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SEQUENCE CORNER

A PCR survey of *Hox* genes in the myzostomid *Myzostoma cirriferum*

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Abstract Using degenerate primers, we were able to identify seven *Hox* genes for the myzostomid *Myzostoma cirriferum*. The recovered fragments belong to anterior class (*Mci_lab, Mci_pb*), central class (*Mci_Dfd, Mci_Lox5, Mci_Antp, Mci_Lox4*), and posterior class (*Mci_Post2*) paralog groups. Orthology assignment was verified by phylogenetic analyses and presence of diagnostic regions in the homeodomain as well as flanking regions. The presence of *Lox5*, *Lox4*, and *Post2* supports the inclusion of Myzostomida within Lophotrochozoa. We found signature residues within flanking regions of *Lox5*, which are also found in annelids, but not in Platyhelminthes. As such the available *Hox* genes data of myzostomids support an annelid relationship.

Keywords Myzostomida · Annelida · *Hox* genes · Platyhelminthes · Phylogeny

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Introduction

Myzostomida comprise a group of marine worms which are either ectocommensals or parasites of echinoderms. The phylogenetic position of this taxon is still controversially discussed. Whereas the presence of many morphological characters is congruent with an annelid affinity (e.g., parapodia-like structures, chitinous chaetae, a ladder-like nervous system, trochophora larva, serial nephridia), some recent molecular analyses point to a platyzoan (Platyhelminthes and relatives) origin of these organisms (Eeckhaut et al. 2000; Dunn et al. 2008). However, a recent analysis using mitochondrial gene order and sequence data strongly supports an annelid affinity of Myzostomida (Bleidorn et al. 2007). Nevertheless, myzostomids show a unique mode of development and the relationship of segment formation in myzostomids (Jägersten 1940) to segment formation, as described for various annelids remains unclear.

The genetical basis of animal development has been a key issue in recent evolutionary investigations to homologize structures across animal phyla (e.g., Tessmar-Raible et al. 2007). *Hox* genes have been found to be important regulators involved in embryonic development, e.g., in the patterning of the anterior–posterior axis or segmentation (Lemons and McGinnis 2006). These genes comprise a gene family of which eight to 14 different paralog groups (PGs) are found in most bilaterians and due to whole genome duplication more copies of each gene are described for vertebrate PGs (e.g., de Rosa et al. 1999). Kulakova et al. (2007) reported *Hox* genes belonging to 11 PGs for the annelid *Nereis virens*. The information on flatworm *Hox* genes is scarce, but a scattering of surveys suggests that the complement might be as in other lophotrochozoans (Olson 2008). Typically, these genes are clustered and respect the colinearity rule, as it has been recently shown for the

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capitellid polychaete *Capitella* (Fröbius et al. 2008). This means that PGs are expressed along the body axis in the same order as they are arranged on the *Hox* cluster (Carroll 1995). However, examples for disrupted *Hox* cluster organization have been described and one case includes a parasitic flatworm (Pierce et al. 2005).

For further exploration of phylogenetic relationships, we conducted a *Hox* gene survey in the myzostomid *Myzostoma cirriferum*. Here, we report the first *Hox* gene sequences for Myzostomida and discuss the phylogenetic significance of these data. Analyses of *Hox* genes will be a prerequisite for future developmental studies (e.g., comparing the expression of *Hox* genes in the course of segmentation between Myzostomida and Annelida) of this enigmatic lophotrochozoan taxon.

Material and methods

Individuals of *M. cirriferum* were collected from its host *Antedon bifida* (Echinodermata, Crinoidea) in Morgat (Britany, France). DNA extraction was performed using the Qiagen DNeasy™ Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

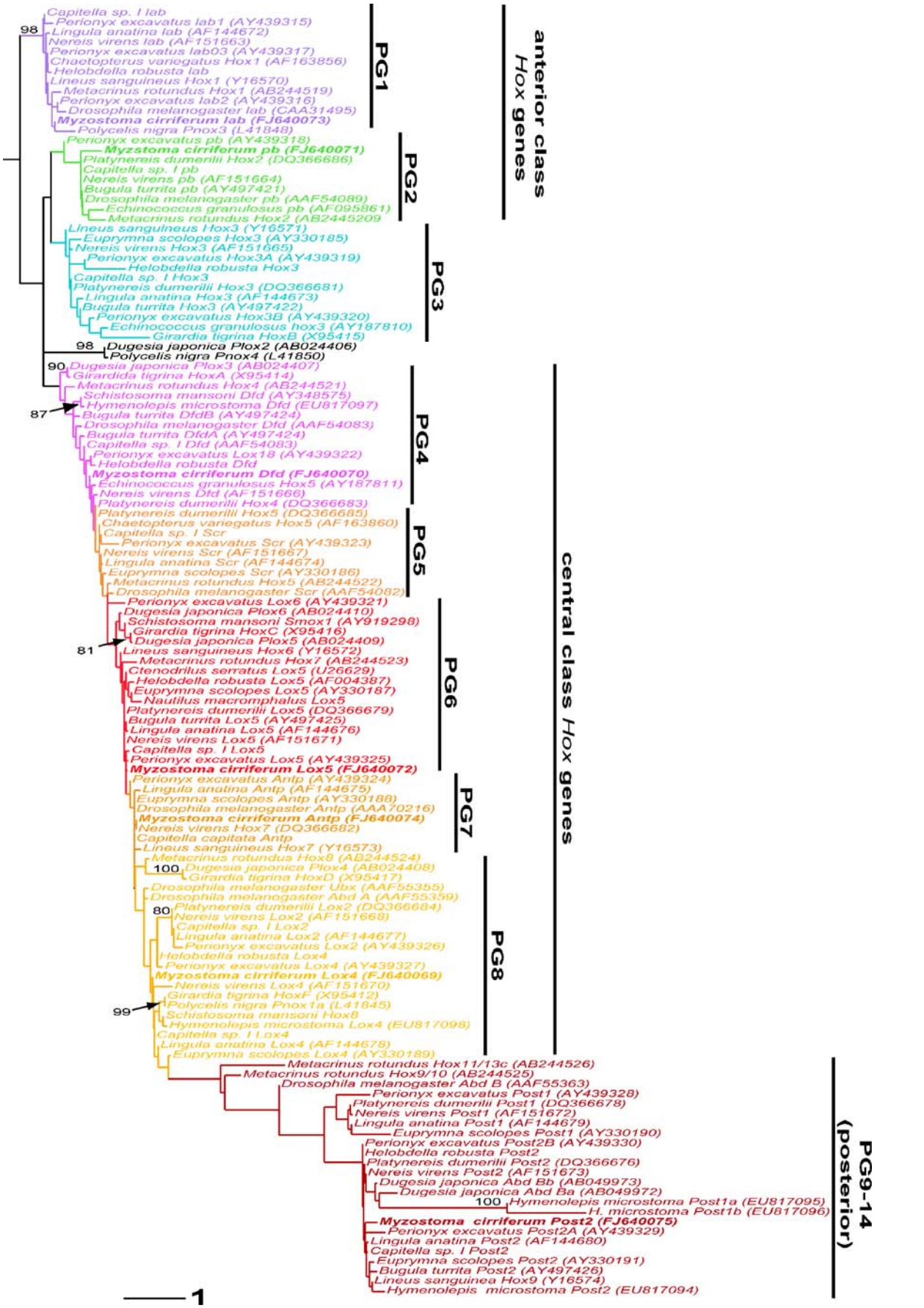
We used two different primer sets for amplifying homeodomains. The first primer pair (pair 1) Hox1F-LELEKE (GCTCTAGARYTNGARAARGARTT) and Hox1R-WFQNRR (CGGGGATCCCKNCKRTYTYGRA ACCA) (Passamaneck and Halanych 2004) was used to screen (mainly) for anterior and medial class *Hox* genes, whereas a second primer set (pair 2) published by Lee et al. (2003) was used to screen for multiple classes of homeodomains. PCR amplifications were carried out in a total volume of 37.5 μl containing 1 mM Tris–HCL, pH 9.0, 5 mM KCL, 0.15 mM $MgCl_2$, 0.05 mM of each dNTP, 0.13 μM of both forward and reverse primers, and 0.75 U Taq polymerase (QBiogene, Heidelberg, Germany). PCR profile for all reactions was as follows: 94°C for 2 min as initial denaturation; 30 cycles with 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min; final extension at 70°C for 10 min. All PCR products were purified using the NucleoSpin Extract II Kit (Macherey & Nagel, Düren, Germany). Purified PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen).

Using the amplified homeodomains as a starting point, we performed genome walking to amplify adjacent flanking regions towards both ends. For this purpose, genomic DNA was digested with restriction enzymes (EcoR V, Dra I, Pvu II, Ssp I) and adaptors supplied by the Genome Walker Kit (Clonetech) were ligated to both ends of the fragments. Two rounds of PCR amplifications were performed with adaptor-specific and gene-specific primer (gene-specific primers are available on request). The first cycle was set as follows: 94°C for 1 min as initial denaturation; seven cycles with 94°C for 25 s and 72°C for 3 min, followed by 32 cycles of 94°C for 25 s and 67°C for 3 min; final extension at 70°C for 7 min. The second PCR, with nested primers, used the product of the first amplification as a template with the same parameters. All PCR products were purified using the NucleoSpin Extract II Kit (Macherey & Nagel, Düren, Germany). Purified PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen).

After a screening using T3/T7 colony PCRs, selected products were sequenced on an AB 3100 multicapillary automatic sequencer (Applied Biosystems, Foster City, CA, USA). Cycle sequencing reactions were performed with the T7 primer using the BigDye version 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). All sequences have been submitted to NCBI GenBank under accession numbers FJ640069 to FJ640075.

The obtained clones were screened for *Hox* genes using BLAST searches against NCBI GenBank. Orthology assignment of gene fragments is based on both phylogenetic analysis and inspection of diagnostic residues in the homeodomain and flanking regions. We compiled a *Hox* gene alignment including representatives of Annelida (*Capitella sp. I*, Cca*; Chaetopterus variopedatus,* Cva*; Ctenodrilus serratus*, Cse*; Helobdella robusta,* Hro*; Nereis virens,* Nvi*; Perionyx excavatus*, Pex*; Platynereis dumerilii*, Pdu) and Platyhelminthes (*Echinococcus granulosus*, Egr*; Dugesia japonica*, Dja*; Girardia tigrina,* Gti*; Hymenolepis microstoma,* Hmi*; Schistosoma mansoni,* Sma), as well as Mollusca (*Euprymna scolopes,* Esc*; Nautilus macromphalus,* Nma), Nemertea (*Lineus sanguineus,* Lsa), Bryozoa (*Bugula turrita,* Btu), Brachiopoda (*Lingula anatina,* Lan), Arthropoda (*Drosophila melanogaster*, Dme), and Deuterostomia (*Metacrinus rotundus*, Mro) (see Fig. 1 for accession numbers). Additional to available data from NCBI GenBank, we screened the *Capitella* sp. I (http://genome.jgi-psf.org/Capca1/Capca1.home.html) and *Helobdella robusta* (http://genome.jgi-psf.org/Helro1/) draft genomes for *Hox* genes using the *Hox* gene complement published for *Nereis virens* (Kulakova et al. 2007) as BLAST query. For phylogenetic analysis, only the homeodomain (no flanking regions) was included. Sequences were easily aligned by eye. ML analysis of the dataset was conducted by using RAxML version 7.0.3 (Stamatakis 2006), using GTR + WAG + P-Invar model parameters. Support values were estimated by 1,000 bootstrap replicates. Maximum parsimony analysis with tree-bisection-reconnection (TBR) branch swapping, random

Fig. 1 Maximum likelihood analysis of *Hox* genes relationships using RAxML with the WAG + Γ + I model of amino acid evolution. GenBank accession numbers are given with names, sequences without accession numbers are from BLAST searches of draft genomes. Bootstrap values >70% are given at the nodes. *Colors* are unique to each *Hox* PG, sequences with uncertain affinities are in *black*


Capitella sp. I lab
Perionyx excavatus lab1 (AY439315)
Lingula anatina lab (AF144672)
Nereis virens lab (AF151663)
Perionyx excavatus lab03 (AY439317)
Chaetopterus variegatus Hox1 (AF163856)
Helobdella robusta lab
Lineus sanguineus Hox1 (Y16570)
Metacrinus rotundus Hox1 (AB244519)
Perionyx excavatus lab2 (AY439316)
Drosophila melanogaster lab (CAA31495)
Myzostoma cirriferum lab (FJ640073)
Polycelis nigra Pnox3 (L41848)
Perionyx excavatus pb (AY439318)
Myzstoma cirriferum pb (FJ640071)
Platynereis dumerilii Hox2 (DQ366686)
Capitella sp. I pb
Nereis virens pb (AF151664)
Bugula turrita pb (AY497421)
Drosophila melanogaster pb (AAF54089)
Echinococcus granulosus pb (AF095861)
Metacrinus rotundus Hox2 (AB2445209
Lineus sanguineus Hox3 (Y16571)
Euprymna scolopes Hox3 (AY330185)
Nereis virens Hox3 (AF151665)
Perionyx excavatus Hox3A (AY439319)
Helobdella robusta Hox3
Capitella sp. I Hox3
Platynereis dumerilii Hox3 (DQ366681)
Lingula anatina Hox3 (AF144673)
Bugula turrita Hox3 (AY497422)
Perionyx excavatus Hox3B (AY439320)
Echinococcus granulosus hox3 (AY187810)
Girardia tigrina HoxB (X95415)
Dugesia japonica Plox2 (AB024406)
Polycelis nigra Pnox4 (L41850)
Dugesia japonica Plox3 (AB024407)
Girardida tigrina HoxA (X95414)
Metacrinus rotundus Hox4 (AB244521)
Schistosoma mansoni Dfd (AY348575)
Hymenolepis microstoma Dfd (EU817097)
Bugula turrita DfdB (AY497424)
Drosophila melanogaster Dfd (AAF54083)
Bugula turrita DfdA (AY497424)
Capitella sp. I Dfd (AAF54083)
Perionyx excavatus Lox18 (AY439322)
Helobdella robusta Dfd
Myzostoma cirriferum Dfd (FJ640070)
Echinococcus granulosus Hox5 (AY187811)
Nereis virens Dfd (AF151666)
Platynereis dumerilii Hox4 (DQ366683)
Platynereis dumerilii Hox5 (DQ366685)
Chaetopterus variegatus Hox5 (AF163860)
Capitella sp. I Scr
Perionyx excavatus Scr (AY439323)
Nereis virens Scr (AF151667)
Lingula anatina Scr (AF144674)
Euprymna scolopes Scr (AY330186)
Metacrinus rotundus Hox5 (AB244522)
Drosophila melanogaster Scr (AAF54082)
Perionyx excavatus Lox6 (AY439321)
Dugesia japonica Plox6 (AB024410)
Schistosoma mansoni Smox1 (AY919298)
Girardia tigrina HoxC (X95416)
Dugesia japonica Plox5 (AB024409)
Lineus sanguineus Hox6 (Y16572)
Metacrinus rotundus Hox7 (AB244523)
Ctenodrilus serratus Lox5 (U26629)
Helobdella robusta Lox5 (AF004387)
Euprymna scolopes Lox5 (AY330187)
Nautilus macromphalus Lox5
Platynereis dumerilii Lox5 (DQ366679)
Bugula turrita Lox5 (AY497425)
Lingula anatina Lox5 (AF144676)
Nereis virens Lox5 (AF151671)
Capitella sp. I Lox5
Perionyx excavatus Lox5 (AY439325)
Myzostoma cirriferum Lox5 (FJ640072)
Perionyx excavatus Antp (AY439324)
Lingula anatina Antp (AF144675)
Euprymna scolopes Antp (AY330188)
Drosophila melanogaster Antp (AAA70216)
Myzostoma cirriferum Antp (FJ640074)
Nereis virens Hox7 (DQ366682)
Capitella capitata Antp
Lineus sanguineus Hox7 (Y16573)
Metacrinus rotundus Hox8 (AB244524)
Dugesia japonica Plox4 (AB024408)
Girardia tigrina HoxD (X95417)
Drosophila melanogaster Ubx (AAF55355)
Drosophila melanogaster Abd A (AAF55359)
Platynereis dumerilii Lox2 (DQ366684)
Nereis virens Lox2 (AF151668)
Capitella sp. I Lox2
Lingula anatina Lox2 (AF144677)
Perionyx excavatus Lox2 (AY439326)
Helobdella robusta Lox4
Perionyx excavatus Lox4 (AY439327)
Myzostoma cirriferum Lox4 (FJ640069)
Nereis virens Lox4 (AF151670)
Girardia tigrina HoxF (X95412)
Polycelis nigra Pnox1a (L41845)
Schistosoma mansoni Hox8
Hymenolepis microstoma Lox4 (EU817098)
Capitella sp. I Lox4
Lingula anatina Lox4 (AF144678)
Euprymna scolopes Lox4 (AY330189)
Metacrinus rotundus Hox11/13c (AB244526)
Metacrinus rotundus Hox9/10 (AB244525)
Drosophila melanogaster Abd B (AAF55363)
Perionyx excavatus Post1 (AY439328)
Platynereis dumerilii Post1 (DQ366678)
Nereis virens Post1 (AF151672)
Lingula anatina Post1 (AF144679)
Euprymna scolopes Post1 (AY330190)
Perionyx excavatus Post2B (AY439330)
Helobdella robusta Post2
Platynereis dumerilii Post2 (DQ366676)
Nereis virens Post2 (AF151673)
Dugesia japonica Abd Bb (AB049973)
Dugesia japonica Abd Ba (AB049972)
Hymenolepis microstoma Post1a (EU817095)
H. microstoma Post1b (EU817096)
Myzostoma cirriferum Post2 (FJ640075)
Perionyx excavatus Post2A (AY439329)
Lingula anatina Post2 (AF144680)
Capitella sp. I Post2
Euprymna scolopes Post2 (AY330191)
Bugula turrita Post2 (AY497426)
Lineus sanguinea Hox9 (Y16574)
Hymenolepis microstoma Post2 (EU817094)
98
98
90
87
81
100
80
99
100
PG1
PG2
PG3
PG4
PG5
PG6
PG7
PG8
PG9-14 (posterior)
anterior class Hox genes
central class Hox genes
1

sequence addition, and holding of trees limited to 10,000 was conducted using PAUP*, version 4.b10 (Swofford 2001). Clade support was assessed with 1,000 nonparametric bootstrap replicates.

Additionally, alignments including flanking regions of a selected set of taxa were constructed for each of the recovered myzostomid *Hox* fragments.

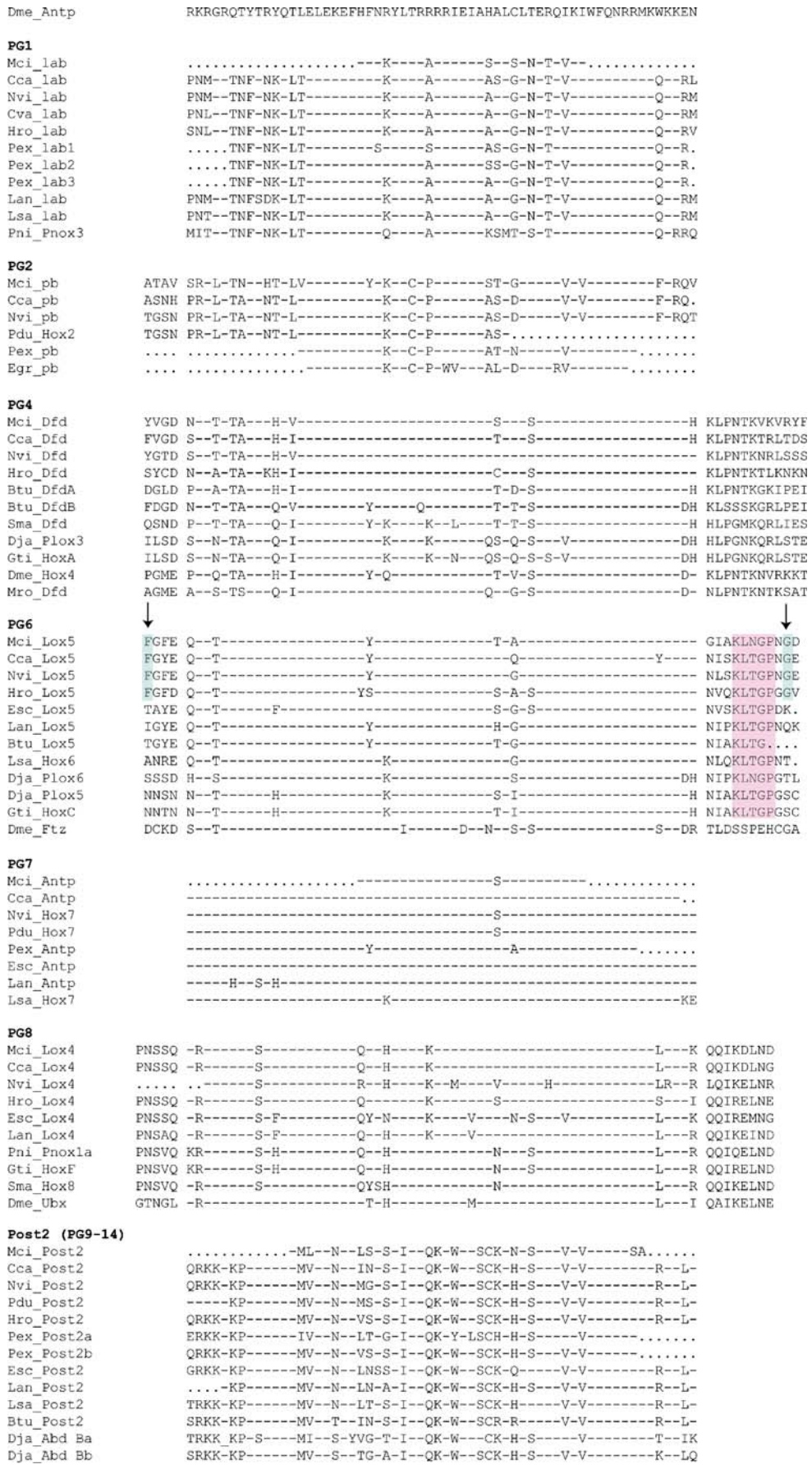
Results and discussion

We screened 192 clones in total, of which 144 and 48 were identified as products amplified by primer pair 1 and pair 2, respectively. All fragments that were subsequently annotated as anterior or central class *Hox* genes were amplified with pair 1 (primers from Passamaneck and Halanych 2004), whereas the posterior class *Hox* gene fragment was amplified with pair 2 (primers from Lee et al. 2003). In total, our PCR survey using degenerated primers recovered seven *Hox* fragments and we were able to sequence flanking regions for four of them following the genome walking approach. Orthology assignment using phylogenetic analysis (ML-tree shown in Fig. 1) and alignments including flanking regions (Fig. 2) revealed that the sequences belong to *Hox* paralog groups (PG) including *Hox1* (*Mci_lab*—*M. cirriferum Hox* genes were designated with the prefix Mci), *Hox2* (*Mci_pb*), *Hox4* (*Mci_Dfd*), *Lox5* (*Mci_Lox5*), *Hox7* (*Mci_Antp*), *Lox4* (*Mci_Lox4*), and *Post2* (*Mci_Post2*). The strict consensus of 10,000 equally parsimonious trees of the MP-analysis revealed a tree that is less resolved, but largely congruent with the ML-tree regarding assignment of myzostomid *Hox* gene fragments (see electronic supplement S1)

The presence of *Lox5*, *Lox4*, and *Post2* orthologs supports the placement of Myzostomida within Lophotrochozoa. The *Lox5* ortholog shows the typical C-parapeptide, except that it bears a "KLNGP" instead of "KLTGP" as usually found in most lophotrochozoans (de Rosa et al. 1999; Balavoine et al. 2002). Moreover, the flanking region upstream of the homeodomain shows a motif similar to that of annelids included in the present study (Fig. 2, "FGFE" motif). Flanking regions obtained for *Mci_Lox4* strongly support its assignment to the *Lox4* PG. *Mci_lab* is a member of the PG 1 (*Hox1*-PG) and typical PG 1 signature residues can be identified in its homeodomain (Fig. 2). *Mci_pb* was identified as a PG 2 (*Hox2*-PG) member. Interestingly, our phylogenetic reconstruction (Fig. 1) recovered unambiguously only one platyhelminth sequence within this group, the tapeworm *Echinococcus granulosus*. However, BLAST searches revealed a vertebrate similarity for this last sequence, which might be due to a contamination by its host's DNA, as *E. granulosus* parasitizes dogs. Moreover, additional BLAST searches within the available *Schistosoma mansoni* genome data (http://www.sanger.ac.uk/Projects/S_mansoni/) have not recovered any sequence that groups within the PG2 cluster. The sequences that Olson (2008) tentatively assigned to a combined PG 2 + PG 3 clade cluster within the PG 3 clade in our analysis or outside both PGs. In summary, the existence of PG 2 genes has still to be proven for Platyhelminthes and in fact might even have been lost in this lineage. A myzostomid PG 4 member (*Mci_Dfd*) has been found. A "LPNTK" motif in the downstream flanking region (Fig. 2) is present in the myzostomid, in the annelids, in one of the bryozoan sequences, and in the arthropod and deuterostome representatives, but not in any of the available flatworm sequences. A parsimonious explanation for this pattern is that annelids and myzostomids retained the plesiomorphic condition, which must have been already available in the last common bilaterian ancestor, whereas flatworms show a derived condition. We obtained a short fragment assigned to *Mci_Antp* belonging to the PG 7, but we were not able to amplify its flanking regions and the homeodomain itself appears to be conserved across the included taxa (Fig. 2).

Fig. 2 Alignment of *Hox* genes homeodomains and flanking regions. *Dashes* represent identity with the *Drosophila melanogaster Antp* homeodomain sequence shown at the *top* of the alignment. Diagnostic signature residues (discussed in the text) are highlighted, signature residues exclusively found in annelids + *Myzostoma* are marked by an *arrow*

The phylogenetic position of myzostomids is still under discussion and most analyses either recover an annelid or platyzoan relationship (Bleidorn et al. 2007; Dunn et al. 2008). *Hox* genes have been repeatedly used for phylogenetic inference (e.g. de Rosa et al. 1999), but the "right" way to analyze these data has been disputed (Telford 2000). One method is to search for so called signatures (a progression of diagnostic residues) in alignments of ortholog genes (e.g. de Rosa et al. 1999). As more sequences are included in this approach, the more diluted the picture becomes and it would be straightforward to combine single sets of ortholog sequences into a supermatrix for subsequent phylogenetic analyses. However, the ambiguous orthology of some *Hox* gene fragments, especially those of many of the flatworms, weakens this approach. We thus limit our analysis to the former method. Myzostomids share signatures with annelids (e.g., *Lox5*, see details above) and a member of the PG 2 (*Mci_pb*) has been identified, which might have been lost in the platyhelminth lineage. The present findings can be interpreted as a (weak) support of the annelid affinity hypothesis and is hence in concordance with morphology and mitochondrial data (Bleidorn et al. 2007). In addition, these first available *Hox* genes sequences for myzostomids form a starting point for future studies on their ontogeny, relative to their putative phylogenetic relationships.


Dme_Antp RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN
PG1
Mci_lab .....---K----A------S--S-N-T-V--.....
Cca_lab PNM--TNF-NK-LT---------K----A------AS-G-N-T-V----------Q--RL
Nvi_lab PNM--TNF-NK-LT---------K----A------A--G-N-T-V----------Q--RM
Cva_lab PNL--TNF-NK-LT---------K----A------A--G-N-T-V----------Q--RM
Hro_lab SNL--TNF-NK-LT---------K----A------A--G-N-T-V----------Q--RV
Pex_lab1 .....TNF-NK-LT--------S-----S------AS-G-N-T----------Q--R.
Pex_lab2 .....TNF-NK-LT--------------A------SS-G-N-T-V----------Q--R.
Pex_lab3 .....TNF-NK-LT---------K----A------A--G-N-T-V----------Q--R.
Lan_lab PNM--TNFSDK-LT---------K----A------A--G-N-T-V----------Q--RM
Lsa_lab PNT--TNF-NK-LT---------K----A------A--G-N-T-V----------Q--RM
Pni_Pnox3 MIT--TNF-NK-LT---------Q----A------KSMT-S-T----------Q-RRQ
PG2
Mci_pb ATAV SR-L-TN--HT-LV-------Y-K--C-P------ST-G-----V-V--------F-RQV
Cca_pb ASNH PR-L-TA--NT-L----------K--C-P------AS-D-----V-V--------F-RQ.
Nvi_pb TGSN PR-L-TA--NT-L----------K--C-P------AS-D-----V-V--------F-RQT
Pdu_Hox2 TGSN PR-L-TA--NT-L----------K--C-P------AS-.....
Pex_pb ....---------K--C-P------AT-N-----V--------.....
Egr_pb ....---------K--C-P-WV---AL-D----RV-------.....
PG4
Mci_Dfd YVGD N--T-TA---H-V----------S---S----------H KLPNTKVKVRYF
Cca_Dfd FVGD S--T-TA---H-I----------T---S----------H KLPNTKTRLTDS
Nvi_Dfd YGTD S--T-TA---H-V---------- KLPNTKNRLSSS
Hro_Dfd SYCD N--A-TA--KH-I----------C---S---------- KLPNTKTLKNKN
Btu_DfdA DGLD P--A-TA---H-I----------T-D-S----------H KLPNTKGKIPEI
Btu_DfdB FDGD N--T-TA---Q-V--------Y-----Q--------T-T-S----------DH KLSSSKGRLPEI
Sma_Dfd QSND P--T-TA---Q-I---------Y-K----K--L----T-T-S----------H HLPGMKQRLIES
Dja_Plox3 ILSD S--N-TA---Q-I----------K----K------QS-Q-S---V----------DH HLPGNKQRLSTE
Gti_HoxA ILSD S--N-TA---Q-I----------K----K--N---QS-Q-S-S-V----------DH HLPGNKQRLSTE
Dme_Hox4 PGME P--Q-TA---H-I--------Y-Q----------T-V-S----------D- KLPNTKNVRKKT
Mro_Dfd AGME A--S-TS---Q-I----------Q--G-S----------D- NLPNTKNTKSAT
PG6
Mci_Lox5 FGFE Q--T----------Y----------T-A---------- GIAKLNGPNGD
Cca_Lox5 FGYE Q--T----------Y----------Q----------Y---- NISKLTGPNGE
Nvi_Lox5 FGFE Q--T----------Y----------G---------- NLSKLTGPNGE
Hro_Lox5 FGFD Q--T----------YS---------S-A-S---------- NVQKLTGPGGV
Esc_Lox5 TAYE Q--T------F----------S-G-S---------- NVSKLTGPDK.
Lan_Lox5 IGYE Q--T----------Y----------H-G---------- NIPKLTGPNQK
Btu_Lox5 TGYE Q--T----------Y----------T-G---------- NIAKLTG....
Lsa_Hox6 ANRE Q--T----------K----------G---------- NLQKLTGPNT.
Dja_Plox6 SSSD H--S----------K----------S----------DH NIPKLNGPGTL
Dja_Plox5 NNSN N--T------H----------K----------S-I----------H NIAKLTGPGSC
Gti_HoxC NNTN N--T------H----------K----------T-I----------H NIAKLTGPGSC
Dme_Ftz DCKD S--T----------I------D--N--S-S----------S--DR TLDSSPEHCGA
PG7
Mci_Antp .....----------S----------.....
Cca_Antp ----------..
Nvi_Hox7 ----------S----------
Pdu_Hox7 ----------S----------
Pex_Antp ----------Y----------A----------.....
Esc_Antp ----------
Lan_Antp -----H--S-H----------
Lsa_Hox7 ----------K----------KE
PG8
Mci_Lox4 PNSSQ -R------S----------Q--H----K----------L---K QQIKDLND
Cca_Lox4 PNSSQ -R------S----------Q--H----K----------L---R QQIKDLNG
Nvi_Lox4 ....------S----------R--H----K--M----V-----H----------LR--R LQIKELNR
Hro_Lox4 PNSSQ -R------S----------Q-------K-------S----------S---I QQIRELNE
Esc_Lox4 PNSSQ -R------S-F---------QY-N----K----V----N-S---V----------L---K QQIREMNG
Lan_Lox4 PNSAQ -R------S-F---------Q--H----K----V----------L---R QQIKEIND
Pni_Pnoxla PNSVQ KR------S-H---------Q--H----------N---S----------L---R QQIQELND
Gti_HoxF PNSVQ KR------S-H---------Q--H----------N---S----------L---R QQIRELND
Sma_Hox8 PNSVQ -R------S----------QYSH----------N----------L---R QQIKELND
Dme_Ubx GTNGL -R----------T-H---------M----------L---I QAIKELNE
Post2 (PG9-14)
Mci_Post2 .....-ML--N--LS-S-I--QK-W--SCK-N-S---V-V-----SA.....
Cca_Post2 QRKK-KP------MV--N--IN-S-I--QK-W--SCK-H-S---V-V--------R--L-
Nvi_Post2 QRKK-KP------MV--N--MG-S-I--QK-W--SCK-H-S---V-V--------R--L-
Pdu_Post2 -----KP------MV--N--MS-S-I--QK-W--SCK-H-S---V-V--------R--L-
Hro_Post2 QRKK-KP------MV--N--VS-S-I--QK-W--SCK-H-S---V-V--------R--L-
Pex_Post2a ERKK-KP------IV--N--LT-G-I--QK-Y-LSCH-H-S-----V------.....
Pex_Post2b QRKK-KP------MV--N--VS-S-I--QK-W--SCK-H-S---V-V------.....
Esc_Post2 GRKK-KP------MV--N--LNSS-I--QK-W--SCK-Q-----V-V--------R--L-
Lan_Post2 ....-KP------MV--N--LN-A-I--QK-W--SCK-H-S---V-V--------R--L-
Lsa_Post2 TRKK-KP------MV--N--LT-S-I--QK-W--SCK-H-----V-V--------R--L-
Btu_Post2 SRKK-KP------MV--T--IN-S-I--QK-W--SCR-R-----V-V--------R--L-
Dja_Abd Ba TRKK_KP-S----MI--S-YVG-T-I--QK-W---CK-H-S-----V--------T--IK
Dja_Abd Bb SRKK-KP------MV--S--TG-A-I--QK-W--SCK-H-S---V-V--------K--LQ

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