

Supplementary information for

*Genes with spiralian-specific protein motifs are expressed in spiralian
ciliary bands*

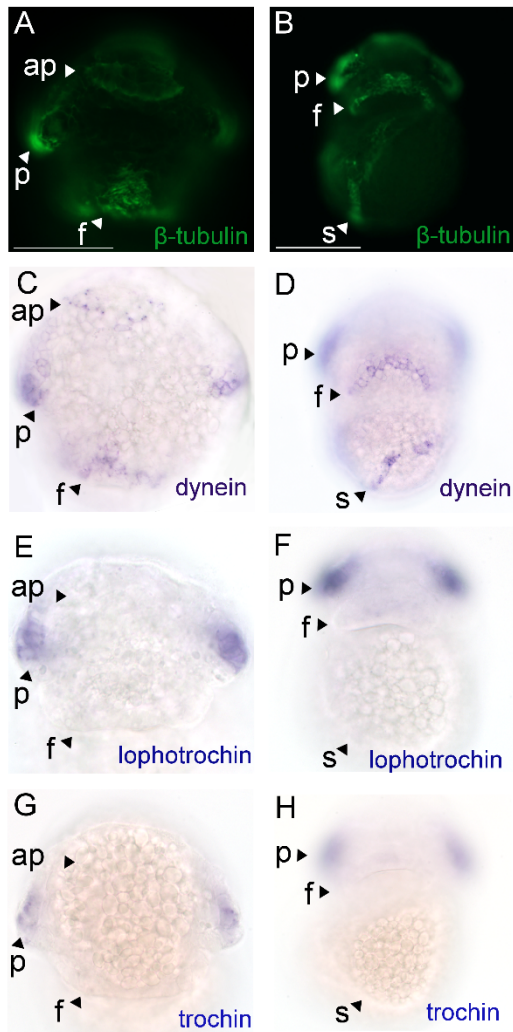
Wu et al.

<i>Antinomermes_californiensis</i>	AEQAAAYILEIAIATQHKVRAMKIME	PRLCSMTCDSESRMWLAVTQSG	NDKVAVLNLSKRVRLDHQSGKVEYVLDAYPFCEQKVRAGWI	-LQTNSDVEITLHAGGHQGYAALG	152			
<i>Maculaura_alaskensis</i>	SFSAQAARILIESIAISQHKVMACQMG	PRLCPHTCDESRMILALVNH	NDKVAVLNLSKRVLCDCQTLGKEWISSYFPYSEDQKYRALRI	-LQTVEDPMNAKTLHAGGHQGYAPLG	153			
<i>Tritia_obsolete</i>	YFSALQASAVLHLSRFPEDLKAAKMLE	PRLCNMSCLEARELISINIQ	NDRLIVLD SKRVRLDQCTLGVEEYLAFSSYFQDQKAKMLNI	-LRTVSESDQDLHAGGHQGYAAAG	152			
<i>Crassostrea_tigula</i>	FYNADQAAHLHLYCANPADKIRLRLVLE	PRLCMTCCREGHIDINAVIH	NDKLLALD CKRVRLDQSGKLGEEYLISAPFFETDQKYRAVQ	-LQTNSFVQDYLHAGGHQGYAALG	153			
<i>Capitella_teiga</i>	FYSASQAHLILAFYNSNDRLRATQVLE	SRLCNMTCSSEITIGFTITIA	NDKIKALNSIKRYLRDVTREGQDIIATFFDQKYEAVQ	-QRVLTARSQDQDLHAGGHQGYAALG	150			
<i>Platynereis_dumerilii</i>	SFTAHQASIIYAFPNSSDKVHWQTLQ	TKLSPMTCDEADLTGSGFPFH	NDKVALQSGVRRVRLDSQDFGVENLISAFPHYEDQKQAFGV	-LRTARTSVHNKVLHAGGHQGYAALG	153			
<i>Terebratalia_transversa</i>	FFHHEVAHRIITYCAVPSDKIRATQIME	PRLCNMTCCDAVDLTGSGVH	NDKLLILD CKIRALKLDSQTFREGAYELISATFEEDQKARAYSI	-LQTVSTVQDKVPLHAGGHQGYAALG	149			
<i>Phoronopsis_hameri</i>	SFNAEQASRVVATYASPSHKIRATQIME	PRLTRMSCSEALGTVASVH	NDKLLIVLD CKMRKALDTCQMRLGQELISAFPHYEDQKMRALS	-LGTVRSDVADKTPHAGGHQGYAALG	153			
<i>Brachionus_manjavecis</i>	FFFAEQASQRLVATFLRPDKITKAVMLE	PKLSTSPCQQAHSLLAALVLP	EDKLEALE YKRRATINDQTEGGQGVYVSTFEEDHKIRAAKI	-LISVVAKQGVQVAGHGLGYAPMG	169			
<i>Macrostromum_lignano</i>	YITPQIAEILITFKDADRVKATRLLE	PRGRYPTCAEAATQGLTFEFS	LGSDRLPMALC CKRLRYIDHGSTAGVEFLISAFDNEDQKQALSI	-LQRTVHYADKLHAGGHQGYGATG	153			
<i>Branchiostoma_belcheri</i>	IIFNHLARVLVTEFKDSDKLKAVQATQ	PRGLYPTCAEAATQGLTFEFS	NTQLTLE IVARINITDGTG	-GHLVQESAFSGHDKTKAMEI	-ANRCSGAGG-VYPAGAPGYVPSAP	143		
<i>Nematostella_vectensis</i>	FFSADQVAELVEKFTFDDEKVEAVKICS	KNLNPVLTCEAVSLVLSFGFD	DRKVKALK LGIDRIIDPEAY	-LQTVDSISFVSQYEVKRLFEAC	QTQCFDPGNNYGATG	142		
<i>Amphimedon_queenslandic</i>	FFFGVQDAVQTLKLKFSNDQLKLEAVE	PRMPLNGQEGDVLTVLFAA	SDKLAVD MIADHYIDYPTTK	-YDV	-LDAIDIFDQDEKARAR	-LDKAVF	-KPTTGCPTSCPTVP	141
<i>Naegleria_gruber</i>	PNISDVLSVILHFGGDDNDKVLKPNQLQ	QTNMIALITGSEAAISVIRTFNFD	NERIRALD AIVNSIYDRKQNS	EDVLAFFMFGFSSDINRKAIRL	-MQTR			215
<i>Manis_javanica</i>	YFSSEQVDLLRYFSFAEPLQKAPKALQ	HKMWAVHVFQSNLNLNCTFIS	KDKLVALE LNASINVDQNS	-RPT	-EDVLAFFMFGFSSDINRKAIRL	-VLEQAFKGGCKAPHAMISSCGTIP		144

<i>Antinomeretes_californiensis</i>	GLH	TQARPLPVPHLYGSLQFQSLQPGQ	GEIETPPAAQTGVIPSVYSGHPSVAYPPQPRQEPYA	---	216		
<i>Macaulaura_alaskensis</i>	GLY	TQSRPLEALLYGSLITQNSSSAGH	GPIAVPPAADPGIKAKYASHPSVAYPIDITYETDY	---	220		
<i>Tritia_obsolete</i>	GLF	TQARPLNGHLGYHLYVQNEAPGH	GKIEVPLTAQGVVPSIYTGHPYSVAYPAKISYEDRAY	-PGP	220		
<i>Crassostrea_gigas</i>	GLY	TQARPMVPHLYGVNADQQLLPGH	GKIEETPEAQGVVPSIYTGHPYSVAYPAKISYEDRAY	-PGN	220		
<i>Capitella_teleta</i>	GLY	TQSRPLVPHLYGSEEGNQKPGV	GRIETPEPAKATLPSVYSGHPSVAYPPGITYADREY	-MTQ	218		
<i>Platynereis_dumerlii</i>	GLY	TQSRPLVPHLYGSRVEAQGVNMGISNDMLPVTARPNTAPFLYTSYPSHSIRGIGDYATDRQY	---	PSN	219		
<i>Terebratalia_transversa</i>	GLY	TQARPLVPQLYGSLIAHQKSLPGQ	GEITAIPQADGVIPSVYTSHPYSVAYPAKISYEDRAY	-PGA	221		
<i>Phoronopsis_harmeri</i>	GLY	TQARMPVPHLYGSLIPEQKLTIPGY	GATEITPEAQGVVPSIYTGHPYSVAYPDRISFAQDRQY	-PGT	220		
<i>Brachionus_manjavacas</i>	CLY	TNAVNPNIHPHYGVLVDLGLNPHRE	YDSSYNRAVPTKSSIYSDNMS	EKKNIWFWSI	232		
<i>Macrostromum_lignano</i>	GLY	TQGNPLVPHLYGSEEGQRLPHG	GEPSLPVHAKPGRTLASVYSHPSVAYPDRISFAATRY	-MSS	221		
<i>Branchiostoma_belcheri</i> _XP_019647158.1	PTT	RPAASGIHIRGPDGLGSLMKGVGAAMGAAMGATGVGAAMAHAGAGFDRIKATGA	PAPGTITVATGYTYPGQTATAYA	QNTNTAYP	GOATTAYP	243	
<i>Nematostella_vectensis</i> _XP_001631054.1		ATNLTGYNPYMGNGG-TLE	YNQSPGVGDG-AGF	IPAEYAYYGYDQ	SAMWT	PAPYGASPEMAPLTAYGPLYGVGQSDQYSGN	224
<i>Amphimedon_queenslandic</i> _XP_011480376.1	PSC	PPGQ	YPPGQYHPG	PGPPGQYTPGPPGQYTPGPPGQYTPGPPGQYTPGPPGQ	GOYT	PGPPGQ	204
<i>Naegleria_gruber</i> _XP_002675533.1							215
<i>Manis_javanica</i> _XP_017503694.1	GNPY		PKGPKSRINGFP				160

Pantionemertes_californiensis	--HI----	DQTTYPMHPYAGAPPLGYHQ--GAPATGFS--	248	AGGHQGYAALGGLGTHQAPRLVPHLYGSLQEQ
Maculaura_alaskensis	NVLLED	EPGVNRSYPTGPPFGYHQ--GNKASFGYPNNEPTPVKLPGGH--	268	AGGHQGYAPLGGLYTQSRPLKALLYGSITRQ
Tritlia_obsolete	VGFPAT	VDGPCSYPGGAPPLGNNT--GAPATGTPRLDSRGFVY	262	AGGHQGYAAMGGLFYQAPRLNGHLYGHLVYQ
Crassostrea_gigas	SSLGQS	LPGDYPKGAPPLGYHS--GAPNPTGFLQLES	255	AGGHQGYAALGGLYTQAPRPMVPHLYGVNAVDQ
Capitella_teleta	APQVA-	PSLPEYPAGAPPLGYHQ--GGPATGFGYLSITLSE	257	AGGHQGYAALGGLYTQSRPLVPHLYGSLVEEQ
Platynereis_dumerilii	VVPNF-	YLDYPTVTLERSY--GNMTVAGNQTHVFTGY	253	AGGHQGYAALGGLYTQSRPLVPHLYGSRVYEAQ
Terebratalia_transversa	RGFPED	VTFGSYYPGAPPMFGHG--GAPATGFGPLGTMQ	259	AGGHQGYAALGGLYTQAPRLVPQLVPQLYGSIAHQ
Phoronopsis_harmeri	EGYPAT	IKAEPTYAGAPVPTGTCGAPATGFGPLKTYIQY	263	AGGHQGYAALGGLYTQAPRPMVPHLYGSLPQEQ
Brachionus_manjavacas	PSFIQD	ITLWRDRIQNKVL	252	AGGHLGYAPMGKLYTNVAPHHTYGPVLDQ
Macrostromum_lignano	VPEPDQ		227	AGGHQGYGATGGLYTNQNPFLVHLYGSLLEEQ
Brachistotoma_belcheri	APPSGGYPPAGTGYPTAGAGYPP--	APGT--GYPPAPGAGYPPPGGP-G-YPPAP-GS--	308	*** * * * * *
Nematostella_vectensis	TSNITYEP	QTFFAPQSDGFPFYW--	282	
Amphimedon_queenslandic	YPP-	GQYPSGPPAQYPPGQYPP--GPPAQYVYPPGPPGQYPPPGGASGQYPPGQYGGQYPAVQYQYPPPPGQYPSYPHP	242	
Naegleria_gruberi_XP			215	
Manis_javanica_XP			160	

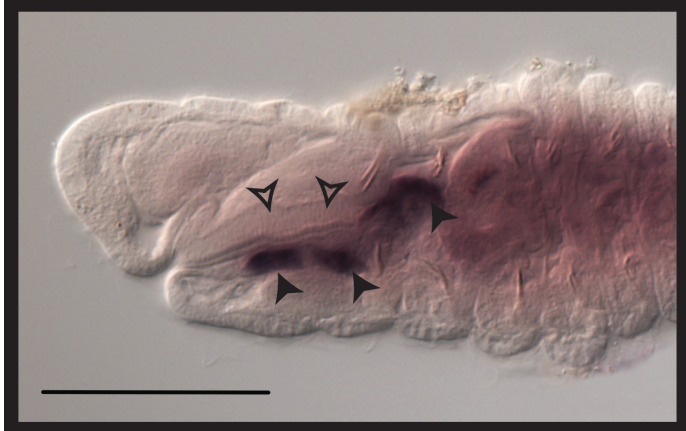
Supplementary Figure 1. Amino acid alignment of *lophotrochin*. Alignment of spiralian *lophotrochin* protein sequences and the non-spiralian sequences with highest similarity (in the grey box). The characteristic *lophotrochin* motif is highlighted in the black box and the alignment of only the spiralian *lophotrochin* motifs is separately shown in the bottom right corner in the red box.



Supplementary Figure 2. Axonemal *dynein*, *lophotrochin* and *trochin* expression in the mollusc *Tritia*. A, C, E, G are the same stage early larvae, and B, D, F, H are the same stage late larvae. A, C, E, G are anterior-ventral view and B, D, F, H are ventral view. (A, B): β -tubulin staining (green) of all ciliary structures. (A): β -tubulin staining showing cilia in the apical plate, primary ciliary band and foot. (B) β -tubulin staining showing cilia in primary ciliary band, foot and shell margin. (C, D) The *dynein* expression (purple). (C) *Dynein* is expressed in all of the ciliary structures, including the apical plate, primary ciliary band and foot. (D) *Dynein* is expressed in the apical plate, foot and shell margin. (E, F) *Lophotrochin* expression (purple) is strong in the primary ciliary band, but not detected in the other ciliary structures: apical plate, foot and shell margin. (G, H) *Trochin* expression (purple) is strong in the primary ciliary band, but not detected in the other ciliary structures: apical plate, foot and shell margin. (A, C, E, F) the imaging focus was on the level of the apical plate and foot. (B, D, F, H) the imaging focus was on the level of foot and shell margin. Abbreviations: ap: apical plate, p: primary ciliary band, f: foot, s: shell margin. Scale bar: 100 μ m. For each *in situ* hybridization, at least 20 animals were stained and all had the pattern shown.

Capitella_teleta	MATYSFEHVNRTKANHINADTRNLLDGKQRAYPNGNYASGDGKSRVWS--GHGFYSPSAR	58
Platynereis_dumerlii	MATYTFEHINRRKATHVNADTRNLLDGRQRAYPNDNYLSGDGKSMVWM--GERYYNPKNR	58
Terebratalia_transversa	MAQYFHERLSG-----RTIKPYPTPTNNYLSGDGKSAVWK--GHSFYVPSE	46
Phoronis_australis	-MQYYHERLSG-----RIMKPPRIYPTDNYLSGDGKSTVWPTRGSNFYVPSE	47
Macrostomum_lignano	-M----AARNCYSKYST---DPAFTGPKIVTPPNNYLSGNGKSEVYQ--GAGWYVPSE	49
Notospermus_geniculatus	MM----AARDCYSKYSL---DKRFSGPKIITPTHNYLSGDWKSFICYE--RSSYYTPSE	50
Crassostrea_gigas	MH-----RPEYGTSAGTRLFDRGPYNSTPVDNYPSGDGKSMIWR--GSHYVPSE	49
Tritia_obsoleta	MQ----TGTTRTSSYNI--KEDLL--KRNHSTPSNNYFSGDGKSFVWS--GAAYYVPSE	50
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Capitella_teleta	KWQKLHEVKALPNDRIPDLIEFQSEDDWRKKWQNRDRVD-SKNCAGSGFRNACPPLKLD	117
Platynereis_dumerlii	DWMKHHDKGAPNARVPGQIEFPHEDAWREWKNRDTPGSGSVCASSGFRYSAPKELKD	118
Terebratalia_transversa	RFVKYHEYRALPPERRADAKDYQREDDWRFQYQRDRPKG--YFVQNYKFTDPELKLH	103
Phoronis_australis	RFIKYHDKHLPPERRADQKEYMSEDEWRAQAKRDAPP---YYPNGKLKTNMPPLRLD	103
Macrostomum_lignano	RWQRYHEYTALPSSSRDAIDFQSEDDFVQFRQARDPRA-PMLTSY-IPGEKPADLKLT	107
Notospermus_geniculatus	RWIKYHDYKHIPREARRDAIDFQSEDKWVEFQRHRDAPRG-DKLNPGGIGHSNIPQLLF	109
Crassostrea_gigas	RWTDYHQYRSLPRDTRRDAKEMQSEDQWDFMRQRDTPSG-YYHKDIGLLQTGVPECRLF	108
Tritia_obsoleta	SWIKYPEHRSPLSDTKRDAIDMQSEDSWVRFMRNRDQPSG-YYFPRVGIRPSNVPELKLS	109
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Capitella_teleta	GYAFNPFNLYRTGVPAQTLNTHTPWPKTDARSFSPSWRGPRGGYGYHEELDIHHNGGYR	177
Platynereis_dumerlii	GYAFNPYNLYRTGTPAVTMYNPNPWPKTDVRQFPTWRGPRGGYGYHEELDVHHNGGYK	178
Terebratalia_transversa	GYCTNPFNLHRTGVEARTLYDP----LPDGINNKSWRGPHGGYGYHERLDARDTGGFR	158
Phoronis_australis	GYCMNPFNLHRTGVQARSVYNP----KPDLI-DPTWRGPHGGYGYHERLDSRDTKGIR	157
Macrostomum_lignano	GYRREDS----VVPEHSQFS---TYERFPR-----WQGSYGYGYHEALDLHDGGRMK	153
Notospermus_geniculatus	GYTRDPA----SYPAVTQYQ---PPARFPQ-----WSGPGYGYGYHENLEHHASGRYR	155
Crassostrea_gigas	GYTRDLP----SMPPRALQD--LSWPKSDAFVPLERSDRGKYGYGYHEAIARERQQRKK	161
Tritia_obsoleta	GYTRTLP----SMPGRELFLL--QPWPKCDAWTPPVRGRGEYGYGYHERIENERRRRREM	162
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Capitella_teleta	FPRDP-KALA---NDEDAMKYEYMRNQPSLHQKQP-----VEFPVLL	215
Platynereis_dumerlii	FPRDP-KVLV---NDEDIYKYEYMRNVPSVHQKLP-----VEFPVLQ	216
Terebratalia_transversa	IT----KQLY---NDEDYLKWEQMRTRDDPNLLGITETARLTLPLV*	198
Phoronis_australis	LY----KQLY---NDEDWLKWEQYKTLATKT-----	181
Macrostomum_lignano	TLPALPRYPGYPLVSSVTGI-----	173
Notospermus_geniculatus	DLPAIRRYPGYIMSNMTGN-----	175
Crassostrea_gigas	ELPESMRVEARDIPML-----	177
Tritia_obsoleta	EMPITQRIDASHVPTFAYNSRWTFDQP-----	189

Supplementary Figure 3. Amino acid alignment of *trochin* protein sequences.



Supplementary Figure 4. Expression of *Ct-trochin* in the juvenile stage of *Capitella teleta*. After metamorphosis, expression is only observed in the ventral face of the pharynx (black arrowheads) but not the dorsal portion (open arrowheads). Scale bar: 100 μ m. For each *in situ* hybridization, at least 20 animals were stained and all had the pattern shown.