FIGURE S1

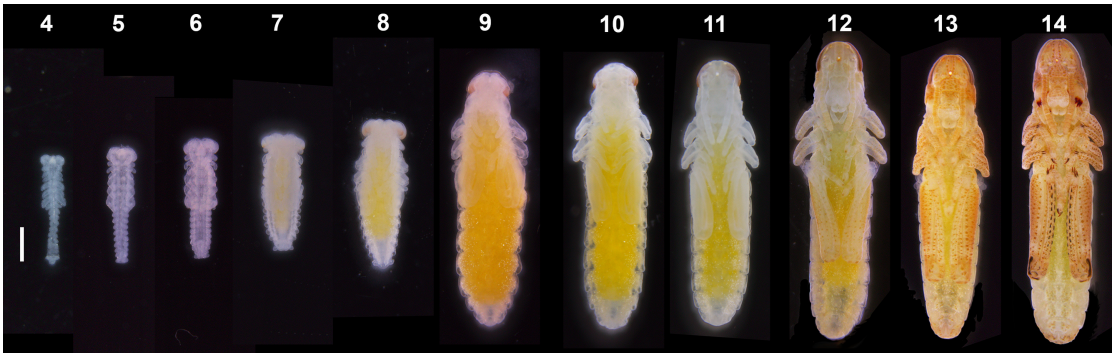

Figure S1. *S. gregaria* embryonic stages used in this study.

Images of live embryos dissected out of the eggs; imaged under a stereomicroscope. Eggs and embryos of *S. gregaria* typically slightly vary in size. Numbers indicate age in days. Scale bar: 1 mm. Background in photos was cleaned (see Methods).

FIGURE S2

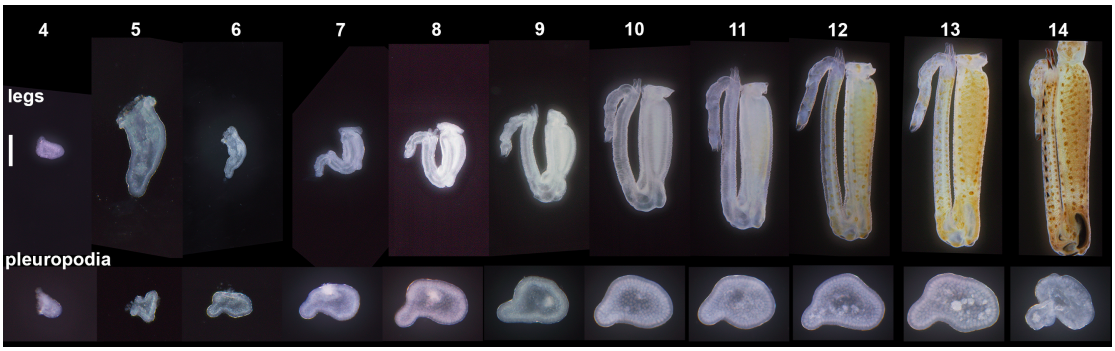

Figure S2. External features of developing hind legs and pleuropodia.

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

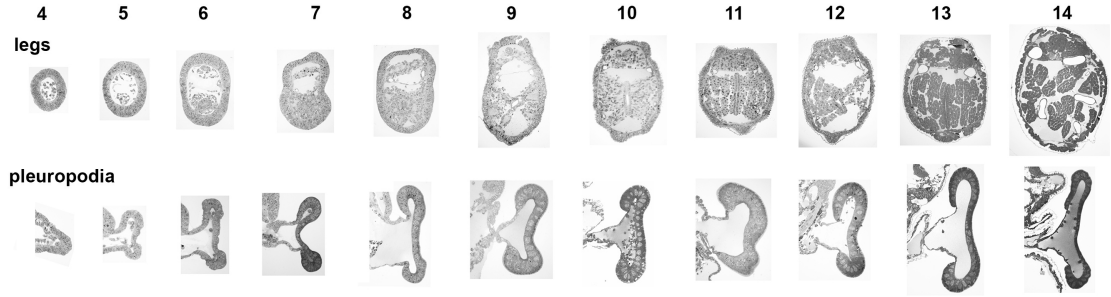

Figure S3. Cross-sections through developing hind legs and pleuropodia. Toluidine blue stained semithin sections of appendages embedded in epoxy resin. Numbers indicate age in days.

FIGURE S4

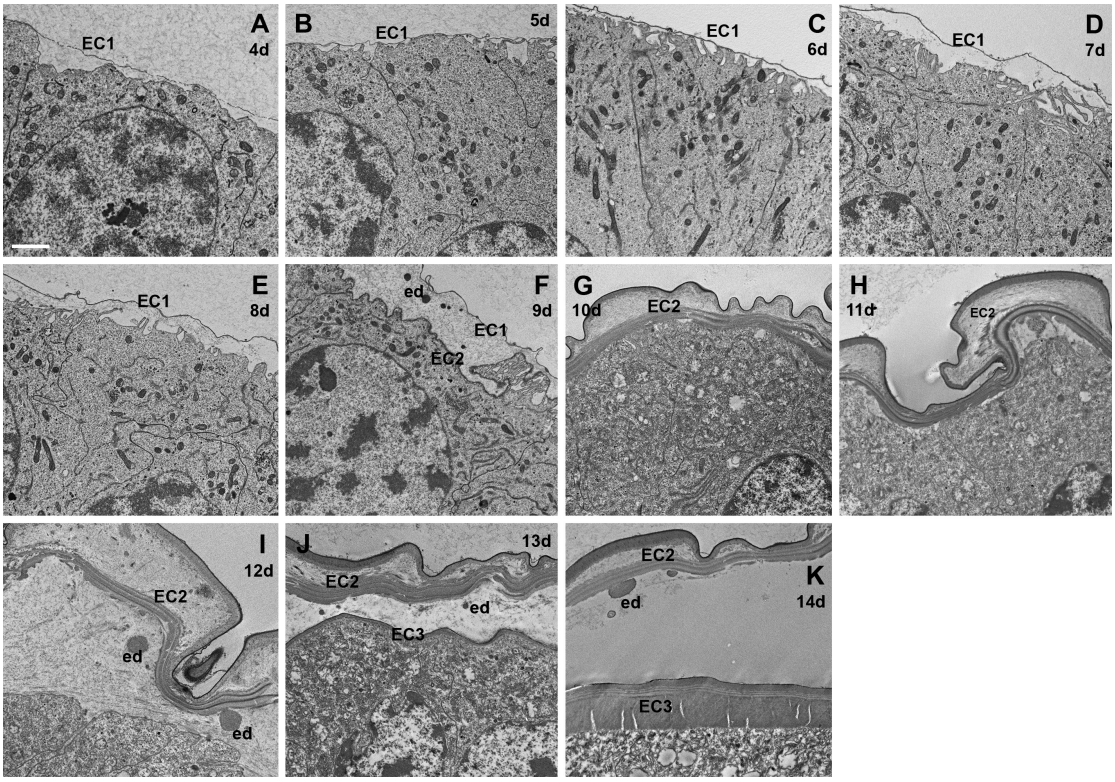

Figure S4. Ultrastructure of epidermal cells in developing hind legs. TEM micrographs. Compare with pleuropodia in Figure 3. Note the three different cuticles and appearance of ecdysial droplets (ed) during embryonic

27 moulting. EC1, EC2, EC3, the first, the second and the third embryonic cuticle,

28 respectively (EC3 becomes the cuticle of the first instar larva). Scale bar: 2 μm.

29

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

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RLTVFVKLDADGSRPTGQLTLDTDESYRLQVRRESQDLQAEIDARSFFGARHALETLSQLAWWDPVSGCVHILDSAIVKDAPKF
RHRGLMVDTARNFIPLEALQRTVDAMASNKLNTLHWHLTDSTSFPYLSRALPTMARYGAYSPEQVYSMEDVSRLAEFARERG
VRLVVELDVPAHAAAGWPTEQVSCSEQRGSAANAPLVQQQQHRQNEDNGLQYRQEERRERRAQHGGEQQPAWWELCGQPP
CGQLPPADEAAFGTLRTLYQELRQASGASDVAHLGGDEVSAECWGGVRGERLWSLWGGFMRRAHRELVAASQGNPPTAVLV
WSSELTAPHNLRRYFDPSTHVVQVWGGSKWNETLPVLLAGFRAVVSHVDAWYLDCGWGDFRSGGPGPCGPVATWQTVYSH
RPWAAFPPGARSRLLGGEACLWSEKVDDQTLDVRLWPRAAALAERLWSDPPAGVHPDLPPPGSPQRDEPTLRRAYQRLSHH
RERLVARGVRAEAMWPRYCHLNPGACF*

Sg-hex

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LGGDEVGFECWESNQDILDFMSEHNITESKDLESYYIQKIVDIASNLNSKSIVWQEVFDNEVRLSADTVVHIWTGDRNEELDSV
TAAGHYTLLSQCYYLDRFRYFGGDWHKFYNCEPLDFSADNVYQYDLVIGGEAAMWSEFVDESNVESRVWPRASAVAERLWSP
MNVTDIDEAATRIEEHYCRLRRRGINAQPPNGPGYCV*

(B)

Sg-cht5-1

MRTSAAWFLAVAGLCVVFCPPLVSGNVGDRGRVVCYFSNWAIYRPGIGRYGIDDVPASMCTHLVYSFIGVSNVTWGVLVIDPE
NDVENHGFANFTALKSKYPGLKTQLAIGGWAEGGRKYSAMAAVPARRRSLIASVVEYMKRYGFDGFDLDWEYPGAADRGGSF
SDKNHFKCFVQELREAFDAEGQGWEITMAVPLAKFRLQEGYHVPELCELVDAIHVMSYDLRGNWAGFADTHSPLYKRPHDQ
WAYEKLNVHDGLKLWQDMGCPAHKLVVGVPFYGRSFTLSAGNKDYKLGTYINKEAGGGKPGNYTQAKGFLAYYEICLEIQEVG
GWTEKWDEAGKVPYAYKGTQWVGFENPKSVQIKMDFIKAKGYGGAMTWAIDMDDFRGVCGPKDALISVMYNNMKDYIVPD
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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
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QFKVVCFVEGWAGYRRDPMRFTTADIDPFACTHIIYAFAVMDPHDLHIKPQDEQYDIIQGGYRSIVGLKRQNPQLKVMISVGG
WPEERRKFAEMTASASTRREFIRSVLHFIDEYGFDGIDLDWEYPGAADMGGSAREKEHFSLLVEELAEAFAPRGSVLSASVSPS
RFRVEDGYDVPRLARRLDFLNLMAFDLLTEQDAAADHHAPLTQRKHDYGLAVFYNVDYAVRYWLRKGARRDQLVVGIPFHG
HSFTLQDEAKNSPGAPVKGLGKEGPYTQEKGFLAYFEILQLLEEGHWMKATDDVGSPYMVKGNQWIGYEDQRSIATKVMYIK

KNLLGGAMVWALDLDDFEGAYGQKWPLLSVVKKGLLETTPQSDQQQASQEPTHVTPPIAGVPVSVDSSQYNCSGRGYVRDSA
SCQIYHRCEWGMKHTYICPEGLHYDSRTQLCDWPQIANCPMDNSSQRIEQENQSEVACNEEGLMEDPKDCNRYYMCHKGVA
QHYSCMLGQYFNVQKGICEYGSCMPKAPQDNIPSSQTRNLVGEDHYKVVCYYASWAWYRKEGGKFVPEHIDPTLCTHIVYAYA
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RHQFGGLHLDWDYPRCWQSNCGRGPTSDKPNFTKLVQELRQAFKKQNPPLALAISISGYHEVIDEAYDLAELGRNTDFMSVM
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MQPGMLAYYEICDRVRNNFWKIGRDRFGATGPFAYAGNQWVSFEDTKSVKEKAKYIKNMGYGGAMTFTLDLDDFENRCCRG
AFPLLRSINRVFGRIPDSAEPSGDDCTRPPPPVTPPPPTYTTGVDSGDHRPTTPISTTHQHPTSPKPSTTEYPWW

Sg-cht10-2
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CAKGPASDKSSFAALVKELRQAFEPKGLLLSSAVSPSKTVIDAGYDVKTLAENLDWIAVMTYDFHGQWDKKTGHVAPLYFHPD
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RLVNSPGARRRFIEDVIDFIEQNNFDGLDLDWEYPKCWQVDCKKGPDSDKEAFAAFVRELRAAFNPKGLLLTAAVSPSKAVVD
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GGMIWALDLDDFRNVCSCEKYPLLKTINRVLRGYPGPGPNCDIEATEKPGSEETDNRIHPTIPPTSSTNNWNVISGGGGIVPKDP
TCGNRLFAPHDKDCNKYYLCQYGDFMEQSCPQGLYWNKDHCDWPSNTDCSKEDSSVINPAPIASTQEPEMSSTTENIHMSES
TVTTSIRPSEPGTSTVMTPSGDYMVVCYFTNWAWYRQGLGKYLPSDIDTSLCTHIAYGFAVLDGNSLTIKPHDSWADLDNEFY
TKVSGLKKKGIKVLLAIGGWNDSLGDKYSRLANNPSARRKFVEHVVKFIEKYGFEGLDLDWEYPKCWQVDCNAGPDSDKQGF
ADLVKELSMAFKPRGLLLSSAVSPSKVVIDSGYDVPVLSQYFDYISVMTYDFHGHWDKQTGHVAPLYYYPGDTYDYFNANFTM
HYWIEKGADRKKLIMGMPMYGQSFSLADAKNHGLNAKSYGPGEAGEFTRAGGFMAYYEICYNVKSKGWTTVRDPEGRIGPYA
YRGNQWVSYDDVSDIRRKTQFIKELGLGGGMIWALDLDDFRNRCGCGTYPLLRTINSELRGLTANTHDCT*

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

Sg-cht7-2
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VSGASLLRARSPSLRTVLGLRLSGAVARAALASPSRRLALARDAARRLYAHHLDGIELSVDDDEAASAAAADAAPAATARQGLV
ALLKALRTALDSHGREKRDYLVSEQVFDDFTTQEYEPTWSDGSSRKSRRRATTTTTTTSTTESPEETAARYLELERDAQNAQL
LLSLPTKPETIAKRYDVKNITRYVDYVVLRTQAMTDDSERGLVYHPSRLMGLDDMLNADAVVDLVTSLGASPAQLVITLPGQA
TAFELRREDRTEPRSPASGAPRTISQPELCRALSRGNWTLERDEDQTAPYAYSGRRWIAFDDALSASIKGKYAVVRGLAGTAVD
AADALDWQGTCGAPASQLRALHSALAQLRRSSRGALLHGLE

Sg-cht7-3
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CSVMYKAKYIASSSYGGAMVFSLNVDDHQGVCAGTTFLLTTQIRNILGVSWQ*

Sg-cht2
MQQLAPLAFVLAFLAAAFAASPLGHNKAVVCYVSSWAVYRPGNGVFTVSDINPNICSHLVYAFAGLNATDNTIITLDKYNDLEE
DYGKGNYKKITGLKNQYPHLKVSIAIGGWNEGSANYSHMASTPTTRQQFIRSVVNFLRKYNFDGLDLDWEYPTQRGGVPSDRE
NFVALVRELRQEFDKNGWLLTAALGASTAVIEKAYDVPMLGKYLDYMHIMCYDYHGTWDKMTGANAPLYGSSPSDTLSVDN
SIRYYLKLGAPAKKLLMGVPLYGRTFMSDANANMGGLGAPAEEKSFQGPYTKEDGYMGYNEICLELKTNSSMWTIMWDDKSS
TPYAVSTNKVIVYDNAKSLTEKVNLAMKLELGGIMVWPLDTDDFRGECSEGIYPLMHTINKAIVQSSQQKSDSSGMKVPDSTA
AASCGCASLIFLSFLYLFQL*

Sg-cht6-1
VCYYTNWSVYRPGTAKFTPQNINPYLCTHLIYAFGGLSRENGLRPFDKYQDIEQGGYAKFTGLKTYNKDLKTMLAIGGWNEGS
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PAGIEYIDKGFDIASMNKHLDFMNILSYDYHSAFEPAVNHHSPLYSMEEDDEYNFDAQLTIDHTVNHYMKSGADRNKLVLGIP
TYGRSYTLFNPLATELGSPADGPGEQGDSTREKGYLAYYEICENLQSDDWKVVQPNPSAMGPYAYKGNQWVSYDDMDIIKKK
AQYVNDNGLGGIMFWAIDNDDFRGKCHGRPYPLIEAGKEAMLKGVKRSNNEIETTPVQNNRQSSRKRNRNRSKGNARGRTRT

TASTSTVVTTTTTTTTTTTAAPLITPSYTTPEPPTTPDPGSDFKCKDEGFFPHPRDCKKYFWCLDSGPSNLGIVAHQFTCPSGLFF
NKAADSCDYARNVVCNKKSKSQGGSSSTLPPIKAATSSTTRFSTSPSTKLTTKLTTTTTTEPPPVLDDDDDDDD

Sg-cht6-2
MNIRVKQPVIIGNCYRGQPNRLWEVFILKWFLVAVACLIAAGAVTVYLAHYFMKTRYTSTNVTGVTGQHSDLNTYKGQLQDM
GDGYSLFKQEDMTQICKTDELTGSQQMRKQSTKLVCYYTFPGPGGLVPDKIDPFLCTHINIAAVGINNSKLEPLCEERKEVIKSL
VGLKTRNKNLKVILSVIGMPGGFGDMVSKSSSRRMFIKDL

Sg-cht8-1
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NRFNALKSSNTKTLVAIGGWNEGSATYSAVMNDASLRAKFVQNVVNFVKTYGFDGFDLDWEYPANRGGSPGDLTAFVELIKE
LRTEFDKYGYLLTAAVGVGRYLIGTAYDVPQISKYLDFINLMTYDLHGSWDGKTGQNAPLYASSADKTEAERQLNVDSSVRYW
IQNGADPSKLVLGMGTYGRTFTLSSAANTGVGAPATAPGTNGPYTMESGMMGYNEICEKINAGGWTVVWDEEQKVPYAVNG
NQWIGYDNEESIRLKSQYVLDMGLAGGMIWSLETDDFKGLCGSKTYPLLSTINEVLRGITSTNSGSSSSSSSSSSSSSSSSSSSSS
SSSSNTAASASSSSGVCSSAGYVRDPSDCGVFYLCTASGSGYTASKFTCPGDLVFDESSSACNYKSLVAC*

Sg-cht8-2
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RFNKLKSSNTKTLVAIGGWNEGSATYSAVMNNAALRQKFVQNVVNFVKTYGFDGFDLDWEYPANRGGSPGDLRAYVELLKEL
RAEFDKHGFILSAAVGVGRYLIGSAYDVPQLSKYLDFINL

Sg-cht8-3
ESGMMGYNEICEKIKAGGWKVTWDDEQKVPYAVSGNQWVGYDNEESIKLKSQYVLDMGLGGGMIWSLETDDFKGVCGAGTF
PLLSAINQVLRGAAATSSAGSSTSGSSSGSSGSSSSSSASSGGSSSGTSSGSSTTSSGASADSSGSSASSGSSSSGSSATSVSSGSNSSG
VCNSAGYARDPSDCGV

Sg-idgf-1
MAELPLLLLLLAAAATCWTSAAALGATRVVCYLDGGALRRPEPHRMLVSEIEPSLTYCTHLIYGYATIDTDSYKAVPRHEGEGT
NYTSVVALKRRFPALNVLLSIGGGSADSGQREKYLHLLESDEHRRTFVKSAKDLLKQYHFDGIDIAWEFPMNKEKKERSTLGSF
WHGFKKVIGLAHSHKDEKADEHRREFSSLIQELKTSLKTENALLTLSVIPYINHTLYYDCSALSPHVDHLHLLAYDYHTPQRTP
NTADYPAPLYVAGKRDPDLTADGNVRWFLERGFPSRKIILGIPTFARTWKLDDDSRVSGVPPIEADGAGDTDNIANTAGIMAF
QTVCMLLPNAGNAGYKTTLSRVTDPTDRLGSYGFRLPSGEVTGLWVGYEDPDVAQYKAAYAKIKSLGGIAFSDLSLDDYHGICT
GDKYPIVRAGTLKLRYK*

Sg-idgf-2
MQSFARLLLLSACCWSAALAATTKVVCYFNTSALKRPESSRMLLSQIEPSFSYCTHLVVGYATINTETYKAVPPSEDEHTTYTNI
VALKRRFPSLKILLSIGGGAADTDTREKYFELLESDEHRTTFVSSAKSLLKQHGFDGIDIAWEFPKNKAKKDRGTFGSIWHGIKK
AVGAAHSHTDEKADEHKSQFSALIRELRTSLRNENALLTLSVIPYINQSLYYDPTALNQQIDELHVLAFDYRNPERDSQGGRLPC
AALPSRAEGLRPLGRREHPLVPRELIPS*

Sg-idgf-3
TDSYKAVPRYQDDTTKYTSLVALKERFPSLKVLLSIGGGGADADQRKKYLELLESDEHRRTFVDSVKELLQQNRFDGIDIAWEFPFSK
EKKDRGNVWHGVKKVLGYAHSHRDENPDEHRRQFSALIRELKSSLKTQNALLTLSVIPYINHSLYYDCASLSPEIDQLHLLAYDYHSP
TRTPKKADYPAPLYRAGERSADLTVDGNVRWFLEKGFPSRKIILGIPTFARTWKLTKDSRITGVPPIDADGPGVAGSIANISGLLAYQT
VCTLLPNDANAAYRTTLRRVTDPTDRLGSYGFRLPTREVSGLWVGYENQHSAEYKAAYARKKSLGGIAFSDLSLDDYNGVCTGEKFP
IVRAGTLKLLSTSV*

Figure S5. Amino acid sequences and conserved domains of *S. gregaria* 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

229 family 20 catalytic domain): grey. In (B): Glyco 18 domain (catalytic domain):

230 light blue, Chitin-binding domain type 2 (ChBD2): green; catalytically critical

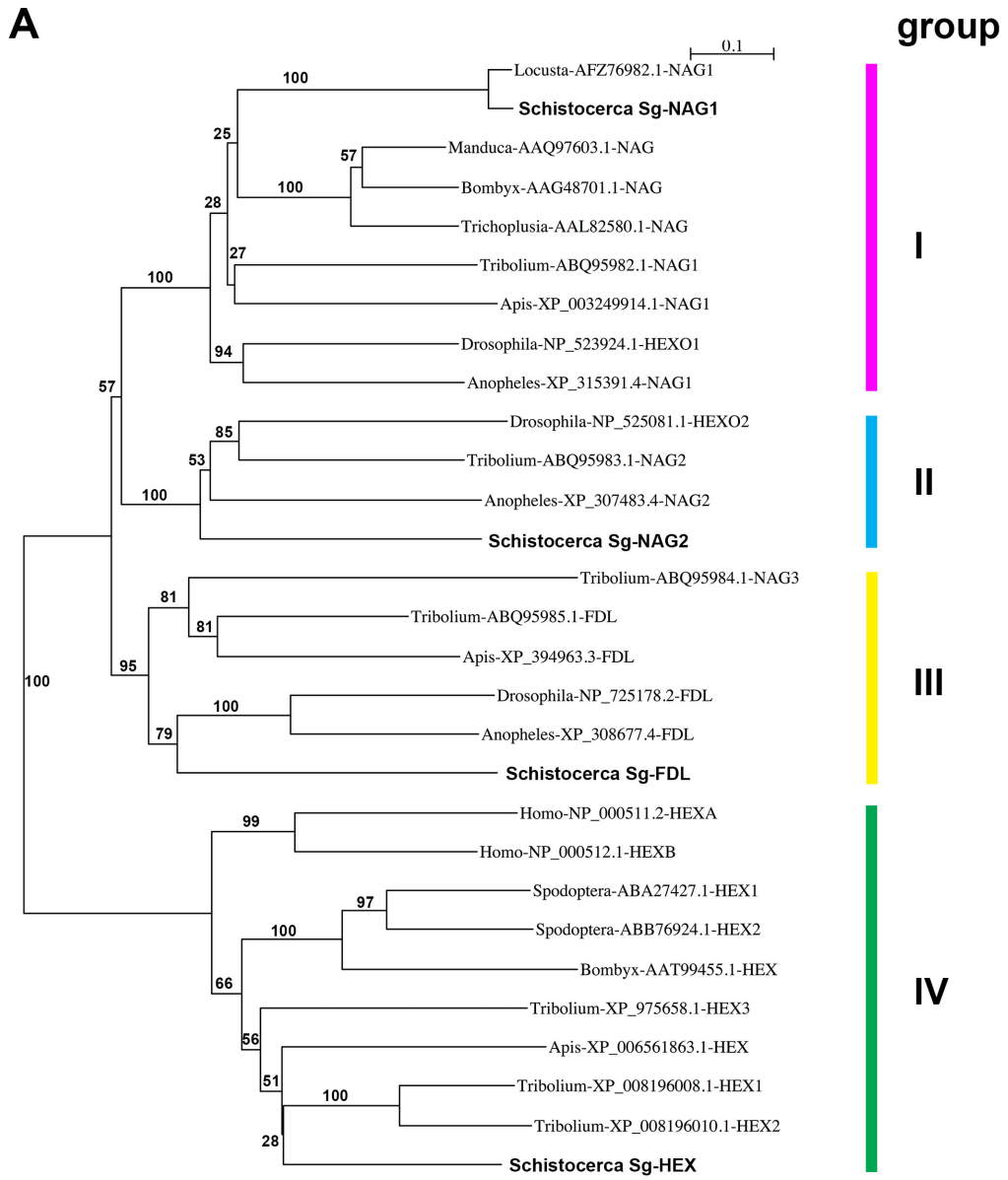
231 consensus sequence in the Glyco 18 domain, FDG(L/F)DLDWE(Y/F)P, is

232 highlighted in yellow and amino acid changes from the consensus are coloured in

233 orange.

234

235 **FIGURE S6**



236

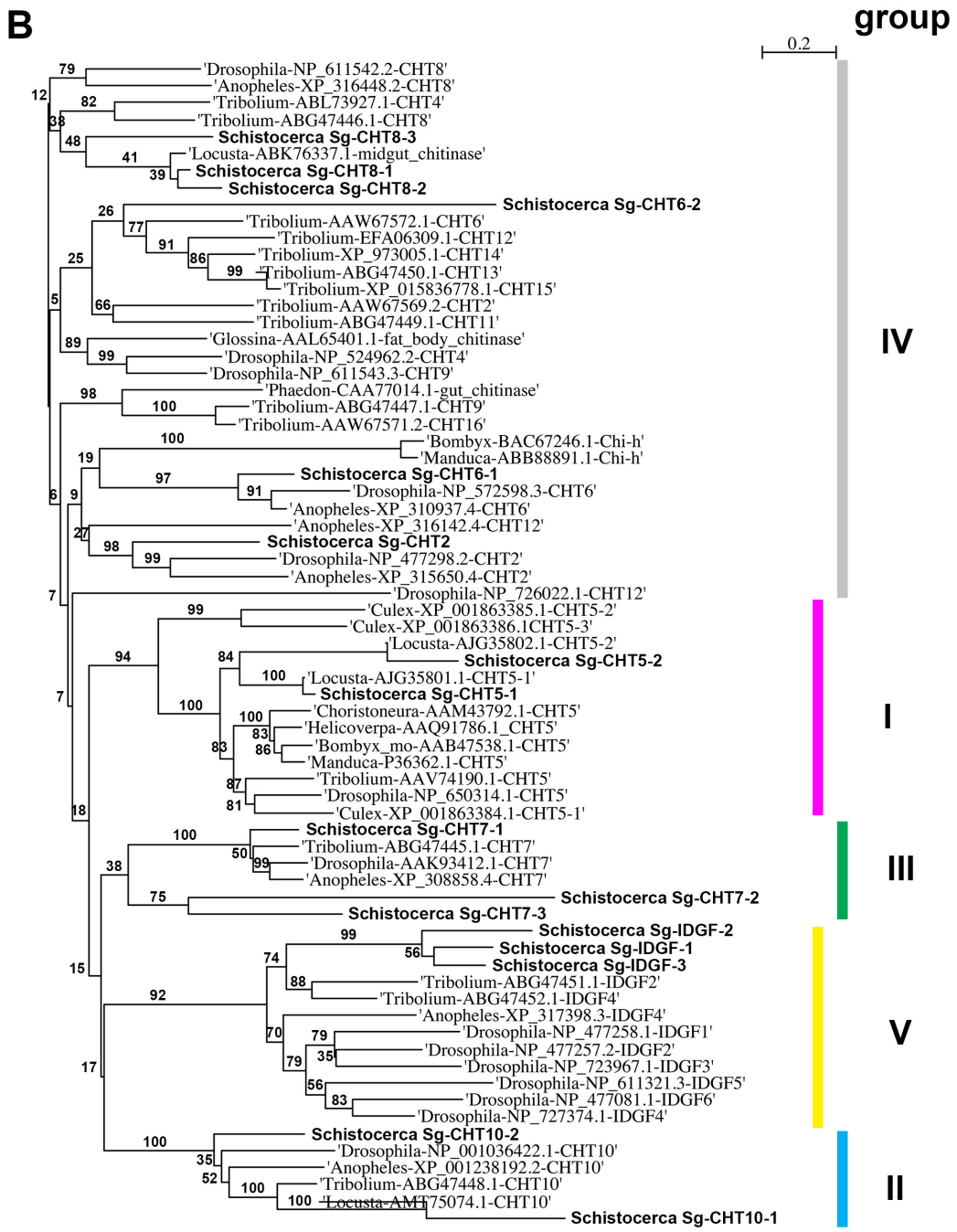
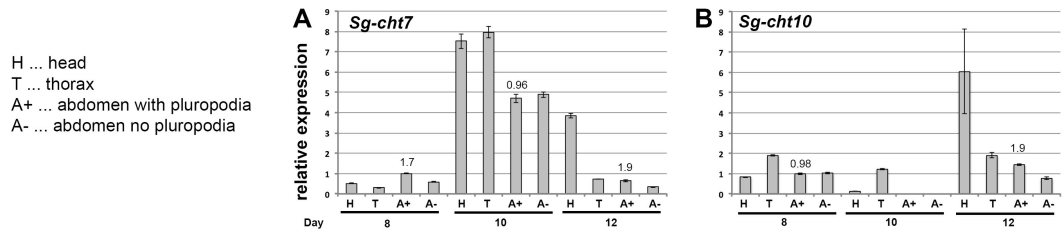


Figure S6. Phylogenetic trees of chitin degrading enzymes in *S. gregaria* and other insects.

240 (A) NAGs, (B) CHTs. *S. gregaria* sequences are in bold. Amino acid sequences
241 were extracted from NCBI GenBank. The numbers above the branches are
242 bootstrap support. The markers show a branch length. Both trees are unrooted.
243 The tree in (A) was prepared using the SeaView software (version 4.6.1; Gouy et
244 al, 2010; http://doua.prabi.fr/software/seaview): alignment with default
245 parameters, tree using the Neighbor Joining method, Poisson distribution, 5000
246 bootstrap replicates. The tree in (B) was prepared using the CLC Sequence
247 Viewer (version 7.8.1; https://www.qiagenbioinformatics.com/products/clc-
248 sequence-viewer/): alignment with default parameters except gap open cost 3.0
249 and gap extension cost 3.0, tree using Neighbor Joining method, Kimura model,
250 1000 bootstrap replicates.
251
252 **FIGURE S7**



253
254 **Figure S7. Real-time RT-PCR expression analysis of *Sg-cht7-1* and *Sg-cht10-***
255 ***1* on cDNA from parts of *S. gregaria* embryos.**
256 cDNA was prepared from mRNAs isolated from parts of embryos at the age of 8,
257 10 and 12 days: H, head; T, thorax; A+, abdomen with pleuropodia; A-, abdomen
258 without pleuropodia. Analysis of 3-4 technical replicates is shown. Expression in
259 A+8 (abdomen with pleuropodia when they first become differentiated) was set
260 as 1. Numbers above A+ expression is fold change from A- of the same age.

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263 (for Additional file 1 and Additional file 2)

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