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Current status of annelid phylogeny

Anne Weigert[1] · Christoph Bleidorn[1,2]

Abstract Annelida is an ecologically and morphologically diverse phylum within the Lophotrochozoa whose members occupy a wide range of environments and show diverse life styles. The phylogeny of this group comprising more than 17, 000 species remained controversial for a long time. By using next-generation sequencing and phylogenomic analyses of huge data matrices, it was finally possible to reach a well-supported and resolved annelid backbone tree. Most annelid diversity is comprised in two reciprocal monophyletic groups, Sedentaria and Errantia, which are named after the predominant life style of their members. Errantia include Aciculata (Phyllodocida + Eunicida) and Protodriliformia, which is a taxon of interstitial polychaetes. Sedentaria comprise most of the polychaete families formerly classified as Canalipalpata or Scolecida, as well as the Clitellata. Six taxa branch as a basal grade outside of this major radiation: Oweniidae, Magelonidae, Chaetopteridae, Sipuncula, Amphinomida, and *Lobatocerebrum*. Oweniidae and Magelonidae form a monophyletic group which we name Palaeoannelida, which constitutes the sister taxon of the remaining annelids. The early splits of annelid phylogeny date back to the Cambrian. The new annelid phylogeny highlights the variability and lability of annelid body plans, and many instances of simplifications of body plan as adaptations to new life styles can be found. Therefore, annelids will be an appropriate model to understand major transitions in the evolution of Bilateria in general. Evolutionary developmental studies are one way to investigate macroevolutionary transition in annelids. We briefly summarize the state of developmental model organisms in Annelida and also propose new candidates on the background of the phylogeny.

Keywords Annelida · Evo-devo · Evolution · Model organism · Phylogenomics · Polychaeta

Annelid phylogeny—a historical perspective

Annelida, commonly referred to as segmented or ringed worms, is an ecologically diverse phylum within the Lophotrochozoa whose members occupy a wide range of environments including terrestrial, limnic, and marine habitats (Rouse and Pleijel 2001; Aguado et al. 2014). Several species are highly specialized to distinct ecological niches such as intertidal and pelagic zones or hydrothermal vents. In addition, annelids exhibit a huge diversity of body and larval forms, life modes, and feeding strategies and possess true segmentation, which is otherwise found only in Arthropoda and Chordata (Seaver 2003). The combination of these features makes Annelida a subject of intense phylogenetic research to understand their evolution. Up to date, around 17, 000 described species are recognized (Zhang 2011), but their overall systematics were unclear in the past. Formerly, the Annelida were classified into four groups: Archiannelida, Polychaeta, Oligochaeta, and Hirudinea (Grube 1850; Hatschek 1878; Lamarck 1818). Additionally, several invertebrate taxa like Echiura, Pogonophora, Myzostomida, and Sipuncula were regarded as closely related to Annelida, but

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mainly considered as own separate phyla, and therefore excluded from the annelid radiation (Graff 1877; Johansson 1937; Newby 1940). The classification of annelids has changed drastically many times, resulting in competing hypotheses based on different character sets. Here, we summarize the main changes and developments in the phylogenetic history of Annelida.

Morphology-based classifications

Polychaete phylogeny: A problem in protostome evolution was the title of a review article by Kristian Fauchald published in 1974, summarizing the history and foreshadowing future problems of reconstructing the phylogeny of Annelida (Fauchald 1974). We will briefly summarize morphology-based classifications concentrating on the two main groups which have been distinguished: polychaetes and clitellates. For more detailed reviews please refer to Struck (2013b and Fauchald and Rouse (1997).

Polychaeta

The Polychaeta or bristle worms comprise most of the diversity of annelids. They mostly live in marine habitats and are characterized by parapodia-bearing chitinous chaetae, and were first separated from clitellate annelids by Grube (1850). The most widely used system of polychaete classification during the twentieth century was the division into two groups, mainly based on the observed lifestyle and feeding strategies (Quatrefages 1865; Fauvel 1923): Errantia, uniting the more or less free moving and predatory forms, and Sedentaria, comprising the often sessile and tube-dwelling forms. This classification did not intend to depict evolutionary relationships and was replaced by the first thorough morphological cladistic analysis by Rouse and Fauchald (1997), who subdivided Polychaeta into two separate taxonomic clades: Palpata and Scolecida (Fig. 1). The Palpata were characterized by the possession of palps and were further subdivided into two distinct groups: Canalipalpata, which feature the presence of peristomial grooved palps and include mainly burrowing deposit feeders or sessile tube dwelling filter feeders (Sabellida, Spionida, Terebellida), and Aciculata, which are characterized by the presence of aciculae (internalized supporting chaetae) and include members with a more mobile life style, actively foraging and predating (Eunicida, Phyllodocida). The Aciculata resembled more or less the Errantia adapted by Fauvel (1923). The Scolecida completely lack palps and head appendages and include mostly burrowing and deposit feeding taxa, as, e.g., Orbiniidae, Arenicolidae, or Opheliidae. A group of difficult to place polychaete worms were the "Archiannelida". They comprise a number of interstitial worms, which are characterized by the lack of body or head appendages, which is regarded as an adaptation to the interstitium (Hermans 1969). Formerly, they were considered as a monophyletic group within Annelida, either as part of Polychaeta or forming the basal radiation of annelids, or even their own phylum, and included five families: Dinophilidae, Nerillidae, Polygordiidae, Protodrilidae, and Saccocirridae (Westheide 2008). However, during the twentieth century this hypothesis of a common ancestor of the interstitial annelids remained controversial. Consequently the "Archiannelida" were regarded as an artificial grouping with an independent adaptation to the interstitium of several members leading to a simplified body plan either by miniaturization or progenetic evolution (Fauchald 1977; Purschke and Jouin 1988).

Clitellata

Characterized by the presence of a cocoon-forming clitellum during part of their life cycles, hermaphroditism, and the absence of parapodia, the clitellates traditionally comprised the Oligochaeta, possessing a few chaetae, and leeches (Hirudinea), which completely lack chaeta (Brusca and Brusca 2003). According to their simplified appearance they were regarded as a separate class from other annelids and their monophyly was convincingly supported (Grube 1850; Dales 1963; Fauchald 1977; Rouse and Fauchald 1997).

Molecular phylogenetics of Annelida

By the end of the twentieth century, the first molecular phylogenetic analyses focusing on annelid relationships were published. A short overview of the development and achievements of annelid molecular phylogenetics follows.

Single gene analyses

The first molecular studies including annelid taxa usually analyzed 18S rRNA or elongation factor 1α gene sequences (Winnepenninckx et al. 1995; Kim et al. 1996; McHugh 1997; Kojima 1998; Winnepenninckx et al. 1998; Eeckhaut et al. 2000; McHugh 2000; Struck et al. 2002a; Bleidorn et al. 2003a, b). At first, the availability of data was increasing slowly and taxon sampling was low, covering only a fraction of the annelid diversity. Higher ranked polychaete taxa as postulated by Rouse and Fauchald (1997) (Aciculata, Canalipalpata, Scolecida), or even Annelida and Polychaeta could not be recovered as monophyletic, since outgroups were branching between annelid taxa. Furthermore, the resolution of relationships among families was poor and highly inconsistent between analyses. Whereas the monophyly of many annelid families could be corroborated, the relationships among them remained largely obscure (Bleidorn 2009). Currently, molecular data for the 18S rRNA is available for almost all major annelid families, but unfortunately this marker alone

Fig. 1 Annelid phylogeny
according to Rouse and Fauchald
(1997) based on morphology.
Annelida are split into Polychaeta
and Clitellata. Polychaeta com-
prise Palpata (Canalipalpata and
Aciculata) and Scolecida
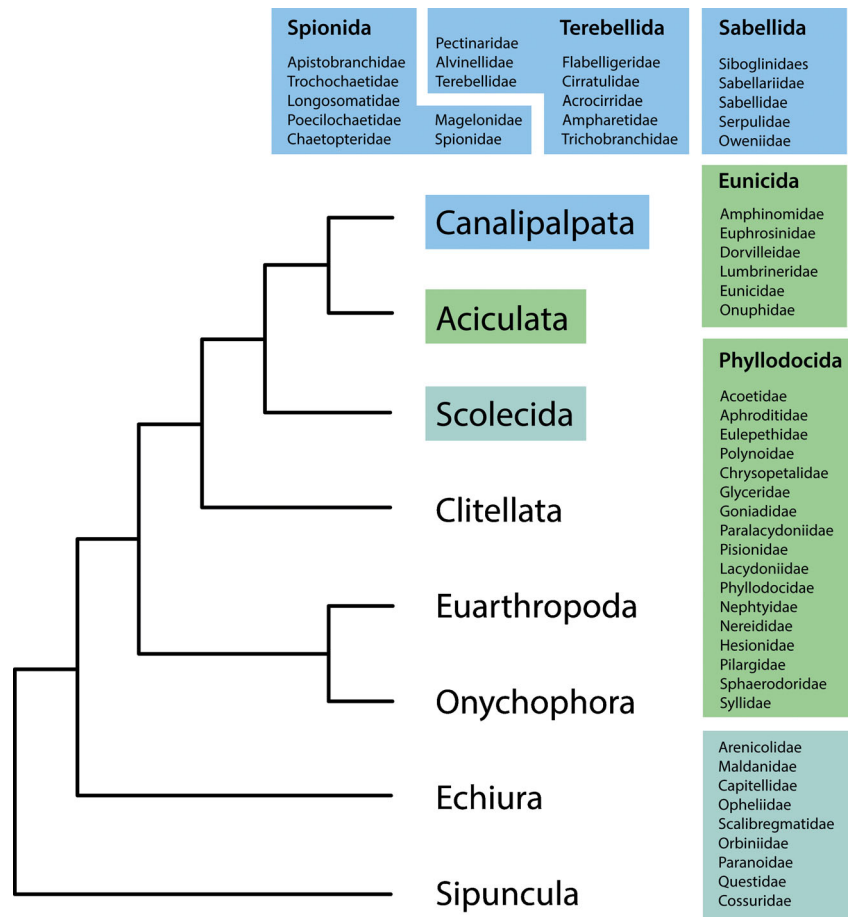
has proven to be insufficient for resolving annelid relationships, especially for deeper nodes. However, the incorporation of molecular data provided substantial evidence for the inclusion of Echiura and Siboglinidae within Annelida and additional support for the hypothesis that Clitellata should be nested within polychaetes (McHugh 2000; Bleidorn et al. 2003b). Moreover, the in-group relationships of many annelid families could be resolved with such an approach (Bleidorn 2009).

Multiple genes and combination of morphological and molecular markers

As molecular techniques became more accessible, the coverage of molecular data for 18S rRNA and elongation factor 1α increased and additional genes (28S rRNA, 16S rRNA, histone H3, U2 snRNA ,and cytochrome oxidase I) were incorporated in larger studies to achieve a higher resolution and consistent family relationships (Brown et al. 1999; Zrzavý 2001; Rousset et al. 2004; Struck and Purschke 2005; Colgan et al. 2006; Struck et al. 2007; Rousset et al. 2007; Struck et al. 2008; Worsaae and Rouse 2008; Zrzavy et al. 2009). The benefit of multiple genetic markers and (in some

instances) their combination with morphological characters was an increase of the recovery of several monophyletic annelid groups (e.g., Eunicida, Terebellida) and to some degree increase of nodal support. Although the position of Echiura and Siboglinidae within Annelida could be confirmed as well as the inclusion of Clitellata within polychaetes, Annelida are consistently not supported as a monophyletic group, as various other lophotrochozoan taxa are scattered between them. In the analysis conducted by Struck et al. (2007), monophyletic annelids were recovered for the first time with a larger taxon sampling, but the support was weak, especially for the deeper nodes. Additionally, in all studies the phylogenetic assignment of certain groups with putative annelid affinities (e.g., Myzostomida, Diurodrilidae, Sipuncula) was still problematic and the root of the annelid tree remained unknown (Rouse and Pleijel 2006).

Mitochondrial genomics

The analyses of complete mitochondrial genomes and comparison of their gene arrangements became a powerful tool for solving phylogenetic questions. As is typical for most animals, annelid mitochondrial genomes are circular molecules,

possess only limited intergenic sequences apart from one large non-coding region, and encode for 13 protein-coding genes, 2 ribosomal RNAs, and 22 transfer RNAs. It was observed that closely related taxa often exhibit an identical order of these genes and that rearrangements may contain phylogenetic signal (Boore and Brown 1994; Boore 1999). Using Sanger sequencing, the analyses of complete mitochondrial genomes, which usually range in size around 16 kbp for annelids, was laborious. However, the advent of next-generation sequencing techniques dramatically increased the speed to recover mitochondrial genomes from whole-genome sequencing approaches (Richter et al. 2015). Over the years, several annelid mitochondrial genomes were completed and their investigation revealed a relatively conserved gene order within annelids in comparison to other lophotrochozoan groups (Bleidorn et al. 2007; Bleidorn et al. 2009b; Mwinyi et al. 2009; Bernt et al. 2013; Boore and Brown 2000; Boore and Staton 2002; Jennings and Halanych 2005; Struck et al. 2007; Shen et al. 2009; Wu et al. 2009; Dordel et al. 2010; Golombek et al. 2013; Li et al. 2015; Richter et al. 2015). A recent study focusing on two Syllidae (Errantia) recovered two highly rearranged gene orders for *Ramisyllis multicaudata* and *Trypanobia cryptica*, suggesting a higher variability in some taxa (Aguado et al. 2015b). Nevertheless, most investigated annelids belonging to Errantia or Sedentaria bear an identical order of protein-coding and ribosomal RNA genes, which allows the reconstruction of a common ground pattern for these taxa. Interestingly, a recent study suggests that several annelids which are now regarded as being placed outside Errantia and Sedentaria (see below) strongly deviate from this pattern: Magelonidae, Oweniidae, Chaetopteridae, Amphinomidae, and Sipuncula (Weigert et al. 2016). Nevertheless, the reconstruction of annelid relationships using mitochondrial sequence data yielded similarly unsatisfying results as observed with multi-gene analyses, particularly in resolving deeper splits. At least in the case of taxa with an uncertain phylogenetic position (e.g., Myzostomida, Diurodrilidae), the application of mitochondrial data provided additional support affiliating them with Annelida (Bleidorn et al. 2007; Golombek et al. 2013).

Transcriptomics using Sanger-based sequencing of expressed sequence tags (ESTs)

To recover robust annelid relationships and elucidate their evolutionary history, further investigations apparently would have to rely on a higher amount of molecular data. Randomly sequenced clones of mRNA transcripts became a way to assemble big datasets for non-model organisms (Dunn et al. 2008). In an extensive study by Struck et al. (2011), a large molecular data set for these so-called expressed sequence tags (ESTs) of annelids was compiled, comprising 230 genes (47, 953 amino acids with a coverage of 42 %) and 34 annelid species (24 polychaete and 5 clitellate families). As a result, a well-supported tree was obtained, dividing monophyletic Annelida into two large subgroups and some early branching species. For the two groups which comprised the main radiation of annelid diversity, Struck et al. (2011) readapted the classification after Quatrefages (1865) and Fauvel (1923) with some modifications and named them Sedentaria and Errantia. The composition of Sedentaria is similar to Canalipalpata + Scolecida proposed by Rouse and Fauchald (1997) with the exclusion of Chaetopteridae and Orbiniidae, but the inclusion of clitellates. The Errantia contain the Aciculata as proposed by Rouse and Fauchald (1997) with the addition of Orbiniidae and the exclusion of amphinomids. The basal branching part of the annelid tree is formed by Sipuncula, Myzostomida, and Chaetopteridae. Despite the notably high support of internal nodes, the deeper nodes still lacked significant support and were influenced by the choice of outgroup taxa. Furthermore, due to the use of Sanger sequencing only low gene coverage per taxon was achieved, thereby introducing high amounts of missing data in the analyzed datasets, and the misassignment of paralogous genes resulted in misplacement of Orbiniidae and Amphinomidae (Kvist and Siddall 2013; Struck 2013a, b).

Annelid phylogeny—state of knowledge

Next-generation sequencing techniques dramatically improved the amount and output speed of sequences, while at the same time being cost-effective (McCormack et al. 2013). Basically, the characterization of complete transcriptomes and at least lowly covered genomes became affordable for non-model organisms and individual research projects. With the help of these technical advancements, a well-resolved and supported annelid phylogeny finally became available (Fig. 2).

The most comprehensive analyses up to date, based on large molecular data sets generated by Illumina sequencing, were conducted by Weigert et al. (2014), Struck et al. (2015), and Andrade et al. (2015), and congruently resulted in a robust backbone of annelid phylogeny (Fig. 2). With the main focus on the basal annelid radiation, Weigert et al. (2014) compiled data sets including up to 622 orthologous genes, increasing the number of amino acid position to 170,497 and gene coverage per taxon to 49 % and included in total 60 annelid species (34 polychaete and 5 clitellate families). Furthermore, crucial taxa which were missing in the previous phylogenomic study by Struck et al. (2011) were added (e.g., Oweniidae and Magelonidae) and groups with low gene coverage and with an uncertain phylogenetic position were represented by more species and more data (Sipuncula, Chaetopteridae, Amphinomidae, and Myzostomida). According to this analysis, the Annelida are divided into the
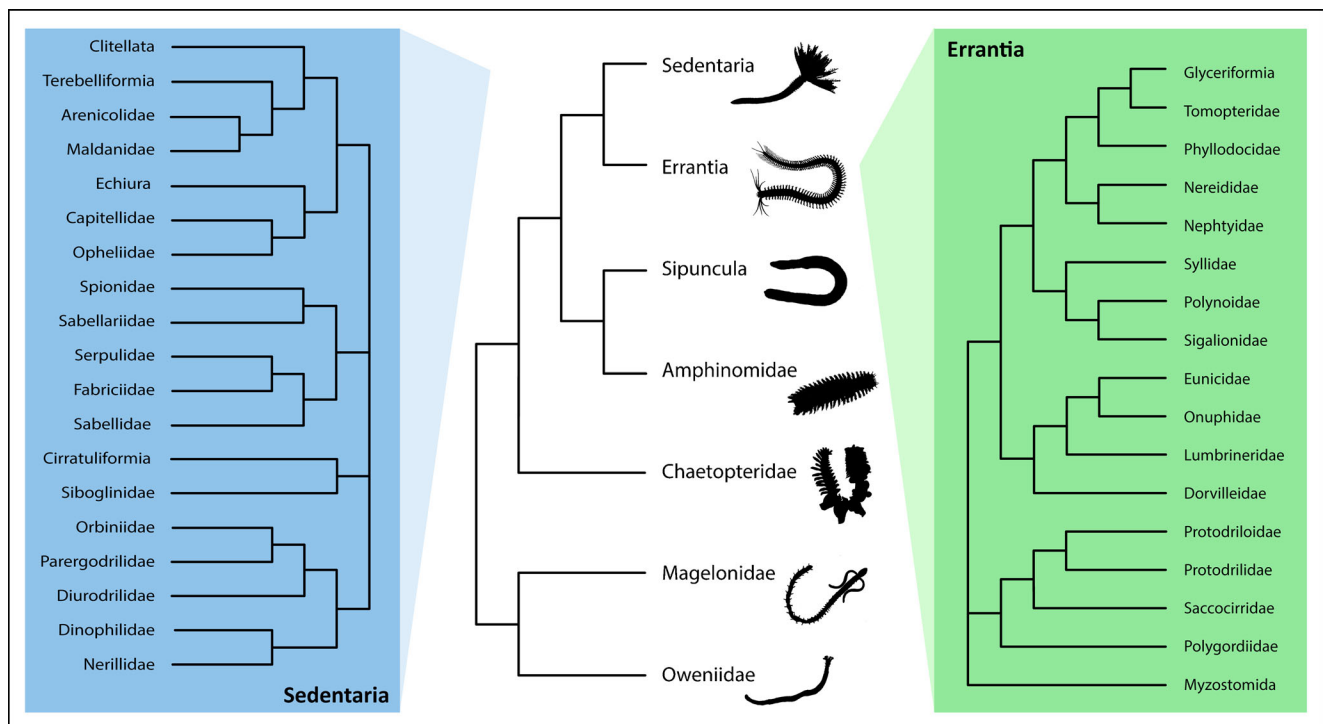
Fig. 2 Annelid relationships based on the current knowledge. The tree combines results from larger phylogenomic studies based on molecular data (Weigert et al. 2014; Struck et al. 2011; Struck et al. 2015). Placements of families which are not covered in these studies were incorporated from other molecular studies if consistent results with identical sister group relationships were recovered (Struck et al. 2007; Struck et al. 2008)

two major clades of Errantia and Sedentaria (together forming Pleistoannelida) and the five basal branching lineages Sipuncula, Amphinomida, Chaetopteridae, Magelonidae, and Oweniidae. Based on the data set from Weigert et al. (2014), Struck et al. (2015) recently investigated the possible existence of an archiannelid clade by adding several interstitial annelid species to the analysis. The largest data set compiled 679 orthologous genes from 80 annelid species, with 189,193 amino acid positions and gene coverage per taxon of 41.2 %. As a result, the monophyly of "Archiannelida" was not supported and former archiannelid taxa grouped within Errantia or Sedentaria.

Finally, Andrade et al. (2015) compiled a completely new dataset for deeply sequenced transcriptomes of annelids. Their taxon sampling is broadly overlapping with that of Weigert et al. (2014) and Struck et al. (2015), but they also added previously unsampled families, e.g., Sternaspidae, Fauveliopsidae, and the symbiotic Sphinteridae. Due to their reliance on deeply sequenced transcriptome libraries they were able to strongly reduce the amount of missing data as compared with previous analyses. Their analyzed data matrices contained up to 1473 orthologous genes comprising ~337,000 amino acid positions, while showing a matrix completeness of >95 %. Analyses using Bayesian Inference and Maximum Likelihood implementing different models largely supported the results of the previous analyses, confirming the composition of taxa

of the basal branching grade of annelids, as well as the monophyly of Sedentaria and Errantia. As noticed in previous analyses, myzostomids form a long branch and are difficult to place in phylogenetic analyses (Bleidorn et al. 2014). The same was found for *Spinther*, a genus of annelids which lives in symbiosis with sponges.

The affiliation of investigated annelid families up to date are summarized below based on Weigert et al. (2014). Insights into *archiannelid relationships* are discussed separately.

Sedentaria

The following taxa are assigned to Sedentaria based on Weigert et al. (2014): Clitellata, Pectinariidae, Alvinellidae, Terebellidae, Arenicolidae, Opheliidae, Echiura, Capitellidae, Spionidae, Sabellariidae, Serpulidae, Sabellidae, Siboglinidae, Acrocirridae, Flabelligeridae, Cirratulidae, and Orbiniida. Moreover, *Spinther*, Fauveliopsidae and Sternaspidae should be further included in Sedentaria according to Andrade et al. (2015). In general, relationships of Sedentaria are well-supported for most nodes and are consistent across analyses including different numbers of genes. Terebellomorpha (including Alvinellidae, Ampharetidae, Pectinaridae, Terebellidae, and Trichobranchidae) and Cirratuliformia (including Acrocirridae, Cirratulidae, Ctenodrilidae, Fauveliopsidae,

Flabelligeridae, and Sternaspidae) appear monophyletic, even though not all members were included, yet. A clade of Terebellida comprising all these taxa (Rouse and Fauchald 1997) could not be recovered. Terebellomorpha are more closely related to Clitellata and Cirratuliformia cluster with Siboglinidae. The taxa Sabellaridae, Serpulidae, Sabellidae, Siboglinidae, and Oweniidae, which were formerly combined in a clade called Sabellida (Rouse and Fauchald 1997) are now dispersed throughout the tree: Oweniidae are part of the basal radiation, Siboglinidae group with Cirratuliformia and Orbiniidae and Sabellariidae are more closely related to Spionidae than to Sabellidae and Serpulidae (Weigert et al. 2014). The Orbiniidae, which were regarded as intermediate forms of errant and sedentary living polychaetes (Fauvel 1927) or part of Errantia (Struck et al. 2011) are firmly placed within Sedentaria. However, the definite position either as sister group to all remaining Sedentaria or to Siboglinidae together with Cirratuliformia depends on the number of characters and the taxon sampling used in phylogenomic analyses and therefore still an open question (Weigert et al. 2014; Struck et al. 2015). For the following families, an affiliation to Sedentaria is likely as they consistently grouped with sedentarian taxa in analyses based on a single or few genes (Struck et al. 2007; Struck et al. 2008): Trichobranchidae (Terebellomorpha), Maldanidae, and Trochochaetidae.

Errantia

The clade Errantia comprises the following taxa based on Weigert et al. (2014): Lumbrineridae, Eunicidae, Onuphidae, Glyceridae, Tomopteridae, Phyllodocidae, Nephtyidae, Nereididae, Sigalionidae, Polynoidae, Syllidae, and Myzostomida. The monophyly of Eunicida (including Lumbrineridae, Eunicidae, and Onuphidae) and Phyllodocida (including Glyceridae, Tomopteridae, Phyllodocidae, Nephtyidae, Nereididae, Sigalionidae, Polynoidae, and Syllidae) is strongly supported in all analyses. The paraphyly of Phyllodocida in analyses conducted by Struck et al. (2011) was mainly due to missing data and the shared paralogous genes of three species (Phyllodocida—*Sthenelais boa*, Orbiniidae—*Scoloplos armiger*, Amphinomida—*Eurythoe complanata*). Amphinomidae, which were formerly assigned to Eunicida (Rouse and Fauchald 1997) or Errantia (Struck et al. 2011; Fauvel 1927) are now excluded from Pleistoannelida and branch off in the basal part of the tree together with Sipuncula. Additionally, Errantia include the interstitial Protodriliformia (see discussion under 2.4), which are regarded as sister taxon to Eunicida + Phyllodocida. The latter two taxa are united as the newly combined Aciculata by Andrade et al. (2015).

The position of Myzostomida remains controversial. By increasing the number of molecular characters in the phylogenetic analyses, their position becomes more stable, switching

from outside of the annelid tree, to within the early branching annelid radiation, to an affiliation with errant annelids. Moreover, mitochondrial gene order comparisons strongly suggest an inclusion within Pleistoannelida, most likely as part of Errantia (Weigert et al. 2016). However, long branch attraction (LBA) artifacts, choice of molecular markers, and outgroups still impede a robust placement in the tree (for review, see Bleidorn et al. (2014)). Additionally, Goniadidae and Aphroditidae are likely part of Errantia as supported in previous analyses (Struck et al. 2007; Struck et al. 2008).

Basal branching lineages

Sipuncula

The peanut worms were traditionally considered as their own phylum and not as part of Annelida (Rouse and Fauchald 1997), due to the absence of segmentation and head and body appendages. Even in the light of new molecular data, their affiliation to annelids was debated. Depending on the molecular markers used, their phylogenetic position switched from derived and deeply nested annelids over early branching annelids to the sister taxon of all annelids (Struck et al. 2007; Sperling et al. 2009; Mwinyi et al. 2009; Dordel et al. 2010; Struck et al. 2011). However, by increasing both the number of data and species, the position of Sipuncula nested within Annelida as sister taxon of Amphinomida received convincingly strong support and a sister group relationship to Annelida can be rejected (Weigert et al. 2014; Andrade et al. 2015).

Amphinomida

The Amphinomida comprise the Amphinomidae and Euphrosinidae (Borda et al. 2015) and were formerly considered as closely related to errant polychaetes: as part of Eunicida within Aciculata (Rouse and Fauchald 1997), as sister group to Eunicida and Phyllodocida within Aciculata (Rouse and Pleijel 2001), or placed with various other annelid taxa (Rousset et al. 2007; Zrzavy et al. 2009), or as a basal branching lineage (Struck et al. 2007; Struck et al. 2008; Hausdorf et al. 2007). Since gene coverage was low in all previous molecular analyses, no stable phylogenetic affiliation of this annelid group could be assessed. Even in larger data sets including numerous genes their position was unstable, affected by erroneous assignments of orthologous genes (Struck et al. 2011; Kvist and Siddall 2013; Struck 2013a, b). In contrast to Struck et al. (2011), who found Amphinomida as part of Errantia together with Eunicida, paraphyletic Phyllodocida and Orbiniidae, Weigert et al. (2014) could circumvent these problems and provide additional strong support for an early branching of amphinomids. Together with the Sipuncula, they form the sister group of

Pleistoannelida (Sedentaria and Errantia), a result which also found strong support in the phylogenomic analysis of Andrade et al. (2015).

Chaetopteridae

The affiliation of the tube dwelling chaetopterids to sedentarian annelids (especially to Spionida and Canalipalpata respectively by Rouse and Fauchald (1997)) was proposed by many researchers and a more basal branching position indicated by different molecular studies was discussed and believed to be an artifact (Struck et al. 2007; Bleidorn et al. 2009b; Zrzavy et al. 2009; Dordel et al. 2010; Eibye-Jacobsen and Vinther 2012). However, the basal branching position became stable and robustly supported once more species and molecular data were incorporated in the analyses (Struck et al. 2011; Weigert et al. 2014; Andrade et al. 2015).

Magelonidae

Like Chaetopteridae, Magelonidae were considered as part of Spionida within Canalipalpata based on morphological characters (Rouse and Fauchald 1997, 1998). The few conducted molecular studies could not substantiate this position, placing them either with outgroups like Brachiopoda and Mollusca or in close relationship to various annelids like Orbiniidae and Scolecida (Bleidorn et al. 2003b; Rousset et al. 2004; Rousset et al. 2007; Zrzavy et al. 2009). In the latest phylogenomic study, a robust placement at the base of the annelid tree together with Oweniidae could be achieved (Weigert et al. 2014). This close relationship is additionally supported by morphological characters like the presence of a monociliated epidermis and lack of nuchal organs (Capa et al. 2012).

Oweniidae

The phylogenetic assessment of Oweniidae had been challenging using both molecular and morphological data (Bartolomaeus 1995; Rouse and Fauchald 1997, 1998; Eeckhaut et al. 2000; Bleidorn et al. 2003a, b; Rousset et al. 2004; Rousset et al. 2007; Struck et al. 2007; Struck et al. 2008; Zrzavy et al. 2009). Oweniidae bear several unusual characteristics, like the presence of a largely intraepidermal nervous system (Bubko and Minichev 1972), the special type of larva (Mitraria) with deuterostome-like features (Smart and Von Dassow 2009), and the possession of monociliated epidermis cells (Gardiner 1978). Based on cladistic analyses of morphological data, they were grouped deeply in the annelid tree in close relationship to Sabellida and Siboglinida within Canalipalpata (Rouse and Fauchald 1998; Fauchald and Rouse 1997). Analyses based on only few molecular markers failed to resolve the position of Oweniidae within the Annelida, placing them either with Apistobranchidae, outgroups, in a basal branching position, or Siboglinidae and other sabellimorphs, but always with low support values (Kojima 1998; Eeckhaut et al. 2000; Rousset et al. 2004; Bleidorn et al. 2003b; Rousset et al. 2007; Struck et al. 2007; Struck et al. 2008; Zrzavy et al. 2009; Struck 2013a, b). As ascertained for other basal branching annelids, increasing the number of genes used in molecular analyses not only improved the reconstruction of their phylogenetic position, but also increased support values resulting in a highly robust phylogeny (Weigert et al. 2014; Andrade et al. 2015). Together with Magelonidae they form the sister group to all remaining annelids, which has been strongly supported in all recent phylogenomic analyses. For an easier communication, we propose the name Palaeoannelida for the taxon comprising Oweniidae and Magelonidae.

Interstitial annelids formerly known as "Archiannelida"

Archiannelids

Morphological as well as molecular studies could not substantiate the hypothesis of the "Archiannelida" and recover them as a monophyletic group (Rouse and Fauchald 1997; Struck et al. 2002a, b; Struck and Purschke 2005; Worsaae and Kristensen 2005; Rousset et al. 2007; Struck et al. 2008; Zrzavy et al. 2009). However, molecular analyses mostly lacked the inclusion of all archiannelid taxa and, due to low gene coverage and number of molecular markers, did not recover relationships between polychaete families. To assess the phylogenetic position of Dinophilidae, Nerillidae, Polygordiidae, Protodrilidae, and Saccocirridae, a phylogenomic framework was needed. This was achieved by Struck et al. (2015), who recovered that these interstitial annelids do not form a monophyletic group, but two monophyla which are robustly placed separately within the annelid tree. The evolution of the first group named Orbiniida is consistent with the hypothesis of a progenetic origin and contains the former archiannelid taxa Diurodrilidae, Dinophilidae, and Nerillidae, as well as the Orbiniidae and Parergodrilidae. Interestingly, the name-giving taxon of this clade (Orbiniidae) includes several lineages which likely evolved by progenesis (Bleidorn et al. 2009a). The second group named Protodriliformia comprises the former archiannelid taxa Protodrilidae, Saccocirridae, and Polygordiidae and likely evolved by gradual transition to smaller size (miniaturization). Since all investigated interstitial annelids group deeply within the annelid tree and are not part of the basal branching lineages, the hypothesis that the "Archiannelida" or interstitial annelids with a simple body organization represent the ancestral annelid condition can be rejected.

Diurodrilus and Lobatocerebrum

The genus *Diurodrilus* currently contains only six described species, and members are characterized as unsegmented, worm-like interstitial organisms, with a flattened body and minute size (Worsaae and Kristensen 2005). Several characteristics support a placement among Annelida, e.g., the ultrastructure of the cuticle. However, *Diurodrilus* lacks typical annelid features such as segmentation, coelomic cavities, chaetae or nuchal organs and furthermore bear some similarities with Micrognathozoa (Lophotrochozoa, Gnathifera), such as the paired trunk ciliophores and the position of the protonephridia (Kristensen and Niilonen 1982; Worsaae and Rouse 2008). Until recently, molecular data was limited and 18S and 28S rRNA data resulted in different phylogenetic positions either with Micrognathozoa or other lophotrochozoan taxa (Worsaae and Rouse 2008). Mitochondrial genome data supported an inclusion within Annelida and placed them either with Orbiniidae or in the basal part of the tree (Golombek et al. 2013). Due to the limited amount of molecular data and the unique morphological features their phylogenetic position could not be assessed, but showed a clear trend towards Annelida (Worsaae and Kristensen 2005; Worsaae and Rouse 2008; Golombek et al. 2013). In the light of new molecular data and the incorporation of *Diurodrilus* within a large phylogenomic framework, the support for an annelid affiliation is substantiated (Struck et al. 2015). As indicated with mitochondrial data, *Diurodrilus* groups within Sedentaria in close relationship with Orbiniidae together with Dinophilidae, Nerillidae, and Parergodrilidae in the newly composed clade Orbiniida (Struck et al. 2015). A close relationship to orbiniids was also recovered in a phylogenomic analysis by Laumer et al. (2015). In summary, these new results settle the discussion and clearly support an annelid origin for *Diurodrilus*. As for other members of Orbiniida, an origin by progenetic evolution may explain the many reductions which have to be assumed for this taxon (e.g., loss of segmentation) (Struck et al. 2015).

Another enigmatic taxon with uncertain affinities is *Lobatocerebrum*, which also lack segmentation and chaetae. Based on the structure of its organ systems, *Lobatocerebrum* had originally been described as an annelid lineage (Rieger 1980, 1981). Recent investigations of the epidermis, nervous and muscular system of *Lobatocerebrum riegeri* using transmission electron and confocal microscopy are in line with this initial assessment (Kerbl et al. 2015). As typical for annelids, a mid-ventral nerve could be found; even though as typical for interstitial groups the overall morphology was not only similar to other interstitial annelids, but also meiofaunal members of Platyhelminthes or Gnathostomulida. However, a placement within annelids was confirmed by a phylogenomic analysis, which groups them as sister taxon of Amphinomida + Sipuncula (Laumer et al. 2015). Interestingly, they do not

group with any of the other interstitial annelid taxa, but rather seem to represent a convergent case of inhabiting this environment.

Sister group of Clitellata

Within the last decades, the view of major annelid relationships has changed substantially. Instead of a sister group relationship of the monophyletic groups Clitellata and Polychaeta, an inclusion of Clitellata within a paraphyletic assemblage of polychaetes became likely. Henceforth, the Clitellata were regarded as a derived annelid taxon with several reductions of typical polychaete features. To gain insight into the loss and gain of these traits during the evolution of Annelida and adaptation to a terrestrial life, this raised the question which of the polychaete species was the closest relative to Clitellata (Purschke et al. 2014). So far, there is no consensus on which taxon might be the sister group of Clitellata. Potential candidate species that have been discussed are *Hrabeiella periglandulata*, *Parergodrilus heideri,* and *Stygocapitella subterranea* (Parergodrilidae), as well as Questidae, and *Aeolosoma* (Aeolosomatidae).

H. periglandulata is one of the only two known non-clitellate annelids which have successfully adapted to the terrestrial environment (Dozsa-Farxas and Schlaghamersky 2013). *H. periglandulata* shares many characteristic traits with clitellates, like hermaphroditism, the lack of parapodia, possession of simple chaeta, and a dorsally positioned pharynx, which are widely regarded as convergently evolved features rather than homologous structures (Purschke 1999, 2003). So far, molecular data are limited and the few studies including 18S rDNA and/or COI and 28S rDNA sequences of *H. periglandulata* support no robust close relationship with Clitellata (Rota et al. 2001; Struck and Purschke 2005; Struck et al. 2008).

The two members of Parergodrilidae have similar features in common with clitellates as *H. periglandulata* (Purschke 1999). *Parergodrilus heideri*, which is the second terrestrial non-clitellate annelid, lacks nuchal organs and *Stygocapitella subterranea*, inhabiting zones at the transition between the marine and terrestrial environment, has displaced them in the body (Purschke 1999). However, a sister group relationship to Clitellata based on molecular data could not be resolved, since studies incorporating only a few genes found a close relationship to Orbiniidae or various other taxa (Struck et al. 2008; T. Struck et al. 2002a, b; Rousset et al. 2007), but a larger phylogenomic approach could further substantiate these results (Struck et al. 2015).

Questidae are a group of interstitial polychaetes that resemble Clitellata in features of their reproductive biology. These gonochoristic marine worms bear a structure termed *clitellum* and their gonads are restricted to some segments in the anterior part. Some authors used this superficial resemblance as a

support for a sister group relationship of questids and clitellates (Almeida et al. 2003). However, the homology of the clitellum-like structure in questids and the clitellum of the Clitellata has been rejected in previous studies (Giere and Erseus 1998; Giere and Riser 1981). By analyzing mitochondrial gene order and a multi-gene dataset, it could be convincingly shown that questids are nested in the polychaete taxon Orbiniidae (Bleidorn et al. 2009a).

Aeolosomatidae are very small and simply organized annelids which mostly inhabit limnitic environments (Hessling and Purschke 2000) and their phylogenetic position is still discussed. They were considered as closely related to Oligochaeta based on several clitellate-like features similar to those of *H. periglandulata* and Parergodrilidae, and were placed either as sister group to Clitellata, or at the base of the Clitellata, or considered as highly derived clitellates (Moon et al. 1996). Nevertheless, morphological investigations of the central nervous system and sense organs support an exclusion of Aeolosomatidae from Clitellata (Hessling and Purschke 2000). Molecular analyses based on a few genes found inconsistent placements, but no support for an inclusion within Clitellata or only weak support for a sister group relationship (Moon et al. 1996; Struck and Purschke 2005; Rousset et al. 2007; Struck et al. 2007; Struck et al. 2008; Zrzavy et al. 2009).

Larger phylogenomic studies found either Echiura together with Capitellidae or a clade comprising Terebelliformia and Arenicolidae as sister group to Clitellata (Struck et al. 2011; Weigert et al. 2014; Struck et al. 2015). However, these analyses did not include some of the species discussed above and further analyses incorporating these taxa in combination with a larger amount of molecular data could resolve the closest relative to Clitellata.

### Naming higher-ranked annelid taxa

The new annelid phylogeny led to many changes regarding the classification of annelids. Annelida have been newly combined to include Sipuncula, Echiura, and Myzostomida. The placement of Clitellata highly derived in the annelid tree basically renders Polychaeta synonymous to Annelida. However, we usually use "*polychaetes*" to distinguish non-clitellate annelids from clitellates, referring to their traditional name. Similar practice is common for paraphyletic groups like "*fishes*", "*reptiles*" or "*crustaceans*": We abandoned the system by Rouse and Fauchald (1997), due to the non-monophyletic nature of their proposed higher ranking taxa. Instead, we classify the majority of annelids in either Errantia or Sedentaria which together were named as Pleistoannelida by Struck (2011). However, Andrade et al. (2015) reject this name due to problems with its original definition. Following Andrade et al. (2015), within Errantia we use the name Aciculata for the monophyletic group containing

Phyllodocida and Eunicida. Sister taxon to Aciculata is Protodriliformia. Within Sedentaria several taxa classified above the family rank seem to be monophyletic, as found for, e.g., Orbiinida, Terebelliformia, or Clitellata. However, increasing the taxon sampling is necessary for a better understanding of Sedentaria phylogeny. Relationships within the basal branching grade of annelids remained unnamed so far. We propose the name Palaeoannelida for the taxon uniting Oweniidae and Magelonidae.

Macroevolution, evo-devo, and annelid model organisms

Is there science after the tree? This question was discussed by Jenner (2014), who emphasized the problems of interpreting phylogenies in terms of macroevolutionary transitions. One way to study macroevolution is by evolutionary developmental biology (evo-devo). Evo-devo investigates the evolution of developmental processes, thereby aiming to understand the genetic background of phenotypic change (Rudel and Sommer 2003). One principle question in the field of evo-devo is how major transitions of body plan evolution of animals can be explained (Jenner 2014; De Robertis 2008). Current issues which are investigated in various animals are the evolution of the nervous system and formation of eyes, pathways involved in early development and gastrulation, or the emergence of segmentation (Martindale and Hejnol 2009; Oakley and Speiser 2015; Arendt et al. 2008; Tautz 2004). To investigate these and further questions a broad range of model species dispersed over the animal tree are needed (Jenner and Wills 2007; Milinkovitch and Tzika 2007). Well-studied model systems are established for Deuterostomia and Ecdysozoa (e.g., *Mus musculus*, *Danio rerio*, *Caenorhabditis elegans*, and *Drosophila melanogaster*). Unfortunately, the third big bilaterian clade, the Lophotrochozoa, is still underrepresented regarding model systems. The choice of a suitable model organism representing Lophotrochozoa is no easy task and is additionally limited by the unresolved phylogenetic relationships between members of this group (Dunn et al. 2014). However, a resolved and solid phylogeny of the chosen animal groups is indeed a prerequisite for comparing the evolution of important signaling pathways or the expression pattern of certain genes (Telford and Budd 2003). Aside from that, the choice of an appropriate model organism is additionally influenced by its cultivation effort in the laboratory, the time span of a generation, the ease of collecting suitable samples and potential genome size (Bolker 1995). In the following, we briefly introduce current annelid models for evo-devo research and propose new candidates for future studies (Fig. 3).

Fig. 3 Current annelid model organisms and new candidate species for evo-devo studies. Sedentaria: **a** *Capitella teleta* (Capitellidae), **b** *Helobdella austinensis* (Glossiphoniidae), courtesy of David A. Weisblat. Errantia: **d** *Platynereis dumerilii* (Nereididae), courtesy of Stephanie Bannister. Basal branching lineages: **c** *Owenia fusiformis* (Oweniidae), **e** *Chaetopterus variopedatus* (Chaetopteridae), courtesy of Alexander Semenov, **f** *Magelona johnstoni* (Magelonidae)
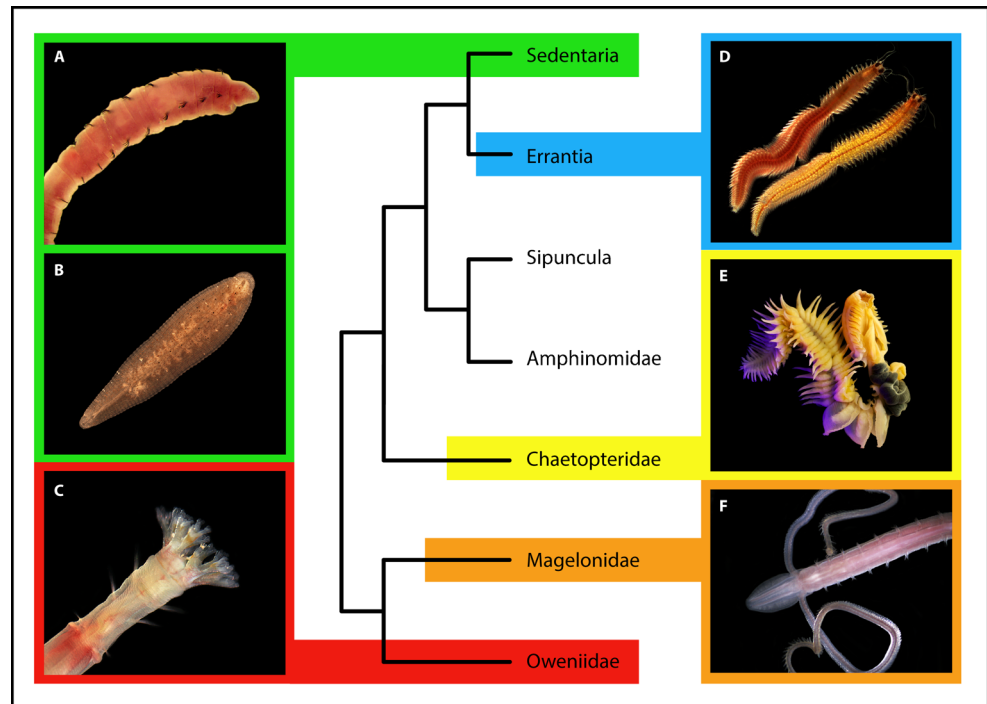
Already established annelid model systems

With *Platynereis dumerilii* within Annelida, there is already the best established lophotrochozoan animal model and several other species have also been investigated to a greater extent (Bleidorn et al. 2015). In the light of recent molecular studies (Weigert et al. (2014); Struck et al. 2015; Andrade et al. 2015), which improved the resolution of the tree and provided a robust annelid phylogeny, the selection of a set of appropriate model species representing annelid diversity should be reconsidered (Ferrier 2012).

Errantia

The Errantia are well-represented by a comprehensive amount of studies carried out on the nereidid *Platynereis dumerilli*, supported by a few studies on the nereidids *Nereis* (*Alitta*) *virens* and *Neanthes arenaceodentata* and species of the dorvilleid genus *Ophryotrocha* (Arendt et al. 2001; Prud'homme et al. 2002; Prud'homme et al. 2003; Tessmar-Raible and Arendt 2003; Arendt et al. 2004; Tessmar-Raible et al. 2005; Kerner et al. 2006; Kulakova et al. 2007; Bergter et al. 2008; Kulakova et al. 2008; Dray et al. 2010; Fischer et al. 2010; Pfeifer et al. 2012; Winchell and Jacobs 2013). Aguado et al. (2015a) propose to study the syllid *Typosyllis antoni* as a model for this species-rich group, which shows a remarkable variety of reproductive modes. The emergence of *P. dumerilii* as a thoroughly investigated model species is mainly due to the well-established culture conditions of the life cycle in the laboratory (Fischer and Dorresteijn 2004). The reproduction cycle of these animals is synchronized with the moon phases, and they display a high ability of regeneration, a hormonal system which is comparatively alike to vertebrates, very simply organized eyes, and a comparatively slow rate of molecular evolution (Tessmar-Raible and Arendt 2003; Zantke et al. 2014). Genes involved in segment formation and regeneration, nervous system and eye development, photoreception, cleavage patterns, and embryogenesis are studied extensively (see Zantke et al. 2014 for review). Several important techniques for studying the development of these worms have been established, as e.g., in situ hybridization, RNA interference, Morpholino knockdowns, transgenic lineages, or gene modification by transcriptional activatorlike effector nucleases (TALENs) (Bannister et al. 2014; Zantke et al. 2014; Backfisch et al. 2013; Backfisch et al. 2014). Moreover, it is possible to obtain gene expression data in a cellular resolution for complete individuals (Achim et al. 2015). Therefore, *P. dumerilii* is an ideal study object for comparisons of developmental mechanisms with other bilaterian animals. Interestingly, with *Platynereis massiliensis*, a closely related species is available, which shows pronounced differences in development (direct vs. indirect) and also their reproductive system (hermaphroditism vs. separate sexes). Initial studies regarding their development have been published for this taxon (Helm et al. 2014).

Sedentaria

Most of the annelid species that have been investigated extensively in various evolutionary developmental studies fall

within the clade of Sedentaria. These include the leech *Helobdella robusta* and the capitellid *Capitella teleta* (formerly *Capitella* sp. I), but there is also some work available for the leech *Hirudo medicinalis*, the common earthworm *Lumbricus terrestris*, the serpulids *Hydroides elegans* and *Spirobranchus* (formerly *Pomatoceros*) *lamarcki*, and the oligochaete *Tubifex tubifex* (Iwasa et al. 2000; Seaver and Shankland 2001; Werbrock et al. 2001; Song et al. 2002; Tessmar-Raible and Arendt 2003; Seaver et al. 2005; Seaver and Kaneshige 2006; Arenas-Mena 2007; Oyama and Shimizu 2007; Weisblat and Kuo 2009; Rivera and Weisblat 2009; Cho et al. 2010; McDougall et al. 2011; Simakov et al. 2013).

As representatives of the Clitellata, members of *Helobdella* are small freshwater leeches belonging to the Glossiphoniidae, which do not feed on blood from other animals. Due to their small body size and ease of rearing in the laboratory, species of *Helobdella* became excellent candidates for experimental studies (Weisblat and Kuo 2009). Investigations of the cleavage and cell lineage patterns in *Helobdella* are not only key components that aid our understanding of the embryogenesis of other spiralian animal groups, but also of changes in development connected to a transition to a terrestrial life in Annelida, since Clitellata are deeply nested within polychaetes. Moreover, the availability of the whole genome of *H. robusta* enables comparisons of genome organization and gene content between annelids and other Bilateria (Simakov et al. 2013).

Other Sedentaria which are currently studied in evo-devo studies are members of the genus *Capitella*. Their simple body organization, small size, short generation time, and cosmopolitan distribution, in addition to the fully sequenced genome of *C. teleta*, are important arguments qualifying capitellids as annelid model species. The embryonic and larval development has been described in detail, as well as regenerative abilities, development of the nervous system, segment formation, and genomic organization (Werbrock et al. 2001; Seaver et al. 2005; Fröbius and Seaver 2006; Seaver and Kaneshige 2006; Frobius et al. 2008; Hill and Savage 2009; Cho et al. 2010; Giani et al. 2011; Seaver et al. 2012; Simakov et al. 2013; Meyer et al. 2015). Moreover, their phylogenetic position could help us to understand the evolution of segmentation within Annelida, with the non-segmented Echiura as their sister taxon. Comparative developmental and gene expression studies in both taxa might shed light on the formation and loss of segments within Annelida.

Basal branching annelids

Within the basal branching lineages, gene expression or developmental studies are available for members of the genus *Chaetopterus* and the peanut worms *Phascolion strombus*, *Nephasoma minuta*, and *Themiste lageniformis* (Irvine et al. 1997; Irvine and Martindale 2000; Seaver et al. 2001; Ferrier

and Holland 2001; Wanninger et al. 2005; Kristof et al. 2008; Boyle and Seaver 2010; Kristof et al. 2011; Boyle and Rice 2014). But the amount of data is limited in comparison to Pleistoannelida and focuses mainly on segment formation and the development of the muscle and nervous system. A main reason for this underrepresentation was the unresolved root of the annelid tree and thereby the lack of interest in taxa like *Oweniidae* and *Magelonida*, which were regarded as derived members of the Canalipalpata. Additionally, chaetopterids and sipunculids differ extremely in their morphology from what was to be believed a typical annelid body plan and the focus of evolutionary and developmental studies within Annelida shifted to species which were regarded to show more plesiomorphic conditions, like *P. dumerilii* (Tessmar-Raible and Arendt 2003). Nevertheless, the placement of the non-segmented Sipuncula within the segmented Annelida provides an interesting insight into the mechanisms responsible for segment formation and loss as is the case in Capitellidae and Echiura. Since annelid relationships became more stable in recent analyses, accompanied with a clearer picture of the root of the annelid tree, it is crucial to expand our knowledge of developmental mechanisms of the early branching lineages.

New candidates of annelids for evo-devo studies

To reconstruct the ancestral features of Bilateria and compare the annelid ground pattern to other lophotrochozoans like molluscs, nemerteans or brachiopods, our knowledge of the basal branching lineages has to be extended. Only Sipuncula and Chaetopteridae are covered scarcely and additional investigations in species of Amphinomida, Oweniidae, and Magelonidae should be the main focus of new evo-devo studies. Potential candidates are the fire worms *E. complanata* and *Hermodice carunculata* (Amphinomidae), the tube dwelling worm *Owenia fusiformis* (Oweniidae), and species of the genus *Magelona*.

E. complanata comprises a complex of closely related species with a wide distribution (Barroso et al. 2010; Arias et al. 2013). Moreover, members of this complex seem to be frequently introduced in sea water aquaria, where they are easy to keep. This species can regenerate anteriorly and posteriorly and has been used in regeneration studies to understand the redevelopment of the nervous and muscular system (Müller et al. 2003; Weidhase et al. in press). It mostly reproduces asexually by fission, but sexual reproduction occasionally occurs (Kudenov 1974). Another potential candidate is the cosmopolitan species *H. carunculata* for which a detailed transcriptome analysis has been published (Mehr et al. 2015). An amphinomid model system could aid our understanding of the evolution of segmentation, since the non-segmented Sipuncula are the direct sister group to the segmented Amphinomida. Fossils of Sipuncula can already be found in

the early Cambrium and provide an interesting insight into the unchanged morphology of these animals for more than 520 Mio years with no obvious hints of segmentation (Huang et al. 2004). Developmental studies conducted so far support the loss of segmentation within Sipuncula, which point out that mechanisms involved in the progress of segment formation and body axis patterning are very old (Kristof et al. 2008; Kristof et al. 2011).

Choosing species from Palaeoannelida, which represent the sister taxon of all remaining annelids, is obvious, since character states observed in this taxon have a greater influence on the reconstruction of the ground pattern of the whole phylum. Nevertheless, it should not been overlooked that of course also these basal branching taxa bear a combination of plesiomorphic and apomorphic characters. Oweniidae were already suggested by Rieger (1988) to resemble ancestral annelid traits and should be found at the base of the annelid tree. His assumptions were based on the presence of characters considered as plesiomorphic, like the already mentioned monociliated epidermis or the intraepidermal position of the nervous system (Bubko and Minichev 1972; Gardiner 1978). However, this hypothesis received neither broad acceptance nor attention. At least in part, this may be due to the fact that no conclusive hypotheses about the evolution of annelids were available. The first step in the direction of an emerging model organism should be the establishment of a life cycle in the laboratory and protocols for developmental studies. Oweniids develop via a so-called mitraria larvae (Smith et al. 1987) and its development has been observed and described in the laboratory for *Owenia collaris* (Smart and Von Dassow 2009). Another species, *O. fusiformis*, is reported worldwide, but likely represents a species complex (Koh and Bhaud 2001; Jolly et al. 2006). They are easy to collect and to keep in aquaria. Lauri et al. (2014) already included juveniles of this species in a comparative evo-devo study on notochord evolution. Larval development has also been described for three species of *Magelona* (Wilson 1982). For two further *Magelona* species, maintenance in a laboratory aquarium is described to study feeding and behavior (Mortimer and Mackie 2014).

Evolutionary implications of the new annelid phylogeny

The new annelid phylogeny reflects the immense variability but also plasticity of body plans found in this taxon. Annelids show many different life styles and also new environments were independently invaded. At least clitellates and parergodrilids adapted to terrestrial habitats convergently (Purschke 1999). Limnitic environments were colonized by clitellates, but also by members of diverse polychaete families, such as ampharetids,

nereidids, sabellids, or serpulids (Glasby and Timm 2008; Kupriyanova et al. 2009). The interstitium was colonized several times and adaptation to this environment took place via progenetic evolution or miniaturization, both resulting in simplified morphologies (Struck et al. 2015; Laumer et al. 2015). The proposed new annelid phylogeny makes it possible to trace such convergent adaptations to different life styles. Therefore, annelids will be an appropriate model to understand major transitions of the evolution of Bilateria in general. Especially the evolution of body plans in Lophotrochozoa (sometimes called Spiralia) remains an open question (Dunn et al. 2014). Recent phylogenomic analyses strongly support a phylogeny where Gnathifera (comprising Micrognathozoa, Gnathostomulida, and Rotifera) and Plathelminthes + Gastrotricha form separate early branches in Lophotrochozoa (Struck et al. 2014; Laumer et al. 2015). This renders the "Platyzoa" paraphyletic and suggests that macroscopic and coelomate taxa like Annelida, Mollusca, or Brachiopoda evolved from a microscopic and acoelomate ancestor with a meiobenthic lifestyle. However, annelid phylogeny suggests that microscopic taxa often evolve from a macroscopic ancestor. Already Rieger (1980) proposed that for example the interstitial taxon *Lobatocerebum may become an important example for trying to understand the origin and evolution of acoelomate flatworms from coelomate ancestors*. Repeated simplification of body plans is frequently found across the annelid tree. As noted by Andrade et al. (2015) many members of Annelida do not show the morphological characters which have been regarded as its synapomorphies, e.g., segmentation or the presence of chitinous chaetae. Character loss seems frequent across annelids and might partially explain the difficulties of reconstructing the phylogeny of this group by morphological data matrices alone (Bleidorn 2007; Purschke et al. 2000). However, the occurrence of several convergent events of major body plan transitions makes annelids a key taxon to develop and test ideas about major transitions in animal evolution. For example, we have to assume at least four convergent instances of loss of segmentation, as seen in Sipuncula, *Lobatocerebrum*, *Diurodrilus*, and Myzostomida, and many more taxa show a highly reduced segmentation (Halanych et al. 2002). In many of these cases, the segmented annelid sister taxon is known. Comparative evo-devo studies will help to elucidate which changes in the genetic machinery controlling the development of these body plans are necessary.

The new annelid phylogeny also highlights the immense age of the basal splits within the tree. Well-preserved fossils of Sipuncula from the Lower Cambrian suggest that their morphology has remained conserved over the last 520 million years. Logic predicts that lineages branching off earlier in

the annelid tree, such as Palaeoannelida (Oweniidae + Magelonidae) or Chaetopteridae, separated from the remaining annelids even before this time. Members of the basal grade of Annelida and also known Cambrian annelid fossils show a remarkable diversity of body plans (Eibye-Jacobsen 2004; Parry et al. 2014; Weigert et al. 2014; Parry et al. 2015; Liu et al. 2015), making it difficult to deduce the ground pattern of the last common annelid ancestor (Purschke 2002; Struck 2011; Arendt 2011; Eibye-Jacobsen and Vinther 2012; Purschke et al. 2014). However, there seems at least to be a consensus that the ancestor was a segmented macroscopic organism, had chaetae-bearing parapodia, and an anteriorly located pair of palps (but see Parry et al. 2015 for a different view regarding head appendages). This contradicts those scenarios where the last common ancestor resembles recent clitellates (Clark 1964). The direction of evolution took different routes in Errantia and Sedentaria, which comprise the main diversity of annelids. These clades show adaptations to either an errant or a sedentary lifestyle, with the modification of traits such as peristaltic movement, parapodia, and sensory perception (Struck et al. 2011). Up to date, no molecular clock analyses of annelid relationships are available; therefore, there is no information about the age of Pleistoannelida. However, given that jaw fossils (scolecodonts) strongly resembling mandibles of Eunicida (Errantia) firstly appeared in the Late Cambrian–Early Ordovician, we have to assume that this group also has an age of at least ~485 million years (Hints and Eriksson 2007).

Conclusion

After century-long discussions, a well-supported and resolved annelid backbone tree is available. Three large-scale phylogenomic analyses employing a wide array of phylogenetic methodologies, but also two independently constructed data sets, strongly support the here proposed view of a new annelid phylogeny. Several polychaete families still have to be investigated, but morphology and molecular phylogenetic analyses on single or a few genes predict that most of these will be placed either in Sedentaria or Errantia. However, many questions are still open. Clitellate relationships have (mostly) not been investigated in a phylogenomic framework and also the question about their sister taxon remains unclear. Some long-branched annelid taxa such as Spintheridae or Myzostomida remain difficult to place, but are likely part of either Errantia or Sedentaria. Molecular clock analyses are missing, which could give an estimate about the age of annelid taxa. Nevertheless, the phylogeny presented here opens the possibility of tracing common evolutionary trends across annelids and presents a long-missing framework for evolutionary developmental studies.

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