Appendix A Species and protein sequences used in this study.

Protein name	Species	Accession number
Drosha	*Pteromalus puparum*	PpDrosha*
	Nasonia vitripennis	XP_031778770.1
	Ceratosolen solmsi	XP_011505854.1
	Copidosoma floridanum	XP_014213264.1
	Apis mellifera	XP_016766928.1
	Bombus terrestris	XP_003394274.1
	Solenopsis invicta	XP_025986293.1
	Tribolium castaneum	XP_008199088.1
	Agrilus planipennis	XP_018332033.1
	Drosophila melanogaster	NP_477436.1
	Aedes aegypti	XP_001653338.1
	Bombyx mori	XP_012547603.1
	Papilio xuthus	XP_013172751.1
	Tetranychus urticae	XP_015787469.1
Pasha	*Pteromalus puparum*	PpPasha*
	Nasonia vitripennis	XP_003424309.3
	Ceratosolen solmsi	XP_011498331.1
	Copidosoma floridanum	XP_014204775.1
	Apis mellifera	XP_006559676.1
	Bombus terrestris	XP_003397039.1
	Solenopsis invicta	XP_011166887.1
	Tribolium castaneum	XP_015836202.1
	Agrilus planipennis	XP_018333701.1
	Drosophila melanogaster	NP_001263150.1
	Aedes aegypti	XP_021710923.1
	Bombyx mori	XP_021208180.1
	Papilio xuthus	XP_013167918.1
	Tetranychus urticae	XP_015793480.1
Exportin-5	*Pteromalus puparum*	PpExp-5*
	Nasonia vitripennis	XP_008203379.1
	Ceratosolen solmsi	XP_011499457.1
	Copidosoma floridanum	XP_014204789.1
	Apis mellifera	XP_396789.2
	Bombus terrestris	XP_003398965.1
	Solenopsis invicta	XP_011163732.1
	Tribolium castaneum	XP_974696.2
	Agrilus planipennis	XP_018329940.1
	Drosophila melanogaster	NP_608339.2
	Aedes aegypti	XP_001660023.2
	Bombyx mori	XP_021204862.1
	Papilio xuthus	XP_013174923.1
	Tetranychus urticae	XP_015794798.1

Loquacious	*Pteromalus puparum*	PpLoq*
	Nasonia vitripennis	XP_016839605.1
	Ceratosolen solmsi	XP_011500654.1
	Copidosoma floridanum	XP_014211571.1
	Apis mellifera	AmLoq*
	Bombus terrestris	XP_012169164.1
	Solenopsis invicta	XP_025989733.1
	Tribolium castaneum	XP_008198430.1
	Agrilus planipennis	XP_025832115.1
	Drosophila melanogaster	NP_001285894.1
	Aedes aegypti	XP_021701497.1
	Bombyx mori	XP_012550850.1
	Papilio xuthus	XP_013164367.1
	Tetranychus urticae	XP_025017170.1
Dcier-1	*Pteromalus puparum*	PpDcier-1*
	Nasonia vitripennis	XP_001605287.1
	Ceratosolen solmsi	XP_011502152.1
	Copidosoma floridanum	XP_014210593.1
	Apis mellifera	NP_001116485.2
	Bombus terrestris	XP_020723074.1
	Solenopsis invicta	XP_025991666.1
	Tribolium castaneum	XP_008199045.1
	Agrilus planipennis	XP_018328602.1
	Drosophila melanogaster	NP_524453.1
	Aedes aegypti	XP_001659747.2
	Bombyx mori	KWMTBOMO06302†
	Papilio xuthus	XP_013163421.1
	Caenorhabditis elegans	NP_498761.2
Ago-1	*Pteromalus puparum*	PpAgo-1*
	Nasonia vitripennis	XP_031783914.1
	Ceratosolen solmsi	XP_011505420.1
	Copidosoma floridanum	XP_014207835.1
	Apis mellifera	XP_006571833.1
	Bombus terrestris	XP_012170889.1
	Solenopsis invicta	XP_025990461.1
	Tribolium castaneum	XP_008196652.1
	Agrilus planipennis	XP_018333020.1
	Drosophila melanogaster	NP_001246314.1
	Aedes aegypti	XP_021704825.1
	Bombyx mori	NP_001095931.1
	Papilio xuthus	XP_013176493.1
Dcier-2	*Pteromalus puparum*	PpDcier-2*
	Nasonia vitripennis	XP_031782799.1
	Ceratosolen solmsi	XP_011494459.1

	Copidosoma floridanum	XP_023246881.1
	Apis mellifera	XP_016773223.2
	Bombus terrestris	XP_012163127.1
	Solenopsis invicta	XP_025991526.1
	Tribolium castaneum	XP_008201496.1
	Agrilus planipennis	XP_018331371.1
	Drosophila melanogaster	NP_523778.2
	Aedes aegypti	XP_001652212.1
	Bombyx mori	XP_021207858.1
	Papilio xuthus	XP_013173437.1
	Caenorhabditis elegans	NP_498761.2
Ago-2	Pteromalus puparum	PpAgo-2a*
		PpAgo-2b*
		PpAgo-2c*
	Nasonia vitripennis	XP_032455160.1
		XP_031786529.1
		XP_031786440.1
	Ceratosolen solmsi	XP_011505420.1
	Copidosoma floridanum	XP_014218615.1
		XP_023245295.1
	Apis mellifera	XP_395048.4
	Bombus terrestris	XP_012168271.1
	Solenopsis invicta	XP_025997806.1
	Tribolium castaneum	NP_001107842.1
		XP_008192985.1
	Agrilus planipennis	XP_018319532.1
	Drosophila melanogaster	AAO39550.1
	Aedes aegypti	XP_011493002.2
	Bombyx mori	XP_021205714.1
	Papilio xuthus	XP_013179888.1
R2D2	Pteromalus puparum	PpR2D2-1*
		PpR2D2-2*
	Nasonia vitripennis	XP_031785356.1
		XP_008214050.1
	Ceratosolen solmsi	XP_011504752.1
	Copidosoma floridanum	XP_014215309.1
		XP_014205151.1
	Apis mellifera	XP_006560091.1
	Bombus terrestris	XP_003395928.1
	Solenopsis invicta	XP_011156338.1
		XP_011157520.1
	Tribolium castaneum	XP_015835139.1
		NP_001128425.1
	Agrilus planipennis	XP_018328507.1

		XP_018330227.1
	Drosophila melanogaster	NP_001162903.1
	Aedes aegypti	XP_001655660.2
	Bombyx mori	NP_001182007.1
	Papilio xuthus	XP_013174949.1
	Tetranychus urticae	XP_025017170.1
Sid1	*Pteromalus puparum*	PpSid1*
	Nasonia vitripennis	XP_031783168.1
	Ceratosolen solmsi	XP_011495242.1
	Copidosoma floridanum	XP_014207455.1
	Apis mellifera	XP_006565236.1
	Bombus terrestris	XP_012170554.1
	Solenopsis invicta	XP_025995259.1
	Tribolium castaneum	NP_001099012.1
		XP_015837738.1
		NP_001099128.1
	Agrilus planipennis	XP_018319198.1
	Bombyx mori	XP_004930736.1
		NP_001106735.1
	Papilio xuthus	XP_013177205.1
		XP_013177321.1
	Penaeus vannamei	ROT74464.1
Ago-3	*Pteromalus puparum*	PpAgo-3
	Drosophila melanogaster	NP_001036627.2
Piwi	*Pteromalus puparum*	Pp-Piwi-like1
		Pp-Piwi-like2
		Pp-Piwi-like3
		Pp-Piwi-like4
	Drosophila melanogaster	AAD08705.1
Aub	*Drosophila melanogaster*	NP_476734.1

*：Newly identified proteins in this study; †: Protein from silkbase;

Appendix B Sequences of the newly identified proteins in this study.

>PpDrosha

MDNVPLDTEYQYYWQADSSQAQNCPYYPNSSVSYPPSALPSQPPYNAPPPDYNGFNYSVPPPSYFSVPPP
NSSIAYPAGTVTYSSYEYQSHTSVQENKDYYTTSYAQAQSSTYNYNNWDPKCPYQQDSKTDNTQWVAE
TSSWLSHTAVNSKSQNSSADKGYKSVSNGLRRSKSPDSKFHSSKFTSRRKYDDYSNRYGDRNKERKSSR
DRFYKRRSRSLDRKSYASNNSYTNHSSARSERSERLERSESRSRSRRSISRSRSKRRSRSQESHTSLQTSRST
NNIAKRKSLSEREMLLEKYRQNYCATSEDIKRKLNELESDGVLERKIWTRTAPADLYYSRDEKNNKLMK
ATGRLIQLCDDFKKILIDRASSARELQPPYEPPPRKNRARLCRHKSEACSSSSSDSDDSVDEDDRTMEELM
AKKQHPQRLHPEMWFNDPGEMNDGPLCRCSAKSRRSGIRHGIYAGEGAIEKCDPHTNNADKLYHYRITIS
PPTNFLTKTPTIIKHDEHEFIFEGFSMFSHFPLVKLPTCKVIRFNIEYTILYMEEKMPENFTIQELDYFQDYLF
KEILELVDFDLQAAQDKKGCAQFHFMPRFVRDLPDNGQEILSMNEVLSYLIKCSTLLIDPDELPKLVEMPQ
FQWQNFADEVKGMVVTYPGKKPCSVRVDQLDRNQADQPPGVIAYPEIVHFGIRPPQLSYAGNTDYQRA
WRDYVKFRHLLANMPKPSFEDKRKLEAKENKLQELRTQSKMKRDVTVDVTSEGFYRTGIMCDIVQHAM
LIPVLVCHLRFHKSLDMLEQTLNYKFKNRYLLQLALTHPSYRENFGTNPDHARNSLTNCGIRQPEYGDRRI
HYMNTRKRGINTLINIMSRFGAKKETESSIAHNERLEFLGDAVVEFLTSIHLFHMFPDLEEGGLATYRAAIV
QNQHLAVLAKKLNLEQYMLYAHGSDLCHDLELRHAMANCFEALMGALFLDGGIRIADTVFGETLFKKE
LDLLNIWVNYPKHPLQEQEPTGDRQWIPSFELLQKLTKFEEGIGVEFSHIRLLARAFTDRSIGYTNLTLGSN
QRLEFLGDTVLQLIVSEYLYKFFPEHHEGHLSLLRSSLVNNKTQAVVCDDLGMTQYALYGNPKAELKTK
DRADLLEAFLGALYVDKGLLYCQVFCNVCFFPRLQDFIMNQDWNDPKSKLQQCCLTLRTMDGGEPDIPV
YKVIECKGPTNTRVYTVAVYFQGKRLAKASGHSIQEAEMNSAKEALEKSQDLFPQLDHQKRVIAKSMKM
QSWSQGGLKFRTIQSSKFDDRSEDRNSIRYEDRRHDGRNEGRRRDYKYEDRQRDNRHSSDSDSDSHRKR
RSRSRERAKRSSRSRDRYDSVDRRLRDKPRSSSCSSSNSSNSNARSIRNKSAERHSSSDDSTSNCTENIAAA
RGDGNGEDKELKGANRKRRSSVLSNASSNDASSSNNISETDKKCVLPFSEKKMKRLE

>PpPasha

MEVDVQKSDVVVEKKSEDETNSEEMLTQESMDTCTTVLQEVQPSQSKETENPLISEDATDDDLRQFDVL
DDLERHTNQDGSDSDGCSNDSMDSDIPDDEIEAMLEEGLPDEFKGKRSDKRSDMLYEEKEKLVLDEIGHN
HFDVLPEGWVQVTHNSGMPLYLHKQSRVCTLSKPYFLGPGSVRKHEVPVSAIPCLQYKRALKEEEEEKQ
RDKERMQAAQACSLPNAKIETIQENRATQSLDGENLRNYCQSLFRFKSIKVMRFKSWSQRRKFTKNKKQ
RKQLERPTLPDGTKLITFPVGNSAVYSGNSTQEDESASRPPKHWIMNPSGKSYVCILHEYVQHALKKQPT
YKFKELENAATPYSAVVCINDMEYGSGFGSSKKQAKANAARKTLEILIPQMRDKISGDTTTDNNSANSSQ
TIKASKVNSDADLSFFDEISINDPRVAEFCAKTTEPLPHAILITCLQRNFGLGDMEINYSVNTLKHQRNEFT
MRVGKHEATVICRNKKDGKQRAAQAILQRLHPHIHSWGSLLRLYGSRSVKSFKEKKQLEQEITLLQGKA
AVNQPNHAILEKLRQEMRKLSDQRQAVKPIGKFIPPDMPTGSSSNLDNVDL

>PpExp-5

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FGLQLMEHCVKYRWTQMSQTEKVFIKENAMKLLQEGTEPLLQEENHIKDALSRIVVEMIKREWPQQWPQ
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QKQSTLGRTTEAAAHGRVVQVVLLTLSGFVEWVPINHIMEDKGRLLQILCILLSHPTFRCPSAECLQQVVN
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NKRHANGMASYFVDYDTEEEFRMFFHRLRMDLLEGFRNATMVAPLVTFAYVQQWLTVKIRKGSENLAY
KSDPLDPEYLEWEALALALDSVMTRILLVSERPNVQTGLQLLELCLGYSPQDPWILSALLSCISALFVFMS
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GLDRPALENNRDERTGSNRSDLMYCINTVLSVVKRCAIPEDPDRAARGGFVAALSESGNPIYRNPATPHII
PVLPPLFDLLRTMNALFTPAALGLLSEGYRNVHGIIESEKANLLGLHTSNNNENSADSELQSAPLARMQGF
LTTIHDNCYHMLGSGCHTIGRDLYQLPGLAAALLNSVFSNMEVIPDHRLRPIIRVFMKPFIYSCPSAFYETV
LVPVLAHVSTHMYQRLSAKWQHMNSLYEAGSLDDDNTDAQEVIDDMLNRNLTRDFVDVLKVALVGGA
ASDASPPDSMEQDSGGMAVDSPPARGSNSIVAEVVSELGSFILRHSSTFQSVVLCILGALSWNDSNASLRA
TMLTGPVVRALAAAEGILNPGMAAHIMMAILQGLQLHGQHEANQGSLITLGAQVYECLRPKFPNIIEVMQ
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>PpLoq
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RLEMKALPNKTPVSVLQELLSRRGTTPKYELVQIEGAIHEPTFRYRVTVADVVAMGTGRSKKEAKHAAA
KAVLDKLIGVNTENAEAPLPNSIPDENFPLSSQNIQEIQSGYGEEKVVNNPIGSLQEMCMSRHWPPPKYSM
EGEEGLPHERQFTIVCSILKYRQVGQGKSKKLAKRQAAHKMWQSLQDTNSNRTQGVDEDEIVQRNANV
NAHYADLKDSKIATLTTPHSHKVSQFHKNLKSSTGVKLFELQTHLFQNTCLNGDVNLVQFLQEIASEQQ
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LLAYKMEDEKCVQNDVKEFTYSTLNFLFDHRYDPTEIYSEEFLEDIKKIPNPVAKPCEMIYEFLHILNTLGL
WAADRAALVLLMLIEKLKIKTPYERHYLLFGVVATLFLKIRAYCDDIFSGLTEAEKILKFSTPKIHRLIEVIK
TFSPPPKEEKEENVTVNNDVEKSDSANSKDQNHDKSVKNFHKKTDGGFKKLRTFRHSRGLTDPDLLCGVI
FVDNAVTAKVLFYLLNEMSKCDESLQFLSPLYTSEKTNDELFCGRDLELEYKKQEEVLKKFRIHECNLLIS
TAILEEGIDIPKCNFVMRFEFPKNYQSYVQCKSRARATDALHVLMVPEIESEVYIQRLAHYHYIEKILLSKC
TSKEADECEETEADAYDSLLPEYKPLNEADAPKVTLNSAISLVNRYCAKLPSDTFTKLTPEWKINNITVEN
KQMYICSLRLPINSPLKYSISSYPMPNKALAKRMAALQLCVCLHKENEIDDYLLPVGKENFKAHPEDSEVP
TLPDDENMNLTEARPGTTKRRQYYYKKIADALIDCKPELEKPCYLYHINMVLSCPLPEEQNTRGRKIYPPE
ESDIGFGILTRKKIPNVCPFPIYTRSGEVRVDLKLSKETVVLDESKIDKVVSFLNYTFTNVLRLQKYLMLFD
PNVSENSYIIVPVKKQPDASDICVDWDFVECIYANRDSMPNCVSEKNRQNFVFEANKYHDAVIMPWYRN
QDQPQYFYVAEICNHLNPKSSFPGADYSTFEEYYSKKYNIQIQNLEQPLLDVDHTSARLNFLTPRYVNRKG
VALPTSSEETKRAKRENLEQKQILIGELCAIHPFPASLWRQAVCLPCILYRINALLLADQIRRHVAQSISLGQ
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DDEEGLTIAYTGDNVAEAFDKKGKAFEAEKEKQSCVERQEKSELMHWNFKGDLSDEFYQLHRLAHIDK
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HEGKLSHLRSKQVSNLNLYRLGRQKMLGESMIASKFEPHDNWLPPCYYVPKELEQALIESGVPSAMWNQ
ADIPPFSRFSEINDLVRETEHKLEIIKSELSQSDHSPLNGPNGLTGDPLRSFIPYNLITQHSIPDKSIADCVEALI
GAYLIACGPRGALLFMSWLGIHVLPSEEVTVISDSKPTERPPGSTPYVEREEEDGRTKWTQLRYKKLQEPP
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RHLYEDPRQHSPGALTDLRSALVNNTIFASLAVRCGFHKYFRHLSPGLSTVIDRFVRIQEENGHSISEEYYL
IGEKEFEEAEEAEDVEVPKALGDVFESLAGAIYLDSNMSLDAVWAVYFEIMQSEIEQFSANVPKSPIRELL
ELEPETAKFGKPEKLADGRRVRVTVDVFGKGSFKGIGRNYRIAKCTAAKCALKKLKKSQSCIKGRK
>PpDicer-2

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VVQQTAFLSRHTGLVCKGYSGDMGVDFWSNTEWRNEIDTNQVLVMTAQIFLNLLIHGCMSLDKINLLIF
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VTDYATNPKEYVEYYDTPQNISVLSEISSIVEYASSILKYVDLPNRMENPASSAIFKPVSKTIKLNRILSDVE
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LKLLEVLKTFYNNMENKTNFCCIIFVKRRFTAKVLYHLLLKLSSSDKNFEFLKPQYMVGYNNDPYKNARE
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YVQEFGLTISNNMMEVRGRILPPPKLQYGGRVSSLSGQTKQQALPNQGVWDMRGKQFFTGVEIRVWAIA
CFAPQRTVREDALRAFTSQLQKISNDAGMPIIGQPCFCKYATGPDQVEPMFRYLKSTFQALQLVCVVLPG
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ILYRDAVSEGQFLHVLQHELTAIREACIKLEADYRPGITFIVVQKRHHTRLFCADKKEQSGKSGNIPAGTT
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PMFFV

>PpAgo-2b

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LKPSQSILDNKTWTILNLDNRTKDDRLYKLVDILKRMAISVSLPIGEPFTPFTKIQLKGHDIRGLMEYFNKM
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>PpAgo-2c

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>PpAgo3

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QRRQVIRKFIQQVNGNEVTKKLLSDWGLEIDNDTLDLSGRVLDPEELMFGNDYKTKSPNADWGKAATNS
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>PpPiwi-like1

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>PpPiwi-like2

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>PpPiwi-like3

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HRSLISVF
>PpR2D2-1
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>PpR2D2-2

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>AmLoq
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KSKKVAKRHAAHKMWQALHYMTTENPNIDEDEVLQRNANVSARYADLKGSKISTLTTIHSLKVSQFHKS
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VTSKDAQASAAQNALEYLKIMTKK

Appendix C Primers used in this study.

Primer name	Forward sequence	Reverse sequence
qDrosha	TCTCAAGGCGGACTCAAGTT	TCGGAGGAATGTCGGTTATC
qPasha	AAACCACCGAGCCTTTACCT	CAGTCGCTTCATGCTTACCA
qExp-5	GTCTGGATCGACCAGCTCTC	GGCGGTAGCACAGGTATGAT
qLoq	GTCGAGCGCAAAGGATTTAG	TGTGATAGCTTGCAGGATCG
qDicer-1	GCTGGAGCTAGAGCCTGAGA	AGCTCTGCGACTTCTTGAGC
qAgo-1	AGTTGGTAGTATGGACGCCC	CAAGCTTCACGAATGGCAGT
qDicer-2	CTGACAAGCAGCAATCCGTA	CCTCTCCGCAGGTTGAATAA
qR2D2-1	AGTCACCAGCTAACGTTCCA	TGCTTTGCCTGTTTCTTGGT
qR2D2-2	ACCTTGTCAGTTTGCCACAC	CGACAGAAGAATGGCAAGGG
qAgo-2a	GCTGACATTGCCAACAGAGA	CACCGGCCTCATCAAGTATT
qAgo-2b	GCTGACACTGCCAACAAAGA	CCGTCAAATGCTGGATTTTT
qAgo-2c	TTCTCTGCCACATGTTCTCG	CATTGGGCTTCCCTTCAGTA
qSid1	GCGAGGCATCTGGAACTTTT	CGCTTCGTACCACAATCCTG
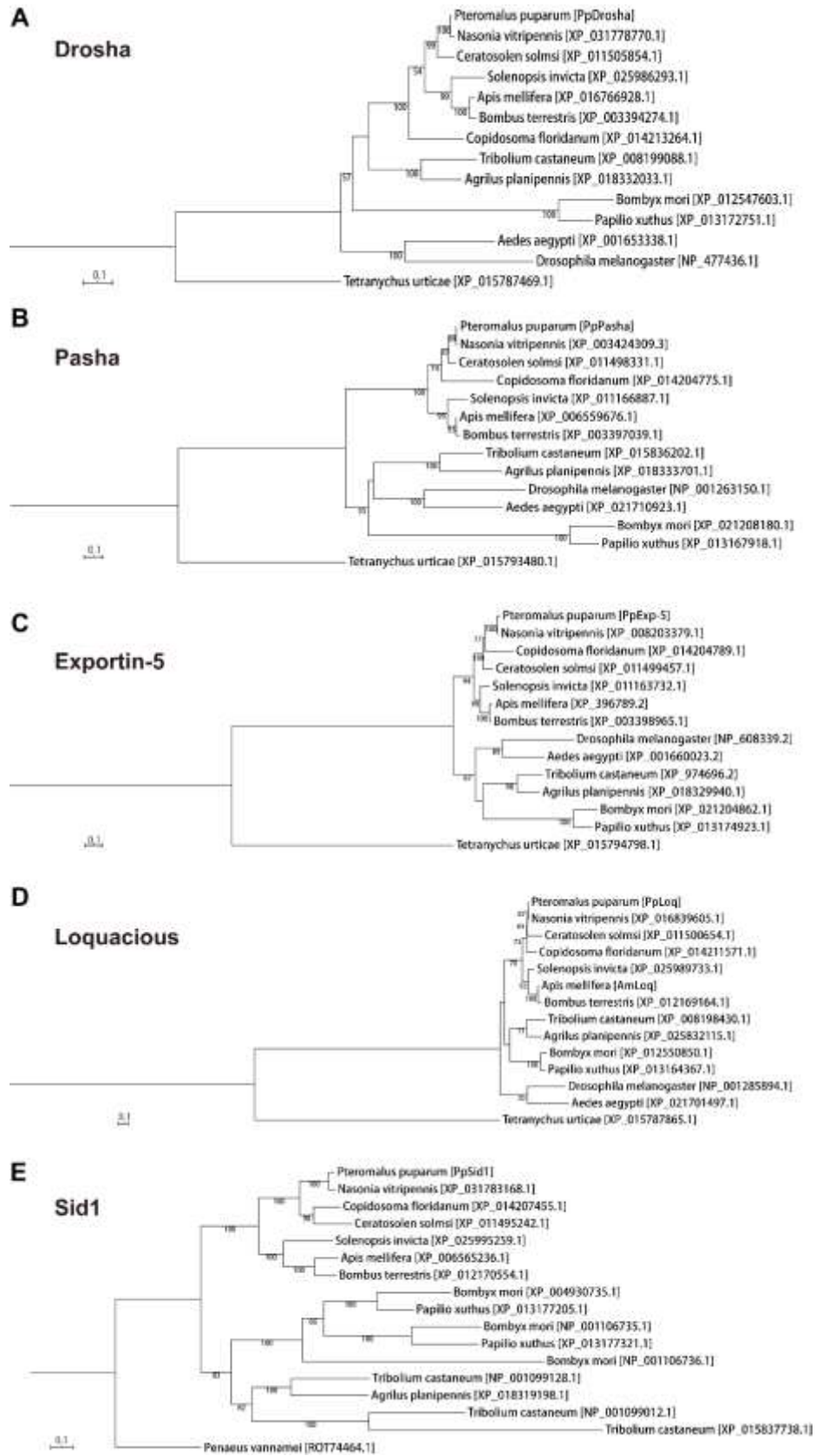
Appendix D Maximum likelihood tree of multiple sequence of alignment of Drosha (A), Pasha (B), Exportin-5 (C), Loquacious (D) and Sid1 (E) proteins. The tree is rooted at *Tetranychus urticae* or *Penaeus vannamei*. Pteromalus puparum proteins are pointed out by pentagram.
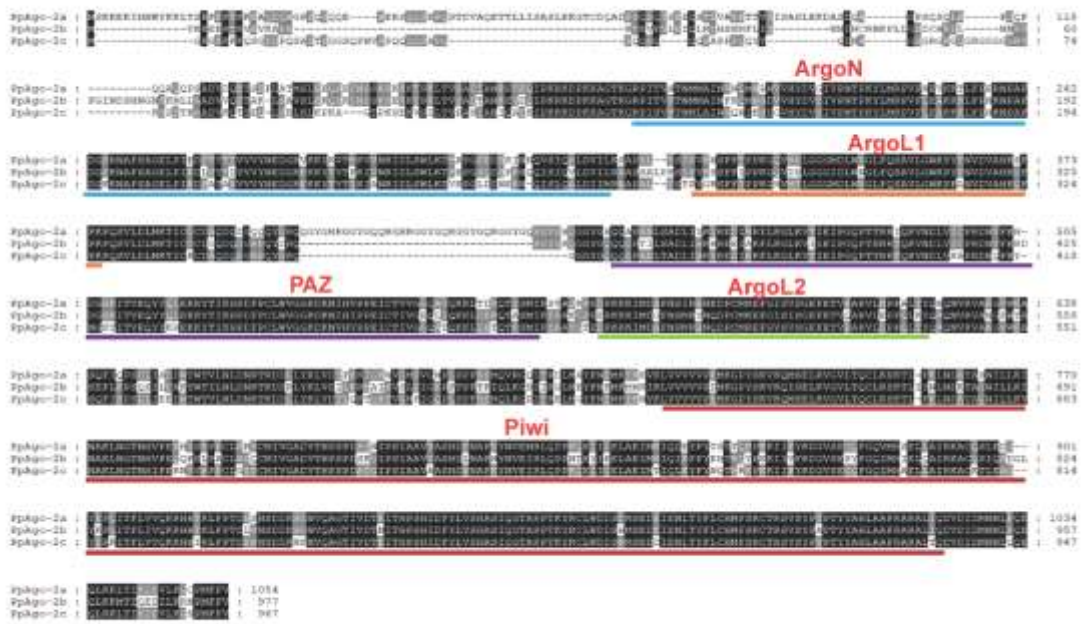
Appendix E Multiple sequence alignment of Ago-2 (A) and R2D2 (B) proteins. The lines below sequences indicate the ranges of predicted domains and the domain names are listed above the sequences. ArgoN: Protein argonaute, N-terminal; ArgoL1: Argonaute, linker 1 domain; ArgoL2: Argonaute, linker 2 domain; Piwi: Piwi domain; dsRBD: Double-stranded RNA-binding domain.

Appendix F Parameter estimation for hymenopteran Dicer and Argonautegenes under free-ratio model using codeml.

Gene	Species	dN	dS	ω
Dicer-1	*Pteromalus puparum*	0.01059	0.11687	0.0906
	Nasonia vitripennis	0.00289	0.30766	0.0094
	Ceratosolen solmsi	0.03529	0.86671	0.0407
	Copidosoma floridanum	66.91498	0.07331	0.0011
	Apis mellifera	0.00922	0.13854	0.0666
	Bombus terrestris	0.00725	0.18267	0.0397
	Solenopsis invicta	0.01744	0.31744	0.0549
Dicer-2	*Pteromalus puparum*	0.01383	0.23354	0.0592
	Nasonia vitripennis	0.02244	0.05807	0.3865
	Ceratosolen solmsi	0.17033	1.134	0.1502
	Copidosoma floridanum	0.24268	1.63735	0.1482
	Apis mellifera	0.05915	0.12048	0.491
	Bombus terrestris	0.06887	0.35928	0.1917
	Solenopsis invicta	0.15945	1.46459	0.1089
Ago-1	*Pteromalus puparum*	0.00189	0.16145	0.0117
	Nasonia vitripennis	0.00001	0.10114	0.0001
	Ceratosolen solmsi	0.00188	0.96204	0.002
	Copidosoma floridanum	0.0041	0.74658	0.0055
	Apis mellifera	0.00124	0.11114	0.0112
	Bombus terrestris	0.00002	0.20237	0.0001
	Solenopsis invicta	0.01275	1.29289	0.0099
Ago-2	*Pteromalus puparum*	0.03996	0.26469	0.151
	Pteromalus puparum	0.05908	0.1959	0.3016
	Pteromalus puparum	0.03928	0.18797	0.2089
	Nasonia vitripennis	0.0425	0.22782	0.1865
	Nasonia vitripennis	0.08655	0.35726	0.2423
	Nasonia vitripennis	0.01077	0.18949	0.0569
	Ceratosolen solmsi	0.12709	1.02614	0.1239
	Copidosoma floridanum	0.05783	0.51774	0.1117
	Copidosoma floridanum	0.04508	0.53012	0.085
	Apis mellifera	0.07145	0.27662	0.2583
	Bombus terrestris	0.07728	0.25616	0.3017
	Solenopsis invicta	0.27479	1.28383	0.214