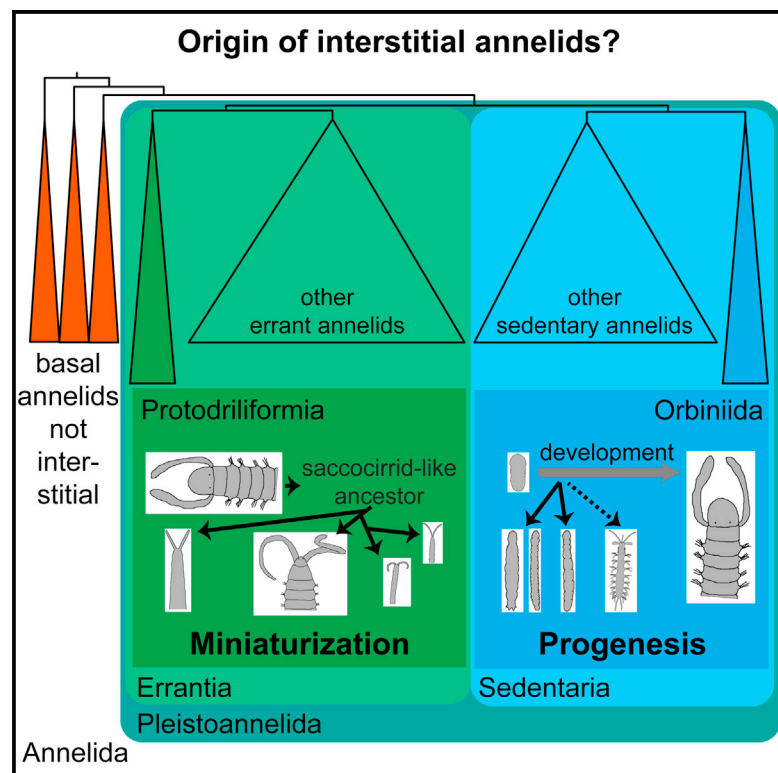


Current Biology

The Evolution of Annelids Reveals Two Adaptive Routes to the Interstitial Realm

Graphical Abstract



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In Brief

Numerous animal taxa inhabit the interstitium, the space between sand grains. Our knowledge of how these species adapted to it is limited. Using a phylogenomic approach, Struck et al. showed that interstitial annelids arose by secondary adaption from larger ancestors due to either progenesis or miniaturization. The importance of miniaturization was underestimated so far.

Highlights

- Interstitial annelid taxa are secondarily derived contra the archiannelid hypothesis
- Several annelid clades adapted to the space between the sand grains by progenesis
- Other interstitial annelids evolved by miniaturization of ancestral adult stages
- Miniaturization is as important as progenesis in the adaptation to the interstitium

Accession Numbers

SRP058117



The Evolution of Annelids Reveals Two Adaptive Routes to the Interstitial Realm

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<http://dx.doi.org/10.1016/j.cub.2015.06.007>

SUMMARY

Many animals permanently inhabit the marine interstitium, the space between sand grains [1, 2]. Different evolutionary scenarios may explain the existence of interstitial animals [3, 4]. These scenarios include (1) that the interstitial realm is the ancestral habitat of bilaterians [5, 6], (2) that interstitial taxa evolved from larger ancestors by miniaturization, or (3) progenesis [3]. The first view mirrors the former hypothesis that interstitial annelids, called archiannelids, were at the base of the annelid radiation [7]. Based on morphological data, however, progenesis is generally favored for interstitial annelids today [3, 4, 8]. Herein, our phylogenomic approach revealed that interstitial archiannelids are robustly placed into two groups nested within the annelid tree. Evolution of the first group comprising among others Dinophilidae is best explained by progenesis. In contrast, the second group comprising Protodrilida and Polygordiidae appears to have evolved by stepwise miniaturization adapting from coarser to finer sediments. Thus, in addition to progenesis [3, 4], miniaturization, thought to be too slow for an adaptation to the interstitium [3], is an important second route allowing adaptation to interstitial environments. Both progenesis and miniaturization should be considered when investigating evolution of interstitial taxa [1, 3].

RESULTS AND DISCUSSION

Marine interstitial environments are inhabited by numerous metazoan taxa (e.g., platyhelminths, nematodes, kinorhynch, copepods, gastrotrichs). These also include various annelid taxa, which among others comprise the so-called archiannelids (Protodrilida, Polygordiidae, Dinophilidae, Diurodrilidae, Nerillidae, and *Apharyngtus*) [8, 9]. Hypotheses regarding evolution of interstitial species can be categorized into three scenarios [3]. First, inhabiting the interstitium is assumed to be ancestral

for Bilateria, as shown by gastrotrichs and gnathostomulids [5, 6]. Likewise, the “Archiannelida” concept proposed that the annelid taxa mentioned above exhibit the ancestral condition of Annelida [7, 10]. However, recent phylogenomic studies [11] placed the non-interstitial lineages Oweniidae and Magelonidae at the base of the annelid tree, but these analyses did not include any archiannelid taxon. The second scenario suggests that progenesis was the evolutionary process for adapting to the interstitium [3]. That is, larval or juvenile stages of a larger ancestor temporarily inhabiting the interstitium arrested somatic development and became sexually mature. Thus, they inhabited the interstitium permanently. The third, often neglected, scenario suggests that the interstitium was colonized by miniaturization via gradual, step-by-step decrease in body size from a much larger adult ancestor, which had an infaunal or epibenthic life history [3]. Whereas miniaturized species should resemble their adult ancestors, progenetic species should resemble the larval or juvenile stages of their ancestors [3].

Progenesis is currently assumed for all archiannelid taxa (taxa labeled with an A in Figure 1) [3, 4, 8, 9, 13–15]. However, the conclusion of progenesis based on morphological data alone entails the risk of circular reasoning [4]. Characters of the adult stage of a taxon are compared with larval or juvenile characters and not with adult characters of another taxon, invoking progenesis a priori [16], followed by the subsequent conclusion of progenesis. In contrast, some molecular-phylogenetic studies found archiannelids as part of the basal annelid radiation, albeit given weak nodal support [10, 17], pointing to the possibility of an interstitial ancestry of Annelida. Hence, we investigated whether these interstitial annelids still show a putatively ancestral condition of Annelida, or whether they evolved by progenesis or miniaturization.

We applied a phylogenomic approach, generating transcriptome sequence data for 12 interstitial archiannelid species representing Protodrilida, Polygordiidae, Dinophilidae, Diurodrilidae, Nerillidae, and *Apharyngtus* and two additional interstitial species (i.e., Parergodrilidae) as well as nine additional annelid taxa using Illumina HiSeq2000 sequencing technology and a modified RNA amplification method [6]. All sequence data were deposited in NCBI SRA and are available via the BioProject SRP058117. These data were complemented with data of 77 annelid and lophotrochozoan outgroup species of a previous study [11].

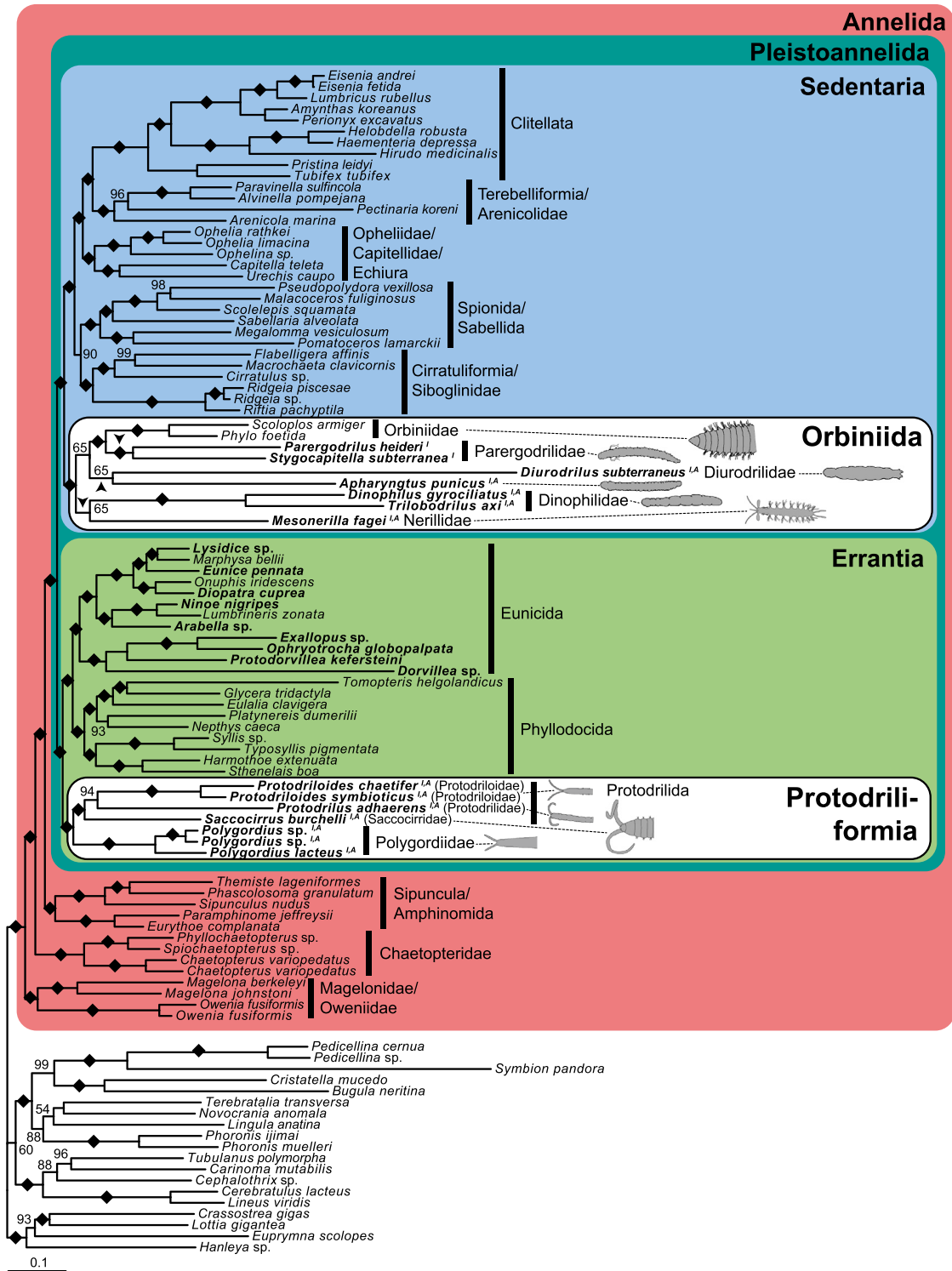


Figure 1. Tree of Maximum Likelihood Analysis of the Largest Dataset, Dataset 1

Tree of maximum likelihood (ML) analysis of the largest dataset (dataset 1) with 100 species, 189,193 amino acid positions, and 41.2% sequence coverage using RAXML [12]. Bootstrap values above 50 are shown at the branches, with values of 100% depicted as diamonds. Higher taxonomic units are indicated, and species for which new data have been generated are in bold. Drawings of relevant taxa are displayed but are not to scale. Superscript I indicates interstitial species, and superscript A indicates former archannelids. Arrowheads indicate possible events of progenesis. For testing of alternative hypotheses, see Table S1, and for density plots of different bias measurements, see Figure S3.

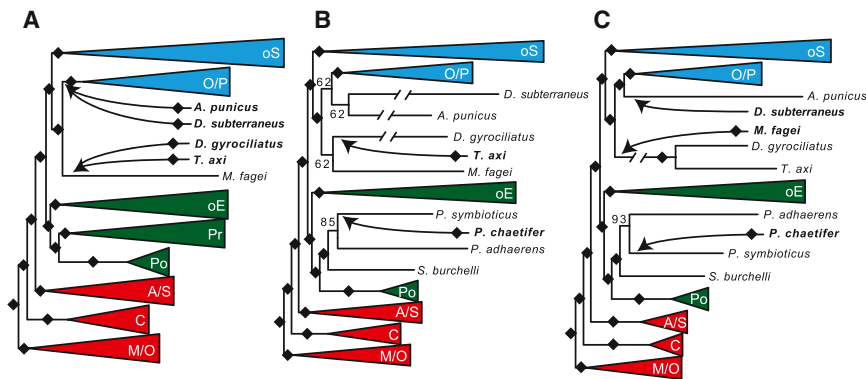
Table 1. Characteristics of the Datasets Generated Based on Different Criteria and Bootstrap Support for the Three Hypotheses, Orbiniida, Protodrilliformia, and Protodrilliformia Placed within Errantia

Dataset (#)	MARE (d)	Sensitivity Criterion	Threshold	Species Re-included	Position (#)	Species (#)	Coverage (%)	Orbiniida	Protodrilliformia	Protodrilliformia within Errantia
1	1.0	none	n/a	n/a	189,193	100	41.23	100	100	100
2	1.0	contamination and paralogy	n/a	n/a	189,193	100	41.21	100	100	100
3	1.5	missing data per partition	n/a	n/a	116,990	100	44.87	94	95	95
4	2.0	missing data per partition	n/a	n/a	80,669	100	47.33	76	77	75
5	2.0	without <i>Mesonerilla</i>	n/a	n/a	80,669	99	47.32	98	100	100
6	2.0	without <i>Protodrilloides</i> and <i>Protodrillus</i>	n/a	n/a	80,669	97	47.10	99	100	93
7	1.0	branch length heterogeneity	72.78	n/a	143,036	100	42.95	100	100	100
8	1.0	evolutionary rate	1.335	n/a	169,392	100	41.88	100	100	100
9	1.0	LB score	50.89	n/a	189,193	98	41.77	100	100	100
10	1.0	LB score	29.24	n/a	189,193	93	42.34	99	100	99
11	1.0	LB score	16.89	n/a	189,193	86	42.00	100	100	100
12	1.0	LB score	16.89	<i>Apharyngtus punicus</i>	189,193	87	42.04	100	100	100
13	1.0	LB score	16.89	<i>Dinophilus gyrotiliatus</i>	189,193	87	42.62	100	100	100
14	1.0	LB score	16.89	<i>Diurodrillus subterraneus</i>	189,193	87	41.67	100	100	100
15	1.0	LB score	16.89	<i>Trilobodrilus axi</i>	189,193	87	42.46	100	100	100
16	1.0	compositional heterogeneity	0.00121	n/a	189,193	97	42.44	100	100	100
17	1.0	compositional heterogeneity	0.00087	n/a	189,193	92	44.50	100	100	100
18	1.0	compositional heterogeneity	0.00064	n/a	189,193	80	49.24	100	100	100
19	1.0	compositional heterogeneity	0.00064	<i>Protodrilloides chaetifer</i>	189,193	81	48.69	100	100	100
20	1.0	compositional heterogeneity	0.00064	<i>Trilobodrilus axi</i>	189,193	81	49.64	100	100	100
21	1.0	missing data per species	0.566	n/a	189,193	44	75.60	100	100	100
22	1.0	missing data per species	0.566	<i>Diurodrillus subterraneus</i>	189,193	45	74.23	100	100	100
23	1.0	missing data per species	0.566	<i>Mesonerilla fagei</i>	189,193	45	74.88	100	100	100
24	1.0	missing data per species	0.566	<i>Protodrilloides chaetifer</i>	189,193	45	74.03	100	100	100

Exclusion of partitions and species was based on density plots. Partitions or species, which were part of the skewed right tails, were excluded. If interstitial species were among the excluded (i.e., biased) species, each excluded interstitial species was re-included. The number of species and positions, the percentage of sequence coverage, the d value of MARE [18], and the sensitivity criterion and the corresponding threshold values determined using TreSpEx [19] and BaCoCa [20], respectively, are provided for each dataset. Moreover, it has been indicated when species were re-included. Bootstrap values are indicated in the last three columns. n/a, not applicable. For more detailed results of the cross-contamination and paralogy screening, see Table S2, and for the position of Diurodrilidae, see Table S3.

Phylogenetic reconstructions based on the largest dataset (dataset 1 in Table 1) resulted in significantly supported phylogenetic relationships within Annelida generally congruent with pre-

vious analyses [11, 21] (Figure 1), except for placement of Cirratuliformia/Siboglinidae. In our analyses, this clade is sister to Sabellida/Spionida, while in most analyses of Weigert et al.



re-included species plus bootstrap support for this position are indicated by arrows. Higher taxonomic units except for the interstitial annelids are collapsed, and outgroups are not shown. Bootstrap values >50 are shown, with values of 100% as diamonds. A/S, Amphinomidae/Sipuncula; C, Chaetopteridae; M/O, Magelonidae/Oweniidae; oE, other Errantia; O/P, Orbiniidae/Parergodrilidae; oS, other Sedentaria; Po, Polygordiidae; Pr, Protodrilida. For the results of the clustering analyses, see Figures S1 and S2.

[11], it is sister to Orbiniidae. Although the reconstructed topology of annelids is generally robust among studies, this change illustrates that increased taxon sampling may be necessary to place all annelid taxa securely.

In our analyses, interstitial annelid taxa were placed into two major groups with significant nodal support of 100% for each placement (Figure 1). First, Protodrilida and Polygordiidae were placed together as sister to the other Errantia. In contrast to morphology-based hypotheses, neither Protodrilida nor Polygordiidae were closely related to any sedentarian taxon, specifically to either Spionida or Opheliidae, respectively, suggesting progenesis [4]. Hypothesis testing significantly rejected these morphology-based hypotheses (Table S1). Second, Dinophilidae, Diurodrilidae, *Apharyngtus*, and Nerillidae were placed in a clade together with Orbiniidae and Parergodrilidae within Sedentaria. For these four archiannelid taxa, a close relationship to Errantia or its subtaxon Eunicida had been proposed based on morphological data, again assuming progenesis [4, 10]. For example, Dinophilidae and Diurodrilidae were considered proge-netic Eunicida due to their resemblance to eunicidan polytroch larvae [3, 4, 8, 9, 13–15]. Such a relationship is not supported by our data and was significantly rejected by hypothesis testing (Table S1). Previous analyses using nuclear rRNA genes also challenged a close relationship of these taxa to Eunicida but lacked strong support for alternative placements [14, 15, 22].

We also thoroughly checked whether reconstruction artifacts affected tree topology and placement of interstitial taxa [6, 23, 24]. To this end, influence of erroneously assigned paralogous sequences, cross-contamination, branch-length heterogeneity, overall evolutionary rate, amino acid composition, compositional heterogeneity, and the degree of shared missing data was assessed. First, we showed that paralogous sequences or cross-contamination [6, 23] was not influencing placement of interstitial taxa as exclusion of potentially affected sequences (Table S2) did not alter results (dataset 2 in Table 1). Second, interstitial species were not clustered as in Figure 1 when hierarchical clustering based on metric values of potential biases was applied (e.g., degree of missing data per species) (Figures S1 and S2). Third, we generated more conservative but smaller datasets by

Figure 2. Trees of ML Analyses from Which Biased Species Were Excluded

Exclusion of species based on density plots and species, which were part of the skewed right tails of normal distributions of these biases, were excluded (Figure S3). If interstitial species were among the excluded (i.e., biased) species, each excluded interstitial species was re-included to determine the effect of the bias on its position (i.e., if this species was placed differently in analyses when all other biased species were excluded). (A–C) Long-branched species using the long branch (LB) score (dataset 11, 86 species) (A), compositional heterogeneity using relative composition frequency variability (RCFV) values (dataset 18, 80 species) (B), and degree of missing data (dataset 21, 44 species) (C). Positions of

excluding biased gene partitions or species (Figure S3; Table 1). If interstitial species were among the excluded (i.e., biased) species, they were re-included to determine the effect of the corresponding bias on the topological position. Phylogenetic reconstructions based on these datasets recovered the same results with respect to the placement of interstitial annelid taxa as shown in Figure 1 with generally significant support of 100% (Figure 2; datasets 3–24 in Table 1). Hence, in contrast to previous studies [3, 4, 8, 9, 13–15, 22], our analyses robustly placed interstitial annelid taxa.

Relationships within the two clades including interstitial taxa were also stable across different analyses, including exclusion of biased interstitial annelid taxa such as those with long branches (e.g., datasets 9–15 in Table 1). Monophyly of both Polygordiidae and Protodrilida was recovered, usually with significant bootstrap support $\geq 95\%$. Within the other clade, Parergodrilidae is always sister to Orbiniidae with maximal support. *Apharyngtus* is sister to Diurodrilidae, and together they are sister to Orbiniidae/Parergodrilidae in all analyses. Dinophilidae and Nerillidae is always sister to these four taxa (Figures 1 and 2). Moreover, re-included biased interstitial species, like the long-branched *Apharyngtus*, were always in the same positions on the tree as they were in the analysis with all taxa (e.g., Figure 2A). However, nodal support was low at some nodes in Figure 1, separating Diurodrilidae from Dinophilidae. This situation is possibly due to biased base composition drawing Diurodrilidae toward Dinophilidae, reducing bootstrap support at associated nodes (Table S3). Excluding *Diurodrilus* increased bootstrap support to 100% for both clades Dinophilidae/Nerillidae and *Apharyngtus*/Orbiniidae/Parergodrilidae.

These results have interesting implications for annelid evolution as interstitial taxa are not part of the basal radiation of lineages. Hence, inhabiting the interstitial realm and possessing a simple body organization are not ancestral traits of Annelida. The “Archiannelida” concept has to be rejected, thereby confirming previous studies [3, 10].

Nerillidae, Dinophilidae, Diurodrilidae, and *Apharyngtus* are closely related (with strong nodal support) to Orbiniidae and Parergodrilidae in our analyses. Therefore, we name this new clade

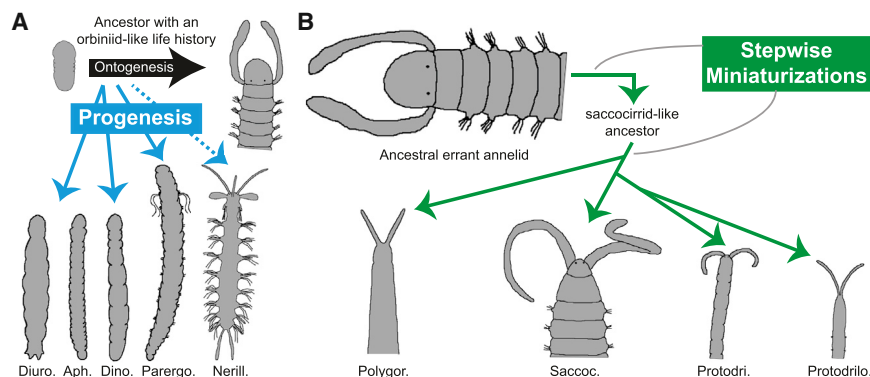


Figure 3. Two Different Scenarios Explain the Evolution of Interstitial Annelids

(A and B) Progenesis (A) and stepwise miniaturization (B). Drawings are not to scale. The dashed line indicates weak evidence only due to size. Aph., *Apharyngtus*; Dino., Dinophilidae; Diuro., Diurodrilidae; Nerill., Nerillidae; Parergo., Parergodrilidae; Polygor., Polygordiidae; Protodri., Protodrilidae; Protodriilo., Protodriloidae; Saccoc., Saccocirridae. For the results of ancestral state reconstruction using maximum likelihood mapping and a modified morphological data matrix of Weigert et al. [11] in Mesquite [27], see Table S4 and Figure S4.

Orbiniida as the first described species of this clade was an orbinid (Figure 1). Ancestral state (AS) reconstructions of Orbiniida (Table S4; Figures S4A and S4B) differed only in two characters from the reconstruction of the last common ancestor (LCA) of Sedentaria, which is remarkably similar to reconstructions of the large LCA of Annelida [11]. Prostomial palps were absent, and the head encompasses two or more rings behind the prostomium. However, both characters have to be critically reviewed as given the AS reconstruction, reversions to the original state occurred. In Nerillidae, prostomial palps are present, and in Orbiniidae, the prostomium is followed by one ring as adults [8, 9]. Although several small-sized orbinid species with two rings in the adult stage are known, recent analyses [25] supported the hypothesis that these species evolved independently by progenesis within Orbiniidae [25, 26]. Therefore, the adult stage of the large LCA of Orbiniida resembled the LCA of Sedentaria by possessing palps and only one ring in addition to parapodia and chaetae.

Parergodrilidae, Dinophilidae, Diurodrilidae, and *Apharyngtus* are of small size (0.3–2.5 mm), comprise only very few segments, and lack palps, parapodia, and chaetae (except for Parergodrilidae possessing chaetae). Hence, these taxa do not resemble the adult stage LCA of Orbiniida. Moreover, Dinophilidae, Diurodrilidae, and *Apharyngtus* possess ciliary rings and bands resembling polytroch larvae of Orbiniidae, which temporarily inhabit the interstitial realm [22, 26]. Moreover, heads of Parergodrilidae, Dinophilidae, and *Apharyngtus* incorporate the prostomium and more than one ring as is also observed in larval and juvenile stages of Orbiniidae. Thus, given that several larval or juvenile characters of orbinids persist in these four taxa, evolution by progenesis seems likely (Figure 3A). The AS reconstruction revealed an LCA for Orbiniida more similar to the LCA of Sedentaria and Orbiniidae. Orbiniidae and the other sedentary annelid taxa have body lengths in the range of several centimeters and not millimeters. Therefore, independent progenesis leading to Dinophilidae, Diurodrilidae/*Apharyngtus*, and Parergodrilidae from a larger ancestor (arrows in Figure 1) is more likely than a single progenesis event at the base of Orbiniida followed by subsequent reversion to large size. This conclusion is further substantiated by the fact that several independent progenesis events are known within Orbiniidae [25]. Dinophilidae and Diurodrilidae/*Apharyngtus* likely evolved by progenesis from an earlier developmental stage than Parergodrilidae (Figure 3A) as the latter more or less resemble juvenile orbinids. Whereas resem-

blance to polytroch larvae and size support the hypothesized progenesis of these taxa, except for their very small (<1 mm) in the range of larval stages [3], strong evidence is lacking for Nerillidae (Figure 3A) [9, 28].

In contrast, Polygordiidae and Protodrilida do not resemble developmental stages of related taxa, and progenesis seems unlikely. Polygordiidae, Saccocirridae, and certain species of Protodrilidae are several centimeters long and usually comprise numerous segments [9, 29]. Instead, we propose that miniaturization explains evolution of these taxa (Figure 3B), thereby contradicting the previous hypothesis of progenesis [4]. Similar to the annelid LCA [11, 21, 30], the errant LCA was reconstructed to be a larger epibenthic or infaunal annelid consisting of a prostomium with palps and eyes followed by one ring, homonymous segments bearing parapodia with simple chaetae, and a pygidium with cirri (Table S4). Saccocirridae (Protodrilida) possess a prostomium with palps and eyes followed by one ring and homonymous segments with parapodia bearing simple chaetae similar to the reconstructed ancestor. Like saccocirrids, Polygordiidae species inhabit coarse gravel sediments [31] but show only internal signs of segmentation. Protodriloidae (Protodrilida) are considerably smaller with a body length of about 1 cm and without obvious external segmentation except for chaetae in *Protodriloides chaetifer* [9]. Similarly for Protodrilidae (Protodrilida), a body length of about 1 cm or more inhabiting coarser sediments as well as complete reduction of parapodia and chaetae are regarded as ancestral traits [29]. During the course of evolution within Protodrilidae, species decreased further in size to only one or a few millimeters and reduced characters like eyes [9, 29]. Whereas interstitial annelids usually are too small to produce a sufficient number of oocytes for development via a planktonic larva, Saccocirridae, Polygordiidae, and certain Protodrilidae can produce enough oocytes for such a mode of development, and only in smaller species does direct development occur [9, 32].

Reconstructions of the LCA of Polygordiidae and Protodrilida were similar to recent Saccocirridae except for the absence of parapodia and presence of pygidial cirri (Table S4). However, parapodia would have to be regained in Saccocirridae. As absence of parapodia was not well supported (proportional likelihood p of 0.56; Figure S4C), regaining a complex character like parapodia seems less plausible than independent losses in Polygordiidae and Protodrilidae/Protodriloidae. Therefore, the LCA was most likely saccocirrid-like evolving from an infaunal ancestor that inhabited coarse sediments. In the lineage that

lead to Polygordiidae, character traits were further reduced [31], while within Protodrilida, additional size reduction occurred, allowing the habitation of finer sediments [8, 9, 29] (Figure 3B). As the LCA resembles the protodrilidan Saccocirridae, we name this clade Protodriliformia (Figure 1).

The most prominent characteristic of interstitial environments is the small space available to organisms, thus enforcing a rigid requirement for small body size [3, 4]. The probability of a successful permanent invasion is likely much higher starting from a stage already in part adapted to the interstitium (e.g., juveniles) as in progenesis than from a larger stage adapted to an infaunal life as in miniaturization [3]. Therefore, explaining evolution of interstitial annelids progenesis has generally been favored over miniaturization [3, 4]. Besides cases of progenetic evolution in Orbiniida, several interstitial species, which independently originated by progenetic evolution, can also be found in Eunicida and possibly Hesionidae [3, 4, 13]. Stepwise miniaturization occurs within Protodriliformia (contra [4]) and Pisionidae [3]. In light of our results, the interstitial species of Syllidae [33] also more likely evolved by miniaturization as they are morphologically very similar to adult stages of non-interstitial syllids. Hence, miniaturization might be another evolutionary trajectory whose importance is similar to the one of progenesis. Thus, there are two different evolutionary routes to adapt to the interstitium from larger ancestors.

In view of our findings on annelids, miniaturization should be taken into account more often when investigating evolution of any interstitial taxa from larger ancestors. Another explanation proposed for some taxa (e.g., Platyhelminthes, Gastrotricha, and Gnathifera) is the idea that they descended from an interstitial bilaterian ancestor [5, 6], but it has been argued that these interstitial taxa are secondarily derived [34]. The debate about taxa like Platyhelminthes, Gastrotricha, and Gnathifera raises the question of how likely secondary reductions in body size (to the point of being interstitial) are over the course of evolutionary history. From a parsimonious perspective, recent phylogenomic analyses were most congruent with the hypothesis of retaining the ancestral interstitial bilaterian lifestyle [5, 6]. For support of the latter hypothesis of secondary reductions, additional evidence, like traces of larval, juvenile, or adult character traits suggesting that they evolved from a larger ancestor, is needed. To date, such unequivocal evidence is lacking for these taxa (i.e., Platyhelminthes, Gastrotricha, and Gnathifera). By contrast, in the case of annelids, our phylogenomic analyses provided a robust evolutionary framework allowing testing alternative hypotheses of body size evolution. When combined with knowledge of larval, juvenile, or adult character traits, both progenesis and miniaturization emerge as important evolutionary processes in Annelida.

ACCESSION NUMBERS

The accession number for the sequence data of the 23 annelid species reported in this paper is NCBI SRA: BioProject SRP058117.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.06.007>.

AUTHOR CONTRIBUTIONS

T.H.S. conceived this study. A.G. and K.M.H. took the lead on data collection. G.P. and W.W. aided in the data collections. A.G., A.W., F.A.F., and K.M.H. generated the RNA-seq libraries. T.H.S. took the lead on data analyses. A.W. and C.B. aided in the data analyses. T.H.S., G.P., C.B., and K.M.H. mainly contributed to writing the manuscript.

ACKNOWLEDGMENTS

This work was funded by the Deutsche Forschungsgemeinschaft (grants DFG-STR 683/5-2 and DFG-STR 683/8-1 to T.H.S.; grant DFG-BL 787/5-1 to C.B.) as well as by the USA National Science Foundation (DEB-1036537). We also thank Birgit Nickel and Matthias Meyer (Max Planck Institute for Evolutionary Anthropology) for their assistance in sequencing using Illumina Hi-Seq 2000. This is contribution #133 to AU Marine Biology Program and contribution #41 to The Molette Biological Laboratory.

Received: March 24, 2015

Revised: May 7, 2015

Accepted: June 2, 2015

Published: July 23, 2015

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