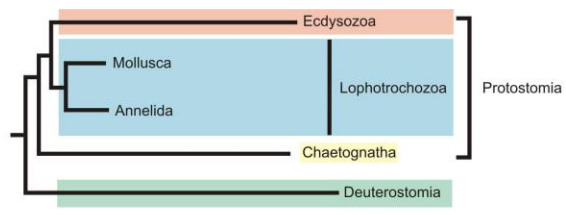
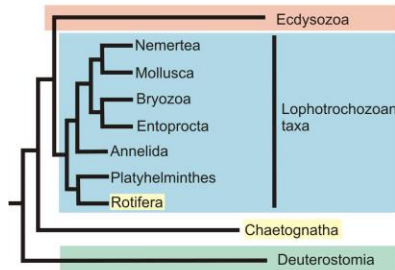


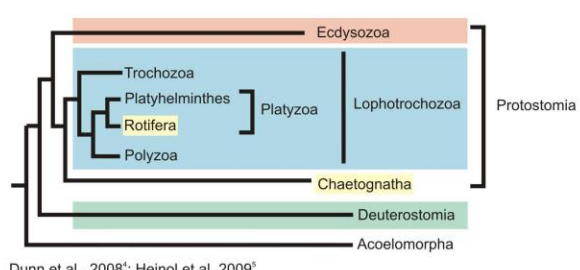
Rotifera not included in dataset
Matus et al. 2006¹



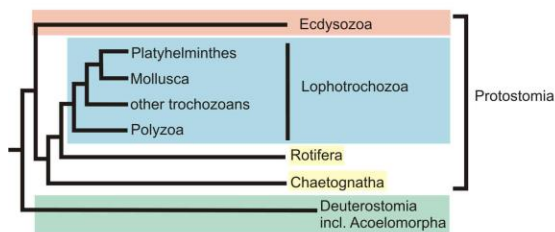
Rotifera not included in dataset
Marlétaz et al. 2006²



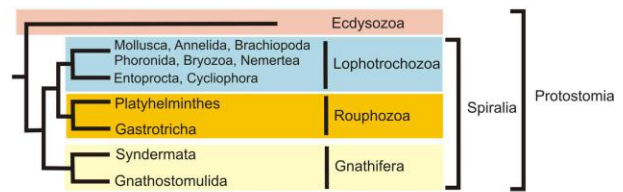
Marlétaz et al. 2008³



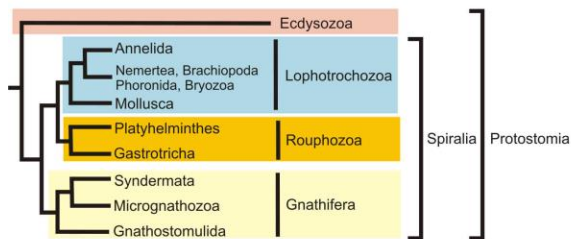
Dunn et al., 2008⁴; Hejnal et al. 2009⁵



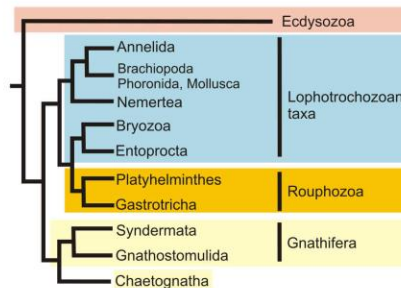
Philippe et al. 2011⁶



Chaetognatha not included in dataset
Struck et al. 2014⁷



Chaetognatha not included in dataset
Laumer et al. 2015⁸

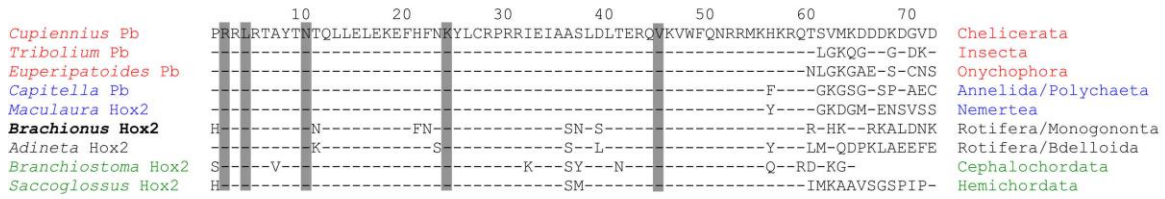


Kocot et al. 2016⁹

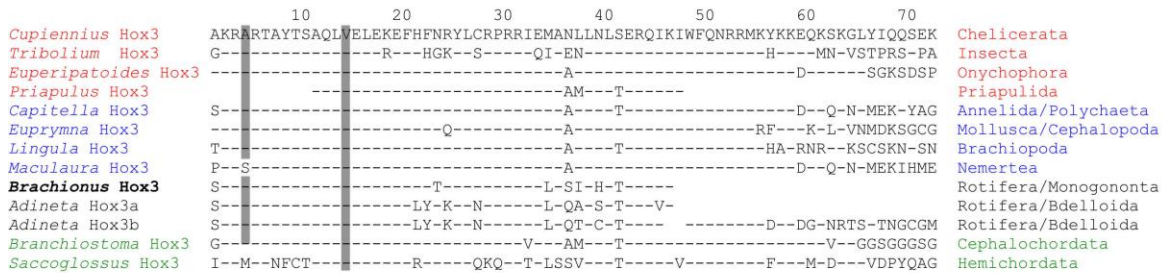
Only one of several phylogenetic trees is depicted here. All trees in this publication exhibit variations of the positions of Rousphozoan and Gnathiferan taxa.

Supplementary Figure 1 | Phylogenetic position of Rotifera and Chaetognatha based on recent large scale phylogenomic studies.

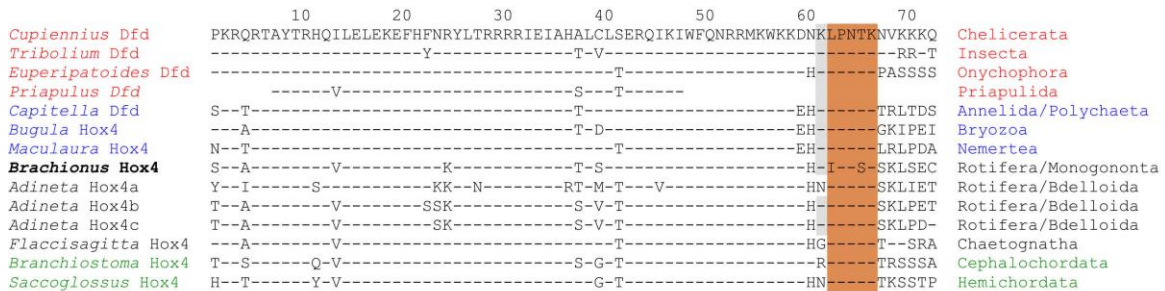
PG2: Hox2/Proboscipedia



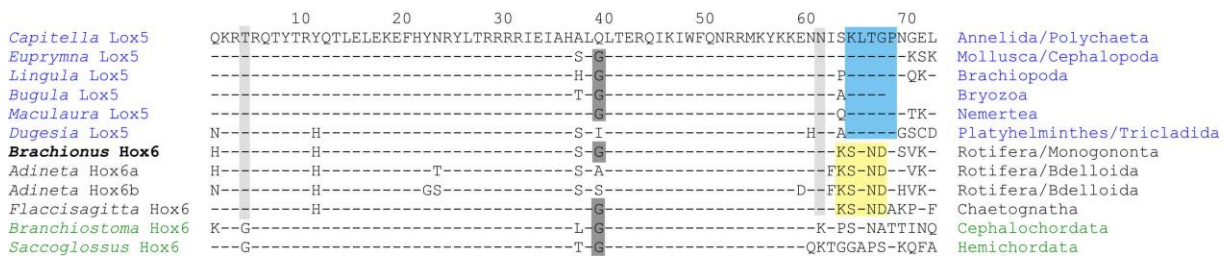
PG3: Hox3



PG4: Hox4/Deformed

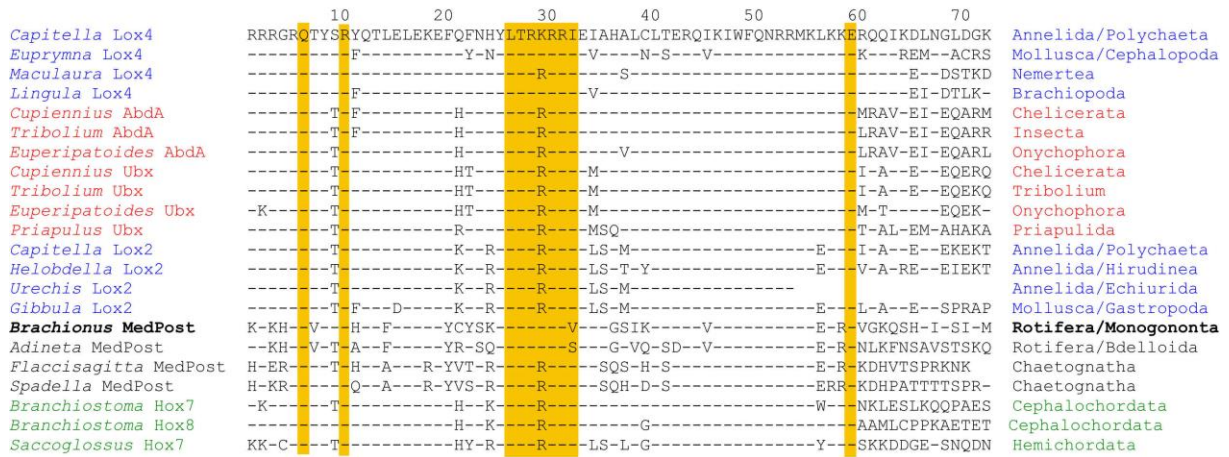


PG6: Hox6/lox5



Supplementary Figure 2 | Alignment of *Hox* gene homeodomains and 3' flanking regions of paralog group 2, 3, 4 and 6. Dashes represent identity with respective *Hox* paralogs from *Cupiennius salei* shown in the top line of each group. Ecdysozoan genes listed in red, platytrichochozoan genes listed in blue and deuterostome genes listed in green respectively. The following *Hox* signatures are marked: *Hox4/Dfd* motif highlighted in orange, *Lox5* parapeptide motif in blue and gnathiferan peptide in yellow. Residues diagnostic for different paralog groups shared by sequences recovered from *Brachionus majavacas* are highlighted in grey: dark grey boxes mark residues found in paralogs across bilaterians and residues boxed in light grey are found in protostomes only.

PG8: Hox8/Lox4/Lox2/AbdA/Ubx



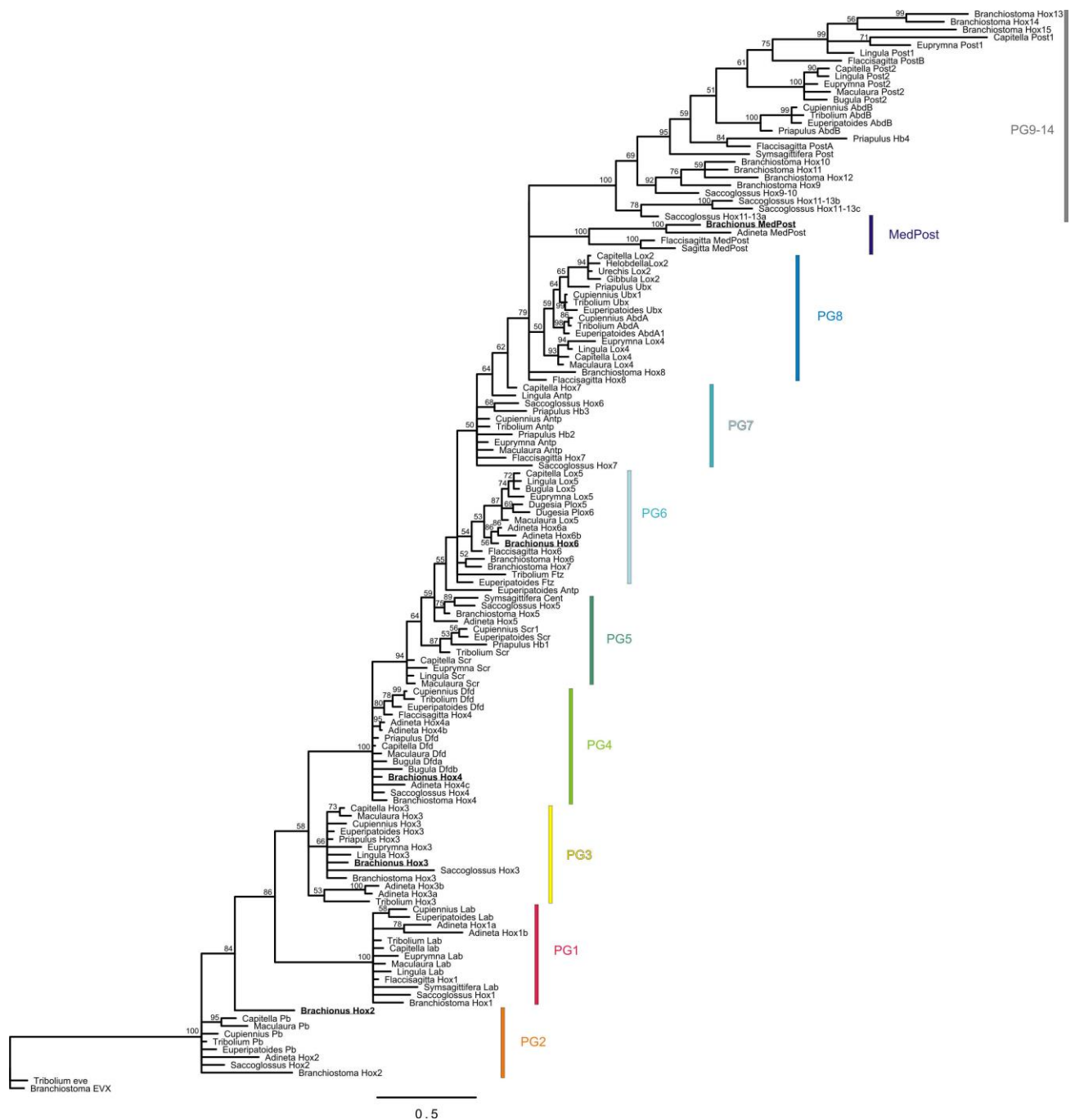
PG9-15: Hox9-15/Post1/Post2/AbdB



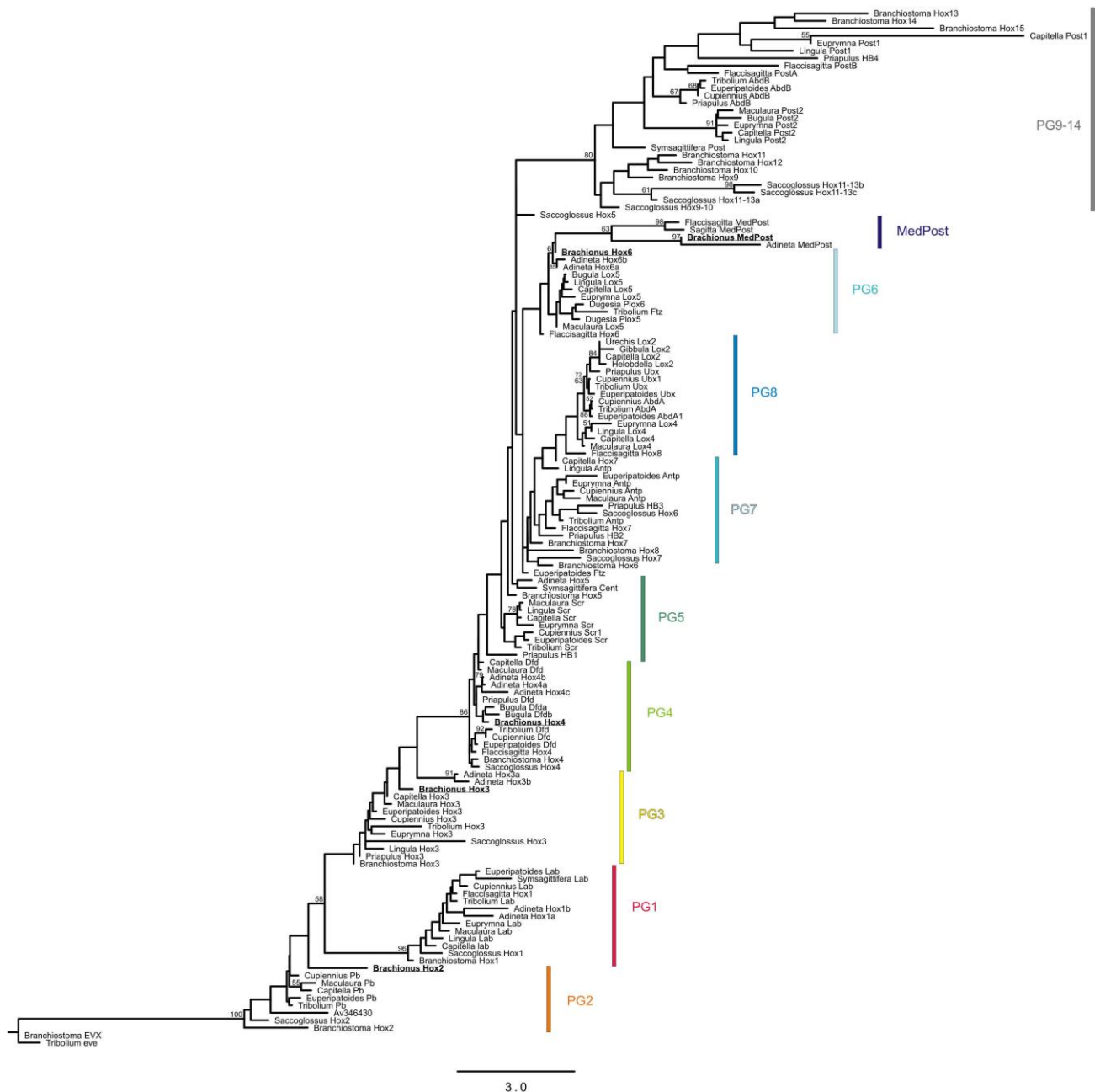
Summary



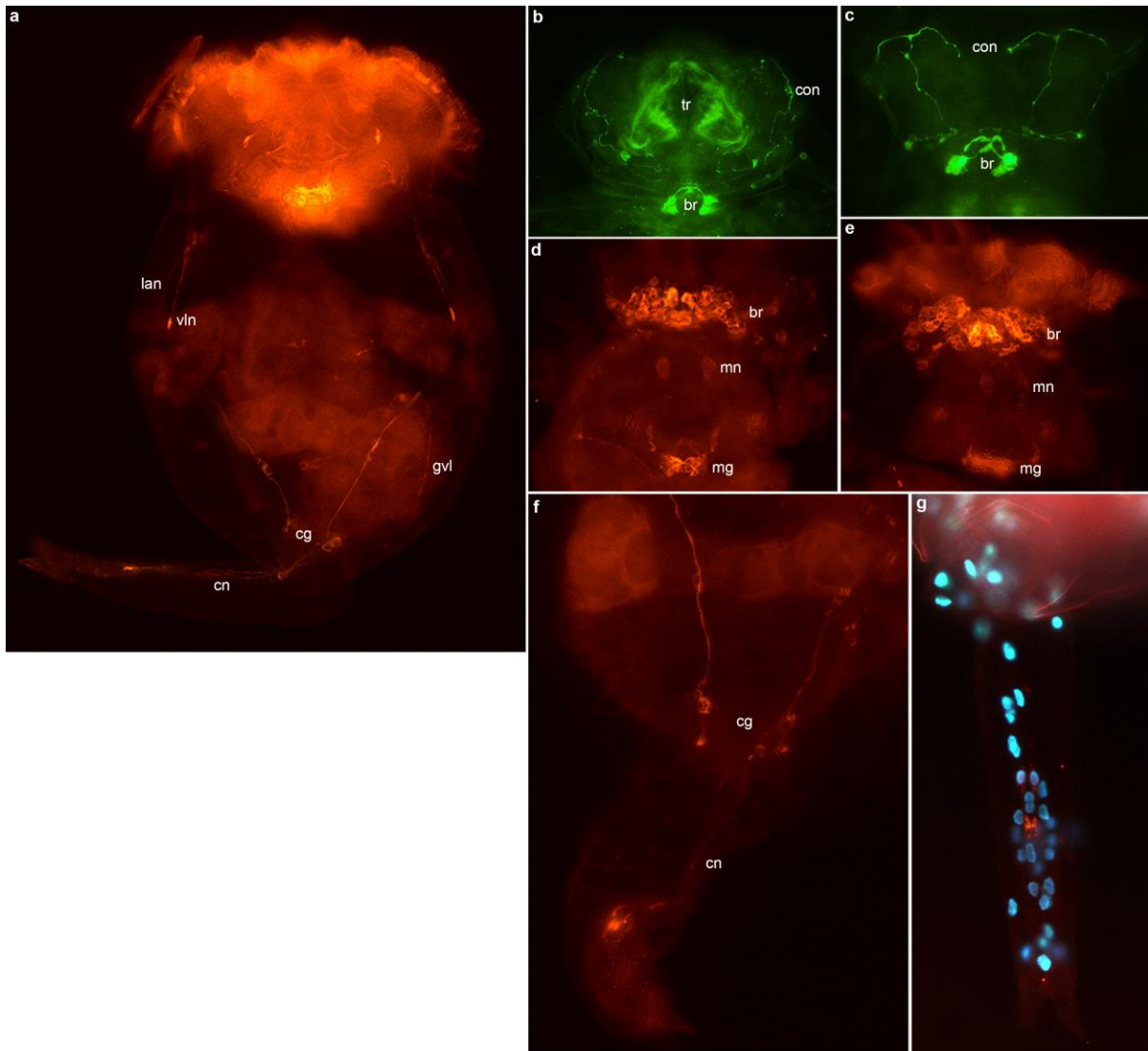
Supplementary Figure 3 | Alignment of *Hox* gene homeodomains and 3' flanking regions of paralog group 8 and 9-15 in comparison with the MedPost sequence recovered from *Brachionus manjavacas*. Dashes represent identity with respective *Hox* paralogs from *Capitella teleta* and *Cupiennius salei* shown in the top line of each group. Ecdysozoan genes listed in red, lophotrochozoan genes listed in blue and deuterostome genes listed in green respectively. Residues diagnostic for central class *Hox* genes shared by sequences recovered from *Brachionus manjavacas* are highlighted in dark yellow and those representing residues diagnostic for posterior class *Hox* genes are enclosed in green boxes. In the summary residues recovered in rotifers or chaetognaths only are marked in lighter colors respectively.



Supplementary Figure 4 | Orthology assignments for *Brachionus plicatilis* Hox genes. Alignments of the 60 amino acid homeodomain and the 12 amino-acids 3' of the homeodomain of representative bilaterian *Hox* genes were used to conduct a Bayesian phylogenetic analysis based on 400,000 trees from 40,000,000 stationary generations. Bayesian posterior probabilities are shown above the branches. Sequences used for this analysis had been isolated from the following species: *Saccoglossus kowalevskii* (Hemichordata) and *Branchiostoma floridae* (Chordata), *Cupiennius salei* (Chelicerata), *Tribolium castaneum* (Insecta), *Euperipatoides kanangrensis* (Onychophora) and *Priapulus caudatus* (Priapulida), *Capitella teleta* (Annelida/Polychaeta), *Helobdella robusta* (Annelida/Hirudinea), *Urechis unicinctus* (Annelida/Echiurida), *Euprymna scolopes* (Mollusca/Cephalopoda), *Gibbula varia* (Mollusca/Gastropoda), *Maculaura alaskensis* (Nemertea), *Lingula anatina* (Brachiopoda), *Bugula turrita* (Bryozoa) and *Dugesia japonica* (Platyhelminthes), *Symsagittifera roscoffensis* (Xenacoelomorpha), *Flaccisagitta enflata* and *Spadella cephaloptera* (Chaetognatha) and *Brachionus manjavacas* and *Adineta vaga* (monogont and bdelloid rotifers respectively). Sequences from *B. manjavacas* are shown underlined and in bold. *B. manjavacas* possesses orthologs from *Hox* paralogy groups (PG) 2, 3, 4 and 6 and an ortholog of *MedPost*.



Supplementary Figure 5 | Orthology assignments for *Brachionus manjavacas* Hox genes. Alignments of the 60 amino acid homeodomain and the 12 amino-acids 3' of the homeodomain of representative bilaterian Hox genes were used to conduct an maximum-likelihood analysis with RAXML-HPC V8.2.9 under the LG+Γ+I model. Bootstrap support values >50 from 1000 iterations are shown above the branches. Sequences used for this analysis had been isolated from the following species: *Saccoglossus kowalevskii* (Hemichordata) and *Branchiostoma floridae* (Chordata), *Cupiennius salei* (Chelicerata), *Tribolium castaneum* (Insecta), *Euperipatoides kanangrensis* (Onychophora) and *Priapulus caudatus* (Priapulida), *Capitella teleta* (Annelida/Polychaeta), *Helobdella robusta* (Annelida/Hirudinea), *Urechis unicinctus* (Annelida/Echiurida), *Euprymna scolopes* (Mollusca/Cephalopoda), *Gibbula varia* (Mollusca/Gastropoda), *Maculaura alaskensis* (Nemertea), *Lingula anatina* (Brachiopoda), *Bugula turrata* (Bryozoa) and *Dugesia japonica* (Platyhelminthes), *Symsagittifera roscoffensis* (Xenacoelomorpha), *Flaccisagitta enflata* and *Spadella cephaloptera* (Chaetognatha) and *Brachionus manjavacas* and *Adineta vaga* (monogonont and bdelloid rotifers respectively). Sequences from *B. manjavacas* are shown underlined and in bold. *B. manjavacas* possesses orthologs from Hox paralogy groups 2, 3, 4 and 6 and an ortholog of *MedPost*.



Supplementary Figure 6 | Morphological features of the nervous system of the monogonont rotifer *Brachionus manjavacas* revealed by immunohistochemical staining on adult females. Red staining: labeling of neurons with anti-FRMFamide, green staining: labelling of neurons with anti-5HT. Blue staining: labelling of nuclei with Hoechst 33342. Anterior to the top. Ventral views except for an anteroventral view in **b**. br, brain; cg, caudal ganglion; cn, caudal nerves; con, coronal nerve; gvl, germovitellarial nerve loop; lan, lateral nerves; mg, mastax ganglion; mn, mastax nerve; tr, trophi; ven, ventrolateral nerves.

Supplementary Table 1 | Primers used for isolation of *Hox* gene sequences of *Brachionus manjavacas*.

Primer	Sequence 5'->3'	
ELEKEF	GAR YTN GAR AARG AR TT	universal forward <i>Hox</i> primer
WFQNRR	CKN CKR TTY TGR AACCA	universal reverse <i>Hox</i> primer
KLARTAYT	AAG CTT GCC MGN ACN GCN TAY AC	PG1-3 <i>Hox</i> primer
AQLVELEKE	GCB CAR YTN GTH GAR YTV GAR AARG	PG3 primer
RGRQTY	MGN GGN MGN CAR ACN TA	PG4-8 <i>Hox</i> primer
BmHox2_RACEfw	TGT GTC GGC CTA GAC GTG TTG AAA TAG CGT CC	<i>Brachionus Hox2</i> 3'RACE-primer
BmHox2_RACErev	CAA ATT GGA CGC TAT TTC AACACG TCT AGG CCG AC	<i>Brachionus Hox2</i> 5'RACE-primer
BmHox3_RACEfw	CTT TAC CGC CTA CTT GTG CAG ACC GCG CCG CAT C	<i>Brachionus Hox3</i> 3'RACE-primer
BmHox3_RACErev	TAG TTC GAT GCG GCG CGG TCT GCA CAA GTA GC	<i>Brachionus Hox3</i> 5'RACE-primer
BmHox4_RACEfw	CAC CAG GTC CTG GAG CTG GAA AAA GAG TTC CAC	<i>Brachionus Hox4</i> 3'RACE-primer
BmHox4_RACErev	GCC TCT CGG ACA AACTGA GTG TGT GCG CGA TCT C	<i>Brachionus Hox4</i> 5'RACE-primer
BmHox6_RACEfw	TGA CTA GAA AGA GGC GAG TTG AAA TTG CCG GAA GC	<i>Brachionus Hox6</i> 3'RACE-primer
BmHox6_RACErev	TGC TTC CGG CAA TTT CAA CTC GCC TCT TTC TAG TC	<i>Brachionus Hox6</i> 5'RACE-primer
BmMedPost_RACEfw	GCG CCG AATTGA GATAGC GCA CTC GTT GGG	<i>Brachionus MedPost</i> 3'RACE-primer
BmMedPost_RACErev	ATC TTG ATC TGA CGC TCA GTC AAA CCC AACGAG TGC	<i>Brachionus MedPost</i> 5'RACE-primer

Supplementary Table 2 | Genome accession numbers of *Adineta vaga* genes and Genbank accession numbers of all remaining sequences used in this study.

Species name	Gene	GenBank #. /Genome Acc. #	Species name	Gene	GenBank #. /Genome Acc. #
<i>Adineta vaga</i>	<i>Adineta Hox1</i>	Av 303240 Av 041050	<i>Eupryma scolopes</i>	<i>Eupryma Post1</i>	AAL25811
<i>Adineta vaga</i>	<i>Adineta Hox2</i>	Av 346430	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta Hox1</i>	ABS18809
<i>Adineta vaga</i>	<i>Adineta Hox3</i>	Av 567830 Av 638170	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta Hox3</i>	ABS18810
<i>Adineta vaga</i>	<i>Adineta Hox4</i>	Av 436520 Av 117920 Av 439300	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta Hox4</i>	ABS18811
<i>Adineta vaga</i>	<i>Adineta Hox5</i>	Av 294680	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta (Hox5)Hox6*</i>	ABS18812
<i>Adineta vaga</i>	<i>Adineta Hox6</i>	Av 170420 Av 045650	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta (Hox6)Hox7*</i>	ABS18813
<i>Adineta vaga</i>	<i>Adineta Medpost</i>	Av 586970	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta Hox8</i>	ABS18814
<i>Brachionus manjavacas</i>	<i>Brachionus Hox2</i>	KT989538	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta MedPost</i>	ABS18817
<i>Brachionus manjavacas</i>	<i>Brachionus Hox3</i>	KT989539	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta postA</i>	ABS18815
<i>Brachionus manjavacas</i>	<i>Brachionus Hox4</i>	KT989540	<i>Flaccisagitta enflata</i>	<i>Flaccisagitta postB</i>	ABS18816
<i>Brachionus manjavacas</i>	<i>Brachionus Hox6</i>	KT989541	<i>Gibbula varia</i>	<i>Gibbula Lox2</i>	ADJ18238
<i>Brachionus manjavacas</i>	<i>Brachionus MedPost</i>	KT989542	<i>Helobdella robusta</i>	<i>Helobdella Lox2</i>	CAA78665
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox1</i>	ACJ74382	<i>Lingula anatina</i>	<i>Lingula Lab</i>	AAD45587
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox2</i>	ACJ74381	<i>Lingula anatina</i>	<i>Lingula Hox3</i>	AAD45588
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox3</i>	ACJ74380	<i>Lingula anatina</i>	<i>Lingula Scr</i>	AAD45589
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox4</i>	ACJ74383	<i>Lingula anatina</i>	<i>Lingula Antp</i>	AAD45590
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox5</i>	ACJ74385	<i>Lingula anatina</i>	<i>Lingula Lox5</i>	AAD45591
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox6</i>	ACJ74384	<i>Lingula anatina</i>	<i>Lingula Lox4</i>	AAD45593
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox7</i>	ACJ74388	<i>Lingula anatina</i>	<i>Lingula Lox2</i>	AAD45592
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox8</i>	ACJ74387	<i>Lingula anatina</i>	<i>Lingula Post2</i>	AAD45595
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox9</i>	ACJ74386	<i>Lingula anatina</i>	<i>Lingula Post1</i>	AAD45594
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox10</i>	ACJ74389	<i>Maculaura alaskensis</i>	<i>Maculaura Lab</i>	AKE07580
<i>Branchiostoma floridae</i>	<i>Branchiostoma Hox11</i>	AAF81909	<i>Maculaura alaskensis</i>	<i>Maculaura Pb</i>	AKE07582
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox12</i>	ACJ74391	<i>Maculaura alaskensis</i>	<i>Maculaura Hox3</i>	AKE07579
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox13</i>	ACJ74390	<i>Maculaura alaskensis</i>	<i>Maculaura Dfd</i>	AKE07586
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox14</i>	ACJ74393	<i>Maculaura alaskensis</i>	<i>Maculaura Scr</i>	AKE07583
<i>Branchiostoma lanceolatum</i>	<i>Branchiostoma Hox15</i>	ACJ74394	<i>Maculaura alaskensis</i>	<i>Maculaura Lox5</i>	AKE07585
<i>Bugula turrita</i>	<i>Bugula Pb</i>	AAS77225	<i>Maculaura alaskensis</i>	<i>Maculaura Antp</i>	AKE07577
<i>Bugula turrita</i>	<i>Bugula Hox3</i>	AAS77226	<i>Maculaura alaskensis</i>	<i>Maculaura Lox4</i>	AKE07581
<i>Bugula turrita</i>	<i>Bugula Dfd</i>	AAS77227	<i>Maculaura alaskensis</i>	<i>Maculaura Post2</i>	AKE07584
<i>Bugula turrita</i>	<i>Bugula Dfdb</i>	AAS77228	<i>Priapulus caudatus</i>	<i>Priapulus Lab</i>	AF144884
<i>Bugula turrita</i>	<i>Bugula Lox5</i>	AAS77229	<i>Priapulus caudatus</i>	<i>Priapulus Pb</i>	AF144885
<i>Bugula turrita</i>	<i>Bugula Post2</i>	AAS77230	<i>Priapulus caudatus</i>	<i>Priapulus Hox3</i>	AF144886
<i>Capitella teleta</i>	<i>Capitella Lab</i>	EU196537	<i>Priapulus caudatus</i>	<i>Priapulus Dfd</i>	AF144887
<i>Capitella teleta</i>	<i>Capitella Pb</i>	EU196538	<i>Priapulus caudatus</i>	<i>Priapulus HB1</i>	AF144888
<i>Capitella teleta</i>	<i>Capitella Hox3</i>	EU196539	<i>Priapulus caudatus</i>	<i>Priapulus HB2</i>	AF144889
<i>Capitella teleta</i>	<i>Capitella Dfd</i>	EU196540	<i>Priapulus caudatus</i>	<i>Priapulus HB3</i>	AF144890
<i>Capitella teleta</i>	<i>Capitella Scr</i>	EU196541	<i>Priapulus caudatus</i>	<i>Priapulus HB4</i>	AF144892
<i>Capitella teleta</i>	<i>Capitella Lox5</i>	EU196542	<i>Priapulus caudatus</i>	<i>Priapulus HB5</i>	AF144894
<i>Capitella teleta</i>	<i>Capitella Antp</i>	EU196547	<i>Priapulus caudatus</i>	<i>Priapulus Ubx</i>	AF144891
<i>Capitella teleta</i>	<i>Capitella Lox4</i>	EU196543	<i>Priapulus caudatus</i>	<i>Priapulus AbdB</i>	AF144893
<i>Capitella teleta</i>	<i>Capitella Lox2</i>	EU196544	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox1</i>	AAP79296
<i>Capitella teleta</i>	<i>Capitella Post2</i>	EU196545	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox2</i>	ABK00018
<i>Capitella teleta</i>	<i>Capitella Post1</i>	EU196546	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox3</i>	AAP79286
<i>Cupiennius salei</i>	<i>Cupiennius Lab</i>	CAA07497	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox4</i>	AAP79297
<i>Cupiennius salei</i>	<i>Cupiennius Pb</i>	CAL91855	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox5</i>	ABK00019
<i>Cupiennius salei</i>	<i>Cupiennius Hox3</i>	CAA06645	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox6</i>	ABK00020
<i>Cupiennius salei</i>	<i>Cupiennius Dfd</i>	CAA07498	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox7</i>	AAP79287
<i>Cupiennius salei</i>	<i>Cupiennius Scr</i>	CAL91856	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox9/10</i>	ABK00021
<i>Cupiennius salei</i>	<i>Cupiennius Antp</i>	CAA07499	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox11/13a</i>	ABK00022
<i>Cupiennius salei</i>	<i>Cupiennius Ubx1</i>	CAA07500	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox11/13b</i>	ABK00023
<i>Cupiennius salei</i>	<i>Cupiennius AbdA</i>	CAA07502	<i>Saccoglossus kowalevskii</i>	<i>Saccoglossus Hox11/13c</i>	ABK00023
<i>Cupiennius salei</i>	<i>Cupiennius AbdB</i>	CAB40807	<i>Spadella cephaloptera</i>	<i>Spadella MedPost</i>	AAO16206
<i>Dugesia japonica</i>	<i>Dugesia PLOX5</i>	BAA77405	<i>Symsagittifera roscoffensis</i>	<i>Symsagittifera Lab</i>	AAN11404
<i>Dugesia japonica</i>	<i>Dugesia PLOX6</i>	BAA77406	<i>Symsagittifera roscoffensis</i>	<i>Symsagittifera Central</i>	AAN11405
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Lab</i>	CCK73369	<i>Symsagittifera roscoffensis</i>	<i>Symsagittifera post</i>	AAN11406
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Pb</i>	CCK73370	<i>Tribolium castaneum</i>	<i>Tribolium Lab</i>	AAF64148
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Hox3</i>	CCK73371	<i>Tribolium castaneum</i>	<i>Tribolium Pb</i>	AAF03888
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Dfd</i>	CCK73372	<i>Tribolium castaneum</i>	<i>Tribolium Zen</i>	NP_001036813
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Ftz</i>	CCK73374	<i>Tribolium castaneum</i>	<i>Tribolium Dfd</i>	AAK16423
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Scr</i>	CCK73373	<i>Tribolium castaneum</i>	<i>Tribolium Ftz</i>	AAK16421
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Antp</i>	CCK73375	<i>Tribolium castaneum</i>	<i>Tribolium Scr</i>	AAK16422
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides Ubx</i>	CCK73376	<i>Tribolium castaneum</i>	<i>Tribolium Antp</i>	EEZ99250
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides AbdA</i>	CCK73377	<i>Tribolium castaneum</i>	<i>Tribolium Ubx</i>	NP_001034497
<i>Euperipatoides kanangrensis</i>	<i>Euperipatoides AbdB</i>	CCK73379	<i>Tribolium castaneum</i>	<i>Tribolium AbdA</i>	AAB70263
<i>Eupryma scolopes</i>	<i>Eupryma Lab</i>	published	<i>Tribolium castaneum</i>	<i>Tribolium AbdB</i>	AAF36721
<i>Eupryma scolopes</i>	<i>Eupryma Hox3</i>	AAL25806	<i>Urechis unicinctus</i>	<i>Urechis</i>	AAW72807
<i>Eupryma scolopes</i>	<i>Eupryma Scr</i>	AAL25807			
<i>Eupryma scolopes</i>	<i>Eupryma Lox5</i>	AAL25808			
<i>Eupryma scolopes</i>	<i>Eupryma Antp</i>	AAL25809			
<i>Eupryma scolopes</i>	<i>Eupryma Lox4</i>	AAL25810			
<i>Eupryma scolopes</i>	<i>Eupryma Post2</i>	AAL25812			

* Matus et al. (2007)¹⁰ isolated *Flaccisagitta enflata* orthologs of *Hox6* and *Hox7* genes as shown in Fig.3 and Fig.4 of that publication. The sequences however were submitted to Genbank as *Hox5* and *Hox6* respectively even though a *Flaccisagitta Hox5* had not been isolated. All of our phylogenetic analyses identify these genes as the original *Hox6* and *Hox7* orthologs. The assignments shown in this study reflect the original assignments.

Supplementary References

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