Supplementary Figures and Tables

Supplementary Figure 1. Expression heatmap for all miRNAs conserved in platypus and echidna brain, kidney, testis, lung, heart and liver with a normalized cloning multiplicity of at least 10 in each tissue.

Supplementary Figure 2. Expression heatmap for miRNAs conserved in platypus and human brain, testis, liver and heart with a normalized cloning multiplicity of at least 10 in each tissue.

Supplementary Figure 3. Expression heatmap for miRNAs conserved in platypus and mouse brain, testis, liver, heart, kidney and lung with a normalized cloning multiplicity of at least 10 in each tissue.

Supplementary Figure 4. Expression heatmap for miRNAs conserved in platypus and chicken brain, testis, liver, heart, kidney and lung with a normalized cloning multiplicity of at least 10 in each tissue.

Supplementary Table 1. Ensembl gene identification numbers for RNAi genes from platypus.

Supplementary Table 2. Partial sequence for platypus *PIWIL4* used to construct phylogenetic tree.

Supplementary Table 3. Platypus and echidna miRNAs. 332 miRNAs were identified in platypus and echidna. MiRNAs with orthologs in other species were named accordingly; novel miRNAs were given new names (oan-miR-1325 to -1422). All miRNAs have been deposited in miRBase. Chromosomal or contig coordinates are listed. Cloning multiplicities for each putative miRNA are shown for each tissue in platypus and echidna. Conservation category (Monotreme/mouse/human/chicken; Monotreme/mouse/human; Monotreme/chicken; Monotreme/fish; Monotreme only; Platypus only; Echidna only) and the name of the orthologous miRNA(s) is indicated. Homology and orthology relationships with platypus, mouse, human and chicken were determined by BLAST against the Rfam collection, requiring at least 16 nucleotide identity. Platypus homologs were determined by BLAST against the platypus set. Orthologous echidna miRNAs were identified by perfect identity in the mature miRNA. MiRNAs cloned only in echidna but which perfectly match the platypus genome were named oan- due to lack of availability of an echidna genome sequence. MiRNA mature and star sequences of chicken orthologs from a set of 999 predicted and cloned miRNAs are indicated. In cases where orthologous miRNAs were cloned from chicken but were not predicted, either due to missing genome sequence or prediction problems, the sequence and cloning multiplicities of the mature cloned miRNA is listed. Cloning multiplicities refer only to the shown annotated mature miRNA and do not include multiplicities of miRNA variants with variable 3’ ends or of miRNA* strands. Seed sequences in nucleotide positions 2-8 for each mature platypus miRNA is listed, and miRNAs sharing seeds in platypus, mouse, human and chicken are indicated. Conservation category for each platypus seed (Monotreme/mouse/human/chicken; Monotreme/mouse/human; Monotreme/chicken; Monotreme only) is shown. Hairpin sequences for each platypus miRNA are listed, and miRNAs from testis clusters are highlighted in gray.

Supplementary Table 4. piRNA clusters in platypus. The position and length of each cluster is indicated, as well as the number of piRNAs mapping to each cluster. List may not be complete. Coordinates are from the March 2007 v5.0.1 (ornAna1) *Ornithorhynchus anatinus* draft assembly of the draft platypus genome. Only piRNAs mapping 1 to 5 times to the genome were used in this analysis.

Supplementary Table 5. Annotation of platypus piRNAs. The number of sequences mapping to each category is indicated. piRNAs were annotated according to the ornAna1 platypus genome assembly. In the first column, all the clones were taken into account. In the second column, the clone set was collapsed to non-redundant sequences.