Supplemental Tables and Figure Legends

Deep Small RNA Sequencing from the Nematode Ascaris Reveals Conservation, Functional Diversification, and Novel Developmental Profiles

SUPPLEMENTAL TABLES

Table S1. *Ascaris* genome and cDNA: genome sequencing and assembly statistics, reads and functional coverage, and cDNA sequencing and assembly statistics.

Table S2. *Ascaris* small RNA libraries: sequencing statistics, reads normalization, and types of analyzed small RNAs.

Table S3. *Ascaris* miRNAs: mature miRNA, miRNA*, and miRNA hairpin sequences; seed families, polycistronic clusters, other features of miRNAs, miRNA/miRNA* read frequencies, and expression profiles and quantification of miRNA Northern blots.

Table S4. *Ascaris* miRNA seed families: miRNA seed family comparison between *Ascaris* and *C. elegans*.

Table S5. *Ascaris* miRNA compared to other organisms: comparison of *Ascaris* miRNAs to miRNAs from other nematodes, flies and humans.

Table S6. *Ascaris* miRNA editing and 3' addition: *Ascaris* miRNAs with editing or 3' addition (modifications that exceed 5% of the mature miRNA).

Table S7. *Ascaris* siRNA targets: curated mRNA targets of *Ascaris* 26G-RNAs and 22G-RNAs. Targets were identified as a minimum of 100 reads of small RNA/mRNA/million reads in at least one of the 5'-all-phosphate libraries.

Table S8. *Ascaris* siRNA read frequency: comparison of the read frequency of different types of siRNAs in *Ascaris*.

Table S9. *Ascaris* small RNA machinery: *Ascaris* proteins involved in different small RNA pathways and a comparison to those in *C. elegans* and other organisms.

SUPPLEMENTAL FIGURES

Figure S1. Size distribution of analyzed *Ascaris* 5' monophosphate small RNAs. Each figure provides the size distribution of the **raw small RNA reads** derived from different samples.

Figure S2. Size distribution of *Ascaris* 5' monophosphate miRNAs, siRNAs, and 28-32 nt RNAs. Each figure provides the size distribution of the **raw small RNA reads** derived from different samples.

Figure S3. Size distribution of analyzed *Ascaris* 5' All-phosphate small RNAs. Each figure provides the size distribution of the raw small RNA reads derived from different samples.

Figure S4. Size distribution of *Ascaris* 5' All-phosphate miRNAs, siRNAs, and 28-32 nt RNAs. Each figure provides the size distribution of the raw small RNA reads derived from different samples.

Figure S5. *Ascaris* miRNA hairpins with small RNA reads. Each miRNA hairpin sequence is illustrated with the Mfold predicted structure with the lowest free energy. These are followed by information on mapped small RNA's position, length, and frequency (raw small RNA reads from all stages). miRNAs are shown in red and miRNA* in blue. For simplicity, only small RNAs with read frequency either over 0.05% of the mature miRNA or over 100 are shown, with the exception for those low-frequency miRNA*s that have read numbers lower than 0.05% of the mature miRNAs.

Figure S6. *Ascaris* miRNAs expressed during zygote maturation and 1-4 cell embryos. A. Northern blots of 4 miRNAs expressed during intrauterine zygote maturation. Note the absence of pre-miRNAs in stages preceding the appearance of the

mature miRNAs suggesting the new miRNAs are derived from new transcription. **B.** Transcription of many miRNAs begins as early as the 1-4 cell embryos. Note the scale is logarithmic and the reads are normalized.

Figure S7. *Ascaris* polycistronic loci and miRNA expression. Each miRNA is illustrated as a box containing a red area indicating whether the miRNA is derived from the 5' or 3' arm of the hairpin, 5p or 3p, respectively. The sequence above the miRNA box is the seed sequence for the miRNA; red seed sequences represent those expressed at the same time during a developmental stage. The number below the box is the total of the raw miRNA reads in all stages examined.

Figure S8. *Ascaris* miRNA 5p and 3p shift with miRNA duplication. Illustration of parologous asu-miR-9 and asu-miR-79 apparently derived from gene duplication. **A.** alignment of hairpin sequences of asu-miR-9 and asu-miR-79. **B** and **D.** Small RNAs mapped to asu-miR-9 (**B**) and asu-miR-79 (**D**) as described in Figure S5 (reads are from Fig. S5). **C** and **E.** Predicted secondary structure of asu-miR-9 (**C**) and asu-miR79 (**E**) hairpins.

Figure S9. Frequency and distribution of 22G/26G-RNAs on 26G-RNA targets. Note that all small RNAs are antisense. Red, 22G-RNAs; blue, 26G-RNAs. Vertical dashed lines indicate the start and stop of the ORF. Numbers on left indicate the normalized read frequency of the gray line. (see separate FigS9.26G-RNA_targets.pdf)

Figure S10. Frequency and distribution of 22G/26G-RNAs on 22G-RNA targets. Note that all small RNAs are antisense. Red, 22G-RNAs; blue, 26G-RNAs. Vertical dashed lines indicate the start and stop of the ORF. Numbers on left indicates the normalized read frequency of the gray line. (see separate FigS10.22G-RNA_targets.pdf).

Figure S11. Larger *Ascaris* small RNAs derived from atypical genomic hairpins.

A. Illustration of the lowest free energy structures predicted by Mfold for atypical hairpins. Blue shading represents the major and yellow the minor small RNA derived from the hairpin. See Figure S12 for small RNAs and their total raw read frequencies from the atypical hairpins.

B. Small RNA read expression profiles (normalized) for small RNAs derived from atypical hairpins.

C. Northern blots demonstrating small RNAs derived from atypical hairpins.

Figure S12. *Ascaris* small RNAs derived from atypical hairpins. *Ascaris* atypical hairpins and alignment of derived small RNAs and their total raw read frequencies described in Figure S11. Blue represents the major and yellow the minor small RNA derived from the atypical hairpin.