

A

GH31-1 MKIRDFIKSFGIRKGRQISTTNLVSFEAF-----YDDRGNKFP
GH31-2 MA-----
GH31-3 MERSD-----
GH31-4 MA-----
GH31-5 MDRKKYRNATKYNKVPQEIPKIVLAAPSTENLSLLDSEMDSKSRNDNNSPDKTDDVIQRDTRVPPMRRKNSISMPNLDDLKVFIEQEGDGYDTSTEKIN
GH31-6 ME-----
GH31-7 M-----
GH31-8 MK-----
GH31-9 MS-----
GH31-10 MT-----TFIQQQ

GH31-1 DIKESCTKSNPSQNEEYIGGYVRFKETSSEAKPVFRP-----SLPIILVIFILISVF
GH31-2 -----QN-----LLYISLILFIVLYAS
GH31-3 -----KM-----LRPLLALLIASYVS
GH31-4 -----EM-----TRYAILLVLATTA
GH31-5 TTIQEPEPDEPQNISYPRSVRRKSVLSPRMLKPPEDTELGGSPSNSITSVNSIASLLKEKIQNLPQTFRKKKSPEYKTKVFGVGLFTTIIIVLIVTAYF
GH31-6 -----LTKICFNVLT-L
GH31-7 -----ILK-----Y-----LLAIAIAINFATAKY
GH31-8 -----CTTSSAVYSIIKGMNCWRAI-----LTAILLVGVNQV--L
GH31-9 -----KGCHKLF-----TFLIFLTLHSIRGI
GH31-10 NELREDEDETATASVKWTVVWTLCKTTIVGWFF-----TFAFALIVPVLTYYL

GH31-1 IILPIIYLVNCFRFTFDNQDFYHRNAPAIYSDTSTKEPKDQTKRPWKLHDLVPPAPDYNQCKLLTDLKDFCFPEDGANAECCEARGCCWIIPRKNKSVL
GH31-2 AV-----DKNNFKTCEQSSFCRRLRS--VKP
GH31-3 CA-----NHTIYKDCSRIPFCKKLR--TIE
GH31-4 AI-----DKTVFKSCSERPFCNRLRNVVD
GH31-5 LY-----QQKVL
GH31-6 VF-----
GH31-7 TY-----
GH31-8 VQ-----
GH31-9 VV-----
GH31-10 VV-----NLGTYEKVDFTTCFVNSTFRVPCGKVNISEHECVKLACCYDKPTDSCY

GH31-1 ----SQAPLDVPYCFYPPNYNTYKVVNVTETSFGLIAFWRRQYRTAYPDDVDELK-MIVKYETETRLR-VKFVDPSS-----FRFEPYPYEV--
GH31-2 GE--SKYELDLBESLEITDNSVESKLLN-----TEAGILFKFSLTAISGNI FR-LQVDEASPLYARYRPQF-ALRGEPPQVAKLKL
GH31-3 NH--SKFTAQLDKLSQTGNVISIPLTN-----SDSQELI-LQISLLVHETAR-IKIAEKGS--KRYQLAD-VLAEFPQTLEISA
GH31-4 DK--SKYSADVVSASIKDNILTVPLKN-----ENGNLSL-LLVSMFEGNKIR-VKVEDPDS--KRFE LGDIVIEELKELPLVS
GH31-5 KVFYFGNMKLNKAK----RTVKIFN-----DEGEDVVKLHLGTTLNVDNVLNCLPADNR-----
GH31-6 --SFGCAKIDLKP---TTNGLSISVFN-----DDNEVTLQGTIGFGIDFTVS-DCQNHYTQ-----
GH31-7 --KLGDEIDFTA---TSNGEIIYIRD-----AEIGSQLYGTIGVG--KNA-SCIDFPR-----
GH31-8 --CSTVNEIILKP---SSNGLAIEVNQ-----KE-EKKLKGITIGVIGDFTNI-NCYQES-----
GH31-9 --KNGNITLQLEP---KSNGISFAAYD-----GK-IKKLEGILGRGIFNSNV-DCIGTQV-----
GH31-10 HYLPSRYSYDLAGSSYKASRSSPFFNT-----TAVEEMQ- ISVNEISINKVS-IILHKPS-----

GH31-1 ----PIVDKASLALSIIQIN-----TFKSGFKVLRKSDN-----VTIFDSNNFLNLI-----YSNQ--
GH31-2 LE--RNKEYVSI---EYNNKIV-----HARP-FKIEVFDKDELISVNVGRGLFKFEHFRKKPEQNNEGEQQVEADPGTWEENFKSHHS
GH31-3 KT--SERE-VSIAPTNASYSYKVVVT-----TGTS-FKVEFYDD-----NTKEIALNGEQLV-----FDKEDN-
GH31-4 DTLDGTGDS--LLVTPKDES LPKVVID-----AGPP-LNITFYG-G-----GLKQVVL DGERLV-----YESTDES
GH31-5 -----DDGSLCLEWMHRARLYMNFDDLGS DVKCYNVQWIALS-----ESL-----
GH31-6 -----CAFNGSSLSIN-----EVDSGYQIVWNSVY-----TDG-----
GH31-7 -----CKVGDNDVAFRF-----LPGTSLSVISTNN-----TQE-----
GH31-8 -----CQVGDADFSVK-----QSEDFGHKWTNN-----LTS-----
GH31-9 -----CVINDSNFSVE-----PISAGFRLKWKTFN-----TRR-----
GH31-10 -----TSIAENTVNVREYVVKK-----AAEK-LMVEIFRPN-----GDRIFSTAKGPLI-----ASEN--

GH31-1 ----MLQISSKL---PSKYIYGIGEHRSNL-LLSTE-----WSKFTLFNHDMVP-----TDNKNLYGSHPPFYLMENSTKSHGVFLLNS
GH31-2 KPKGPSAVGLDISF---PGALRVYGLPEHADRL-PLRRTGPGG---VDPYRLYNLDVFE-----YELDSTMAIYGAI PV-VYAHSPKRTIGAFWLNA
GH31-3 ----SFAFATEF---INAKRLYGLHHHPVKL-ELPSTRDVGKVLMDPYRNRNADNDH-----YEVGSPMAVYGSVPV-VYGHSENQTSIGIFLHNA
GH31-4 K----AFTFKADF---PEANRLYGLLDHAWSL-ALGDTNNGTAES-GDPLRLRNSDSWG-----YEANSPMALYGARPV-VYGHSAKNTSGIFLHNA
GH31-5 ----APTDCFD---SHSHWYGGGQTAENAWPLEKGSHF---YQPFITGNIETHEWGNVLRKRYFINS-----KGAAIIVVDNE
GH31-6 ----EFVDKFQL---NSGNSYWFGGPERYLQN-PLEKLVLNQ---NDPYVIKKA DNFA---VAERYWLSN-----KGTFIIFIDDK
GH31-7 ----VLKDCFFTA FPSASTQWYGGPERRVQTWPLQHMRI SQ---SSPYISRKDDNHA---VAERYWLSN-----LGIYIYLSSEE
GH31-8 ----VFQDCFD---EEGVHWYGGPERKKQSWPIEKLEIES---YQAYVLHQLDNFA---VAERYWLSN-----KGLYIYLNSK
GH31-9 ----AFQDCYDL---TEGWQWYGGPTIFSLWIPIQTNEIDG---SSPYVLDNGKGM---YAERYWLSN-----KGSYIFLDDD
GH31-10 ----YWEWTVHL---TDHSLFGLDKT---LLQRHKNST---ISKVYFKKNNDHS-----NFPV-IWAYHRGQFHGLTVKHD

GH31-1 NAMDVILQ-----PTPAITFRTIGGI--FDYFFLGTPTS-----DVISQYTDLIGRPYMP-----PYWGL--GFHLCRFG
GH31-2 AETWVDINNSKDGVMSSIVNLVSGSKSENNVDVHFMSSESV--ADLFILLGPTPK-----AAVKQYASLTGVHPLP-----QYTYL--AYHQCRWN
GH31-3 AEQWVDIWDYDEES-----TSGWSYFMVESGT--LDLFILLGNQPK-----NVVRQFTKLTGVAHMP-----QLWTL--GYHQCRWS
GH31-4 AEQWIDASSGSEG-----KDPVYFIVERAA--FDLFI LLGPTPK-----EIVRQFTGLTGKAHLP-----QI WAL--GYHQCRFS
GH31-5 TPLYISIRDSP-KKEFCLRAQ-----YDNFAFVNKFTSTAQLNYSICTSANMSQLHVFLSEHTLWDGLKKEDSNIIDYFLTEPVWEM--PDMKEALTQ
GH31-6 VPLFIQNNNE-NGALLLIAKCI-----NP---YVNRQRNI--LKYDLVTKKNAKEAHL---HAVNNYLKGPPTGYPNENMIRKPIWTTWPYKQPVQD
GH31-7 NPLFVSNQEDDSKDQVCFISKVQ---SP---YINRDQNI--LEYVIYFSDDPKTAHL---NAVNTYLKGPNGYPNPKMIKEPIWTTWAKYKAPITD
GH31-8 VPLYVDQNISN-KNRVCFIAKLE-----GP---YINRHKNF--LEYDIVIKDDPREAHV---HAVKTFLGKPSGYPDERMI TEPIWTTWAKYKTKISD
GH31-9 VPLYVDQNKLR--NNSVCFKSYIR-----DPFHAYFNRTNRV--LQYYLIFKDDVKQAHL---HAVNKFLGKPKDHPNEAMVAEPIWTTWAKYKTKIND
GH31-10 GPLEVSILT-----SNLIIKGLLGLET---IELVLYTGPTPA-----DLHQQHLEDSEQLDV-----PEWLL--KTHMCRKN

GH31-1 YKTLNNTTRHV---MQRNIDAGIPLDTQWNLDYMNDSNDFTYNQVNFKD-LPSFVRELHSGRMHYIPLIDPGVSASEKPGTYPPYDVGIEMDIFIKNSSG
GH31-2 YNDEDDVTTV---VQTFDANDMPVDFMWLDEIYTNKKYFTWDPKIFPH-PEIMVNNLTATGRKLIVIDPHYKRESG---YFVHEDCLANDYVVKTKDN
GH31-3 YKTQEDTKFV---VAEMNANNPMDAIWLDDIYTDGKRYFTWDPETYS-PEELQRNLSSFNKKLVVLDPHIKVDED---YPVYTGA-KGKYFVKKPNG
GH31-4 YKSQDDVKDV---VARLDENDFPWDAIWLGDGHTDDYKWFHWNHTTYTD-PVELQONISATGRVCSVSDPHIKVDDD---YDVYAGA-KGKYFTKWANG
GH31-5 DIVIDNVTTKITNSVAVLKQGHIILINEFW-----QEIQIGDFELDQSREFPD-FDKLIEKLKRRGRFVVFITQFFISTESF-----NFAEV-VRKLLVSEKRS
GH31-6 NKVNEFVKSIVDH--NFLGGQIEIDENW-----EVCFGSHVFNKDKFPD-ENGLIDSIKSGQFKTALWINPFPVNNDCQ-----NYSNEGLDKGYFVKDVNG
GH31-7 DIVIEFAKSIREN--GFENGOLEIDDW-----EKCYGAQEFSEKFSK-ISDTVKQLKDMDFRVTLWVHFPVNECK-----YRDEGIQGGDFVKGVNG
GH31-8 EIVLDFAKDIRDN--GYEKGQIEIDDYW-----EKCYGAQEFPTPTFPD-ITNTIKTLKSWYRVTLWIHPFVNSDCQ-----DNSKIGLEKGYFVLDQNG
GH31-9 SVVLLKFAKDIRNH--GYEKGQIEIDENW-----ERCYGAQEFQF-HNFTN-IKDTVKILKSNFRVTLWIHPFVNDNCN-----NNSQIGLEKGYFVLDKYG
GH31-10 EKFLNVSTLVSNFVLDSEADSFCDENL-----LMGILAEARANDTSYQDAVQALITPLREKGGKFLLSVPPQVLTNSD-----FYNNASSLDDLLYTYLKN

GH31-1 Q---PFVGVVWNRV-STVWPDFTPNPNVVDYWTLM---LKNLHKE-----IQFDGAWIDMNEPSNLSGAFNGCNPSTLETPPYFPDIDGGVINY
GH31-2 N---VYEGWCWPGS-SS-YIDFYKPEAAEYKLYRLENFKGTS-----HDVY-IWNDMNEPSVF-----NGP-EITMPK-----
GH31-3 E---DFKDCWPGL-SS-YIDFLDPEARDYASWYSYKFKKST-----PVLAGVWDMNEPSVF-----DDELEKTLFP-----
GH31-4 S---EYVADCWPGA-SS-WIDYLNPEAADYSTWFSEKFKNGST-----PTLAGIWNMNEPAVF-----DDSTKTMFW-----
GH31-5 DKRIPALTRYKSLQ-SAGVLDITNVQITISWLLHK---LKKVMNT-----YKIDSFYLDIGVA-----YNNPH-----
GH31-6 D---T-RAIWESA-NAHQVDFTPNPAKWFSDK---IKALADD-----PGLDGFKFDAGETDY-----AIPPH-----
GH31-7 L---N-NATWNGNGNGHQIDFTNPKAAEWYDDR---LKKLQTN-----PGIDSFKFDAGEADY-----AEQPA-----
GH31-8 R---A-NGSWWNGN-DSYQIDFTNPEAAEWWSAR---LKKLQON-----PGIDSFKFDAGETDY-----SPQFS-----
GH31-9 R---H-TGWWNSN-DAYVIDFTNPAQATEWQLR---VKKLQKD-----FGFDSFKFDAGETDF-----CPQPA-----
GH31-10 T---IYQKGKYLQD-VS-YIDYSHENIQKYMVEFGTFLEDFNHTIDGLVLTDNWPANEEFKMDNDSFPYF-----TKALQDAMS-----

GH31-1 KTVCMASAKQYAGLHYNVHNLGLTEAIVTNFALAEIRG--KRPMVISRSSFGLGHYAGHWSGDVWSSW---DDLKFSVPTILSFSLYGVPLMGADICGF
GH31-2 -DTI---HHGGWEHRDVHNEYALAHYATFQGLLQRTFN-KRPFILTRGHFPGSQRYTVVWTGDNAAEW---SHLAVSYPMCLSEALGGMSFCGADVGGF
GH31-3 -DVL---HHGNVPHRDIHNIYGLFQTMATHQGLMQRDNQTRKPFILTRSHFAGSQRYTAMWTGDNTADW---PFLQVSYSECLSNIMGLVFCGADVGGF
GH31-4 -EVQ---HYGGVEHGDHNIYGLLHVKSTHKGMLMARQNAKRFVLTFRSNFAGSQRYAAMWTGDNATW---EHFANSISECMANMLGMVFCGADVGGF
GH31-5 -YYQ-----CEKALLNPEYKTIFTNQLG-----EVPLFGVNSAIERPRSPSFVVLPEFEFSW---DGLRKIIPTILTYGILGYPFLIPGAVGG
GH31-6 -QFD---HVE---DQEEVFNITFKKYVETCAQ-----FGDLIEVRSARWTONLQVFLRMIKDKSLWMTDDGLESVLTLLIQMNLNGYPLVLPDMVGG
GH31-7 -VFP---YVSRAEQNLNLLTTEYIKTCAN-----FGDLIEVRSARFNQNSNFVRMLDKDSRWDMQNLPTLVTLFQLNLNGYPLVLPDMIGG
GH31-8 -VYH---GV---NQEDVFNILSESYVRTCAK-----FGPLVEVRSASRTQDLPIFIRMIKDKSNWGDNSGLYTLITLTLQMNINGYTLVLPDMIGG
GH31-9 -VYK---TV---DQELIPNIFSGKYLRTCAT-----FGNLIEVRTGWRTQDLPIFTRMLDKTSNWAYRNLKSLVTLTLIQMNVGTYMVLVLPDMIGG
GH31-10 -TIQWNTTANDILHIQKHNHYGGYQYKSLKDYFKS---KEIFILSATKISQVEP---MIENVETSW---TNLRKYLDSVLFDSIIIGNHLVSI PVCGD

GH31-1 -----NGNSTAPLCNRWMLGAFYPPSRNHTDDGIDQDPVAFGP---QVVAHSV-KAL-TARYYLLPYLYTLFHKAHVTGETVARPLFFE
GH31-2 -----FNNPDTQLRQWYQAAVWLPFFRAHAHIDTRRREPYLEFPD---DVKNRIRATLRLRYAHIPLFYTLFWQHEISGEPVIRPLFYQ
GH31-3 -----FHNPETELVQRWYQAGVWLPFYRGAENNTDRREPIYIST---EVQDVIRNAL-RLRYKHIPVWYTLFYEHTRTGDPVVRPIFYD
GH31-4 -----IGDPSDELLQRWYQGAWVWTFPRGHSSRESKRREPYLEFSK---DVQDVIRNAL-RLRYQHIPIAIYTLFYEHTVTGDPHISPLYYQ
GH31-5 DYAVPTEMLVTNGTENVLADPELYIRWLQLATFLPVVRYRHL-----PNSYSQN---NIAGLAKELAKKRHESITPKLKKFARVSNLNLGLPIRPLWML
GH31-6 -----NSY-RQAPTAELIIRWTQANALMPALQFGL-----PWFYPSDKFDTEIVRKYT-NFHEEYGDKIIIEAMKASMDKGTVPVNPPIWVV
GH31-7 -----NGYGGNVPTAELIVRWTQANTFMPMQFSYL-----PWFNSDEFNTLEIVKYYV-SLHEQYSVDVIKAMERSVSDGSPVNPPIWVI
GH31-8 -----NGYAGKLPADELLVVRWTQANTFMPAMQFSYL-----PWEITSTKFNVAKIVKKEV-DLHEKYADHIIIRAMKNSVEKGSVPVNPPIWVI
GH31-9 -----NGY-GVQPDALFVVRWTQANTFMPAMQFSYL-----PWFNETKFDVPAIAKSCV-KLHKEYSHI IIDAMKKSVLVYGSVPVNPPIWVI
GH31-10 -----TNVFDTSIQMKLCLRWYLIATMPMPFIRISAPK-----PWRNPD---DLSAKYDQQAQSALDTRNMLLAYNGLISSNEPVIIRPMFYD

GH31-1 FPQDKNTYQIDTHFLWGSDDLIVPVLKENDVNVTAYIPKGN-----WYNYFTLEG-FYSKGEN---F-TLDAPLDTIPLLVRRGGAIVPLQ---
GH31-2 YPNENDNVVDIDNEILVGSVLAAMIAEPGASVGVYLPGGANBY-----WYDIED---FRLYQGTGN---Y-NIPVSLDKSLAFYRGGSIIPRKD--
GH31-3 YPG---SVDQDYHILLGSNILARPVMEKGARSVTVNFPGGSDNQ-----WYRVDDGSKFYNGQSS---A-ILPVTILTSPPYFKAGSIIITRRD--
GH31-4 YPR---VNRDRDTQTMIGADIMSAVTKPGVKSVEVYFPGGYSDTDPFDKQTDWFYRADQKQFSQYRAGLV---E-QIEVDITSSPFFYRAGSIVFVLE--
GH31-5 DSDDPACHLAVDDEFISGDEIIVAPVLSHGAREEIIYLPAGV-----WKGID---GSLRKGSRW---IHDYRVEEDNVAYFERMP-----
GH31-6 DPTDSFSYTYIDFELLEDVLPVAVLVEGATSRNIYLPKGN-----WKDGN---GETFVGLN---VTNYDAPIDVLPYFVKTD-----
GH31-7 DPTDPVALACNDEYLVGEDILVAPVLEEKSRNVLYLPRGR-----WRDQGN---GTVYEGPLS---F-DYPADIDILPFFIKED-----
GH31-8 APKDVQALGCDDYLVGEEILVAPVKEGATSRDVLVLPAGK-----WVDGNN---GDIYQGPVT---V-DYDAGIDILPFFILSQ-----
GH31-9 DPTDQKALKCDDFVLEGEKILVAPVLTQGATRDVLYLPKGN-----WKDGN---GTYYQGPIT---L-DYDADITTI PFFILEQ-----
GH31-10 FYENTTTFSLYEQYMGKNMLVHPLTADRTRKMNVLPAIDV-----WYEFWG---GDIYKPTKTNPWV---SISVTEADFAVAPKGSVIPLIDGN

GH31-1 -----KPKNTTDSRKSLELL-----IANDERKSASGDLYWDDGDSLNSYKEKQ---YSSLMELEENTLRSY---ATFI
GH31-2 -----RPRRASPLMKNDPFTLY-----IALDSNKKASGYLVDDNESFE-YKNQK-YLYLHFSF---EDNTF---N---SVLV
GH31-3 -----EERSSTAEMQDDPFVLY-----VNVDKENTASGNLYVDDYTSFE-YQNNQRYFYANLAY---QSNQI---D---ISIV
GH31-4 -----TPRKS SAVEAKDDFYSIY-----VNVDRRNSAKGRLYKDDLYSFN-YQKDN-YFYSEAKWIPGSEKM---Y---MTTI
GH31-5 -----
GH31-6 -----
GH31-7 -----
GH31-8 -----
GH31-9 -----
GH31-10 TVNLIVALNFTSSKRTRSFIESSGSATGFMKTGDISITFTANIKSVDISSSSDKKWSLHLYGKVKYQYLSNKPAYYDVNRSIDQDTQNMPYEPPTTTT

GH31-1 GSE----IPPNLDRVAVLGIEDSVKEVVNGAS---HKNFDYNSKNKYLNDKLDIPLQSP-----VISWK*
GH31-2 DKT-DYPTKEWVERLIVLGGPKIGAKLTSKSLGTVELDTSYSHEDRVLVVRKPGVSIREKF-----TIKL-Y*
GH31-3 DGN-FSGMTVTLGQVIVHQ---QSDKDEKRYTRTYTHLADGTPKLS-----INIVEKL-NGNMVSLKL*
GH31-4 SGPDPQSRVYVYVVKVHELLESETGELQIKRSMFTKTSGGIPLHK-----IDIKALREGNGTLELFS*
GH31-5 -----DDTRF*
GH31-6 -----*
GH31-7 -----S*
GH31-8 -----*
GH31-9 -----*
GH31-10 ESTSSTTTTSTTASTVTESTEDTGTIISSTPTMESTGTGNTGSSSLISEITADTGTSSSTPTRESTTEE

B



Figure S6. GH31 family gene sequences identified from the *D. v. virgifera* transcriptome (A) and the maximum-likelihood phylogeny of GH31 family proteins (B). In the alignment, the labels for partial sequences are shown in italics. In the phylogeny, labels for the coleopteran species belonging to the superfamily Curculionoidea, *D. v. virgifera*, and other beetle sequences are shown in olive, red, and orange, respectively. Their species abbreviations are found in Table S5. Arthropod, other metazoan, nematode, fungal, plant, and bacterial sequences are indicated by black, purple, grey, cyan, green, and brown, respectively. The accession numbers shown in parenthesis are from NCBI except for: BGIBMGA012077-PA and BGIBMGA013995 from SilkDB (<http://www.silkdb.org>), DPOGS202361 from MonarchBase (<http://monarchbase.umassmed.edu>), and Px016165 from Diamondback moth Genome Database (<http://59.79.254.1/DBM/>). The numbers at internal branches show the bootstrap support values (%) for the maximum-likelihood and neighbor-joining phylogenies in this order. Supporting values are shown only when higher than 60%. The scale bar represents the number of amino acid substitutions per site.