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Expression of *Abdominal-B* in the brine shrimp, *Artemia franciscana*, expands our evolutionary understanding of the crustacean abdomen

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ABSTRACT

The brine shrimp, *Artemia franciscana*, has a body plan composed of 11 thoracic segments, followed by 2 genital segments, and then 6 additional abdominal segments. Previous studies of *Artemia* reported that expression of the posterior-most Hox gene, *Abdominal-B* (*Abd-B*), is restricted to the genital segments and is not observed posteriorly in the abdomen at any developmental stage. This report was remarkable because it suggested that the *Artemia* abdomen posterior to the genital segments was a novel body region of 6 segments that bore no homology to any region in other crustaceans and was unique amongst arthropods in being a Hox-free segmented domain outside of the head. In this study, we used RT-PCR, antibody staining, and *in situ* hybridization on various stages of *Artemia* nauplii to show that *Abd-B* mRNA and protein are in fact expressed throughout the abdominal segments during *Artemia* development, but this expression later retracts to the two genital segments (G1, G2) and the T11 appendages. This suggests that *Abd-B* does play a role in specifying abdominal segment identity in all crustaceans that have been examined and suggests a common evolutionary origin for the crustacean abdomen.

1. Introduction

Within the arthropods, the group Pancrustacea, which is comprised of the paraphyletic groups Crustacea and Hexapoda, display a remarkable level of variation in their body plans, highlighted by the morphological and functional diversity of their appendages (Schram, 1986; Lozano-Fernandez et al., 2019). The study of Hox gene expression and function has provided some insights into how this diversity is created during development and changes during evolution (reviewed in Abzhanov and Kaufman, 2000a; Hughes and Kaufman, 2002a; Sun and Patel, 2019). These studies reveal that these genes are well conserved during evolution and have retained an overall role in determining regional identity along the anterior-posterior axis, but the domains in which they are expressed, and the downstream developmental pathways that they regulate, may vary considerably from species to species. Several additional studies suggest that evolutionary changes in the Hox protein coding region have also played an important role in the way in which Hox proteins interact with downstream targets (Galant and Carroll, 2002; Ronshaugen et al., 2002; Hsia et al., 2010).

Here we focus on three Hox genes, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*), which in *Drosophila* comprise the Bithorax Complex (BX-C) (Struhl and White, 1985). These genes have been particularly well studied in Malacostraca crustaceans, which includes crayfish, amphipods, and isopods, both in terms of expression (Brena et al., 2005; Serano et al., 2016; Abzhanov and Kaufman, 2000b), and more recently from a functional perspective (Liubicich et al., 2009; Pavlopoulos et al., 2009; Martin et al., 2016). Malacostraca have a general body plan composed of a head, followed by 8 thoracic segments and 6 abdominal segments and a telson, but within this framework there is considerable variation of the morphology and function of the segments and associated limbs. Detailed studies in the amphipod crustacean, *Parhyale hawaiensis*, combined with comparative expression data has

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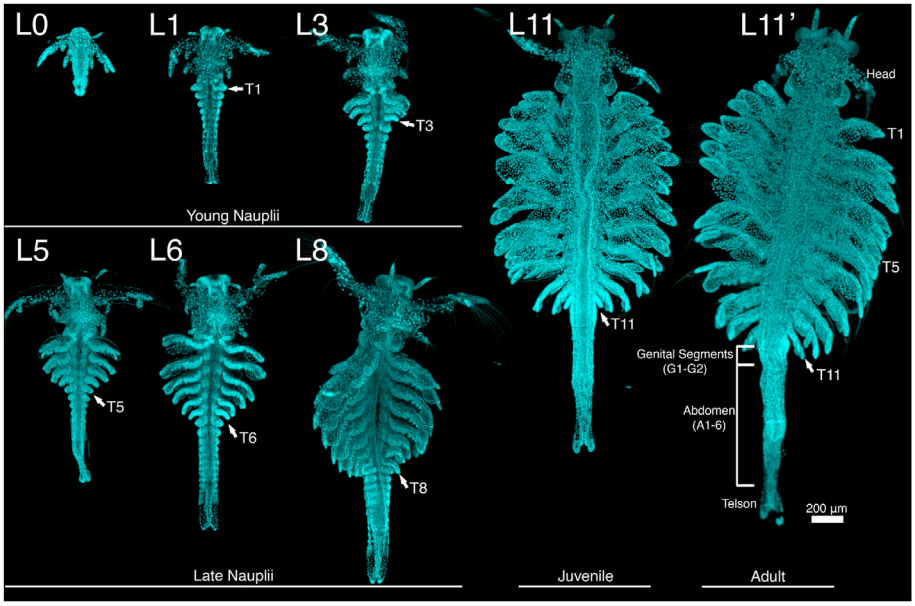

Fig. 1. Developmental stages of Artemia franciscana, nuclei stained with DAPI.
L0-L3 represent young nauplii, which rely on antennae for locomotion. L5-8 represent late nauplii, where the transition from antennae to thoracopods for locomotion begins, completed by L8. L11 is a juvenile, containing all body segments, but in which limbs are still growing. L11′ is an adult with all body segments and limbs fully formed. Anteroposterior axis: head, thoracic segments (T1-T11 with thoracopods on each), genital segments (G1-G2), abdominal segments (A1-A6), and telson. Arrows point to the thoracic limb that was used for staging. Scale bar 200 µm.

revealed how changes in Hox expression may have led to evolutionary transitions in the crustacean body plan. In particular, shifts in *Ubx* expression along the anteroposterior (A/P) axis have been implicated in evolutionary changes in the number and position of maxillipeds (thoracic feeding appendages) (Averof and Patel, 1997; Liubicich et al., 2009; Pavlopoulos et al., 2009), whereas changes in *abd-A* expression along the A/P axis are responsible for the evolutionary shifts in the limb types present in the posterior thorax and anterior abdomen (Martin et al., 2016).

The Branchiopoda are a different lineage of crustaceans that includes the genera *Daphnia*, *Artemia*, and *Triops*, which possess body plans distinct from one another and from the Malacostraca. Members of Branchiopoda can have a carapace, as seen in *Daphnia* (Cladocera) and *Triops* (Notostraca), or no carapace, as seen in *Artemia* (Anostraca). Some members of Branchiopoda, including that of *Artemia*, undergo a naupliar stage in which their segments develop sequentially along the A/P axis during an extended post-embryonic period. The adult body plan of *Artemia* is comprised of a head, 11 thoracic segments (T1-T11), 2 genital segments (G1-G2), 6 abdominal, or post-genital segments (A1-A6), and a telson (Fig. 1 L11'). In *Artemia*, the abdominal segments lack appendages, while each thoracic segment possesses a pair of multi-branched limbs.

Previously studies showed that *Artemia Ubx* is expressed ectodermally throughout the thoracic region from T1 to T11 (Averof and Akam, 1995; Averof and Patel, 1997), *abd-A* is expressed ectodermally in the thoracic region from T2-T11 (Hsia et al., 2010), and *Abd-B* is expressed in only the two genital segments (Averof and Akam, 1995; Copf et al., 2003), thus making the *Artemia* abdomen a Hox-free region. This led to the suggestion that the thoracic and genital segments of *Artemia* are homologous to the entire trunk of other arthropods, and the *Artemia* abdomen was proposed to be a unique body region that bore no homology to any other body regions in crustaceans (Averof and Akam, 1995; Williams and Nagy, 1995; Copf et al., 2003). At the time, this discovery was remarkable because previous studies had not identified a segmented region, posterior to the head, devoid of Hox gene expression, and this observation of a Hox-free region was also put forward as an explanation for the leg-less abdomen in *Artemia* (Averof and Akam, 1995; Copf et al., 2003).

To test whether the *Artemia* abdomen is truly Hox-free, we revisited this question with more sensitive techniques to examine *Abd-B* expression throughout development in *Artemia,* using RT-PCR, immunohistochemistry, and *in situ* hybridization. Here we show that *Abd-B* mRNA and protein are expressed in the genital segments of adult *Artemia*, but we also show that they are expressed posterior to the genital segments in the six segments of the abdomen during development while these segments are forming and differentiating.

2. Materials and methods

2.1. Animal culture

Artemia franciscana were obtained as either diapausing cysts (Brine Shrimp Direct, Utah, USA) or as adults (Carolina Biological Supply, North Carolina, USA). Cysts, were hatched in an Artemia cone, then transferred to an aerated tank to continue development to adulthood, where they were allowed to reproduce without diapausing. All life stages of *Artemia* were fed either a diet of dried spirulina powder, yeast, and wheat germ flour, or RG complete (Reef Nutrition, California, USA), a concentrated algal mix, three times a week with biweekly water changes.

To stage nauplii (Fig. 1), we examined the morphology of the thoracic limbs. As *Artemia* develop, segments are progressively added along the A/P axis, and shortly after each thoracic segment becomes morphologically distinct, limb outgrowth begins. We staged individual nauplii based on the number of distinct thoracic limbs that were present, using the criteria that the limb had to have grown out far enough so that it is was angled posteriorly (Fig. 1, arrow pointing to most posterior limb counted). Some previous methods of staging have relied on delineating nascent segmental divisions (Criel and Macrae, 2002), but this morphology is often difficult to see in specimens that have been processed for immunostaining and *in situ* hybridization, hence our use of limb morphology. We have also maintained the convention of distinguishing the genital segments as G1-G2, with an abdomen of A1-A6 (Averof and Akam, 1995; Copf et al., 2003), instead of the alternative of referring to this entire region as eight abdominal segments A1-A8 (Criel and Macrae, 2002).

2.2. Nauplii fixation

Artemia of various stages were collected and fixed in artificial seawater containing 3.2% paraformaldehyde for 30–40 min. Samples were washed 3 × 5min in PT (1x PBS and 0.1% Triton x-100), then dehydrated through a methanol series, as described in Rehm et al. (2009). Embryos were stored in 100% MeOH at −20 °C. After rehydration in PT, these nauplii were used for either immunofluorescence staining or *insitu* hybridization.

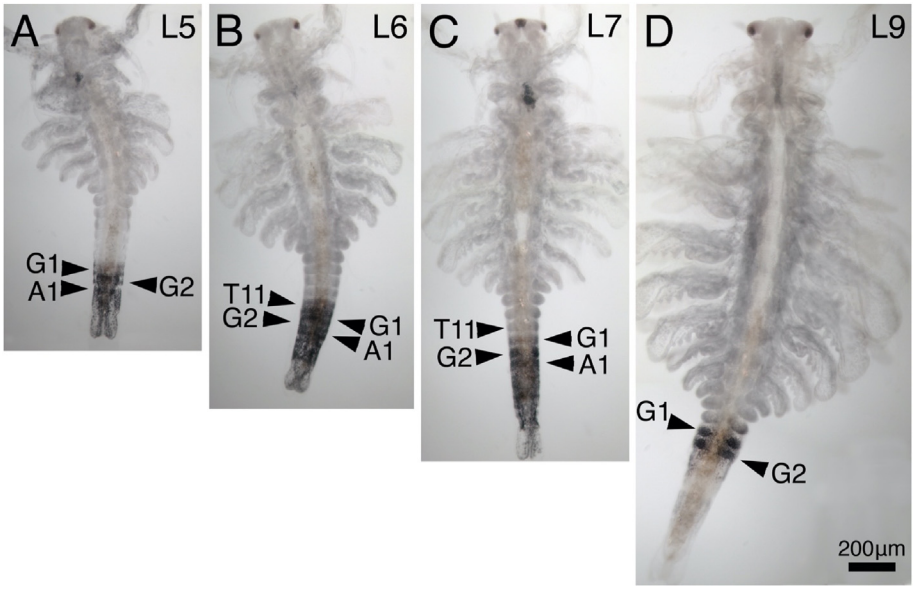

Fig. 2. Antibody series. Artemia Abd-B is expressed in abdominal/post-genital segments.
A-C, Immunocytochemistry using a previously described cross-reactive Abd-B antibody (Copf et al., 2003). For each animal, T11 (the last thoracic segment), G1 and G2 (the two genital segments) are marked with arrowheads. A, Stage L5 in which Abd-B staining is observed in developing segments posterior to A3. B, Stage L6 in which Abd-B staining persists in post-genital segments. C, Stage L7 in which Abd-B expression is detected in G1 and G2, but also post-genitally. D, Stage L9 in which Abd-B is not expressed post-genitally. Scale bar 200 µm.

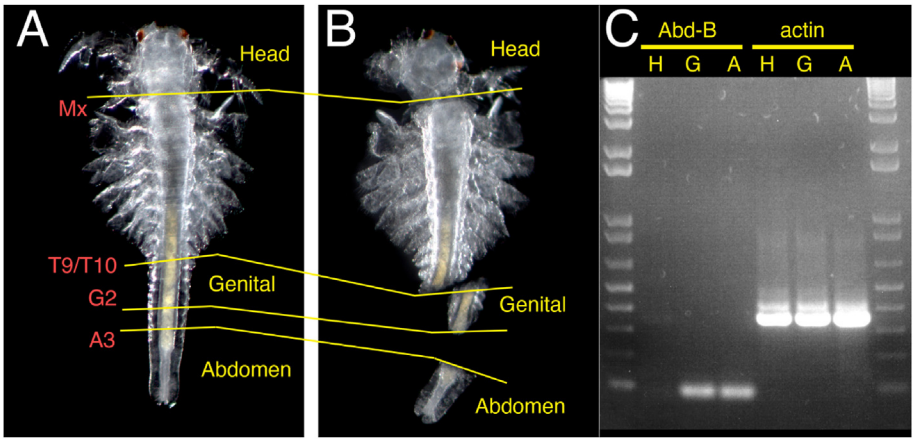

Fig. 3. Artemia RT-PCR analysis.
A, Stage L8 Artemia illustrating the sections performed to isolate the head, genital and post-genital (abdominal) segments. B, Same animal after sectioning. In order to prevent any contamination of the abdominal section with tissue from the genital section, two to three segments were avoided between both sections. In the figure, the genital sections include T11, G1, G2 and part of A1 while the abdominal section includes only segments A3 and posterior. C, Electrophoresis (1.5% agarose) gel showing the amplification of Artemia Abd-B and actin in the head, genital and abdominal fragments by PCR. Loading order: (lane 2) head section cDNA with Af-Abd-B primers; (lane 3) genital fragment cDNA with Af-Abd-B primers; (lane 4) abdominal section with Af-Abd-B primers; (lane 5) head section with Af-actin primers; (lane 6) genital section with Af-actin primers; (lane 7) abdominal section with Af-actin primers; (lane 1 and 8) ladder (1 Kb Plus DNA from Invitrogen).

2.3. *Artemia* immunocytochemistry

Artemia immunocytochemistry was carried out as described in Patel (1994) with the following modifications to improve antibody penetration through the cuticle; after fixation, animals were sonicated with 2–4 brief pulses with a probe sonicator, and then treated for 30–60 min at room temperature with a detergent solution composed of 0.3% Triton x-100 and 0.3% sodium deoxycholate in 1X PBS. For the detection of Abd-B protein in *Artemia*, we used the same mouse antisera raised against *Drosophila* Abd-B that was described in Copf et al., (2003) (originally produced by, and kindly provided by, Sue Celniker). Detection of the primary antibody was with an HRP-coupled goat anti-mouse secondary (Jackson ImmunoResearch) incubated overnight at 4 °C, and final detection was with nickel enhanced DAB (Patel, 1994). We have found that the combination of sonication and detergent treatment allows for improved permeabilization and more robust antibody staining.

2.4. *Artemia* RT-PCR

Staged nauplii were incubated in ice water until they ceased moving, and then cut apart on a dissecting microscope using forceps and a microsurgical knife. Nauplii were dissected transversely at four different positions along the A/P axis (1) through the gnathal segments, (2) T9/T10, (3) G2, and (4) A3. This allowed us to isolate three separate body regions which we refer to as head, genital, and abdominal fragments (see Fig. 3). In order to obtain enough material for RNA extraction, cDNA synthesis, and PCR amplification, tissue from the same section of different individuals was pooled. Total RNA from each domain was extracted using Trizol, purified with chloroform, and precipitated with isopropanol. The cDNA synthesis was performed using SuperScript III First Strand kit (Invitrogen).

The *Artemia Abd-B* sequence corresponding to accession number X87250 was used to design primers to amplify a 68 bp fragment of the gene (Averof and Akam, 1995). Nested PCR amplification was performed with annealing at 50 °C for 30 s and extension at 72 °C for 30 s, using the following sets of primers: first round of PCR with TTCGAAAGAA-GAGGAAGCCATA (outer forward primer) and AAATTTCGTGC-TAGTTCCCATC (outer reverse primer), followed by a second round of PCR with AGGAAGCCATATTCCAAGTTCC (inner forward primer) and TAGTTCCCATCGTTTCTGCTTT (inner reverse primer). As a positive control for the PCR amplifications, a 317 bp fragment from the *Artemia actin* gene (GenBank: EU142254.1) was amplified from each section

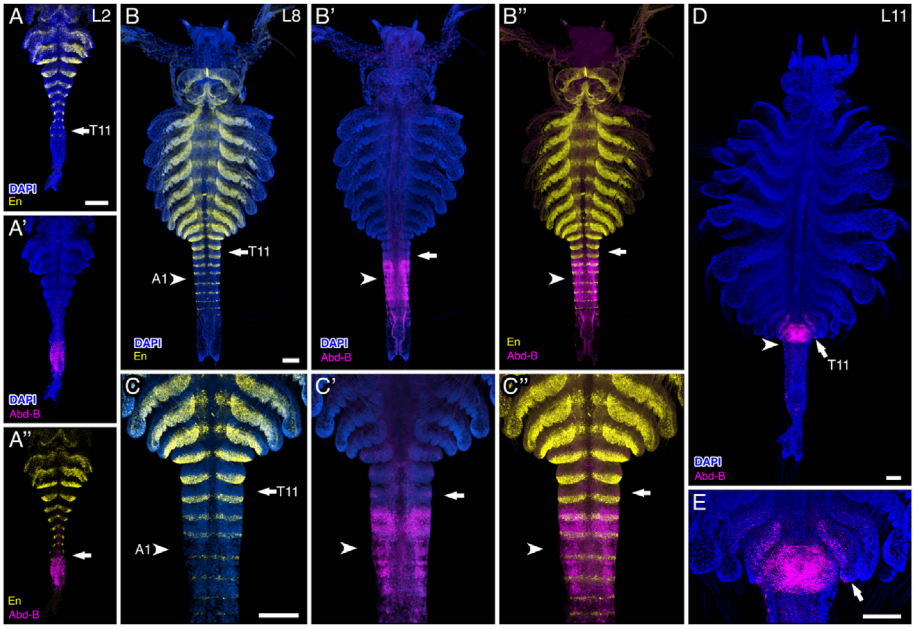

Fig. 4. Abd-B mRNA expression throughout development of Artemia.
A-A″, L2 nauplius. Abd-B is expressed posterior to thoracic segment 11 (T11). B–B″, L8 nauplius. Abd-B expressed posterior to T11, through the genital segments (G1-G2), and into the developing abdomen. C–C″, images taken at a higher magnification of B–B″ individuals. D, L11 adult. Abd-B expression has retracted anteriorly and is localized to the T11 thoracopods and G1-G2. E, higher magnification of the same individual shown in D. T11 represented by arrow, A1 by arrowhead. Scale bars 100 μm.

using the following set of primers: first round of PCR with GACAATGGTTCTGGCATGTG (outer forward primer) and AAGTCACGACCAGCCAAGTC (outer reverse primer), followed by a second round of PCR with ATGGTTGGAATGGGTCAAAA (inner forward primer) and GGTGTGGGAGACACCATCTC (inner reverse primer). The PCR fragments were cloned using the QIAGEN PCR Cloning Kit. The fragments cloned were sequenced using M13 primers and the sequence identities were confirmed via BLAST search.

2.5. *In situ hybridization chain reaction (HCR)*

In situ hybridization chain reaction was carried out as described in Bruce et al. (2021) with the same modifications of sonication and detergent permeabilization used for immunocytochemistry. Probes were designed by Molecular Instruments with the sequences for *en* (X70939), *Ubx* (AF435787.1), *abd-A* (GQ141056.1), and *Abd-B* (OK095359.1).

2.6. *Mounting and imaging*

Samples were generally mounted ventrally, with the exception of adults, which were mounted dorsally to minimize the obstruction from the limbs. All samples were mounted on microscope slides with two #1 coverslips as support and a #1.5 coverslip over the sample, and were imaged using a Zeiss LSM880, with linear unmixing used to assist in removing cuticle autofluorescence. Images were processed using Zen-Black (Zeiss) and ImageJ, and figures assembled using Photoshop (Adobe).

3. Results and discussion

3.1. *Artemia Abd-B is expressed in the abdomen during early stages*

Within insects *Abd-B* has been found to be expressed in the posterior most segments of the abdomen (Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988; Kelsh et al., 1993; Peterson et al., 1999; Hughes and Kaufman, 2002a; Angelini et al., 2005; Tomita and Kikuchi, 2009). Within Crustacea *Abd-B* has been found to be expressed throughout the abdomen along the A/P axis (Hughes and Kaufman, 2002a; Blin et al., 2003; Brena et al., 2005; Serano et al., 2016), with the one notable exception being *Artemia* (Averof and Akam, 1995; Copf et al., 2003). *Artemia Abd-B* expression was reported to be restricted to the genital segments (G1-G2) by stage L9 and not expressed in the remaining posterior abdominal segments (A1-A6) (Averof and Akam, 1995; Copf et al., 2003).

With the permeabilization techniques described here, we performed immunocytochemistry using the same cross-reactive anti-Abd-B antibody as Copf et al. (2003). In our hands, we first detected Abd-B protein in stage L5 (Fig. 2A). At this stage, Abd-B was observed in the genital segments and throughout the developing abdomen. Abd-B expression persisted in the abdomen through stage L8 (Fig. 2B and C), but was no longer detectable in the abdomen shortly thereafter (Fig. 2D). As adults, Abd-B expression was restricted to the T11 appendages and the two genital segments.

To further confirm these results, we performed RT-PCR on various portions of a stage L8 *Artemia* using primers specific to the *Artemia Abd-B* gene (Averof and Akam, 1995). We isolated the anterior head segments (head fragment), the genital segments G1 and G2 (genital fragment) and the post-genital segments posterior to A3 (abdominal fragment) (Fig. 3A and B). For both the genital and abdominal fragments, our RT-PCR experiments amplified a DNA fragment of the correct size, which was confirmed as *Abd-B* by subsequent sequencing. No amplification of *Abd-B* was observed for the head fragment (Fig. 3C). As a positive control, we amplified the *Artemia actin* gene, which was present in each of the analyzed fragments (Fig. 3C). These RT-PCR experiments show that *Artemia Abd-B* transcripts are present not only in the genital segments, but also in the abdominal segments.

Utilizing the same permeabilization techniques described in combination with *in situ* hybridization chain reaction (HCR), as opposed to traditional *in situ* methods using digoxigenin-labelled riboprobes, we were able to more precisely define the expression domains of the posterior Hox transcripts in *Artemia*. Here we used *engrailed* (*en*) to identify each new segment as it formed. At stage L2, *en* was expressed up to the last thoracic segment, T11 (Fig. 4A). At this stage, *Abd-B* expression was observed in T11 and extended posteriorly (Fig. 4A'-A″). By L8, the combination of *en* and *Abd-B* revealed that *Abd-B* mRNA was present in T11 and extended posteriorly to A6 (Fig. 4B-C″). At this time point, *Abd-B* was expressed in its maximal number of segments and as observed in the last thoracic segment (T11), both genital segments (G1 and G2), and throughout all abdominal segments (A1-A6). By L9, *Abd-B* expression had decreased in the abdomen (Fig. 5A'' and 5B″). By L11, *Abd-B* expression was restricted to the T11 appendages and the two genital segments, G1 and G2 (Fig. 4D and E).

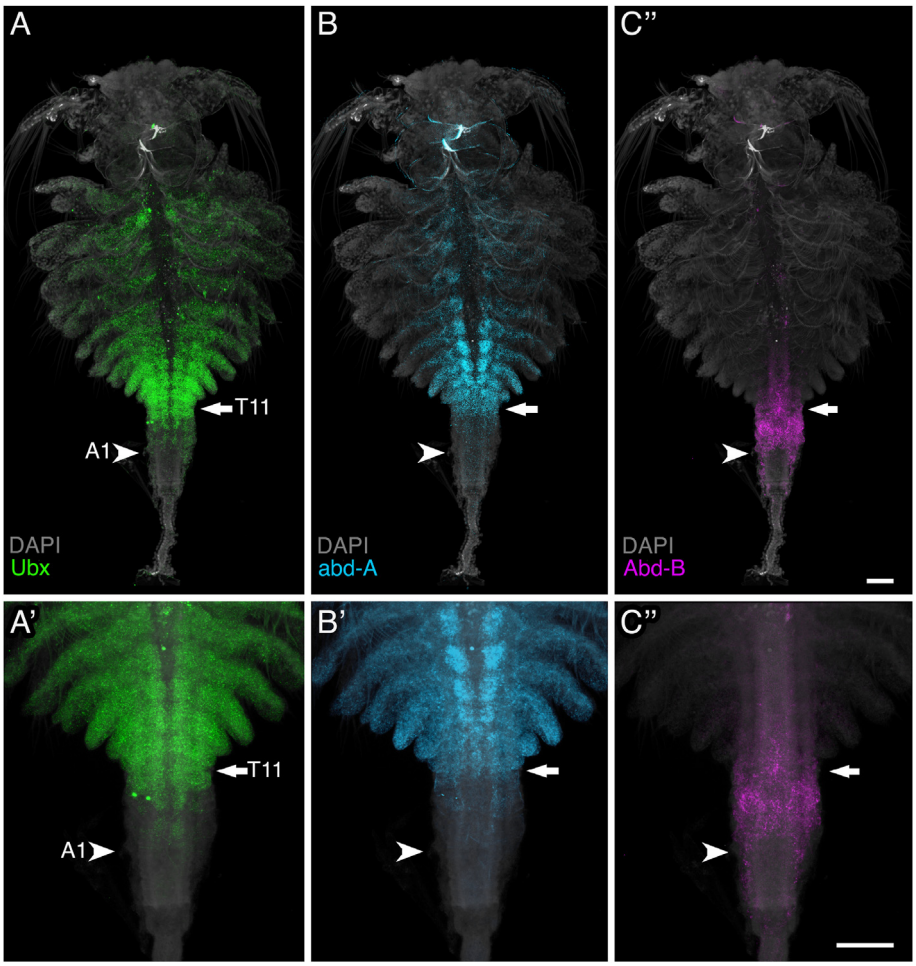

Fig. 5. Posterior boundaries of Ubx, abd-A, and Abd-B mRNA expression in an L9 juvenile.
A-C, Stage L9 individual. A′- C′, closeup of A-C. A-A′, Ubx, expressed up to T11. B– B′, abd-A, expressed up to T11. C–C′, Abd-B, expression retracted to T11-G2. T11 represented by arrow and A1 by arrowhead. Scattered signal within the gut itself comes from autofluorescence of gut contents. Scale bar 100 μm.

To further understand the potential role of Hox genes in defining the boundaries between thoracic, genital, and post-genital segment identities in *Artemia*, we compared the expression domains for *Ubx, abd-A,* and *Abd-B*. Our results showed that *Ubx* was expressed in T1-T11 throughout development and *abd-A* was expressed in T2-T11, confirming previous descriptions (Averof and Akam, 1995; Averof and Patel, 1997). At stage L9, when *Abd-B* had retracted from the abdomen (Fig. 4C and C′), neither *Ubx* nor *abd-A* extended into abdominal segments (Fig. 5A and B, 5A′ and B′). Instead, *Ubx and abd-A* expression at this stage were limited to the thoracic segments. This confirms that *Abd-B* is the only Hox gene expressed in the abdomen during development. Thus, the abdomen of *Artemia* is not a Hox-free domain, but rather *Abd-B* mRNA and protein are expressed in this region during development, but eventually become restricted to the genital segments and the T11 appendage at late stages. Given our observation of *Artemia Abd-B* expression and the expression patterns previously reported, we suggest that the common ancestor of all Pancrustacea expressed *Abd-B* throughout its abdomen.

Within Pancrustacea, the expression domains of *Ubx, abd-A,* and *Abd-B* have been well studied and, in some lineages, functionally tested. Particular interest has been paid to the role in specifying not only the type of appendage on a given segment, but whether or not appendage formation is initiated at all. In many cases, a focus has been on the ability of each of these Hox genes to repress the expression of the limb patterning gene, *Distalless* (*Dll*). In Diptera (*Drosophila*) *Ubx*, *abd-A*, and *Abd-B* all repress limb development, resulting in a limb-less abdomen (Vachon et al., 1992; Sánchez-Herrero et al., 1994; Castiell-Gair and Akam, 1995; Estrada and Sanchez-Herrero, 2001). This pattern of repression also appears to hold for Lepidoptera (*Junonia* and *Bombyx*) (Warren et al., 1994; Tomita and Kikuchi, 2009). A different pattern is observed in more distantly related insect lineages such Coleoptera (*Tribolium*) (Lewis et al., 2000), Hemiptera (*Oncopeltus*) (Angelini et al., 2005), and Orthoptera (*Schistocerca*) (Kelsh et al., 1994; Palopoli and Patel, 1998), where only *abd-A* and *Abd-B* repress appendage development, and there is an appendage present on the A1 segment. In Collembola (*Folsomia* and *Orchesella*), springtails, only *Abd-B* represses limb development, and not *Ubx* or *abd-A* (Palopoli and Patel, 1998; Konopova and Akam, 2014). In *Parhyale* (Malacostraca), *Ubx*, *abd-A*, and *Abd-B* do not repress limb development (Liubicich et al., 2009; Pavlopoulos et al., 2009; Serano et al., 2016; Martin et al., 2016).

We can further refine the evolutionary history of Hox gene function with the results we report here. Our data show that in *Artemia* (Branchiopoda), *Abd-B* is expressed in the abdomen throughout development, and we hypothesize that this expression of *Abd-B* may repress limb development in these segments. While the expression is transient, the importance of temporally modulated expression of Hox genes in controlling limb positioning has been well illustrated in *Drosophila* (Castiell-Gair and Akam, 1995) and Lepidoptera (Warren et al., 1994). Likewise, the expression of *Abd-B* in the T11 segment of *Artemia* is at a very low level compared to abdominal segments (Fig. 4), and is highly mosaic during limb formation, possibly explaining why *Abd-B* does not block limb development in this *Artemia* segment, reminiscent of why *Ubx* expression in *Drosophila* T3 does not prevent limb development in this

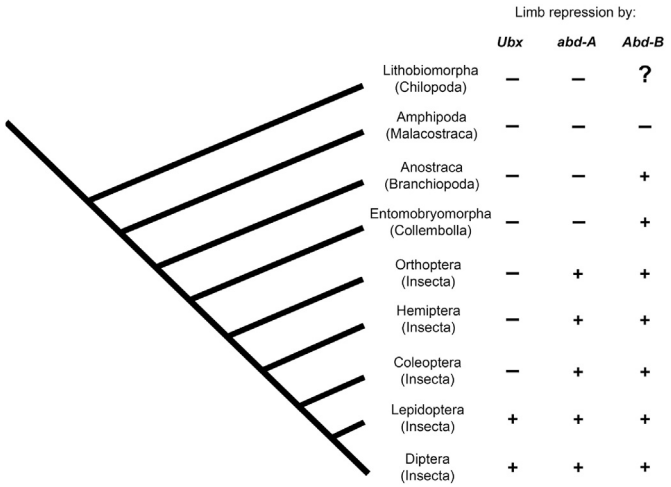

Fig. 6. Hypothesized step-wise accumulation of limb repression in Arthropoda based on functional and expression data.
In Diptera and Lepidoptera (Insecta) *Ubx, abd-A,* and *Abd-B* repress limb development. In Coleoptera, Hemiptera, and Orthoptera (Insecta), *abd-A,* and *Abd-B* repress limb development, but not *Ubx*. For the most basally related group to Insecta, Entomobryomorpha (Collembolla, springtails), only *Abd-B* represses limb development, and this is also the case Anostraca (Branchiopoda crustacean, *Artemia* data shown here). Within Amphipoda (Malacostraca crustacean) *Ubx, abd-A,* and *Abd-B* do not repress limb development. Outgroup are members of Chiliopoda (Myriapoda), where *Abd-B* is weakly present in the trunk segments during development, but is most strongly expressed in the telson (Hughes and Kaufman, 2002b). Relationships within Arthropoda are based on Lozano-Fernandez et al., (2019), references for Hox gene limb repression are given in the main text.

Drosophila segment (Castelli-Gair and Akam, 1995). Even though branchiopods, such as *Artemia*, are crustaceans, their evolutionary relationship is closer to that of Hexapoda than to Malacostraca (Lozano-Fernandez et al., 2019). Looking at Pancrustacea as a whole, we suggest that there is a step-wise acquisition of limb repression during evolution, starting with *Abd-B,* then adding *abd-A,* and most recently *Ubx* (Fig. 6).

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Declaration of competing interest

None.

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References

Abzhanov, A., Kaufman, T.C., 2000a. Crustacean (malacostracan) Hox genes and the evolution of the arthropod trunk. Development (Cambridge, U. K.) 127 (11), 2239–2249.

Abzhanov, A., Kaufman, T.C., 2000b. Embryonic expression patterns of the Hox genes of the crayfish Procambarus clarkii (Crustacea, Decapoda). Evol. Dev. 2 (5), 271–283. https://doi.org/10.1046/j.1525-142x.2000.00066.x.

Angelini, D.R., Liu, P.Z., Hughes, C.L., Kaufman, T.C., 2005. Hox gene function and interaction in the milkweed bug Oncopeltus fasciatus (Hemiptera). Dev. Biol. 287 (2), 440–455. https://doi.org/10.1016/j.ydbio.2005.08.010.

Averof, M., Akam, M., 1995. Hox genes and the diversification of insect and crustacean body plans. Nature 376 (6539), 420–423. https://doi.org/10.1038/376420a0.

Averof, M., Patel, N.H., 1997. Crustacean appendage evolution associated with changes in Hox gene expression. Nature 388 (6643), 682–686. https://doi.org/10.1038/41786.

Blin, M., Rabet, N., Deutsch, J.S., Mouchel-Vielh, E., 2003. Possible implication of Hox genes Abdominal-B and abdominal-A in the specification of genital and abdominal segments in cirripedes. Dev. Gene. Evol. 213 (2), 90–96. https://doi.org/10.1007/s00427-003-0294-z.

Brena, C., Liu, P.Z., Minelli, A., Kaufman, T.C., 2005. Abd-B expression in Porcellio scaber Latreille, 1804 (Isopoda: Crustacea): conserved pattern versus novel roles in development and evolution. Evol. Dev. 7 (1), 42–50. https://doi.org/10.1111/j.1525-142X.2005.05005.x.

Bruce, H.S., Jerz, G., Kelly, S., McCarthy, J., Pomerantz, A., Senevirathne, G., Sherrard, A., Sun, D.A., Wolff, C., Patel, N.H., 2021. Hybridization Chain Reaction (HCR) in Situ Protocol. https://doi.org/10.17504/protocols.io.bunznvf6.

Castelli-Gair, J., Akam, M., 1995. How the Hox gene Ultrabithorax specifies two different segments: the significance of spatial and temporal regulation within metameres. Development (Cambridge, U. K.) 121 (9), 2973–2982.

Copf, T., Rabet, N., Celniker, S.E., Averof, M., 2003. Posterior patterning genes and the identification of a unique body region in the brine shrimp Artemia franciscana. Development (Cambridge, U. K.) 130 (24), 5915–5927. https://doi.org/10.1242/dev.00835.

Criel, G.R.J., Macrae, T.H., 2002. *Artemia* morphology and structure. In: Abatzopoulos, T.J., Beardmore, J.A., Clegg, J.S., Sorgeloos, P. (Eds.), Artemia: Basic and Applied Biology. Biology of Aquatic Organisms, vol. 1. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0791-6_1.

Estrada, B., Sánchez-Herrero, E., 2001 Feb. The Hox gene Abdominal-B antagonizes appendage development in the genital disc of Drosophila. Development 128 (3), 331–339. PMID: 11152632.

Galant, R., Carroll, S.B., 2002. Evolution of a transcriptional repression domain in an insect Hox protein. Nature 415 (6874), 910–913. https://doi.org/10.1038/nature717.

Hsia, C.C., Paré, A.C., Hannon, M., Ronshaugen, M., McGinnis, W., 2010. Silencing of an abdominal Hox gene during early development is correlated with limb development in a crustacean trunk. Evol. Dev. 12 (2), 131–143. https://doi.org/10.1111/j.1525-142X.2010.00399.x.

Hughes, C.L., Kaufman, T.C., 2002a. Hox genes and the evolution of the arthropod body plan. Evol. Dev. 4 (6), 459–499. https://doi.org/10.1046/j.1525-142x.2002.02034.x.

Hughes, C.L., Kaufman, T.C., 2002b. Exploring the myriapod body plan: expression patterns of the ten Hox genes in a centipede. Development (Cambridge, U. K.) 129 (5), 1225–1238.

Kelsh, R., Dawson, I., Akam, M., 1993. An analysis of abdominal-B expression in the locust Schistocerca gregaria. Development (Cambridge, U. K.) 117 (1), 293–305.

Kelsh, R., Weinzierl, R.O., White, R.A., Akam, M., 1994. Homeotic gene expression in the locust Schistocerca: an antibody that detects conserved epitopes in Ultrabithorax and abdominal-A proteins. Dev. Genet. 15 (1), 19–31. https://doi.org/10.1002/dvg.1020150104.

Konopova, B., Akam, M., 2014. The Hox genes Ultrabithorax and abdominal-A specify three different types of abdominal appendage in the springtail Orchesella cincta (Collembola). EvoDevo 5 (1), 2. https://doi.org/10.1186/2041-9139-5-2.

Kuziora, M.A., McGinnis, W., 1988. Different transcripts of the Drosophila Abd-B gene correlate with distinct genetic sub-functions. EMBO J. 7 (10), 3233–3244.

Lewis, D.L., DeCamillis, M., Bennett, R.L., 2000. Distinct roles of the homeotic genes Ubx and abd-A in beetle embryonic abdominal appendage development. Proc. Natl. Acad. Sci. U. S. A 97 (9), 4504–4509. https://doi.org/10.1073/pnas.97.9.4504.

Liubicich, D.M., Serano, J.M., Pavlopoulos, A., Kontarakis, Z., Protas, M.E., Kwan, E., Chatterjee, S., Tran, K.D., Averof, M., Patel, N.H., 2009. Knockdown of Parhyale Ultrabithorax recapitulates evolutionary changes in crustacean appendage morphology. Proc. Natl. Acad. Sci. U. S. A 106 (33), 13892–13896. https://doi.org/10.1073/pnas.0903105106.

Lozano-Fernandez, J., Giacomelli, M., Fleming, J.F., Chen, A., Vinther, J., Thomsen, P.F., Glenner, H., Palero, F., Legg, D.A., Iliffe, T.M., Pisani, D., Olesen, J., 2019. Pancrustacean evolution illuminated by taxon-rich genomic-scale data sets with an expanded remipede sampling. Gen. Biol. Evol. 11 (8), 2055–2070. https://doi.org/10.1093/gbe/evz097.

Martin, A., Serano, J.M., Jarvis, E., Bruce, H.S., Wang, J., Ray, S., Barker, C.A., O'Connell, L.C., Patel, N.H., 2016. CRISPR/Cas9 mutagenesis reveals versatile roles of Hox genes in Crustacean limb specification and evolution. Curr. Biol. : CB 26 (1), 14–26. https://doi.org/10.1016/j.cub.2015.11.021.

Palopoli, M.F., Patel, N.H., 1998. Evolution of the interaction between Hox genes and a downstream target. Curr. Biol. : CB 8 (10), 587–590. https://doi.org/10.1016/s0960-9822(98)70228-3.

Patel, N.H., 1994. Chapter 24 imaging neuronal subsets and other cell types in whole-mount drosophila embryos and larvae using antibody probes. Methods in Cell Biology, 44. Elsevier. https://doi.org/10.1016/S0091-679X(08)60927-9, pp. 445-456, 456a, 456b, 457–487.

Pavlopoulos, A., Kontarakis, Z., Liubicich, D.M., Serano, J.M., Akam, M., Patel, N.H., Averof, M., 2009. Probing the evolution of appendage specialization by Hox gene misexpression in an emerging model crustacean. Proc. Natl. Acad. Sci. U. S. A 106 (33), 13897–13902. https://doi.org/10.1073/pnas.0902804106.

Peterson, M.D., Rogers, B.T., Popadić, A., Kaufman, T.C., 1999. The embryonic expression pattern of labial, posterior homeotic complex genes and the teashirt homologue in an apterygote insect. Dev. Gene. Evol. 209 (2), 77–90. https://doi.org/10.1007/s004270050230.

Rehm, E.J., Hannibal, R.L., Chaw, R.C., Vargas-Vila, M.A., Patel, N.H., 2009. Fixation and dissection of parhyale hawaiensis embryos. Cold Spr. Harbor Protoc. 1. https://doi.org/10.1101/pdb.prot5127.

Ronshaugen, M., McGinnis, N., McGinnis, W., 2002. Hox protein mutation and macroevolution of the insect body plan. Nature 415 (6874), 914–917. https://doi.org/10.1038/nature716.

Sánchez-Herrero, E., Crosby, M.A., 1988. The Abdominal-B gene of Drosophila melanogaster: overlapping transcripts exhibit two different spatial distributions. EMBO J. 7 (7), 2163–2173.

Sánchez-Herrero, E., Guerrero, I., Sampedro, J., González-Reyes, A., 1994. Developmental consequences of unrestricted expression of the abd-A gene of Drosophila. Mech. Dev. 46 (3), 153–167. https://doi.org/10.1016/0925-4773(94)90068-x.

Schram, F.R., 1986. What are Crustaceans? Crustacea. Oxford University Press, New York, USA, p. 3.

Serano, J.M., Martin, A., Liubicich, D.M., Jarvis, E., Bruce, H.S., La, K., Browne, W.E., Grimwood, J., Patel, N.H., 2016. Comprehensive analysis of Hox gene expression in the amphipod crustacean Parhyale hawaiensis. Dev. Biol. 409 (1), 297–309. https://doi.org/10.1016/j.ydbio.2015.10.029.

Struhl, G., White, R.A., 1985. Regulation of the Ultrabithorax gene of Drosophila by other bithorax complex genes. Cell 43 (2 Pt 1), 507–519. https://doi.org/10.1016/0092-8674(85)90180-1.

Sun, D.A., Patel, N.H., 2019. The amphipod crustacean Parhyale hawaiensis: an emerging comparative model of arthropod development, evolution, and regeneration. Wiley Interdis. Dev. Biol. 8 (5), e355. https://doi.org/10.1002/wdev.355.

Tomita, S., Kikuchi, A., 2009. Abd-B suppresses lepidopteran proleg development in posterior abdomen. Dev. Biol. 328 (2), 403–409. https://doi.org/10.1016/j.ydbio.2009.01.040.

Vachon, G., Cohen, B., Pfeifle, C., McGuffin, M.E., Botas, J., Cohen, S.M., 1992. Homeotic genes of the Bithorax complex repress limb development in the abdomen of the Drosophila embryo through the target gene Distal-less. Cell 71 (3), 437–450. https://doi.org/10.1016/0092-8674(92)90513-c.

Warren, R.W., Nagy, L., Selegue, J., Gates, J., Carroll, S., 1994. Evolution of homeotic gene regulation and function in flies and butterflies. Nature 372 (6505), 458–461. https://doi.org/10.1038/372458a0.

Williams, T.A., Nagy, L.M., 1995. Arthropod evolution: brine shrimp add salt to the stew. Curr. Biol. : CB 5 (12), 1330–1333. https://doi.org/10.1016/s0960-9822(95)00261-2.