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Differences in the genetic control of early egg development and reproduction between *C. elegans* and its parthenogenetic relative *D. coronatus*

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Abstract

Background: The free-living nematode *Diploscapter coronatus* is the closest known relative of *Caenorhabditis elegans* with parthenogenetic reproduction. It shows several developmental idiosyncrasies, for example concerning the mode of reproduction, embryonic axis formation and early cleavage pattern (Lahl et al. in Int J Dev Biol 50:393–397, 2006). Our recent genome analysis (Hiraki et al. in BMC Genomics 18:478, 2017) provides a solid foundation to better understand the molecular basis of developmental idiosyncrasies in this species in an evolutionary context by comparison with selected other nematodes. Our genomic data also yielded indications for the view that *D. coronatus* is a product of interspecies hybridization.

Results: In a genomic comparison between *D. coronatus, C. elegans*, other representatives of the genus *Caenorhabditis* and the more distantly related *Pristionchus pacificus* and *Panagrellus redivivus*, certain genes required for central developmental processes in *C. elegans* like control of meiosis and establishment of embryonic polarity were found to be restricted to the genus *Caenorhabditis*. The mRNA content of early *D. coronatus* embryos was sequenced and compared with similar stages in *C. elegans* and *Ascaris suum*. We identified 350 gene families transcribed in the early embryo of *D. coronatus* but not in the other two nematodes. Looking at individual genes transcribed early in *D. coronatus* but not in *C. elegans* and *A. suum*, we found that orthologs of most of these are present in the genomes of the latter species as well, suggesting heterochronic shifts with respect to expression behavior. Considerable genomic heterozygosity and allelic divergence lend further support to the view that *D. coronatus* may be the result of an interspecies hybridization. Expression analysis of early acting single-copy genes yields no indication for silencing of one parental genome.

Conclusions: Our comparative cellular and molecular studies support the view that the genus *Caenorhabditis* differs considerably from the other studied nematodes in its control of development and reproduction. The easy-to-culture parthenogenetic *D. coronatus*, with its high-quality draft genome and only a single chromosome when haploid, offers many new starting points on the cellular, molecular and genomic level to explore alternative routes of nematode development and reproduction.

Keywords: Nematode, *Caenorhabditis*, Parthenogenesis, Embryogenesis, Polarity, Chromosome, Genome, Transcriptome, Hybridization

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Background

Development of the model *Caenorhabditis elegans* has been extensively studied. Although comparative studies in other nematodes revealed considerable variations on the cellular level (for review, see [3]), it seemed self-evident that gene cascades controlling development are conserved across the phylum. However, analysis on the levels of genome and transcriptome suggested major changes in the logic of cell specification and the action of Developmental System Drift [4], i.e., the modification of developmental processes due to altered gene regulatory networks without changing the phenotype of the emerging organism, even between nematodes from neighboring clades [5, 6]. While *C. elegans* can obviously not serve as a general model for nematode development, it has remained unclear how fast the genetic control of development has changed during evolution in the long-branched roundworms.

Therefore, we here analyze molecular and cellular aspects of early development and reproduction in the parthenogenetic species *Diploscapter coronatus*, which has just half the body size of *C. elegans* and whose genome we described recently [2]. *D. coronatus* is a member of the Protorhabditis group, which not only belongs to the same clade as the genus *Caenorhabditis* but is the immediate sister taxon of it [7]. We previously described some idiosyncrasies in early development of *D. coronatus* using microscopic approaches [1, 8].

In the androdioecious hermaphrodite, *C. elegans* oocytes arrest in meiotic prophase and are released sequentially, this way delivering a continuous supply of maturing oocytes [9, 10]. The generation of somatic founder cells via asymmetric germline divisions in *D. coronatus* takes place in the same way as in *C. elegans* despite the absence of sperm-induced polarization prior to first cleavage. In *D. coronatus*, only one polar body is generated during a truncated meiosis explaining the diploid status without fertilization. This suggests differences in the molecular machinery initiating axis polarity.

The control of oocyte maturation in *C. elegans* requires signaling from the sperm via major sperm protein (MSP) [11]. We found earlier that MSP genes are present in parthenogenetic nematodes, including *D. coronatus*; however, MSP protein could not be detected there [12].

Screening the gene and protein sets of *D. coronatus* for regulators of important developmental processes in *C. elegans*, we make comparisons with other members of the genus *Caenorhabditis*, as well as two more distantly related nematodes with gonochoristic and hermaphroditic reproduction. Particularly, we search for peculiarities that can be related to the development of oocyte and early embryo in the context of parthenogenetic reproduction in *D. coronatus*.

In a second approach, we compare the transcriptome of early embryonic stages in *D. coronatus* with the known complement of genes expressed in corresponding stages of *C. elegans* [13] and *Ascaris* [14]. In particular, we are interested to explore to what extent the expression of certain genes in *D. coronatus* can be correlated with its early developmental idiosyncrasies.

Nematodes can follow different modes of reproduction including parthenogenesis. This reproductive mode is a deviation of an original bisexual situation and has been established several times independently within different metazoan taxa. It can arise in several ways including spontaneous mutation, interspecies hybridization or infection with microorganisms and may go along with regular meiosis followed by fusion of gametes, or complete or partial suppression of meiosis [15]. However, no parthenogens have been found in the genus *Caenorhabditis*, despite the rapidly rising number of described species (> 30), while at the same time a shift from gonochoristic to hermaphroditic reproduction took place several times independently in this taxon [16, 17].

With our earlier finding in mind that the genome of *D. coronatus* shows a high degree of heterozygosity [2], we looked for further evidence that parthenogenesis in this species may be the result of interspecies hybridization which is considered a major route to this mode of reproduction in invertebrates and possibly the only one in vertebrates [18].

Methods

Nematode culture and strains

Strains were cultured on agar plates with the uracil-requiring OP50 strain of *E. coli* as a food source, essentially as described by Brenner [19], except that, to reduce contamination with other bacteria, we used minimal medium plates [8]. *D. coronatus* (PDL0010) was kindly provided by Paul De Ley, Dept. Nematology, University of California, Riverside.

To measure brood size, 17 juveniles of *D. coronatus* were isolated and grown individually as described above. When starting to lay eggs, animals were transferred to new culture plates every two days until they died and the total of hatched larvae was counted.

Microscopical analysis and 3-D reconstructions

Development was studied with Nomarski optics using a 100× objective. One-cell stage embryos were collected from agar plates with a drawn-out Pasteur pipette or after dissection of gravid adults. Specimens were placed on microscope slides carrying a thin agar layer as a mechanical buffer and covered with a coverslip sealed on the edges with petroleum jelly. Development was recorded using a 4D microscope with 15–25 optical sections/

embryo and 15–60 s time intervals between scans [20]. Cell behavior was traced with help of the Simi Biocell software (Simi Reality Motion Systems GmbH, Unterschleißheim, Germany). Nuclei were counted in optical sections of DAPI-stained isolated gonads.

Diploscapter coronatus ITS, SSU, LSU rDNA analysis

For each *D. coronatus* rDNA gene, two individuals were picked and lysed. Using single-worm PCR [21], we cloned sequences from each rDNA gene and individual into separate pBluescript KS cloning vectors. For amplification of the ribosomal small subunit (SSU), we used primers described in [22, 23], for the ribosomal large subunit (LSU) primers from [24] and for the ribosomal internal transcribed spacer (ITS) from [25]. For phylogenetic analysis, we used Mr. Bayes [26](version 3.1.2) and RAxML (version 7.2.8) [27] with standard parameters and 100 bootstraps. Resulting trees were collapsed after first node.

OrthoMCL clustering and identification of the presence and absence of orthologs

To reliably compare orthologs, we used the OrthoMCL pipeline (version 2.0.9) [28] to cluster proteomes of five *Caenorhabditis* species (*C. angaria*, *C. briggsae*, *C. elegans*, *C. japonica*, *C. tropicalis* [29–31]; http://www.ebi.ac.uk/ena/data/view/GCA_000186765.1), *D. coronatus*, *Pristionchus pacificus* [32], *Panagrellus redivivus* [33] and *Ascaris suum* [14, 34]. The absence of genes in the *D. coronatus* genome which are present in the genomes of *C. elegans*, *C. briggsae* and *C. remanei* was confirmed by reciprocal BLAST search.

Gene ontology (GO) term analysis

Fisher's exact test for gene ontology (GO) terms of *D. coronatus*-specific clusters and singletons (proteins comprising a species-specific single variant) during early embryogenesis was applied to identify significantly overrepresented GO terms [35, 36] (FDR < 0.05; $p < 0.001$).

Phylogenetic classification and analysis

We here refer to the phylogeny of Holterman [37], dividing nematodes into 12 different clades. Following De Ley and Blaxter [38], we distinguish more basal Enoplea (clades 1 and 2) from more derived Chromadorea (clades 3–12). While *C. elegans*, *D. coronatus* and *P. pacificus* are members of clade 9, other species mentioned in this paper belong to clade 12 (*Meloidogyne* spp.), clade 11 (*Acrobeloides nanus*), clade 10 (*P. redivivus*; *Panagrolaimus* spp.), clade 8 (*A. suum*), clade 6 (*Plectus sambesii*) and clade 2 (*Romanomermis culicivorax*).

In order to visualize the structural differences between the *C. elegans* and the *D. coronatus* LET-99 homologs,

multiple alignments were performed using the program Clustal OMEGA [39]. Outgroup proteins including an N-terminal DEP domain were retrieved from Pfam database [40]. For the phylogenetic analysis, the best amino acid (AA) substitution matrices were identified using the program Prottest3 under the conditions of invariant sites and gamma optimization. Best substitution matrices were identified under the condition of the Bayesian information criterion and the Akaike information criterion [41]. Phylogenetic trees were constructed using RAxML with gamma value optimization and the substitution matrices suggested by Prottest3. Each tree was bootstrapped 100 times.

RNA extraction and RNA sequencing of selected embryonic stages

For RNA sequencing, we collected under the dissecting scope four batches (= 4 independent biological replicates) of approximately 100 eggs each, consisting of 1–8 cell stage embryos. These were placed into 25 μl H_2O, shock-frozen in liquid nitrogen and immediately stored at − 80 °C to avoid RNA degeneration. RNA of each sample was extracted by a slightly modified version of an established protocol [42]. Instead of using 4 M guadiniumthiocyanate (GU) buffer, we used 6 M GU buffer. By adding 175 μl 6 M GU buffer and using a homogenizer (Ultra-Turrax, IKA Werke GmbH), it was possible to lyse the samples under chaotropic conditions. The extracted amount of total RNA was dissolved in 2 μl RNAse-free water and used for RNA amplification using the "Message AMP II" kit (AM1751; Life Technologies Inc.) following the protocol of Hashimshony et al. [13]. This allowed linear amplification (in contrast to exponential amplification methods such as PCR) of the total RNA content, hence significantly decreasing the amplification bias. TruSeq library construction (TruSeq preparation kit version 2; Illumina Inc.) and RNA sequencing were performed on Illumina HiSeq and MiSeq platforms at the local sequencing facility (CCG Cologne). Retrieved paired-end reads ranged from approximately 8,500,000 to 31,000,000, depending on the sequencing platform.

Post-sequencing analysis

Illumina paired-end reads were retrieved in four independent sequencing assays. Illumina adapters and indexes were removed using the program Trimmomatic [43], and 5′ and 3′-prime error-prone reads were removed using the program sickle (github.com/najoshi/sickle). Trimmed reads were used to generate a transcriptome using the de novo assembler Trinity [44]. To identify even scarce transcripts all four libraries were combined, this way a transcriptome with a maximum number of transcripts and the highest median was obtained. To screen

for and eliminate bacterial contamination, assembled transcripts were mapped back to the *D. coronatus* EST library and transcriptome using bowtie2 [45]. For comparison with similar early embryonic stages of *C. elegans* and *A. suum*, the raw data were taken from [13, 46]. In the case of *C. elegans*, sequences showing an average TPM (transcripts per millions) value of > 5 were counted as being expressed. In *A. suum*, significant expression differences between the 1- to 4-cell stages on the one hand and subsequent stages on the other [46] allowed an estimation of the early-stage-specific transcriptome.

The transcriptome of *D. coronatus* was translated into protein sequences using the program Transdecoder [44] with a minimum AA length of 49 residues. Transcripts for which information was only available for the 3′-UTR (untranslated region) or which were shorter than 49 residues were aligned to an EST library to generate extended gene models. Resulting extended contigs were translated into AA sequences following the same procedure as described above. For *C. elegans* and *A. suum*, proteins corresponding to the early transcriptome were downloaded from wormbase.org. The retrieved protein sequences were used for orthologous clustering using OrthoMCL.

Identification of "allelic" gene variants
We determined the intron–exon structure and the positions on the genome for all of the predicted genes. By aligning and clustering all EST libraries making use of CD-HIT [47] at a threshold of 90% identity, we identified ESTs belonging to the same gene. We mapped clustered ESTs against the genome using BLAT [48] this way confirming the exact position of each EST cluster on the genome. EST clusters mapping to open reading frames (ORFs) of genes were translated into AA sequences. Corresponding proteins were cross-compared by an all-versus-all blast [49] approach (at a threshold of 98% identity). We sampled pairwise occurring genes with an amino acid identity of > 98%. These were considered as different copies of the same gene under the condition that they were positioned on different contigs. In the following, we call these pairwise occurring genes with high AA identity "alleles." Taking into account the relative position on the genome, we deduced their numbers by counting positions and contigs.

Identification of *C. elegans* genes in *D. coronatus* with two "alleles" at different loci
Orthologous clusters consisting of only two proteins were extracted from our OrthoMCL clustering. Respective protein sequences were pairwise aligned via clustal OMEGA multiple alignment algorithms. AA identity was calculated by custom Perl scripts. Sequences which are known to exist at two different loci in the *C. elegans* genome and still have an identity of 95% were considered to be genes existing in two distinct "alleles."

Single-copy gene analysis
Single-copy genes present in nematodes of various clades as well as in *Drosophila melanogaster* and *Saccharomyces cerevisiae* were selected based on [50]. *D. coronatus* and *C. remanei* orthologs were identified by OrthoMCL. For *C. remanei*, candidates were retrieved from wormbase.org using the *C. elegans* orthologs described in [50]. Known *C. elegans* orthologs were identified in the *D. coronatus* genome with NCBI Blast. The identity of a *D. coronatus* ortholog was confirmed by OrthoMCL clustering and by using the predicted genes of *D. coronatus* as a query to search for the original ortholog in the *C. elegans* genome (best-reciprocal-blast-hit approach).

By pairwise alignment with clustalW [51], we compared on the one hand *D. coronatus* "alleles" with each other and on the other hand the respective *C. elegans* orthologs with their *C. remanei* orthologs. We also scanned the orthologs of the single-copy genes for conserved protein domains with InterProScan [52]. We counted the number of synonymous and non-synonymous mutations within the respective single-copy genes by pairwise alignment of the sequences on the nucleotide level taking into account the appropriate reading frame and by using the KaKs Calculator (version 1.2) with standard parameters (http://evolution.genomics.org.cn/software.htm; [53]). For a statistics of average nucleotide exchange rates, the one-tailed Welch *t* test for non-equal variances was applied. We tested specifically the null hypothesis, i.e., whether the fraction of non-synonymous mutations is equal or greater than the fraction of synonymous mutations. The null hypothesis was rejected at a significance level of $\alpha = 0.01$.

Identification of expressed *D. coronatus* "alleles" during early embryogenesis
Diploscapter coronatus transcripts expressed during early embryogenesis were mapped back to the *D. coronatus* expressed sequence tag (EST) library using the program Bowtie2. Transcripts mapping to two different genomic contigs were considered as "alleles" (see above). ESTs were clustered using the program CD-HIT with standard parameters for > 94% identity. Mapped reads were used to identify polymorphisms (in particular single-nucleotide polymorphisms; SNPs) by using the programs SAMtools and Bcftools [54] with a minimum sequencing coverage of tenfold per site. The genotype quality (GQ, [55]) of each retrieved SNP was represented by a maximum likelihood for wrong SNP calls of $< 10^{-3}$. Variations which did not meet these criteria were considered as

random variants probably due to sequencing errors. Haplotypes for each variant were inferred by usage of the "vcfgeno2halpo" command of the Vcflib suite (https://github.com/vcflib/vcflib) for a window size of 500 bp. Transcripts with > 99% nucleotide identity were defined as indistinguishable and excluded from the analysis.

Results

Multi-species orthologous clustering

We compared our *D. coronatus* data with other nematodes, including *C. elegans*, to better understand the molecular basis of developmental peculiarities in this species (Table 1). The *D. coronatus* draft genome contains more than 34,000 protein predictions, and we used these to screen for conserved and species-specific genes. In order to identify robust orthologous clusters in comparison with several other species selected for their phylogenetic position (Table 1, Fig. 1), we used OrthoMCL. In total, we found over 8000 orthologous clusters shared between *P. redivivus* (clade 10) and *A. suum* (clade 8). About 80% of these are present in all seven clade-9 species considered here as well, suggesting a core set of shared protein families. However, the majority of the nearly 20,000 clusters are not shared with *P. redivivus* and *A. suum* (Fig. 2).

By analyzing five *Caenorhabditis* species and in addition *D. coronatus*, *P. pacificus* and *P. redivivus*, we found that over 5000 orthologous clusters, or nearly 50% of all clade 9-restricted clusters were specific to the genus *Caenorhabditis*. This suggests that during evolution a considerable number of genes must have newly arisen in the lineage leading to this taxon.

Absence of genes and development in *D. coronatus*

Using our ortholog clustering (Fig. 2), we investigated which genes known to be crucial in development of *C. elegans* are restricted to the genus *Caenorhabditis*. We found an absence of developmental regulators for a variety of biological processes in other nematodes (Fig. 3) and decided to focus on oogenesis and early embryogenesis where we had observed phenotypical idiosyncrasies in *D. coronatus*.
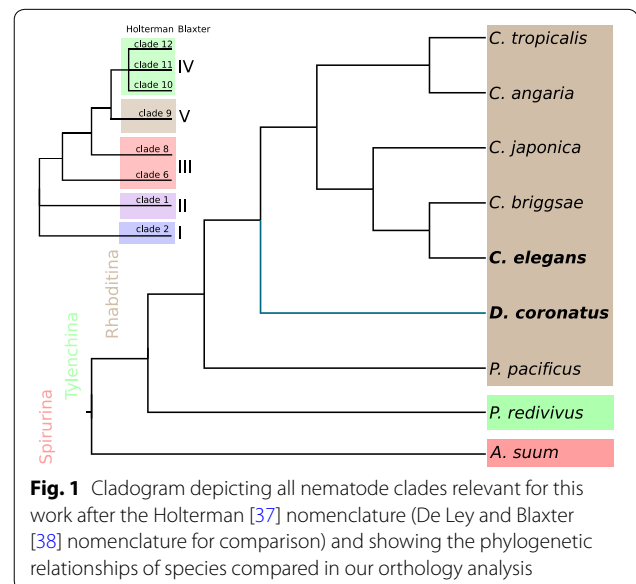
Oocyte development and modified meiosis

In *C. elegans*, the development of germ cells from mitotic oogonia to mature oocytes is arrested in prophase of meiosis I and their subsequent activation by sperm is an elaborate process [56]. We found that each of the two gonadal arms of the mature *D. coronatus* adult is about 5× smaller than in *C. elegans* and contains only 30–100 germ cell nuclei (Fig. 4a, b; $n = 20$) in contrast to the latter where about one thousand is generated [57]. Under our laboratory conditions, individual *Diploscapter*
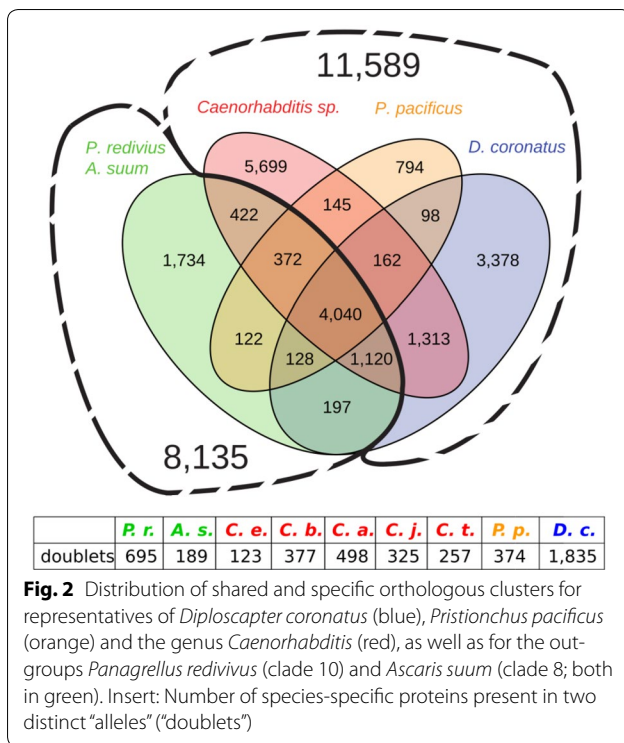
Table 1 Species and proteomes used in this study

Species	Clade[a]	Number of proteins	
		Whole genome	Early embryogenesis
C. elegans	9	22,855	8817
C. angaria	9	28,371	–
C. japonica	9	30,083	–
C. tropicalis	9	24,532	–
D. coronatus	9	31,693	4610
P. pacificus	9	23,750	–
A. suum	8	17,555	3093
P. redivivus	10	26,372	–

[a] For phylogenetic classification, see "Methods"

Fig. 1 Cladogram depicting all nematode clades relevant for this work after the Holterman [37] nomenclature (De Ley and Blaxter [38] nomenclature for comparison) and showing the phylogenetic relationships of species compared in our orthology analysis

females produced less than one-third the number of eggs found in *C. elegans* (on average 80; $n = 17$). Different to the latter, the size of developing germ cells in *D. coronatus* increases only marginally except for the most mature one ("−1 oocyte") which is much larger and densely filled with yolk granules (Fig. 4a; in older adults also the −2 oocyte starts to grow). We wondered whether the same phases of oocyte differentiation as in *C. elegans* can be found in *D. coronatus*.

The analysis of DAPI-stained germline nuclei indicates that this is not the case. In the adult *D. coronatus* ovary essentially all germ cell nuclei appear to be in premeiotic interphase (Fig. 4b) except for rarely observed mitoses in the distal-most region and possibly initiation of meiosis in some late oocytes. Condensed meiotic chromosomes were only found in oval-shaped 1-cell stages in the uterus

Fig. 2 Distribution of shared and specific orthologous clusters for representatives of *Diploscapter coronatus* (blue), *Pristionchus pacificus* (orange) and the genus *Caenorhabditis* (red), as well as for the outgroups *Panagrellus redivivus* (clade 10) and *Ascaris suum* (clade 8; both in green). Insert: Number of species-specific proteins present in two distinct "alleles" ("doublets")

	P. r.	A. s.	C. e.	C. b.	C. a.	C. j.	C. t.	P. p.	D. c.
doublets	695	189	123	377	498	325	257	374	1,835

Fig. 3 Orthologs of genes with essential functions in different processes in *C. elegans* not found in *D. coronatus* and two other species outside the genus *Caenorhabditis*. Green, genes found; red, genes not found. *C.e.*, *C. elegans*; *C.b.*, *C. briggsae*; *C.r.*, *C. remanei*; *D.c.*, *D. coronatus*; *P.p.*, *P. pacificus*; *P.r.*, *P. redivivus*

surrounded by an eggshell (Fig. 4c, d). Thus, in contrast to *C. elegans*, in *D. coronatus* individual germ cells seem to enter meiosis late and one by one without prophase arrest.

Diploscapter coronatus contains only two chromosomes in the diploid status ($2n = 2$ [2, 58]), whereas in *C. elegans* $2n$ equals 12 chromosomes [59]. In accordance with Hechlers report [58], we never observed paired meiotic chromosomes.

Screening the *D. coronatus* genome for genes essential for germ cell development or sex-specific cell differentiation in *C. elegans*, we found orthologs of several of such genes missing (see detailed description below). However, their absence cannot serve as a straight forward explanation for special features of the parthenogenetic *D. coronatus* as they were not detected in *Pristionchus* and *Panagrellus* as well (for phylogeny, see Methods), while present in all three considered *Caenorhabditis* species (Fig. 3). At least, our data indicate that the control of central developmental processes differs between members of the genus *Caenorhabditis* and representatives of neighboring clades and even within the same clade.

Polarity establishment and early embryogenesis

Gonochoristic and hermaphroditic reproduction depends on sperm, which contributes the centriole, essential for mitotic spindle formation, and initiates embryonic polarity as a prerequisite for subsequent asymmetric cell division and soma/germline separation in *C. elegans* [60, 61].

The proper positioning of the first cleavage spindle in *C. elegans* and subsequent movement of one aster toward the posterior pole of the zygote preceding its asymmetric division requires the presence of LET-99. The C-terminal domain of LET-99 appears to be important for its functionality as nonsense mutations lead to strong phenotypes [62]. LET-99 is also the main regulator for the localization of LIN-5 and GPR-1/GPR-2 [63] which act together to generate the necessary spindle pulling force [64, 65]. In the genome of *D. coronatus*, we could not find orthologs of *gpr-1/2* (Fig. 3).

We identified a LET-99 homolog in *D. coronatus* (DcLET-99). However, alignment of the protein sequence with that of other species (Fig. 5) revealed that the last 70 AA of the C-terminal region is absent in the genus *Caenorhabditis*. The *Diploscapter* ortholog thus has more similarity with the protein in other nematodes, like the ones included in our study (Fig. 5). This finding may reflect a non-equivalent function of this protein in *C. elegans* and *D. coronatus*.

With these findings in mind, we compared the establishment of asymmetry between *D. coronatus* and *C. elegans*. Characteristic for *C. elegans* is the migration
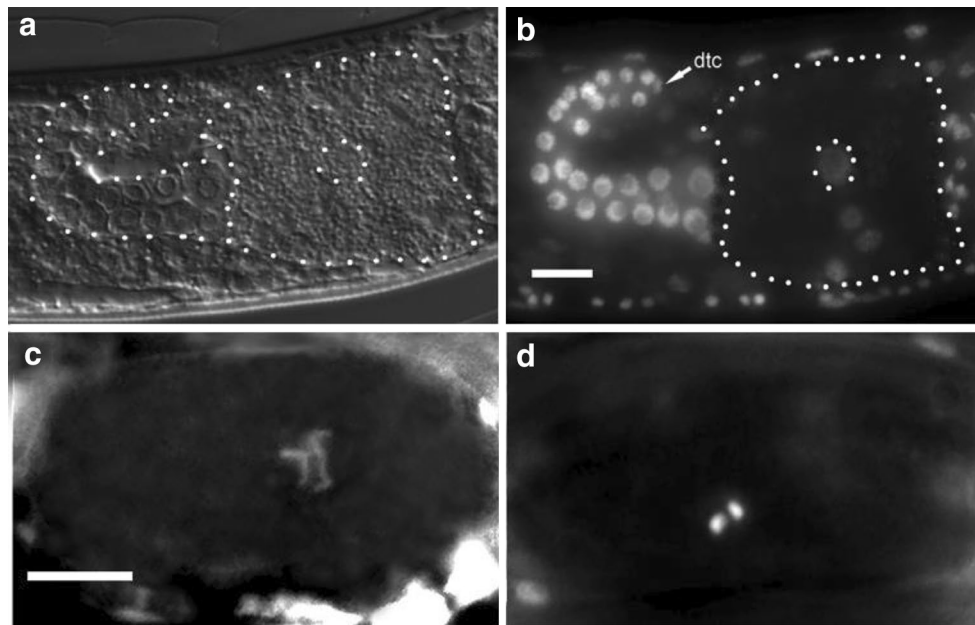
Fig. 4 Gonad and chromosomes in *D. coronatus*. **a** Outline of one gonadal arm including one large oocyte; **b** gonadal arm with DAPI-marked germ cell nuclei; **c**, **d** extended and condensed chromosomes in prophase of meiosis I
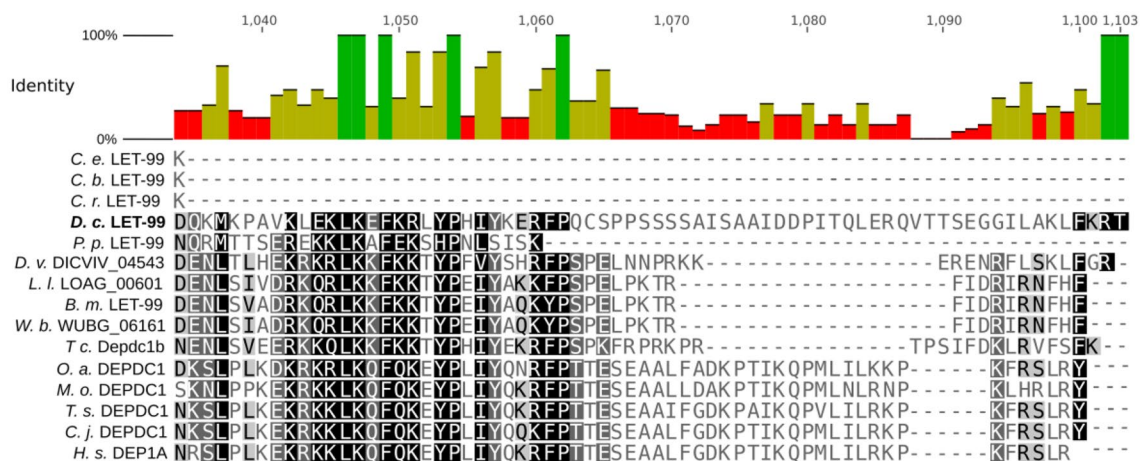

Fig. 5 Sequence alignment of the C-terminal region of the *D. coronatus* LET-99 domain in comparison with *Caenorhabditis,* other nematodes and the homologous mammalian DEPDC1 protein. The C-terminal region is absent in *Caenorhabditis* species (*C. e., C. elegans; C. b., C. briggsae; C. r., C. remanei*) but present in the other tested nematodes (*D. c., D. coronatus; P. p., Pristionchus pacificus; D. v., Dictyocaulus viviparus; L. l., Loa loa; B. m., Brugia malayi; W. b., Wucheria brancrofti; T. c., Toxocara canis*) and vertebrates (*O. a., Ornithorhynchus anatinus; M. o., Microtus ochrogaster; T. s., Tarsius syrichta; C. j., Callithrix jacchus; H. s., Homo sapiens*)

of the two pronuclei to the center of the fertilized egg cell and their subsequent fusion (Fig. 6a–c). Consequently, the posterior aster together with the future P_1 chromosome set is quickly translocated to the posterior, while the anterior aster remains at its original position (Fig. 6c, d). This results in an asymmetric division of the zygote into a larger somatic AB and a smaller P_1 germline cell (Fig. 5e, f). Subsequently, AB divides with transverse and P_1 with longitudinal spindle orientation (Fig. 6g) resulting in a rhomboid 4-cell stage (Fig. 6h). With the division of P_2, a reversal of cleavage polarity (PR) takes place in the germline such that P_3 occupies a more anterior position relative to its somatic sister C (Fig. 6i) [66].
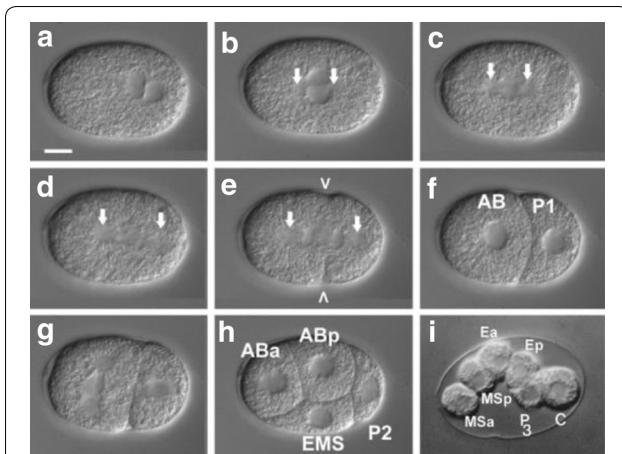
Fig. 6 Early embryogenesis of *C. elegans*. **a** pronuclei meet posteriorly; **b** central rotation of pronuclei; **c** central cleavage spindle; **d** posterior movement of posterior aster; **e** formation of two nuclei, initiation of cytokinesis; **f** 2-cell stage; **g** spindle orientation: transverse in AB and longitudinal in P1; **h** 4-cell stage; **i** polarity reversal with generation of anterior P3 and posterior C. For further details, see text. Arrows, centriolar regions; arrowheads, cleavage furrow; **i** for better visualization of polarity reversal in the germline AB cells had been removed through a laser-induced hole in the eggshell. Scale bar, 10 µm; orientation, anterior, left
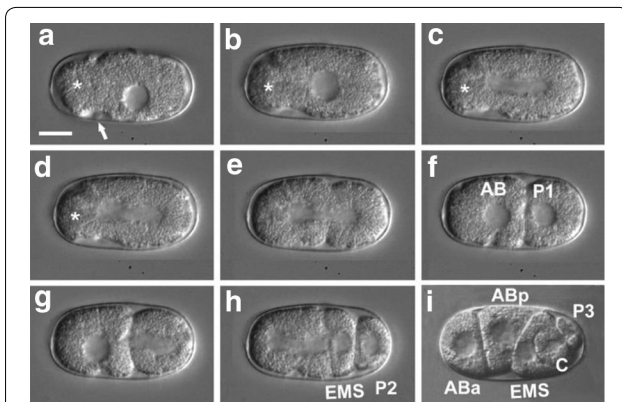
Fig. 7 Early embryogenesis of *D. coronatus*. **a** single pronucleus; **b** formation of anterior constriction; **c** cleavage with central spindle; **d** formation of 2 nuclei, regression of constriction; **e** cytokinesis; **f** 2-cell stage; **g**, **h** a-p-oriented cleavage spindle in P1 and AB; **i** no polarity reversal with generation of anterior C and posterior P3. For further details, see text. Arrow, single polar body; asterisk, separated anterior cytoplasm. Scale bar, 10 µm. Orientation, anterior, left

In the parthenogenetic *Diploscapter*, only one pronucleus is generated during meiosis (Fig. 7a, b; [1]). In contrast to *C. elegans*, a temporary constriction forms at the anterior pole and the maternal pronucleus occupies a slightly eccentric position (Fig. 7b–d). The zygote divides with no shift of the posterior aster (Fig. 6c–e), while the constriction regresses. This way, a larger AB and a

smaller P_1 cell are formed (Fig. 7e, f). Subsequently, AB divides with longitudinal spindle orientation like P_1 (Fig. 7g, h) and a PR in P_2 is absent (Fig. 7i). The absence of *gpr-1* and *gpr-2* plus the considerably diverged *let-99* could give an explanation for the different ways of how the asymmetric division of the zygote is achieved in the two species.

As *gpr-1* and *gpr-2* are absent in *P. pacificus* and *P. redivivus*, too (Fig. 3), we studied formation of asymmetry in 1-cell embryos there and found it to be similar to *D. coronatus*, while *C. briggsae* and *C. remanei* behave like *C. elegans*. This can also be deduced from the video clips accompanying [85]. In addition, we analyzed one representative each of Cephalobidae (*A. nanus*) and Plectidae (*P. sambesii*). With respect to spindle movement, they behave similar to *D. coronatus* (data not shown). These findings indicate that the genus *Caenorhabditis* has developed a special way of how to accomplish the first asymmetric cleavage.

Spindle orientation and germline polarity

As the same a–p spindle orientation in the AB cell of *D. coronatus* was also found in a *par-3* mutant of *C. elegans* [67], we screened the genome of *D. coronatus* for the presence of *par* genes. We found an ortholog of *par-3* but not of *par-2*. The absence of these two genes in *C. elegans* leads to a transverse spindle orientation in P1 [67]. However, in the *par-2/let-99* double mutant the majority of embryos orients the cleavage spindle longitudinally in both blastomeres [68]. As the same genes missing in *D. coronatus* are also absent in *Pristionchus* and *Panagrellus*, which, however (Fig. 3), show a *C. elegans*-like AB spindle orientation, the identified molecular differences between *C. elegans* and *D. coronatus* can at most be considered a prerequisite for an alternative spindle orientation. The visible presence of a central cortical region in P1 and AB rather than in P1 alone has been suggested to be responsible for capturing spindle microtubules in both cells of *D. coronatus* resulting in a–p spindle orientation [8].

Early transcriptome: species-specific orthologous clusters and their expression

To investigate to what extent the initial steps of embryogenesis in *D. coronatus* are reflected on the gene expression level, we sequenced 1–8-cell stages and compared their transcriptome with available data of similar stages from *C. elegans* [13] and *A. suum* [46]. By assembling transcriptomes from four independent biological replicates (Additional file 1: Table S1), we retrieved in total more than 6500 transcripts with a median length of 381 bp (Additional file 2: Fig. S2; Table 2). For around 70% of these, we could identify open reading frames (ORFs) allowing a successful inference of the early proteome.

Table 2 Average transcript lengths and numbers of transcripts of four independently sequenced *D. coronatus* libraries for early developmental stages

Libraries	Median transcript length (bp)	Mean transcript length (bp)	Number of transcripts
1	230.5	244.3	1059
2	269.0	285.9	3151
3	330.0	360.2	4550
1 + 3	329.0	359.1	5218
4	350.0	370.8	5384
2 + 4	358.0	377.0	5531
3 + 4	378.0	432.5	6213
1 + 3 + 4	378.0	400.4	6487
1 + 2 + 3 + 4	381.0	404.0	6550

Fig. 8 Early expressed clusters (protein families) of *D. coronatus* in comparison with the model *C. elegans* and the parasitic *A. suum*. For reference, the number of singletons is given for each species. *C. e.*, *Caenorhabditis elegans*; *D. c.*, *Diploscapter coronatus*; *A. s.*, *Ascaris suum*
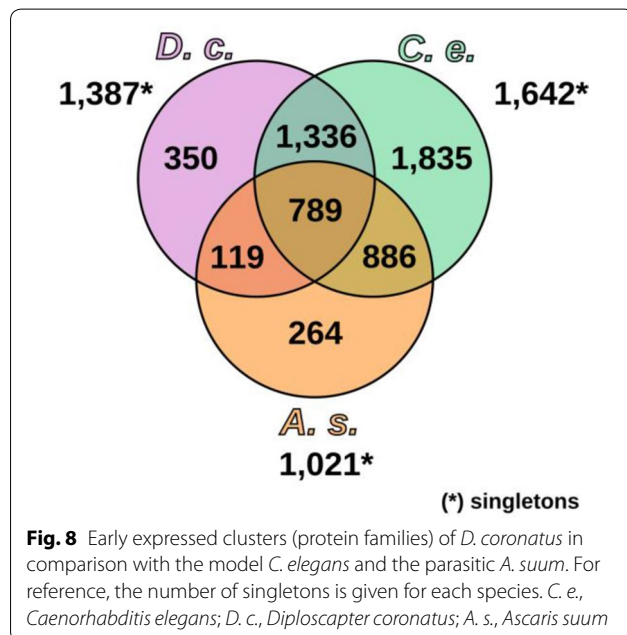
We used our transcriptome data from *D. coronatus* to perform an orthology clustering between the three nematodes. This way, we identified protein clusters that are shared among all three species as well as ones that are exclusively expressed in individual representatives during early development (Fig. 8).

By subtracting orthologs expressed during early embryogenesis in *C. elegans*, we identified genes expressed only in the other two species. We retrieved 119 orthologous clusters shared between *D. coronatus* and *A. suum* as well as 350 (comprising nearly 1500 genes) expressed exclusively in the early *D. coronatus* embryo (Fig. 8). While we found that orthologs of more than 60% of these genes are present in the genomes of *C. elegans* and *A. suum*, they are not transcribed there during early embryogenesis,

suggesting interspecific heterochronic shifts of expression patterns.

Exploring which potential functions these early expressed genes might have in *D. coronatus*, more than 500 were classified as "unknown" as either no homology to any protein could be detected or homologous proteins are also unknown in their respective functions. In addition, we found over 1300 *D. coronatus*-specific transcripts expressed as a single sequence only ("singletons," Fig. 8).

Early transcriptome: functional classification

We searched for significantly overrepresented gene ontology (GO) terms for genes specifically expressed in the early *D. coronatus* embryo (1–8 cell stage) in comparison with the full *D. coronatus* gene set (Additional file 3: Fig. S1, Additional file 4). By far, the most overrepresented was "regulation of centromere complex assembly" (GO:0090230; > 200-fold). Related to this is "CENP-A containing nucleosome assembly at centromere" (GO:0034080; 48-fold). While it must be assumed that CENP-A is ubiquitously essential for mitosis, the lack of expression in *C. elegans* can be explained most easily with a maternal supply of the protein. Other potentially interesting overrepresented terms are "cytokinesis, initiation of separation" (GO:0007108; 64-fold) as well as chromatin remodeling-associated terms such as "histone H2A acetylation" (GO:0043968; 48-fold) and "NuA4 histone acetyltransferase complex" (GO:0035267; sixfold).

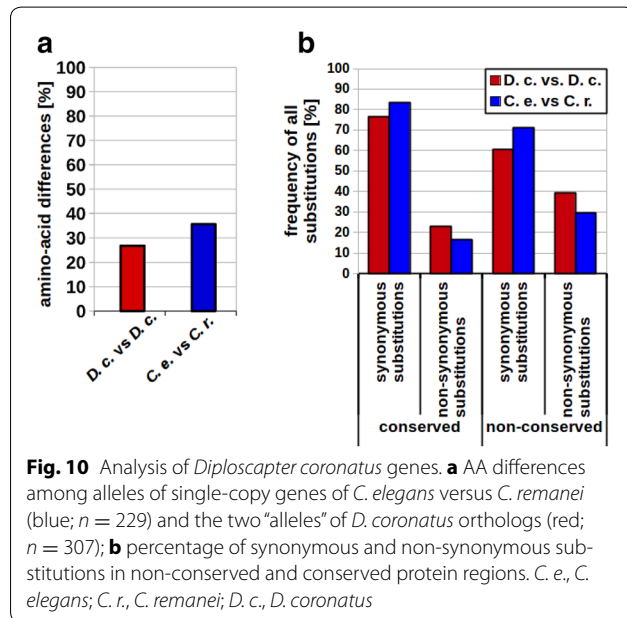NuA4 is involved in the acetylation of H2A in yeast nucleosomes to exchange H2A for H2A.Z [69] which in turn regulates gene expression. In the *C. elegans*, embryo H2A.Z (or HTZ-1) is expressed in every blastomere and essential for normal development [70]. The observed NuA4-dependent acetylation in the early *D. coronatus* embryo is consistent with the assumption that massive zygotic transcription is required.

In search for further genes that could play a role for the unique *D. coronatus* early development, we looked for genes that are expressed in the early embryo but for which no orthologs were found in the genomes of *C. elegans* and *A. suum* and analyzed their expression. In this category, we detected less than 10 genes. As we could not retrieve orthologs of any of these genes in other reference systems like *Drosophila*, zebrafish or mouse, we are presently unable to speculate about their function in *Diploscapter*.

As an alternative approach, we have started to look for protein domains significantly enriched in the early transcriptome of *D. coronatus* in comparison with *C. elegans* and *A. suum*. So far, we found a variety of enriched

domains giving the chance to further investigate the role of certain proteins for developmental peculiarities in this species.

The parthenogenetic *D. coronatus* shows high "allelic" divergence

Analyzing the genome of *D. coronatus*, we had observed an unexpected degree of heterozygosity in the high-quality draft genome (N50 = 1,007,652 bp, number of scaffolds = 511; [2]) resulting in the prediction of two "alleles" per gene in the Augustus pipeline. We observed a similar pattern when using Sanger sequencing methods on cloned PCR products of rRNA genes from individual

Fig. 9 Sequence comparison of the small internal transcribed spacer (ITS) rDNA gene of *D. coronatus*. **a** Sequence alignment of individual clones (*A*–*J*) shows selected regions with distinct single-nucleotide polymorphisms (SNPs). **b** Collapsed maximum likelihood tree representing clustering of sequenced clones. Bootstrap values are shown above and posterior probability beneath branches

Table 3 Genes existing in two distinct alleles identified in the *C. elegans* genome

wormbase ID allele 1	wormbase ID allele 2	AA identity[a]
WBGene00004451	WBGene00012179	97.82
WBGene00001581	WBGene00019017	96.01
WBGene00019254	WBGene00012795	96.20

[a] At a threshold of at least 95% AA identity

Fig. 10 Analysis of *Diploscapter coronatus* genes. **a** AA differences among alleles of single-copy genes of *C. elegans* versus *C. remanei* (blue; $n = 229$) and the two "alleles" of *D. coronatus* orthologs (red; $n = 307$); **b** percentage of synonymous and non-synonymous substitutions in non-conserved and conserved protein regions. *C. e., C. elegans*; *C. r., C. remanei*; *D. c., D. coronatus*

worms. In fact, we retrieved several sequences per rRNA locus per individual (Fig. 9a; Additional file 2: Fig. S2). Aligning these sequences showed distinct single-nucleotide polymorphisms (SNPs), and in a phylogenetic analysis all sequences could be allocated to one of two distinct "alleles" (Fig. 9b; Additional file 2: Fig. S2).

This was on a larger scale reflected in the orthology clustering we performed for this work. Here, we found more than 3000 *D. coronatus*-specific clusters and more than 50% of these contained two in-paralogs ("doublets"), which is a multiple of what has been observed in the other studied nematodes (e.g., 2% in *C. elegans*; Fig. 2). In the complete genome, 66% of all genes in *D. coronatus* were found to exist in doublets [2]. In contrast, the number of clusters consisting of a species-specific single protein ("singletons") was by far the smallest in *D. coronatus* (2727; *C. e.* 6067).

Diploscapter coronatus-specific clusters comprising two proteins are in accordance with our earlier finding that in this species most genes are present as allelic pairs. In contrast, in *C. elegans* we found only three such examples (Table 3).

This pattern can be explained in two ways: (1) the independent accumulation of SNPs in non-recombining alleles (known as Meselson effect) in an old parthenogenetic lineage [71, 72] or (2) a hybrid origin of the parthenogenetic strain, where distinct alleles are inherited from the ancestral sexual species and genomic heterozygosity is maintained.

To investigate to what extent an accumulation of mutations occurred in the *D. coronatus* genome, we compared conserved single-copy genes in the nematode phylum. We found that in *D. coronatus* each of these single-copy genes exists in two distinct "alleles." The number of AA differences between the two *D. coronatus* "alleles" is similar to corresponding AA differences between *C. elegans* and *C. remanei* (Fig. 10a). Calculation of dN/dS ratios in 11 arbitrarily selected single-copy genes revealed a median value of 0.158 (Additional file 1: Table S2) indicating negative selection. To identify potential differences between conserved and non-conserved protein domains, we applied InterProScan [52]. As expected, we found the number of non-synonymous exchanges to be lower than synonymous exchanges. But the ratio of synonymous to non-synonymous substitutions was again not significantly different when comparing the two *D. coronatus* "alleles" with the respective *C. elegans* versus *C. remanei* orthologs (Fig. 10b). This is in line with (ii; see above) and can be easiest explained with a recent interspecies hybridization event.

Differential expression of alleles

Incipient hybrids may face dosage compensation issues, and proteins built from alleles inherited from different genomes might be incompatible or less efficient in molecular machineries. It is therefore possible that hybrid species need to silence one of the parental genomes [73]. Making use of the high-quality *D. coronatus* genome and our RNA-Seq data covering early embryogenesis we asked whether transcripts of one or both "alleles" are generated by screening for single-nucleotide polymorphisms (SNPs) in comparison with an EST library. We found that in the vast majority of genes, both "alleles" are expressed (Additional file 5: Fig. S3).

Discussion

Our previous studies of the parthenogenetic nematode *D. coronatus* focused on early embryogenesis [1, 8] and on molecular regulators important for the oocyte-to-embryo transition [12]. Recently, we sequenced and assembled the genome of *D. coronatus* [2] and here use these data as a reference to revisit these questions in a more comprehensive scope.

Meiosis, *D. coronatus*-specific genes and preservation of heterozygosity

Previously, it has been shown that *D. coronatus* passes through a truncated meiosis generating only one polar body [1]. We find that the gonad of *D. coronatus* differs in several aspects from *C. elegans*. The uniformity of germ cells (Fig. 4a, b) suggests that due to the small size of the gonad the distal tip cell (dtc) prevents entrance into meiosis essentially all along the gonadal tube (in contrast to *C. elegans*, see [74]). Only the most proximal oocyte, possibly due to its translocation into the uterus, has clearly visible escaped its influence (Fig. 4c, d). Another conclusion is that there is no meiotic arrest of germ cells in the gonad as found in *C. elegans* [56, 75], and therefore, no sperm is needed to lift it.

Compared to *C. elegans*, *D. coronatus* seems to follow a different strategy for the control of germ cell development. It would be attractive to study in this respect the role of the dtc in nematodes with particularly small gonads and a low brood size like *D. coronatus* or the parthenogenetic *P. sambesii* [76] and match it with representatives possessing extremely long gonadal arms, like *Ascaris*, producing millions of eggs [77].

The fact that no orthologs were found of crucial genes required for the generation of the synaptonemal complex (e.g., *syp-1/-2/-3*) and chromosome-specific adapters which are also involved in proper meiosis (*zim-1/-2/-3*, *him-8*; also see [2]) could mean that no crossing-over, and thus, no recombination takes place. This is consistent with our inability to detect paired meiotic chromosomes (see also [58]). However, the finding that the genes listed above are absent in *Pristionchus* and *Panagrellus* as well (Fig. 3) does not offer a straight forward explanation. The genetic control of meiosis (and other processes) seems to differ generally between *Caenorhabditis*, and other nematodes and may involve other genes.

We identified more than 3300 *D. coronatus*-specific clusters comprising more than 7500 genes in its genome. However, the role of most of these remains elusive since no orthologs have been found in other model systems (Fig. 2). Looking at early expressed genes alone, we recovered more than 500 genes of unknown function (Fig. 8).

A possible explanation for how the heterozygosity in *D. coronatus* can be preserved while passing through just one meiotic division, including the separation of chromatids rather than homologous chromosomes during meiosis I ("inverted meiosis"; [78]), has been discussed in [2].

Polarity, asymmetry and absence of orthologs

Microscopical analysis of early embryogenesis in *D. coronatus* revealed certain idiosyncrasies [1, 8]. Here we show that the process of initial polarity establishment during the 1-cell stage differs markedly from *C. elegans* (Figs. 4, 5). Based on our ortholog clustering of the *D. coronatus* genome and eight other nematode genomes (Fig. 2), we conclude that certain genes crucial for early embryogenesis in *C. elegans* are absent in *D. coronatus*, *P. pacificus*, *P. redivivus* and *A. suum* (Fig. 3). With respect to polarity establishment, we did not find orthologs of essential genes known from *C. elegans*, such as *gpr-1/-2* in these species indicating differences in how early asymmetry is achieved. This appears particularly plausible for the parthenogenetic *D. coronatus*, where sperm as initial trigger is missing and where orientation of the anterior–posterior egg axis is obviously specified in a random fashion [1]. However, by scanning the *D. coronatus* genome for known GoLoco domain proteins involved in mitosis in vertebrates, we found that the human GPR-1/2 homolog LGN [60] has orthologs in *D. coronatus* (Additional file 6: Fig. S4). This suggests that *C. elegans* acquired new adaptor proteins for division, while *D. coronatus* relies on the established set of proteins known from outgroup species. It remains to be determined whether LGN functionally replaces GPR-1/2 in the *D. coronatus* 1-cell stage and to what extent the modified dcLET-99 homolog (see "Results" and Fig. 5) is involved in the establishment of early polarity.

Looking at conserved protein complexes which are essential for maintaining already established polarity, such as PAR-3/PAR-6/PKC-3 or PAR-1/-2 [63], we found respective orthologs in *D. coronatus*, except for PAR-2 (Fig. 3). This result is in accordance with earlier studies, where it was proposed that the PAR-2/-3 system known from *C. elegans* evolved specifically in the genus *Caenorhabditis* [5]. What could be an alternative mechanism for establishing polarity in other nematodes like *D. coronatus*? It was shown earlier that in *C. elegans* the *par-2* function can be replaced by *lgl-1* (ortholog of the tumor suppressor gene *lethal giant larvae*) if it is overexpressed [79]. *lgl* is known from various animal systems as a regulator of asymmetric cell division [80] indicating its high conservation. Its presence and the simultaneous absence of *par-2* in *D. coronatus* suggest an ancestral molecular mechanism of asymmetric cleavage there and in the other non-*Caenorhabditis* species studied, as known from animals outside the nematodes [81, 82].

Another idiosyncrasy in *D. coronatus* development is that the spindle in AB performs the same rotation as in P_1 resulting in its a–p orientation. In *C. elegans*, spindle microtubules in P_1 seem to compete for attachment to a bleb-like site at the anterior cortex [83, 84]. The presence of a prominent clear cortical region in the cortex of both 2-cell blastomeres in *D. coronatus* may indicate a symmetric distribution of components responsible for capturing spindle microtubules [8]. In the more basal nematode *R. culicivorax* (clade 2), a comparable effect

on spindle behavior appears to be exerted in the 2-cell stage by the "region of the first midbody." Its ablation results in a perpendicular spindle orientation [85]. More data on the gene regulatory networks and intracellular constraints in *D. coronatus* should help us to find the molecular basis for the aberrant orientation of the cleavage spindle in AB. Presently, we speculate that here an original mechanism has been replaced in more derived nematodes, including *C. elegans*, while the phylogenetic branch comprising *Diploscapter* and *Protorhabditis* [7, 16, 86] constitutes an atavism.

GO term analysis

We performed a gene ontology analysis of the "early transcriptome" and found over 70 terms overrepresented specifically in *D. coronatus*. It appears likely that enriched terms inform about important underlying biological processes [87]. While this approach should increase the likelihood for identifying such essential events, it can only play an advisory role in finding the most relevant, enriched annotation terms [88].

Many of the highly overrepresented terms are associated with chromosomal function in *D. coronatus*, for instance concerning centrosomes, chromosome structure, telomeres, DNA replication and gene regulation. This is consistent with the view that in *D. coronatus* these aspects differ considerably from *C. elegans*.

Origin of parthenogenesis and reduction in chromosomes

Different mechanisms and conditions have been described that could lead to the establishment of parthenogenesis [15, 89, 90].

In the parthenogenetic *D. coronatus* where neither males nor sperm have been observed but a high degree of heterozygosity, we conceive two possible scenarios. One is hybridization of closely related gonochoristic species followed by the evolution of parthenogenesis and the other a spontaneous origin of parthenogenetic reproduction followed by independent accumulation of mutations in alleles. In the latter case, the dN/dS ratio in the studied single-copy genes should be high as both non-synonymous and synonymous mutations are expected to accumulate to a similar degree in either of the two alleles. In contrast, a recent interspecies hybridization event should still show the footprints of purifying (negative) selection acting in the sexually reproducing parent species [91].

Comparing the AA sequences of "alleles" of arbitrarily selected single-copy genes, we found a high level of heterozygosity and the fraction of AA exchanges in these *D. coronatus* "alleles" was in fact not significantly different from that of orthologs in two distinct *Caenorhabditis* species (Fig. 10a).

This high genomic heterozygosity with two distinguishable "alleles" per gene, including the rDNA genes (Fig. 8; Additional file 2: Fig. S2) and single-copy genes (Fig. 10), and the low dN/dS ratio, can be easiest explained with a recent event of interspecies hybridization between two closely related representatives where each parental genome has still preserved a major part of its ancestral functionality. In fact, a similar explanation has recently been suggested for more distantly related parasitic nematodes of the genus *Meloidogyne* [72, 92, 93]) and for more closely related parthenogens within the genus *Panagrolaimus* (bioRxiv: [94]).

D. coronatus possesses only a single chromosome in the haploid set, while *C. elegans* and most other studied free-living nematode species of clades 9–12 contain 6 chromosomes or more [95, 96] (our unpublished data). However, a close relative of *Diploscapter*, *Protorhabditis* sp. (laboratory strains JB 122), also contains just a single chromosome, while other members of this genus have six like *C. elegans* (E.S., unpublished data). The most parsimonious explanation for this minimal number in selected species is a comprehensive chromosome fusion. Very recently good evidence has been presented in another *Diploscapter* species that this may be in fact the case [97]. While a reduction in chromosome number due to fusion has been described in a variety of cases [98, 99], integration of all chromosomes into a single one would be a very extraordinary case deserving further attention. A combined detailed phylogenetic and chromosome analysis may reveal whether the assumed fusion has occurred once in a common ancestor or several times independently, whether this has been a stepwise modification or a single total fusion event, and how original chromosomes are arranged in this new construct. While we can only speculate about a possible mechanism for such a dramatic event, it may be worthwhile to investigate whether each chromosome in *D. coronatus* represents a complete haploid parental genome which has remained intact and functional due to the absence of recombination. In this case, chromosome fusion would have taken place most likely prior to the envisaged interspecies hybridization.

A haploid chromosome number of $n = 1$ is neither a necessary prerequisite for parthenogenesis in nematodes nor a consequence of it, since in the bisexual *Ascaris* ($n = 2$) a variant exists with $n = 1$ (*var. univalens*; [100]), and in the parthenogenetic species *A. nanus* and *P. sambesii*, we counted $n = 6$.

It is an exciting question why in contrast to the closely related genera *Diploscapter* and *Protorhabditis* no parthenogenetic representatives have been found among the more than 30 *Caenorhabditis* species isolated so far [101] (NCBI Taxonomy). Taking into account the many

idiosyncrasies of the taxon *Caenorhabditis* concerning the control of development (see, e.g., Fig. 3), it seems not unlikely that its molecular circuitry, allowing for instance a particularly rapid propagation, has not allowed the establishment of parthenogenesis.

The genus *Caenorhabditis* versus *Diploscapter* and other nematodes

Many *C. elegans* genes not found in *D. coronatus* were also absent from other non-*Caenorhabditis* species (Fig. 3). The peculiarities in early development of *D. coronatus* can thus not be explained just by the absence of these genes. It is feasible that in *Diploscapter* certain processes differ from *C. elegans* due to its parthenogenetic mode of reproduction. However, in light of the genomic similarities all studied non-*Caenorhabditis* nematodes may use the same alternative pathways (or at least alternative components) to control essentially identical developmental processes during oogenesis and early embryogenesis (Developmental System Drift [4]). Therefore, it appears more likely that these differences gave the freedom to establish parthenogenesis while preventing it in the genus *Caenorhabditis*. So far only part of the identified differences on the molecular level can be correlated with the described variations on the cellular level where "many roads lead to Rome" [102, 103]. Future studies have to reveal to what extent the developmental characteristics of *D. coronatus* can be explained with variations on the transcriptional and post-transcriptional level.

Even if with improved methods in the one or other case a credible ortholog of a *C. elegans* gene could be excavated that we here counted as being absent with our approach in the non-*Caenorhabditis* representatives, the differences on the level of genes and gene products in comparison with *Diploscapter* (and the other studied nematodes) will still remain remarkable. The particularly rapid evolutionary diversification in the genus *Caenorhabditis* [16] may be related to multifaceted opportunistic life styles. In permanently changing environments, this allows on the one hand short generation times and large progenies whenever food is available in abundance and on the other hand long-term survival under harsh conditions. Extended studies on additional *Caenorhabditis* species and close outgroups will have to reveal whether this genus is really as uniform with respect to its control of development as our limited studies suggest and at which branching points in the phylogenetic tree novelties arose. If the methodology to establish a transcriptional lineage of the early *C. elegans* embryo [104] is applicable to *D. coronatus* and other nematodes as well, evolutionary changes in time and space could even be traced on a single cell level.

Conclusions

The parthenogenetic *D. coronatus* reveals a variety of differences compared to *C. elegans* on the level of cells, chromosomes, genome and transcriptome indicating alternative routes for nematode development and reproduction. Thus, it appears to be an attractive study object to better understand the intricate pathways of evolutionary change among closely related species. Our comparative study further supports the view that the genus *Caenorhabditis* cannot be taken as a blueprint for the genetic control of developmental and reproductive processes in nematodes as it shows a number of idiosyncrasies absent in the other studied representatives. Future avenues to follow in order to reveal further developmentally relevant differences between *D. coronatus*, *C. elegans* and other rhabditid nematodes could be: (1) the role of early transcription versus maternal supply, (2) structure and function of the single chromosome ($n = 1$) in *D. coronatus* (Fig. 4c) assumed to be the result of fusion, (3) meiotic pairing and crossover, apparently absent in *D. coronatus*, (4) the mechanism of chromosome separation.

Additional files

Additional file 1: Table S1. RNA-Seq statistics. **Table S2:** dN/dS values.

Additional file 2: Fig. S2. a, c, Sequence comparison of the small (SSU) and large subunit (LSU) rDNA genes of *D. coronatus*. b, Collapsed Maximum Likelihood (ML) tree representing clustering of sequenced clones for the SSU rDNA gene. (d) Collapsed ML tree representing clustering of sequenced clones for the LSU rDNA gene. Bootstrap values are shown above and posterior probability values beneath branches.

Additional file 3: Fig. S1. GO terms enriched in the *D. coronatus* 1–8 cell transcriptomic proteome in comparison with the complete *D. coronatus* proteome. Associated functional descriptions and test statistics are given in tabular format in Additional file 4.

Additional file 4. 1-8 cell expression-based proteome in comparison to the full *D. coronatus* proteome. Machine (including MS Excel) readable text file in tsv format with Blast2GO-derived statistical test results for enrichment of gene functions.

Additional file 5: Fig. S3. Binning of different combinations of replicates. Combining all four replicates the numbers of expressed sequences appear to saturate at about 6500 transcripts (see Table 2).

Additional file 6: Fig. S4. Phylogenetic tree representing GoLoco (Pfam ID PF02188) domain proteins of *D. coronatus* (*D. c.*), *C. elegans* (*C. e.*), human (*H. s.*), rat (*R. n.*) and mouse (*M. m.*).

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The *D. coronatus* genome and annotation are available through ensembl.caenorhabditis.org. Additional RNA-Seq data are available on SRA under the accession number PRJNA407968.

Consent for publication
All authors declare their consent with publication of the submitted manuscript.

Ethics approval and consent to participate
Not applicable.

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