAKNHGLNAKSYGPGEAGEFTRAGGFMAYYEICYNVKSKGWTTVRDPEGRIGPYA
YRGNQWVSYDDVSDIRRKTQFIKELGLGGGMIWALDLDDFRNRCGCGTYPLLRTINSELRGLTANTHDCT*

Sg-cht7-1
MIAPRCVWRAALWCVVIILLADLVYSASSTGRRRLRRPGGSSSSSTTSSSSSTSTKVRTRDQETSASVNRFRVRNRLTPPGANRK
SGSGSAVAAASDKSGGYKVVCYYTNWSQYRTAHGKFLPEDITPDLCTHIIYAFGWLKKGKLTSFEGNDETKDGKVGLYERVMA
LKKANPKLKVLLALGGWSFGTQKFKAMSETRYTRQTFIYSAIPYLRKHDFDGLDMDWEYPKGTDDKKNFVLLLKELREAFEA
EAQEVKQSRLLLSAAVPVGPDNVRGGYDVPAVASYLDFINLMAYDFHGKWERETGHNAPLYAPSSDSEWRKQLSVDHAATM
WVKLGAPKEKLVIGMPTYGRTFTLSNPSNFKVNAPASGGGKAGDFTKEGGFLAYYEVCDMLKKGATYIWDDEMKVPYAVMG
DQWVGFDDERSIRHKMKWLKEGGYGGAMVWTVDMDDFTGTVCGGGVKYPLIGAIREELRGVSRGPNAKDVDWSKVARTVS
LEATTKPAPIKIDVSEVLNRVRKPTKQAPADLSNEVIDLNSRPAQVFCYMTSWSGKRPGAGKFSPEDVDPSLCTHVVFAFATLK
DHKLAPANDKDDGLYERVIALREKNPQLKVLLAIGGWAFGSTPFKELTSNVFRMNQFVYDAIELLRDFKFDGLDVDWEYPRG
ADDRAAYVSLLKELRMAFEGEAKTAEQPRLLLSAAVPASFEAIAAGYDVPEISKYLDFINVMTYDFHGQWERQVGHNSPLYPLE
SATSYQKKLTVDFSAREWVKQGAPKEKLLIGMPTYGRSFTLVDTSKFDIGAPASGGGAAGRYTAEAGFMAYYEVCDFLHHDNT
TLVWDNEQQVPFAYRGDQWVGFDDERSLKTKMGWLKELGFGGIMVWSVDMDDFRGQCGAGKYPLLTSMRQELRDYRVQLE
YDGPYESRGPLGAYTTKDPTSVSCEEEDGHISYHPDKADCTMYYMCEGERKHHMPCPSNLVFNPNENVCDWPENVEGCMHH
TQAPPAARRR*

Sg-cht7-2
MTWPPPPLLLSLLVLLATSASARFVSTHDVTPCAVEALAPSDKALLCYYEGRLSVYQLDPCLCTHIVFKDAAVVSDNFGLKIVSD
VSGASLLRARSPSLRTVLGLRLSGAVARAALASPSRRLALARDAARRLYAHHLDGIELSVDDDEAASAAAADAAPAATARQGLV
ALLKALRTALDSHGREKRDYLVSEQVFDDFTTQEYEPTWSDGSSRKSRRRATTTTTTTSTTESPEETAARYLELERDAQNAQL
LLSLPTKPETIAKRYDVKNITRYVDYVVLRTQAMTDDSERGLVYHPSRLMGLDDMLNADAVVDLVTSLGASPAQLVITLPGQA
TAFELRREDRTEPRSPASGAPRTISQPELCRALSRGNWTLERDEDQTAPYAYSGRRWIAFDDALSASIKGKYAVVRGLAGTAVD
AADALDWQGTCGAPASQLRALHSALAQLRRSSRGALLHGLE

Sg-cht7-3
DKGMPKNKIIVGIPTYGHSFRLINAENHGWSAPASGYGKIGSKGFVSYPEVCQFLHSTGSKYIFDKNFEVPYAYQGLEWISYDDE
CSVMYKAKYIASSSYGGAMVFSLNVDDHQGVCAGTTFLLTTQIRNILGVSWQ*

Sg-cht2
MQQLAPLAFVLAFLAAAFAASPLGHNKAVVCYVSSWAVYRPGNGVFTVSDINPNICSHLVYAFAGLNATDNTIITLDKYNDLEE
DYGKGNYKKITGLKNQYPHLKVSIAIGGWNEGSANYSHMASTPTTRQQFIRSVVNFLRKYNFDGLQLDWEYPTQRGGVPSDRE
NFVALVREL RQEFDKNGWLLTAALGASTAVIEKAYDVPMLGKYLDYMHIMCYDYHGTWDKMTGANAPLYGSSPSDTLSVDN
SIRYYLKLGAPAKKLLMGVPLYGRTFMSDANANMGGLGAPAEEKSFQGPYTKEDGYMGYNEICLELKTNSSMWTIMWDDKSS
TPYAVSTNKVIVYDNAKSLTEKVNLAMKLELGGIMVWPLDTDDFRGECSEGIYPLMHTINKAIVQSSQQKSDSSGMKVPDSTA
AASCGCASLIFLSFLYLFQL*

Sg-cht6-1
VCYYTNWSVYRPGTAKFTPQNINPYLCTHLIYAFGGLSRENGLRPFDKYQDIEQGGYAKFTGLKTYNKDLKTMLAIGGWNEGS
TRFSPLVADAERRKEFVKNVLRFLRQNHFDGLDLDWEYPAFRDGGKSRDRDNYALLVKELREEFDRESEKTGRPRLLLTMAV
PAGIEYIDKGFDIASMNKHLDFMNILSYDYHSAFEPAVNHHSPLYSMEEDDEYNFDAQLTIDHTVNHYMKSGADRNKLVLGIP
TYGRSYTLFNPLATELGSPADGPGEQGDSTREKGYLAYYEICENLQSDDWKVVQPNPSAMGPYAYKGNQWVSYDDMDIIKKK
AQYVNDNGLGGIMFWAIDNDDFRGKCHGRPYPLIEAGKEAMLKGVKRSNNEIETTPVQNNRQSSRKRNRNRSKGNARGRTRT

TASTSTVVTTTTTTTTTTTAAPLITPSYTTPEPPTTPDPGSDFKCKDEGFFPHPRDCKKYFWCLDSGPSNLGIVAHQFTCPSGLFF
NKAADSCDYARNVVCNKKSKSQGGSSSTLPPIKAATSSTTRFSTSPSTKLTTKLTTTTTTEPPPVLDDDDDDDD

Sg-cht6-2

MNIRVKQPVIIGNCYRGQPNRLWEVFILKWFLVAVACLIAAGAVTVYLAHYFMKTRYTSTNVTGVTGQHSDLNTYKGQLQDM
GDGYSLFKQEDMTQICKTDELTGSQQMRKQSTKLVCYYTFPGPGGLVPDKIDPFLCTHINIAAVGINNSKLEPLCEERKEVIKSL
VGLKTRNKNLKVILSVIGMPGGFGDMVSKSSSRRMFIKDL

Sg-cht8-1

MSHFWLRLAVILGVSLSICGAEDKKVVCYHGSWSAYRNGNGRFEIEYIQPELCTHLIYTFVGITSAGEVRILDEWLDLPSGKNAY
NRFNALKSSNTKTLVAIGGWNEGSATYSAVMNDASLRAKFVQNVVNFVKTYGFDGFDLDWEYPANRGGSPGDLTAFVELIKE
LRTEFDKYGYLLTAAVGVGRYLIGTAYDVPQISKYLDFINLMTYDLHGSWDGKTGQNAPLYASSADKTEAERQLNVDSSVRYW
IQNGADPSKLVLGMGTYGRTFTLSSAANTGVGAPATAPGTNGPYTMESGMMGYNEICEKINAGGWTVVWDEEQKVPYAVNG
NQWIGYDNEESIRLKSQYVLDMGLAGGMIWSLETDDFKGLCGSKTYPLLSTINEVLRGITSTNSGSSSSSSSSSSSSSSSSSSSS
SSSSNTAASASSSSGVCSSAGYVRDPSDCGVFYLCTASGSGYTASKFTCPGDLVFDESSSACNYKSLVAC*

Sg-cht8-2

MSPFLSGLLLLLGVLNICGADEKKVVCYHGSWSAYRNGNGRFEIEYIRPELCTHMIYSFVGITSAGEVRILDEWLDLASGKNAYN
RFNKLKSSNTKTLVAIGGWNEGSATYSAVMNNAALRQKFVQNVVNFVKTYGFDGFDLDWEYPANRGGSPGDLRAYVELLKEL
RAEFDKHGFILSAAVGVGRYLIGSAYDVPQLSKYLDFINL

Sg-cht8-3

ESGMMGYNEICEKIKAGGWKVTWDDEQKVPYAVSGNQWVGYDNEESIKLKSQYVLDMGLGGGMIWSLETDDFKGVCGAGTTF
PLLSAINQVLRGAAATSSAGSSTSGSSSGSSGSSSSSSASSGGSSSGTSSGSSTTSSGASADSSGSSASSGSSSSGSSATSVSSGSNSSG
VCNSAGYARDPSDCGV

Sg-idgf-1

MAELPLLLLLLAAAATCWTSAAALGATRVVCYLDGGALRRPEPHRMLVSEIEPSLTYCTHLIYGYATIDTDSYKAVPRHEGEGT
NYTSVVALKRRFPALNVLLSIGGGSADSGQREKYLHLLESDEHRRTFVKSAKDLLKQYHFDGIDIAWEFPMNKEKKERSTLGSF
WHGFKKVIGLAHSHKDEKADEHRREFSSLIQELKTSLKTENALLTLSVIPYINHTLYYDCSALSPHVDHLHLLAYDYHTPQRTP
NTADYPAPLYVAGKRDPDLTADGNVRWFLERGFPSRKIILGIPTFARTWKLDDDSRVSGVPPIEADGAGDTDNIANTAGIMAF
QTVCMLLPNAGNAGYKTTLSRVTDPTDRLGSYGFRLPSGEVTGLWVGYEDPDVAQYKAAYAKIKSLGGIAFSDLSLDDYHGICT
GDKYPIVRAGTLKLRYK*

Sg-idgf-2

MQSFARLLLLSACCWSAALAATTKVVCYFNTSALKRPESSRMLLSQIEPSFSYCTHLVVGYATINTETYKAVPPSEDEHTTYTNI
VALKRRFPSLKILLSIGGGAADTDTREKYFELLESDEHRTTFVSSAKSLLKQHGFDGIDIAWEFPKNKAKKDRGTFGSIWHGIKK
AVGAAHSHTDEKADEHKSQFSALIRELRTSLRNENALLTLSVIPYINQSLYYDPTALNQQIDELHVLAFDYRNPERDSQGGRLPC
AALPSRAEGLRPLGRREHPLVPRELIPS*

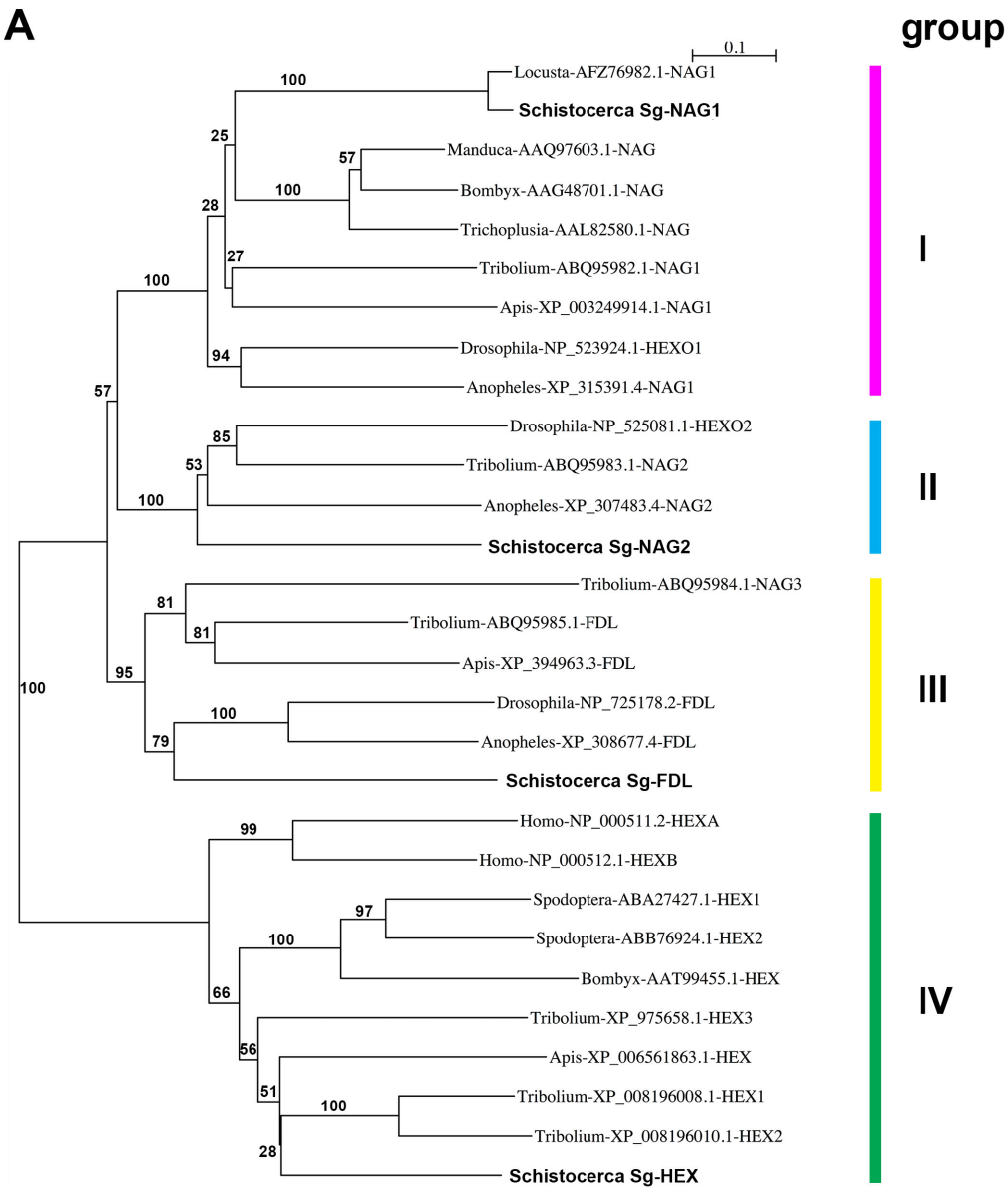
Sg-idgf-3

TDSYKAVPRYQDDTTKYTSLVALKERFPSLKVLLSIGGGGADADQRKKYLELLESDEHRRTFVDSVKELLQQNRFDGIDIAWEFFFSK
EKKDRGNVWHGVKKVLGYAHSHRDENPDEHRRQFSALIRELKSSLKTQNALLTLSVIPYINHSLYYDCASLSPEIDQLHLLAYDYHSP
TRTPKKADYPAPLYRAGERSADLTVDGNVRWFLEKGFPSRKIILGIPTFARTWKLTKDSRITGVPPIDADGPGVAGSIANISGLLAYQT
VCTLLPNDANAAYRTTLRRVTDPTDRLGSYGFRLPTREVSGLWVGYENQHSAEYKAAYARKKSLGGIAFSDLSLDDYNGVCTGEKFP
IVRAGTLKLLSTSV*

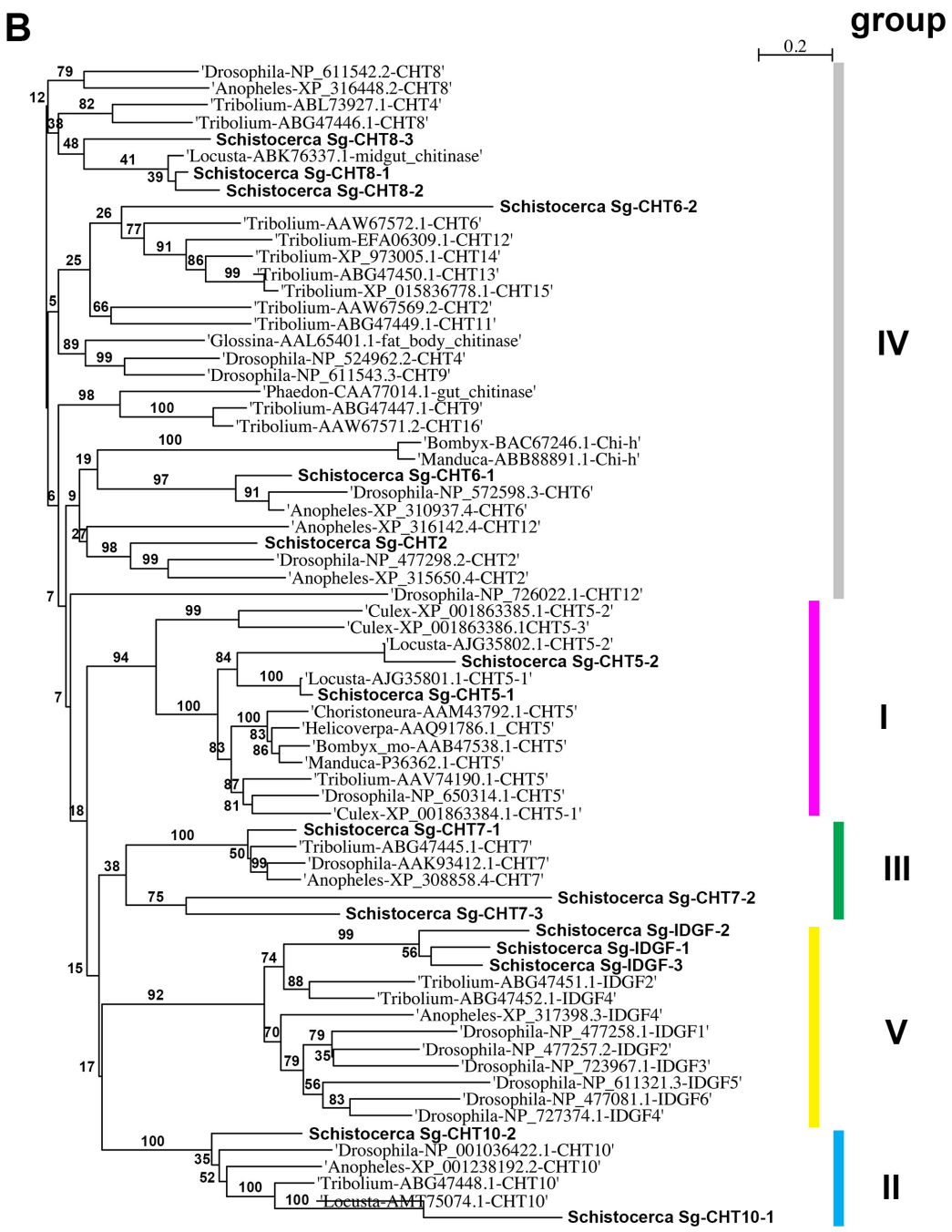
Figure S5. Amino acid sequences and conserved domains of *Schistocerca* chitin degrading enzymes. (A) NAGs, (B) CHTs. Signal peptide and transmembrane region identified by Phobius (http://phobius.binf.ku.dk/index.html) and conserved domains identified by SMART (http://smart.embl-heidelberg.de/) are underlined and coloured. In (A) and (B): signal peptide: magenta, transmembrane region: dark blue. In (A): Glycohydro 20b2 domain (N-terminal domain of the eukaryotic beta-hexosaminidases): light green, Glyco hydro 20 domain (glycoside hydrolase

family 20 catalytic domain): grey. In (B): Glyco 18 domain (catalytic domain): light blue, Chitin-binding domain type 2 (ChBD2): green; catalytically critical consensus sequence in the Glyco 18 domain, FDG(L/F)DLDWE(Y/F)P, is highlighted in yellow and amino acid changes from the consensus are coloured in orange.

242 **FIGURE S6**



243


B
group
0.2
'Drosophila-NP_611542.2-CHT8'
'Anopheles-XP_316448.2-CHT8'
'Tribolium-ABL73927.1-CHT4'
'Tribolium-ABG47446.1-CHT8'
Schistocerca Sg-CHT8-3
'Locusta-ABK76337.1-midgut_chitinase'
Schistocerca Sg-CHT8-1
Schistocerca Sg-CHT8-2
Schistocerca Sg-CHT6-2
'Tribolium-AAW67572.1-CHT6'
'Tribolium-EFA06309.1-CHT12'
'Tribolium-XP_973005.1-CHT14'
'Tribolium-ABG47450.1-CHT13'
'Tribolium-XP_015836778.1-CHT15'
'Tribolium-AAW67569.2-CHT2'
'Tribolium-ABG47449.1-CHT11'
'Glossina-AAL65401.1-fat_body_chitinase'
'Drosophila-NP_524962.2-CHT4'
'Drosophila-NP_611543.3-CHT9'
'Phaedon-CAA77014.1-gut_chitinase'
'Tribolium-ABG47447.1-CHT9'
'Tribolium-AAW67571.2-CHT16'
'Bombyx-BAC67246.1-Chi-h'
'Manduca-ABB88891.1-Chi-h'
Schistocerca Sg-CHT6-1
'Drosophila-NP_572598.3-CHT6'
'Anopheles-XP_310937.4-CHT6'
'Anopheles-XP_316142.4-CHT12'
Schistocerca Sg-CHT2
'Drosophila-NP_477298.2-CHT2'
'Anopheles-XP_315650.4-CHT2'
'Drosophila-NP_726022.1-CHT12'
'Culex-XP_001863385.1-CHT5-2'
'Culex-XP_001863386.1CHT5-3'
'Locusta-AJG35802.1-CHT5-2'
Schistocerca Sg-CHT5-2
'Locusta-AJG35801.1-CHT5-1'
Schistocerca Sg-CHT5-1
'Choristoneura-AAM43792.1-CHT5'
'Helicoverpa-AAQ91786.1_CHT5'
'Bombyx_mo-AAB47538.1-CHT5'
'Manduca-P36362.1-CHT5'
'Tribolium-AAV74190.1-CHT5'
'Drosophila-NP_650314.1-CHT5'
'Culex-XP_001863384.1-CHT5-1'
Schistocerca Sg-CHT7-1
'Tribolium-ABG47445.1-CHT7'
'Drosophila-AAK93412.1-CHT7'
'Anopheles-XP_308858.4-CHT7'
Schistocerca Sg-CHT7-2
Schistocerca Sg-CHT7-3
Schistocerca Sg-IDGF-2
Schistocerca Sg-IDGF-1
Schistocerca Sg-IDGF-3
'Tribolium-ABG47451.1-IDGF2'
'Tribolium-ABG47452.1-IDGF4'
'Anopheles-XP_317398.3-IDGF4'
'Drosophila-NP_477258.1-IDGF1'
'Drosophila-NP_477257.2-IDGF2'
'Drosophila-NP_723967.1-IDGF3'
'Drosophila-NP_611321.3-IDGF5'
'Drosophila-NP_477081.1-IDGF6'
'Drosophila-NP_727374.1-IDGF4'
Schistocerca Sg-CHT10-2
'Drosophila-NP_001036422.1-CHT10'
'Anopheles-XP_001238192.2-CHT10'
'Tribolium-ABG47448.1-CHT10'
'Locusta-AMT75074.1-CHT10'
Schistocerca Sg-CHT10-1
IV
I
III
V
II

244

Figure S6. Phylogenetic trees of chitin degrading enzymes in *Schistocerca* and other insects. (A) NAGs, (B) CHTs. *Schistocerca* sequences are in bold. Amino acid sequences were extracted from NCBI GenBank. The numbers above the branches are bootstrap support. The markers show a branch length. Both trees are unrooted. The tree in (A) was prepared using the SeaView software (version 4.6.1; Gouy et al, 2010; http://doua.prabi.fr/software/seaview): alignment with default parameters, tree using the Neighbor Joining method, Poisson distribution, 5000 bootstrap replicates. The tree in (B) was prepared using the CLC Sequence Viewer (version 7.8.1; https://www.qiagenbioinformatics.com/products/clc-sequence-viewer/): alignment with default parameters except gap open cost 3.0 and gap extension cost 3.0, tree using Neighbor Joining method, Kimura model, 1000 bootstrap replicates.

FIGURE S7

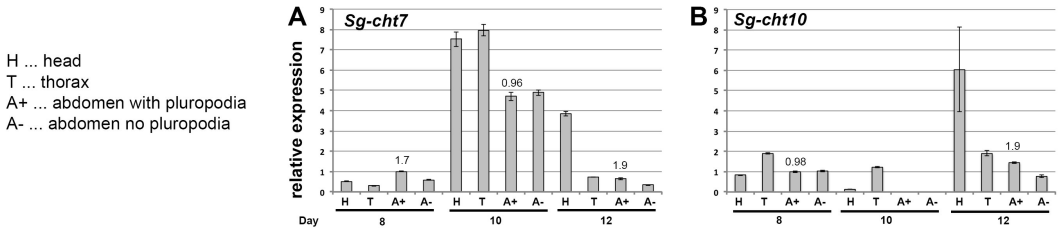


Figure S7. Real-time RT-PCR expression analysis of *Sg-cht7-1* and *Sg-cht10-1* on cDNA from parts of *Schistocerca* embryos. cDNA was prepared from mRNAs isolated from parts of embryos at the age of 8, 10 and 12 days: H, head; T, thorax; A+, abdomen with pleuropodia; A-, abdomen without pleuropodia. Analysis of 3-4 technical replicates is shown. Expression in A+8 (abdomen with pleuropodia when they first become differentiated) was set as 1. Numbers above A+ expression is fold change from A- of the same age.

SUPPLEMENTARY REFERENCES

Angelini DR, Liu PZ, Hughes CL, Kaufman TC. 2005. Hox gene function and interaction in the milkweed bug Oncopeltus fasciatus (Hemiptera). Dev Biol 287:440-455.

Beermann A, Jay DG, Beeman RW, Hülskamp M, Tautz D, Jürgens G. 2001. The Short antennae gene of Tribolium is required for limb development and encodes the orthologue of the Drosophila Distal-less protein. Development 128:287-297.

Bennett RL, Brown SJ, Denell RE. 1999. Molecular and genetic analysis of the Tribolium Ultrabithorax ortholog, Ultrathorax. Dev Genes Evol 209:608-619.

Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221-224.

Hughes CL, Kaufman TC. 2002. Hox genes and the evolution of the arthropod body plan. Evol Dev 4:459-499.

Kelsh R, Weinzierl RO, White RA, Akam M. 1994. Homeotic gene expression in the locust Schistocerca: an antibody that detects conserved epitopes in Ultrabithorax and abdominal-A proteins. Dev Genet 15:19-31.

292 Liu HW, Wang LL, Tang X, Dong ZM, Guo PC, Zhao DC, Xia QY, Zhao P. 2018.
293 Proteomic analysis of Bombyx mori molting fluid: Insights into the molting
294 process. J Proteomics 173:115-125.
295
296 Prpic NM, Wigand B, Damen WG, Klingler M. 2001. Expression of dachshund in
297 wild-type and Distal-less mutant Tribolium corroborates serial homologies in
298 insect appendages. Dev Genes Evol 211:467-477.
299
300 Tear G, Akam M, Martinez-Arias A. 1990. Isolation of an abdominal-A gene from
301 the locust Schistocerca gregaria and its expression during early embryogenesis.
302 Development 110:915-925.
303
304 Wei Z, Yin Y, Zhang B, Wang Z, Peng G, Cao Y, Xia Y. 2007. Cloning of a novel
305 protease required for the molting of Locusta migratoria manilensis. Dev Growth
306 Differ 49:611-621.
307
308 Zhang J, Lu A, Kong L, Zhang Q, Ling E. 2014. Functional analysis of insect molting
309 fluid proteins on the protection and regulation of ecdysis. J Biol Chem
310 289:35891-35906.
311
312 Zhang H, Shinmyo Y, Mito T, Miyawaki K, Sarashina I, Ohuchi H, Noji S. 2005.
313 Expression patterns of the homeotic genes Scr, Antp, Ubx, and abd-A during
314 embryogenesis of the cricket Gryllus bimaculatus. Gene Expr Patterns 5:491-502.