Annelids in Modern Biology

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Part I

Annelids as Model Systems in Biology

Chapter 1

Developing Models for Lophotrochozoan and Annelid Biology

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1.1 INTRODUCTION

Most investigators using model organisms assume that, for the question being asked, the model acts in a similar (or at least predictably different) way compared to the organism of interest (e.g. human, an insect pest, ecologically important taxon). Given the processes of evolutionary change, we can expect that similarity in processes or mechanisms and thus the ability to extrapolate information are often correlated with phylogenetic relatedness. Thus, one might expect that, as a community of scientists, we would try to judiciously choose model organisms to span the diversity of organismal lineages. However, phylogenetic considerations are only one of several factors that determine the suitability of an organism as a model. For example, the ease and cost of propagating lineages in the laboratory, generation time and ability to genetically manipulate are also important considerations.

Nonetheless, model species have historically been heavily skewed toward only two of the three bilaterian lineages (i.e. Ecdysozoa, Deuterostoma). Species including the ecdysozoans *Drosophila melanogaster* and *Caenorhabditis elegans* and the deuterostomes *Mus musculus* and *Danio rerio* dominate the genetic, cellular biology, physiological and developmental literature because they are inexpensive to maintain; rearing and breeding in a laboratory setting is easy; they exhibit short generation times, and tools for genetic manipulation have been well described. Of significance, many of these species were subject to large-scale early mutational screens, thereby making them of interest to a wide range of scientific disciplines. In contrast, these types of screens are lacking for lophotrochozoans, the third bilateran lineage that has been largely ignored for holistic (i.e. multidisciplinary) model organisms.

Because model system choice has been closely tied to the ease of experimentation in a laboratory setting, considerations of evolutionary history have been neglected, resulting

in the most morphologically diverse branch of Bilateria being overlooked (i.e. Lophotro-chozoa). This situation has considerable implications for our understanding of animal evolution and diversity. In particular, studies on the evolution of developmental mecha-nisms have been particularly informative because early studies on ecdysozoan and deuterostome models, and later studies on cnidarians, have suggested conservation of homologous developmental mechanisms, such as axial patterning systems, endoderm formation and neuronal formation (Martindale 2005). However, some genes (e.g. genes related to segmentation) appear to have different roles in the three major bilaterian lineages (Seaver 2003). Without the inclusion of lophotrochozoan data, we cannot fully understand the evolution of these important mechanisms in the context of animal evolution.

Admittedly, choosing a holistic model organism within Lophotrochozoa is not easy. The challenge of selecting model species that will be used by multiple disciplines to rep-resent all of Lophotrochozoa is exacerbated by several factors. Two of these factors are (1) an unresolved phylogeny for Lophotrochozoa, and (2) genetic and development pro-tocols for laboratory experimentation have not been adequately developed. Fortunately, scientists are making considerable advances on both of these issues.

1.2   PHYLOGENETIC CONSIDERATIONS

As mentioned, in order for a model organism to be useful, the information must be extra-polated to other taxa. Thus, a comparative framework is an implicit, but often forgotten, component of studies using model organisms. In biological studies, the comparative framework is phylogeny. Thus, better understanding of evolutionary history allows more refined and accurate comparisons to be made. We now have a reasonable understanding of most major animal lineages. The most basal lineages are likely sponges and placozoans followed by the dipoblastic ctenophores and cnidarians. Within bilaterian animals are three major clades: deuterostomes, ecdysozoan and lophotrochozoans. The deuterostomes consist of Chordata, Hemichordata, Echinodermata and *Xenoturbella*. Major ecdysozoan lineages include Arthopoda, Nematoda, Priapulida, Kinorhyncha and their kin. Sister to ecdysozoans are lophotrochozoans, which include Annelida, Mollusca, Nemertea, Bryozoa, Brachiopoda and their allies. An apparent subgroup of Lophotrochozoa, i.e. Platyzoa, includes Playthelminthes (but not Acoelomorpha) and several meiofaunal taxa, such a gastrotrichs, gnathostomulids and possibly entoprocts. Our current understanding of animal relationships has been recently reviewed elsewhere (Halanych 2004; Giribet et al. 2007; and see Dunn et al. 2008), and thus we will only focus on issues of annelid affinities that are germane to this volume.

The early diversification of Lophotrochozoa presumably started in the late Proterozoic and very early Cambrian (which started 543 mya). Historically, relationships within Lophotrochozoa have been difficult to resolve in part due to the short time between diver-sification events, thus allowing little time for the accumulation of phylogentic signal, relative to the time since such events (Halanych 1998; Rokas et al. 2005). Despite this limitation, recent molecular studies have made progress in unraveling evolutionary rela-tionships within Lophtrochozoa. Large ribosomal subunit (LSU) data and combined small subunit (SSU) + LSU data suggest that platyzoans are among the most derived lineages of lophotrochozoans, with annelids occupying a more basal or central position, respec-tively (Passamaneck and Halanych 2006). Unfortunately, support for internal nodes within these data is weak. In contrast, express sequence tag (EST) data (cDNA libraries generated from expressed messenger RNA that is then sequenced) are becoming a more widely used

tool and hold promise for helping provide some resolution (Telford et al. 2008). Although several interesting EST studies have included lophotrochozoans (e.g. Philippe et al. 2005; Struck and Fisse 2007), the work of Dunn et al. (2008) includes the best taxon sampling of Lophotrochozoa to date (note that all of these studies have large proportions of missing data and the influence of these missing characters on topology reconstruction and robustness of results is not clear). In their analyses, Dunn et al. (2008) found annelids as sister to a brachiopod/phoronid/nemertean clade, which in turn is sister to mollusks. This general cluster of taxa has been loosely observed previously but without nodal support (see Halanych 2004). The close placement of mollusks, annelids and nemerteans is in good agreement with morphological and developmental information based on larval form, cleavage patterns and cell fate (Nielsen 2004; Martindale 2005). The placement of brachiopods and phoronids close to these taxa is puzzling based on morphological or embryological grounds, but note that both annelids and brachiopods have similar chaetal structures (Orrhage 1973).

Traditionally, several presumed phyla have been allied with annelids, including sipunulids, echiurids and siboglinids (formerly pogonophorans; Halanych et al. 2002). A considerable body of evidence shows that all three of these worm groups are within Annelida (McHugh 1997; Rousset et al. 2006; Struck et al. 2007; Dunn et al. 2008). Similarly, myzostomids, an unusual parasitic group of worms that have been variably placed outside annelids, are within the clade (Bleidorn et al. 2007). Several molecular studies (e.g. McHugh 1997; Bleidorn et al. 2003; Rousset et al. 2006; Struck et al. 2007) have placed the Clitellata (Oligochaeta + Huridinea) well within polychaetes rendering the node that defined "Polychaeta" as the same node as Annelida. The following chapter by C. Bleidorn more thoroughly covers the advances in annelid relationships, and therefore this discussion will be forgone here.

1.3 GENETIC AND DEVELOPMENTAL TOOLS

Traditionally, the field of genetics has largely driven the development of "classical" model systems using organisms for which a considerable literature and resources existed. Advances in molecular genetic technology have the potential to change how model organisms can be established. Namely, with the power of genome sequencing and the availability of functional genomic tools, an organism that has received rather limited attention can be very quickly developed into a useful multidisciplinary resource. For example, the sea squirt *Ciona intestinalis* quickly reached prominence as a deuterostome model organism once EST projects began and the genome was sequenced (Satoh et al. 2003).

In the case of lophotrochozoans, such efforts are being made, but as the scientific community interested in such resources tends to be fewer in number, they unfortunately have a smaller collective voice. Poignant illustrations of this fact are recent decisions by the US National Institutes of Health (NIH) to fund major genomic projects on all orders of mammals and tens of species of *Drosophila* before much investment has been made on annelids or mollusks. Nonetheless, the situation is improving as the gastropod limpet *Lottia gigantea*, the leech *Hellobdella robusta*, the capetillid annelid *Capitella* sp. I and the nereidid annelid *Platynereis dumerilii* have been the subject of major genomic efforts. All of these animals are emerging model systems, but additional resources need to be developed. Also, sequencing of the behavioral and neural model gastropod *Aplysia californica*, which has a large genome, is under way. Major genome initiatives also have taken (or are taking) place for some parasitic platyhelminthes (e.g. *Schmidtea mediterranea,*

Taenia solium, Schistosoma mansoni). However, these genomic efforts aim to understand the biology of these parasitic organisms versus developing the resources to be used as a biological model.

Development of these new model systems usually takes into serious consideration the ability of genomic information to be used by disciplines such as evolution of developmental mechanisms or neurobiology. Given that genome projects are getting cheaper and faster, the availability of large amounts of genomic data will not be sufficient to develop a model system. Other resources need to be developed. In the case of lophotrochozoans, several organisms have been used as models within particular disciplines. For example, the squid *Loligo pealei* and nudibranch *Tritonia diomedea* have been used heavily in neurobiology for years (see symposium by McPhie and Miller 2006). Also, the bivalve *Mya arenaria* has served as a model for leukemia; snail species of *Illyanassa* have been used in developmental biology, and *Capitella* species have long been bioindicators in ecotoxicology experiments. However, for all of these models, limitations in developing interdisciplinary resources (e.g. undeveloped methods for surveying gene expression), life history issues (e.g. long generation times) or rearing issues (e.g. hard to maintain in the lab) have hindered the interest in these organisms by other disciplines.

Below we highlight some of the "up-and-coming" annelid systems that may serve as models not only for annelids but for Lophotrochozoa as well. We provide only a brief description of each, as many of these taxa are discussed in more detail in the chapters that follow. For any of these organisms to reach the celebrity status of "model organism," the community must continue to develop resources that have interdisciplinary appeal.

1.4   ANNELID MODEL ORGANISMS

Historically, annelid species have been models for specific biological disciplines, and developing a more interdisciplinary or "holistic" approach has been underway and continues to improve. The combination of remarkable diversity in life history adaptations, form and function has promoted several annelid species that have the potential to become a general model organism. Improved understanding of annelid phylogeny has provided more clues toward identifying models that are broadly representative of other lineages. Although major efforts are being made toward identifying an appropriate model, currently, several annelid lineages hold promise as model systems. The following annelid taxa have already been used extensively as models in molecular and developmental biology (Weisblat and Shankland 1985; Irvine and Seaver 2006; references therein).

1.4.1   "The Leech"

One of the most widely used annelids in developmental biology is the leech. The leech is a conglomeration of several species representing two families: leeches within Glossiphoniidae (i.e. *Helobdella robusta, Helobdella triserialis, Theromyzon tessulatum* and *Haementeria ghilianii*) and the European medicinal leech *Hirudo medicinalis* (Hirudinidae) (Sawyer 1986; Irvine and Seaver 2006). Studies of annelid segmentation and development initially came from leeches (e.g. Sawyer 1986; Shankland 1991). The leech embryo is relatively large and exhibits a stereotypical cleavage program, which has enabled the description of segment formation during development.

Of all leeches, *H. medicinalis* is the most renowned member of Hirudinida. *Hirudo medicinalis* was heavily exploited for its sanguinivorous capacity during the late 19th

century and is one of few invertebrates approved by the U.S. Food and Drug Administration (FDA) as a medical device (Rados 2004). *Hirudo medicinalis* has also been extensively studied with respect to its neurophysiology (e.g. Hagadorn 1966; Brodfuehrer et al. 2008), anticoagulant biochemical properties (e.g. Harvey et al. 1986; Mao et al. 1988), developmental genetics (e.g. Baker and Macagno 2001; Venkitaramani et al. 2004), behavior (e.g. French et al. 2005) and morphology (e.g. Sawyer 1986; Orevi et al. 2000). The lesser-known species of Glossiphoniidae have particularly been amenable to developmental work (Weisblat and Huang 2001). For example, the non-blood-feeding *Helobdella* species have been used for understanding embryogenesis (e.g. Kang et al. 2003), body plan development (Bruce and Shankland 1998; Kuo and Shankland 2004) and segmentation (Seaver 2003). Both *H. medicinalis* and *H. robusta* are subjects of genomic undertakings (ESTs and ESTs and whole genome sequencing, respectively).

Surprisingly, recent application of molecular tools has revealed significant issues with species identification among leeches. Specifically, DNA bar coding and microsatellite markers indicates that the FDA-approved and commercially available *H. medicinalis* is a different species altogether, *Hirudo verbana* (Trontelj and Utevsky 2005; Siddall et al. 2007). Furthermore, the two widely used *Helobdella* species (*H. robusta* and *H. triserialis*) each consist of a species complex (Bely and Weisblat 2006). These identification issues are likely confounded in the literature (see Chapter 3).

1.4.2 The Earthworm

Lumbricus terrestris, the common earthworm, is likely the best-known annelid because it is an easily recognized oligochaete and is widely used as an education tool for invertebrate zoology. Like many annelids, *Lumbricus* is a strong biological indicator due to its sensitivity to soil toxicities and bioaccumulation (e.g. Wright and Stringer 1974; Mahmoud 2007). Although important in agricultural systems, *L. terrestris* is considered a pest of soil communities due to human-assisted introductions that compete with native oligochaete species (Eisenhauer et al. 2007; Holdsworth et al. 2007). In terms of genomics, two species are dominating as model oligochaetes. *Lumbricus terrestris* has been the subject of EST studies (see GenBank) and has a completed mtDNA genome (Boore and Brown 2000), and in 2003, Sturzenbaum and colleagues initiated an EST project for the hummus earthworm, *Lumbricus rubellus*.

1.4.3 *Capitella*

Capitella capitata is an excellent biological indicator (Méndez et al. 1998, 2001) that was thought to be a cosmopolitan species dominating polluted marine environments. Allozyme and life history data reveal that *C. capitata* comprises a species complex with little morphological variation (Grassle and Grassle 1976, 1977; Méndez et al. 2000), suggesting that years of environmental data need to be reevaluated. The work by Grassle and Grassle (1976) identified at least two sibling species, designated *Capitella* sp. I and II as ideal laboratory animals for comparative biology. As a result, speciation (e.g. Grassle 1984; Du et al. 2007), ecology (e.g. Tsutsumi et al. 2005; Martin and Bastida 2006), environmental impact (e.g. Cardoso et al. 2007) and toxicology (e.g. Méndez and Green-Ruiz 2006) of *Capitella* species continue to be of great interest. However, in the recent literature, *Capitella* sp. I is one of the best-developed annelid models being used to understanding body plan formation and segmentation (Seaver et al. 2005; Seaver and Kaneshige 2006).

Capitella sp. I was one of the first lophotrochozoan representatives for which whole genomic sequence was obtained (completed by the Joint Genome Institute).

1.4.4 *Platynereis dumerilii*

Dumeril's clam worm, *P. dumerilii*, is a marine annelid that has been the subject of considerable study. In general, when most think of a polychaete worm, they typically envision members of the family Nereididae (e.g. *Platynereis, Nereis*), despite the amazing diversity of forms across annelid lineages. *Platynereis dumerilii* was chosen as another organism well suited for comparative studies in development (Fischer and Dorresteijn 2004). The study of genes responsible in eye development and photoreception (Arendt et al. 2002) as well as segmentation (e.g. Prud'homme et al. 2003; Kulakova et al. 2007) in *P. dumerilii* has expanded our knowledge of developmental mechanism across bilatarians. Furthermore, *P. dumerilii* has been amendable to culturing in the lab, helping it emerge as a leading annelid model system, especially among European researchers. Arguments that this worm serves as an ancestral or basal model within annelids (Tessmar-Raible and Arendt 2003) are problematic as this position is not supported by molecular (Rousset et al. 2007; Struck et al. 2007) or morphological (Rouse and Pleijel 2001) data. To the contrary, nereidids seem highly derived within Phyllodocid worms.

1.4.5 *Ophryotrocha* sp.

Ophryotrocha species (Dorvilleidae) have been a laboratory model in comparative biology since the late 19th century. Our understanding of *Ophryotrocha* biology has resulted from comparative studies of several different species. Of the 40 described species, *Ophryotrocha labronica, Ophryotrocha puerilis* and *Ophryotrocha diadema* have been heavily studied, particularly with respect to reproduction. *Ophrytrocha* exhibits a diversity of reproductive modes including gonochorism, and both simultaneous and sequential hermaphroditism, which have facilitated comparative studies of reproductive biology (Massamba N'Siala et al. 2006; Prevedelli et al. 2006). *Ophyotrocha* species have also been important in ecology (e.g. Åkesson 1976a, b; Cassai and Prevedelli 1999), behavior (e.g. Schleicherova et al. 2006; Lorenzi et al. 2006), development (e.g. Åkesson 1973; Jacobsohn 1999) and toxicology (e.g. Åkesson 1970; Lee et al. 2006). With respect to the phylogenetic relationships of *Ophryotrocha* species, recent work based on mitochondrial 16S (Dahlgren et al. 2001) in combination with cytochrome c oxidase subunit I (Heggoy et al. 2007) remains incongruent with morphology (Pleijel and Eide 1996) and warrants further study.

1.5 OTHER POTENTIAL ANNELID MODELS

Several other annelids have also been the subject of considerable study and are worthy of mention here. However, a goal of this chapter is not to provide an exhaustive list, but rather to provide insight as to why some species may be emerging as models. Three additional species have been the subject of considerable evolutionary developmental work. These include the serpulid *Hydroides elegans* (Seaver et al. 2005; Seaver and Kaneshige 2006; Arenas-Mena 2007), the chaetopterid *Chaetopterus variopedatus* (Irvine et al. 1997; Irvine and Martindale 2001; Potenza et al. 2003) and the oligocheates *Tubifex tubifex*

(Oyama and Shimizu 2007) and *Paranais litoralis* (Bely 1999). Taxonomic issues concerning identification are certainly problematic for *H. elegans* and *C. variopedatus*, the latter of which has clear tagmosis of the body. *Paranais litoralis* is particularly interesting because of its regenerative properties. *Tubifex tubifex*, a freshwater oligochaete, has been used largely as a food source for cultured animals and as a bioindicator.

Annelids have been playing important roles in comparative biology, and although a single model system for Annelida may not be possible, the emergence of multiple annelid species as models provides a greater advantage in establishing a comparative evolutionary framework for a comprehensive understanding of annelid and lophotrochozoan evolution.

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REFERENCES

ÅKESSON, B. 1970. *Ophryotrocha labronica* as test animal for the study of marine pollution. Helgolander Wiss. Meeresunters. 20, 293–303.

ÅKESSON, B. 1973. Reproduction and larval morphology of five *Ophryotrocha* species (Polychaeta, Dorvilleidae). Zool. Scr. 2, 145–155.

ÅKESSON, B. 1976a. Temperature and life-cycle in *Ophryotrocha labronica* (Polychaeta, Dorvilleidae). Ophelia 15, 37–47.

ÅKESSON, B. 1976b. Morphology and life-cycle of *Ophryotrocha diadema*, a new polychaete species from California. Ophelia 15, 23–35.

ARENAS-MENA, C. 2007. Sinistral equal-size spiral cleavage of the indirectly developing polychaete *Hydroides elegans*. Dev. Dyn. 236, 1611–1622.

ARENDT D, TESSMAR K, MEDEIROS DE CAMPOS-BAPTISTA, M., DORRESTEIJN, A., WITTBRODT, J. 2002. Development of pigment-cup eyes in the polychaete *Platynereis dumerilii* and evolutionary conservation of larval eyes in Bilateria. Development 129, 1143–1154.

BAKER, M.W., MACAGNO, E.R. 2001. Neuronal growth and target recognition: lessons from the leech. Can. J. Zool. 79, 204–217.

BELY, A.E. 1999. Decoupling of fission and regenerative capabilities in an asexual oligochaete. Hydrobiologia 406, 243–251.

BELY, A.E., WEISBLAT, D.A. 2006. Lessons from leeches: a call for DNA barcoding in the lab. Evol. Dev. 8, 491–501.

BLEIDORN, C., VOGT, L., BARTOLOMAEUS, T. 2003. New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences. Mol. Phylogenet. Evol. 29, 279–288.

BLEIDORN, C., EECKHAUT, I., PODSIADLOWSKI, L., SCHULT, N., McHUGH, D., HALANYCH, K.M., MILINKOVITCH, M.C., TIEDEMANN, R. 2007. Mitochondrial genome and nuclear sequence data support Myzostomida as part of the annelid radiation. Mol. Biol. Evol. 24, 1690–1701.

BOORE, J.L., BROWN, W.M. 2000. Sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17, 87–106.

BRODFUEHRER, P.D., McCORMICK, K., TAPYRIK, L., ALBANO, A.M., GRAYBEAL, C. 2008. Activation of two forms of locomotion by a previously identified trigger interneuron for swimming in the medicinal leech. Invert. Neurosci. 8, 31–39.

BRUCE, A.E.E., SHANKLAND, M. 1998. Expression of the head gene Lox22-Otx in the leech *Helobdella* and the origin of the bilateral body plan. Dev. Biol. 201, 101–112.

CARDOSO, P.G., BANKOVIC, M., RAFFAELLI, D., PARDAL, M.A. 2007. Polychaete assemblages as indicators of habitat recovery in a temperate estuary under eutrophication. Estuar. Coast. Shelf Sci. 71, 301–308.

CASSAI, C., PREVEDELLI, D. 1999. Fecundity and reproductive effort in *Ophryotrocha labronica* (Polychaeta: Dorvilleidae). Mar. Biol. 133, 489–494.

DAHLGREN, T.G., ÅKESSON, B., SCHANDER, C., HALANYCH, K.M., SUNDBERG, P. 2001. Molecular phylogeny of the model annelid *Ophryotrocha*. Biol. Bull. 201, 193–203.

DU, H., HAN. J., LIN. K., QU. X., WANG. W. 2007. Characterization of 11 microsatellite loci derived from genomic sequences of polychaete *Capitella capitata* complex. Mol. Ecol. Notes 7, 1144–1146.

DUNN, C.W., HEJNOL, A., MATUS, D.Q., PANG, K., BROWNE, W.E., SMITH, S.A., SEAVER, E.C., ROUSE, G.W., OBST, M., EDGECOMBE, G.D., SØRENSEN, M.V., HADDOCK, S.H.D., SCHMIDT-RHAESA, A., OKUSU, A., KRISTENSEN, R.M., WHEELER, W.C., MARTINDALE, M.Q., GIRIBET, G. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452, 745–749.

EISENHAUER, N., PARTSCH, S., PARKINSON, D., SCHEU, S. 2007. Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biol. Biochem. 39, 1099–1110.

FISCHER, A., DORRESTEIJN, A. 2004. The polychaete *Platynereis dumerilii* (Annelida): a laboratory animal with spiralian cleavage, lifelong segment proliferation and a mixed benthic/pelagic life cycle. Bioessays 26, 314–325.

FRENCH, K.A., CHANG, J., REYNOLDS, S., GONZALEZ, R., KRISTAN, W.B., KRISTAN, W.B. 2005. Development of swimming in the medicinal leech, the gradual acquisition of a behavior. J. Comp. Phys. A Sens. Neur. Behav. Physiol. 191, 813–821.

GIRIBET, G., DUNN, C.W., EDGECOMBE, G.D., ROUSE, G.W. 2007. A modern look at the animal tree of life. Zootaxa 1668, 61–79.

GRASSLE, J.P., GRASSLE, J.F. 1976. Sibling species in the marine pollution indicator *Capitella* (Polychaeta). Science 4239, 567–569.

GRASSLE, J.F., GRASSLE, J.P. 1977. *Temporal Adaptations in Sibling Species of* Capitella in *Ecology of Marine Benthos*. Belle W. Baruch Library in Marine Science, vol. 6, edited by B.C. Coull. University of South Carolina Press, Columbia, South Carolina, pp. 177–189.

GRASSLE, J.P. 1984. Speciation in the genus *Capitella* (Polychaeta, Capitellidae). Prog. Zool. 29, 293–298.

HAGADORN, I.R. 1966. The histochemistry of the neurosecretory system in *Hirudo medicinalis*. Gen. Comp. Endocrin. 6, 288–294.

HALANYCH, K.M. 1998. Considerations for reconstructing metazoan history: signal, resolution, and hypothesis testing. Am. Zool. 38, 929–941.

HALANYCH, K.M. 2004. The new view of animal phylogeny. Ann. Rev. Ecol. Evol. Sys. 35, 229–256.

HALANYCH, K.M., DAHLGREN, T.G., MCHUGH, D. 2002. Unsegmented annelids? Possible origins of four lophotrochozoan worm taxa. Int. Comp. Biol. 42, 678–684.

HARVEY, R.P., DEGRYSE, E., STEFANI, L., SCHAMBER, F., CAZENAVE, J.P., COURTNEY, M., TOLSTOSHEV, P., LECOCQ, J.P. 1986. Cloning and expression of a cDNA coding for the anticoagulant from the bloodsucking leech, *Hirudo medicinalis*. Proc. Natl. Acad. Sci. Biol. Sci. 83, 1084–1088.

HEGGOY, K.K., SCHANDER, C., AKESSON, B. 2007. The phylogeny of the annelid genus *Ophryotrocha* (Dorvilleidae). Mar. Biol. Res. 3, 412–420.

HOLDSWORTH, A.R., FRELICH, L.E., REICH, P.B. 2007. Effects of earthworm invasion on plant species richness in northern hardwood forests. Cons. Biol. 21, 997–1008.

IRVINE, S.Q., MARTINDALE, M.Q. 2001. Comparative analysis of *Hox* gene expression in the polychaete *Chaetopterus*: implications for the evolution of body plan regionalization. Am. Zool. 41, 640–651.

IRVINE, S.Q., SEAVER, E.C. 2006. Early annelid development, a molecular perspective. In *Reproductive Biology and Phylogeny of Annelida*, vol. 4, edited by G.W. Rouse and F. Pleijel. Enfield, New Hampshire, Science Publishers, pp. 93–140.

IRVINE, S.Q., WARINNER, S.A., HUNTER, J.D., MARTINDALE, M.Q. 1997. A survey of homeobox genes in *Chaetopterus variopedatus* and analysis of polychaete homeodomains. Mol. Phylogenet. Evol. 7, 331–345.

JACOBSOHN, S. 1999. Characterization of novel F-actin envelopes surrounding nuclei during cleavage of a polychaete worm. Int. J. Dev. Biol. 43, 19–26.

KANG, D., HUANG, F., LI, D., SHANKLAND, M., GAFFIELD, W., WEISBLAT, D.A. 2003. A hedgehog homolog regulates gut formation in leech (*Helobdella*). Development 130, 1645–1657.

KULAKOVA, M., BAKALENKO, N., NOVIKOVA, E., COOK, C.E., ELISEEVA, E., STEINMETZ, P.R.H., KOSTYUCHENKO, R.P., DONDUA, A., ARENDT, D., AKAM, M., ANDREEVA, T. 2007. *Hox* gene expression in larval development of the polychaetes *Nereis virens* and *Platynereis dumerilii* (Annelida, Lophotrochozoa). Dev. Genes Evol. 217, 39–54.

KUO, D., SHANKLAND, M. 2004. Evolutionary diversification of specification mechanisms within the O/P equivalence group of the leech genus *Helobdella*. Development 131, 5859–5869.

LEE, H.W., BAILEY-BROCK, J.H., MCGURR, M.M. 2006. Temporal changes in the polychaete infaunal community surrounding a Hawaiian mariculture operation. Mar. Ecol. Prog. Ser. 307, 175–185.

LORENZI, M.C., SCHLEICHEROVA, D., SELLA, G. 2006. Life history and sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*: the role of sperm competition. Int. Comp. Biol. 46, 381–389.

MCHUGH, D. 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. Proc. Natl. Acad. Sci. U.S.A. 94, 8006–8009.

MCPHIE, D.L., MILLER, M.W. 2006. Virtual symposium: marine invertebrate models of learning and memory. Biol. Bull. 210, 170–173.

MAHMOUD, H.M. 2007. Earthworm (*Lumbricus terrestris*) as indicator of heavy metals in soils. J. Vet. Res. 11, 23–37.

MAO, S.J.T., YATES, M.T., OWEN, T.J., KRSTENANSKY, J.L. 1988. Interaction of *hirudin* with *thrombin*: identification of a minimal binding domain of *hirudin* that inhibits clotting activity. Biochemistry 27, 8170–8173.

MARTIN, J.P., BASTIDA, R. 2006. Life history and production of *Capitella capitata* (Capitellidae: Polychaeta) in Rio de la Plata estuary (Argentina). Thalassas 22, 25–38.

MARTINDALE, M.Q. 2005. The evolution of metazoan axial properties. Nature Rev. Gen. 6, 917–927.

MASSAMBA N'SIALA, G., PREVEDELLI, D., SIMONINI, R. 2006. Gonochorism versus hermaphroditism: relationship between sexuality and fitness in the genus *Ophryotrocha*

(Polychaeta). Biologia Marina Mediterranea 13, 1104–1108.

Méndez, N., Green-Ruiz, C. 2006. Cadmium and copper effects on larval development and mortality of the polychaete *Capitella* sp. Y from Estero del Yugo, Mazatlan, Mexico. Water Air Soil Pollut. 171, 291–299.

Méndez, N., Flos, J., Romero, J. 1998. Littoral soft-bottom polychaete communities in a pollution gradient in front of Barcelona (western Mediterranean, Spain). Bull. Mar. Sci. 63, 167–178.

Méndez, N., Linke-Gamenick, I., Forbes, V.E. 2000. Variability in reproductive mode and larval development within the *Capitella capitata* species complex. Invert. Reprod. Dev. 38, 131–142.

Méndez, N., Linke-Gamenick, I., Forbes, V.E., Baird, D.J. 2001. Sediment processing in *Capitella* spp. (Polychaeta: Capitellidae): strain-specific differences and effects of the organic toxicant fluoranthene. Mar. Biol. 138, 311–319.

Nielsen, C. 2004. Trochophora larvae: cell-lineages, ciliary bands, and body regions. 1. Annelida and Mollusca. J. Exp. Zool. 302B, 35–68.

Orevi, M., Eldor, A., Giguzin, I., Rigbi, M. 2000. Jaw anatomy of the blood-sucking leeches, Hirudinea *Limnatis nilotica* and *Hirudo medicinalis*, and its relationship to their feeding habits. J. Zool. 250, 121–127.

Orrhage, L. 1973. Light and electron microscope studies of some brachiopod and pogonophoran setae; with a discussion of the 'annelid seta' as a phylogenetic-systematic character. Zeitschrift Morph. Tiere 74, 253–270.

Oyama, A., Shimizu, T. 2007. Transient occurrence of vasa-expressing cells in nongenital segments during embryonic development in the oligochaete annelid *Tubifex tubifex*. Dev. Genes Evol. 217, 675–690.

Passamaneck, Y., Halanych, K.M. 2006. Lophotrochozoan phylogeny assessed with LSU and SSU data: evidence of lophophorate polyphyly. Mol. Phylogenet. Evol. 40, 20–28.

Philippe, H., Lartillot, N., Brinkmann, H. 2005. Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. Mol. Biol. Evol. 22, 1246–1253.

Pleijel, F., Eide, R. 1996. The phylogeny of *Ophryotrocha* (Dorvilleidae: Eunicida: Polychaeta). J. Nat. Hist. 30, 647–659.

Potenza, N., del Gaudio, R., Chiusano, M.L., Russo, G.M.R., Geraci, G. 2003. Specificity of cellular expression of *C. variopedatus* polychaete innexin in the developing embryo: evolutionary aspects of innexins' heterogeneous gene structures. J. Mol. Evol. 57, S165–S173.

Prevedelli, D., N'Siala, G.M., Simonini, R. 2006. Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. J. An. Ecol. 75, 203–212.

Prud'homme, B., de Rosa, R., Arendt, D., Julien, J., Pajaziti, R., Dorresteijn, A.W.C., Adoutte, A., Wittbrodt, J., Balavoine, G. 2003. Arthropod-like expression patterns of engrailed and wingless in the annelid *Platynereis dumerilii* suggest a role in segment formation. Curr. Biol. 13, 1876–1881.

Rados, C. 2004. Beyond bloodletting: FDA gives leeches a medical makeover. FDA Consumer Magazine, p. 38.

Rokas, A., Krueger, D., Carroll, S.B. 2005. Animal evolution and the molecular signature of radiations compressed in time. Science 310, 1933–1938.

Rouse, G.W., Pleijel, F. 2001. *Polychaetes*. Oxford: University Press.

Rousset, V., Pleijel, F., Rouse, G.W., Erseus, C., Siddall, M.E. 2006. A molecular phylogeny of annelids. Cladistics 23, 41–63.

Satoh, N., Satou, Y., Davidson, B., Levine, M. 2003. *Ciona intestinalis*: an emerging model for whole-genome analyses. Trends Genet. 19, 376–381.

Sawyer, R.T. 1986. *Leech Biology and Behaviour*. Oxford: Clarendon Press, pp. 1–418.

Schleicherova, D., Lorenzi, M.C., Sella, G. 2006. How outcrossing hermaphrodites sense the presence of conspecifics and suppress female allocation. Behav. Ecol. 17, 1–5.

Seaver, E.C. 2003. Segmentation: mono- or polyphyletic? Int. J. Dev. Biol. 47, 583–595.

Seaver, E.C., Kaneshige, L.M. 2006. Expression of segmentation genes during larval and juvenile development in the polychaetes *Capitella* sp. I and *H. elegans*. Dev. Biol. 289, 179–194.

Seaver, E.C., Thamm, K., Hill, S.D. 2005. Growth patterns during segmentation in the two polychaete annelids, *Capitella* sp. I, *Hydroides elegans*: comparisons at distinct life history stages. Evol. Dev. 7, 312–326.

Shankland, M. 1991. Leech segmentation: cell lineage and the formation of complex body patterns. Evol. Dev. 144, 221–231.

Siddall, M.E., Trontelj, P., Utevsky, S.Y., Nkamany, M., Macdonald, K.S. 2007. Diverse molecular data demonstrate that commercially available medicinal leeches are not *Hirudo medicinalis*. Proc. Royal Soc. Biol. Sci. Ser. B. 274, 1481–1487.

Struck, T.H., Fisse, F. 2007. Phylogenetic position of nemertea derived from phylogenomic data. Mol. Biol. Evol. 25, 728–736.

Struck, T.H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., Halanych, K.M. 2007. Annelid phylogeny and the status of Sipuncula and Echiura. BMC Evol. Biol. 7, 1–11.

Sturzenbaum, S.R., Parkinson, J., Blaxter, M., Morgan, A.J., Kille, P., Georgiev, O. 2003. The earthworm Expressed Sequence Tag project. Pedobiologia 47, 447–451.

Telford, M.J., Bourlat, S.J., Economou, A., Papillon, D., Rota-Stabelli, O. 2008. The evolution of the Ecdysozoa. Philos. Trans. R. Soc. Lond. Ser. B. 363, 1529–1537.

Tessmar-Raible, K., Arendt, D. 2003. Emerging systems: between vertebrates and arthropods, the Lophotrochozoa. Curr. Op. Gen. Dev. 13, 331–340.

Trontelj, P., Utevsky, S.Y. 2005. Celebrity with a neglected taxonomy: molecular systematics of the medic-

inal leech (genus *Hirudo*). Mol. Phylogenet. Evol. 34, 616–624.

TSUTSUMI, H., TANIGUCHI, A., SAKAMOTO, N. 2005. Feeding and burrowing behaviors of a deposit-feeding capitelid polychaete, *Capitella* sp. I. Benthos Res. 60, 51–58.

VENKITARAMANI, D.V., WANG, D., JI, Y., XU, Y., PONGUTA, L., BOCK, K., ZIPSER, B., JELLIES, J., JOHANSEN, K.M., JOHANSEN, J. 2004. Leech *filamin* and *tractin*: markers for muscle development and nerve formation. J. Neurobiol. 60, 369–380.

WEISBLAT, D.A., HUANG, F.Z. 2001. An overview of glossiphoniid leech development. Can. J. Zool. 79, 218–232.

WEISBLAT, D.A., SHANKLAND, M. 1985. Cell lineage and segmentation in the leech. Philos. Trans. R. Soc. Lond. Ser. B. 312, 39–56.

WRIGHT, M.A., STRINGER, A. 1974. The toxicity of thiabendazole, benomyl, methyl benzimidazol-2-yl carbamate and thiophanate-methyl to the earthworm, *Lumbricus terrestris*. Pesticide Sci. 4, 431–432.