A first look at monotreme meiotic recombination

Master's thesis

Sonia Horan
Supervisors:
Dr. Frank Grützner
Dr. Enkhjargal Tsend-Ayush
Dr. Tasman Daish

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Supplemental Data

S0: Materials and methods:

S0.1: Bioinformatic characterisation

Gene and protein sequences were retrieved using public genome databases (NCBI platypus assembly v5.0.1, Ensembl platypus assembly v61.1o , and UCSC platypus assembly v5.0.1) and using the publicly available sequences, multiple alignments, protein domain predictions, and phylogenetic analyses were performed primarily using Geneious Pro 5.3 (which, in turn, utilised InterPro domain and motif scan databases v4.7 (Hunter et al., 2009), Mr Bayes (Ronquist & Huelsenbeck, 2003), and Muscle alignment v3.9 (Edgar, 2004)). If possible, multiple alignments were generated using protein sequences from chicken, platypus, opossum, mouse and human. Protein multiple alignments yielded two critical pieces of information: the percent identical sites[1] across the (typically) five species analysed and the pairwise percent identity[2] between mouse and platypus. The pairwise percent identity was obtained only after all gaps in the alignment between mouse and platypus sequences are excluded; this was done to eliminate any error that might be contributed by incomplete sequence in the platypus database. After multiple alignment assessments were made, protein domain predictions were assessed to determine which proteins warranted further analysis. Geneious protein domain predictions were confirmed using publicly available software (MyHits Motif scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) and NCBI motif BLAST). Protein domains were typically assessed for mouse, platypus and chicken sequences. Comparative analysis between platypus, mouse and chicken, along with protein domain reference data (from GeneCards, http://www.genecards.org/) allowed for the determination of what, if any, protein domains were missing in the platypus. When protein domains were predicted to be missing in platypus, primers were designed on either side of the putative domain in platypus, and PCR and cDNA sequencing were used to confirm the platypus gene sequences. In the event DNA sequencing yielded a sequence different from the database, the sequencing data was translated and additional protein domain predictions were performed on

[1] Percent identical sites for a five-species multiple alignment is representative of the percentage of amino acid residues that are identical in all analysed sequences at a given site.

[2] Pairwise percent identity is a measure of conservation (Drummond et al., 2011); like percent identical sites, pairwise percent identity is a representation of the number of amino acid residues that are identical at a given site; however, pairwise percent identity is different in that it only analyses two sequences, and therefore is an interpretation of how similar two sequences are to one another. Typically a percent identity greater than or equal to 80% is considered well conserved while, for the purposes of this study, a percent identity below 50% is considered poorly conserved (though it is important to note that divergence time is an important factor as a 50% identity between fly and human is considered to be very well conserved).

the protein sequence. PCR products were sequenced by local sequencing centres (AGRF or IMVS). DNA sequence was re-aligned with the database sequence to confirm the identity of the PCR products.

Phylogenetic analysis was done using a Mr Bayes emulation on Geneious. Gene coding sequences (cds, obtained from Ensembl and NCBI) used in the Bayesian analysis were aligned by codon and all sequence gaps were omitted from all species analysed in order to prevent any bias resulting from inaccuracies in the database.

Table S0.1: Platypus primers used in RT-PCR and expected product sizes and annealing temperatures

Gene	Primers (5' → 3')		Expected cDNA Product Size (bp)	Annealing Temp. (°C)
	Forward	Reverse		
Spo11	TGGCAAGAAACGAAGCAC	AGACCACAGACAACATTTTCA	180	55
Mei4	TTCTCCTCTGCGAAACTAAG	AAACGCAGACGGGTGAAC	311	57
Rec114	GCACAATCAAAAACATCCTG	TCTCCACCTCTTCCACAAAC	660	55
Rad50	AGTAAGGGAGAAGGAGAGGAG	GCTCAATCACTCTTTCCAG	392	55
Rad51	CTCAGTGGCGGAGGAAAC	TCGCTGATGCCCTTGATG	165	57
Rad51 bridge	TTCCTGCGGATGCTGCTG	GTCCCAGACAATGTGAAGGC	230	57
Dmc1	CGTGACCAATCAGATGACTTC	ATTCTCCTCTCCCCTTCC	134	57
Dmc1 Degenerate	ATGGAGGATCAGGTTGTGC	ATTTTCCGCTTCTCGCTG	266	57
Hormad1	CGAAGAAAACAAGCATTACCC	TCACCTCGTCACAGTTTCC	168	57
Blm	TTCCAGCGACTTACCTCACC	CGTTCATCCGCTTGTAGTCC	252	57
Rpa1	GTCTAACCCCATACCAGTCC	TCTCCCCACTTTCATCAACC	132	57
Msh4	GAACGAAAGATGCCAGGAG	GCCGAACATAGTCAGAAAGAC	182	57
MSH4 MutSII domain	ATTGTAGCKGTRGTRGAAG	AARCCCTTTGTTTCRTTG	224	57
Msh4 MutSIIIdomain	ATGATAGAYTCRTCMTCRGC	YTTYARAGGCTCCACAAG	370	57
Msh5	AACCCATCAGACCCCTCAAC	GAAGCCATTCAGGTCCAAG	119	57
Msh5 MutSIII domain	TGARYATAGAYCARGACAC	AGAAGGAASCCAATCARRG	682	57
Mlh1	AGAGACGGAGGAGGAGGAAG	CTCCCTGAGTTTGGTGGTGT	235	57
Mlh1 mismatch repair domain	CAACATCCGCTCCGTCTTC	CGAGGGTAGGAGAGGAGCC	559	57
Mlh3	GAGGACCGTGATGAAACAAG	TGGTGAAACGATAGGGATACA	197	57
Mlh3 MutLC domain	BCAGCAAGTGGAYAAYAAG	TCTAYAAARCASAGYGGCAC	354	57
Rnf212	AGATAAGCAGAGAGGGCAC	TGTTGGAGGCTGAGTAAGG	188	55
Prdm9	GGGATAAGGACAAAGAATGAG	ACGACAGGTGCGATAGTAG	202	55
Prdm9a	GCCAAAGTTACGGTCAAAAAG	CCCTATGGTTTTGGAGTGC	605	55
Prdm9b	TCTTCCTCCTCCTCCATCC	TCCTTCTTCCTTCCACTCC	249	55

S0.2: RT-PCR

RT-PCR was conducted using novel primers (Table S0.1). The PCR cycling conditions for all primers were as follows: a 94°C denaturing step for 2 min, 35 cycles of 94°C for 30 sec, annealing for 30 sec at the specified temperature (Table S0.1), 72°C for 1 min and a final extension at 72°C for 10 min.

S0.3: Pachytene cell spreading

Platypus were captured by netting (AEEC permit R.CG.07.03 and NPWS permit A19 AEEC permit no. S-49-2006) at the Upper Barnard River (NSW, Australia) during breeding season. Specimens were sacrificed with an intraperitoneal injection of pentobarbital (Lethabarb; Virbac, Milperra, NSW, Australia) at a dose of 0.1 mg g^{-1}. Tissue samples from the captured specimens were cryopreserved using the protocol described in Sudman (1989). Initial cell spreading experiments were based on the protocol described in Peters et al. (1997).

Original cell spreading method:

In a microcentrifuge tube, an aliquot of cryopreserved testis tissue (25µl per slide) was added to 500 µl of 1X PBS (with protease inhibitors) in order to clean the sample. This sample was then centrifuged for 5 minutes at 500 rpm and the supernatant was subsequently discarded. The pellet was then re-suspended in a second 500 µl of 1X PBS (with protease inhibitors) and the centrifugation was repeated. Once again the supernatant was discarded and the pellet was re-suspended in 500 µl of 300 mM sucrose (with protease inhibitors) hypotonic solution. The sample was again centrifuged for 5 minutes at 500 rpm, the supernatant was discarded, and the pellet was re-suspended in a small volume of 300 mM the hypotonic solution (10 µl were used for each slide prepared).

Slides were washed in 100% ethanol and were coated in a thin layer of 1% PFA. 10 µl of the prepared testis sample was then dropped on each slide, and the slides were incubated in a humid chamber at 27°C for 2 hours and subsequently allowed to air dry for 30 minutes. Finally, slides were washed using 0.08% photoflow.

Cells spread using this method were not flat when examined under the microscope. To improve spreading of the nuclei, a variety of hypotonic concentrations were used (200 mM and 100 mM sucrose) in an effort to swell the cells enough to overcome this challenge. When these increased concentrations of decreased concentrations of sucrose failed to result in flat cell spreads, alternative protocols were employed. An example cell is can be seen in Fig. S0.1.

Cytospin cell spreading method:

In a microcentrifuge tube, a 40 µl aliquot of cryopreserved testis tissue was added to 500 µl of 1X PBS (with protease inhibitors) in order to clean the sample. This sample was then centrifuged for 5 minutes at 5 rpm and the supernatant was subsequently discarded. The pellet was then re-suspended in a second 500 µl of 1X PBS (with protease inhibitors) and the centrifugation was repeated. Once again the supernatant was discarded and the pellet was re-suspended in 900 µl of 100 mM sucrose (with protease inhibitors) hypotonic solution.

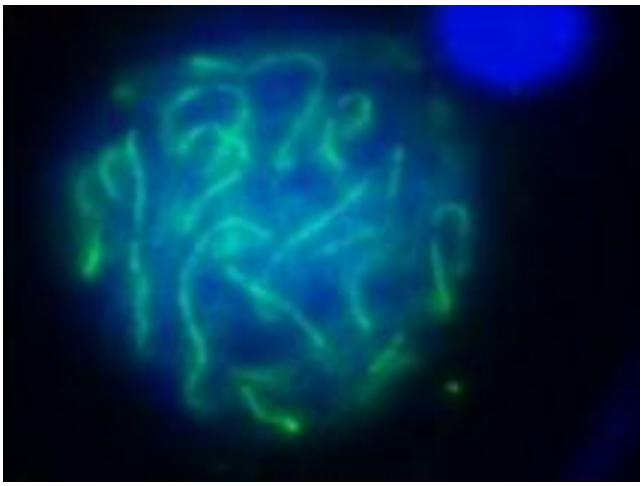

Figure S0.1: Spreading using the standard protocol Peters et al (1997) The above cell shows a platypus pachytene cell. Green staining shows the SCs (specifically the Sycp1 protein) while the blue staining is DAPI. Out of foucs SCs show that the cell has not flattened.

The sample was again centrifuged for 5 minutes at 500 rpm, the supernatant was discarded, and the pellet was re-suspended in a small volume of 100 mM the hypotonic solution (40 µl were used for each slide prepared). Aliquots of the testis sample were loaded into the cytospin chambers and spun onto slides (cleaned with 100% ethanol) for 10 minutes at maximum speed (Shandon Cytospin). 1% PFA was then dropped onto the slides and the slides were incubated in a

humid chamber at 27°C for 10 minutes. Slides were then rinsed with 1X PBS and washed with 0.08% photoflow as described above.

Cell spreading obtained using the cytospin technique was inconsistent. In some instances cells appeared well spread and flat; however, in other experiments cells appeared ruptured and SCs appeared torn or otherwise damaged. The effects of this cell damage were so extensive as to prohibit any consistent and confident scoring of Mlh1 or Dmc1 foci. Given these problems, it was decided not to utilize the cytospin protocol. An example result can be seen in Fig S0.2.

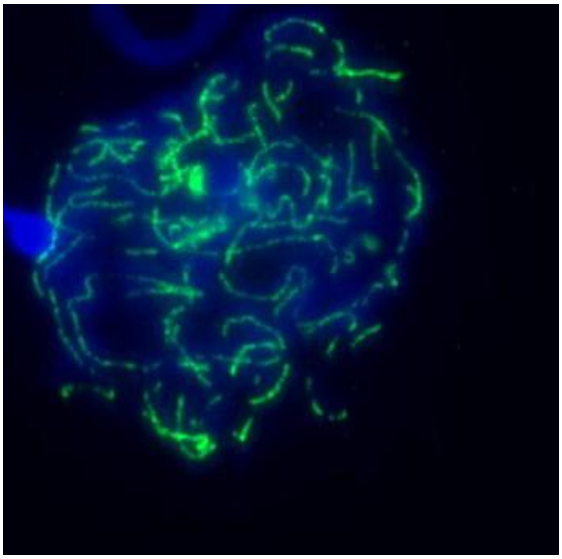

Figure S0.2: Result of the cytospin cell spreading protocol Here a platypus pachytene cell has been stained with DAPI (blue) and an Sycp1 antibody (green). The cell has been spread using the cytospin protocol, and as a result the SCs, while flat, also have a frail and torn appearance. In other experiments (not shown), it was impossible to obtain any reliable antibody staining.

Optimisation of the protocol of Peters et al. (1997)

This, ultimately, was the protocol used in this study. In a microcentrifuge tube, an aliquot of cryopreserved testis tissue (25µl per slide) was added to 500 µl of 1X PBS (with protease inhibitors) in order to clean the sample. This sample was then centrifuged for 5 minutes at 500 rpm and the supernatant was subsequently discarded. The pellet was then re-suspended in 500 µl of 50 mM sucrose (with protease inhibitors) hypotonic solution. The re-suspended pellet was allowed to incubate in the hyptonic solution on ice for 10 minutes before the sample was again centrifuged for 5 minutes at 500 rpm. The supernatant was discarded, and the pellet was re-suspended in a small volume of 50 mM the hypotonic solution (10 µl were used for each slide prepared).

Slides were washed in a 90% methanol, 1% HCl solution and were coated in a thin layer of 4% PFA. 10 µl of the prepared testis sample was then dropped on each slide, and the slides were incubated in a humid chamber at 27°C for 1 hour and subsequently allowed to air dry for 30 minutes. Finally, slides were washed using 0.08% photoflow.

S0.4: Immunofluorescence staining

As with the cell spreading protocol, immunofluorescence staining of platypus pachytene cells required optimization. Serial antibody incubations were performed. Slides were first washed in 1X PBS for 5 minutes and subsequently 500 µl of primary blocking solution (0.5% BSA / 0.5% milk powder / PBS) was added. The slides were then covered with a parafilm cover slip and incubated in a 27°C humid chamber for 1 hour. A 1:200 dilution of primary antibody was made in 10% BSA (diluted in 1X PBS) and 200 µl of dilute antibody was dropped onto each slide after the blocking incubation (Several antibody dilutions—1:100, 1:200, and 1:400—were attempted, and the optimization process showed the 1:200 dilution worked best). The primary antibody was left to incubate on the slides overnight in a 27°C humid chamber (other incubation times and conditions were tests—including 37°C overnight and 37°C for 2 hours; however, 27°C overnight worked best). After this incubation the slides were washed 3 times in 1X PBS for 5 minutes each wash. 500 µl of secondary blocking solution (10% goat serum / 5% milk powder / PBS) was then added to each slide; slides were covered with a parafilm cover slip and incubated for 1 hour in a 27°C humid chamber. The secondary antibodies were diluted 1:500 in secondary blocking solution; 200 µl of dilute antibody was dropped onto each slide after the blocking incubation and slides were left to incubate for 1 hour in a 27°C humid chamber. After the secondary incubation, slides were washed 3 times in 1X PBS for 5 minutes each wash at 27°C. Last, slides were incubated for 1 minute in DAPI, rinsed 3 times using DI water.

S0.5: Fluorescent *In Situ* Hybridisation (FISH)

BAC clones for FISH labeling were co-precipitated using the following procedure:

12.5 µl of each probe was added to a microcentrifuge tube containing 10 µl Salmon Sperm, 20 µl competitor sonicated platypus genomic DNA, and 400 µl ice cold ethanol. Samples were incubated for 1 hour at -80 °C and were subsequently centrifuged for 30 minutes at 14,000 rpm at 4°C. Ethanol was removed and the pellet was allowed to air dry for 15 min. The pellet was then dissolved in 10 µl deionized formamide and shake for 15 min at 37°C. 10 µl of hybridisation

mixture was added, and the sample was shaken for an additional 15 min at 37°C. The probe was denatured for 10 min at 80°C and centrifuged for 2-5 sec afterward. Finally the probe was preannealed for 30 min at 37°C.

Pretreatment and hybridisation of slides:

Slides were washed with 2X SSC for five minutes before undergoing an RNase (198 uL 2X SSC add 2 uL RNase A (10mg/mL stock)) incubation for 30 min in a 37°C humid chamber. Slides were then washed three times for 5 min in 2X SSC. Washed slides were incubated in Pepsin (25 uL of a 10% stock of Pepsin in 50 mL of 0.01 M HCl) at 37°C. After 10 min slides were washed twice for 5 min in 1X PBS and once for 5 min in 1X PBS/50mM MgCl2 at 27°C. Slides were fixed using 1% formaldehyde (incubated for 10 min at 27°C) and were subsequently dehydrated using a serial ethanol incubation (70%, 90%, 100%) for 5 min each. Finally, slides were incubated in a 70% formamide/30% 2X SSC mixture for 3 min at 70°C before a second serial ethanol dehydration series incubation (as described above). FISH probes were added to the slides and left to incubate overnight in a 37°C humid chamber.

Following hybridisation, slides were washed in: 50% Formamide/2X SSC solution at 42°C (three times for 5 min); 2X SSC at 42°C (once for 5 min); 0.1X SSC at 60°C (once for 5 min); and 2X SSC at 42°C (once for 5 min). Slides were last stained with DAPI solution.

S0.6: Cell Measurements

Cell surface calculations were measured using 35 cells from platypus animal P09, slide number 4 (i.e. P09.4) as this experiment yielded the clearest Dmc1 and FISH staining. All surface areas were calculated using Zeiss AxioVision LE Image Analysis Software. DAPI and FISH signals as well as Dmc1 foci were carefully outlined using the "Outline" measuring tool; completing the outline immediately prompted the generation of a surface area. Dmc1 foci surface areas did provide a small challenge given the variation in size. In order to minimise any undue influence of this variation, a variety of Dmc1 foci sizes were analysed; however, only one Dmc1 focus was measured for each cell resulting in a final average of 35 Dmc1 foci across 35 cells. Dmc1 foci were selected to measurement based on an apparent average size (i.e. obviously large and small foci were excluded from consideration in this calculation). This was done to ensure the probabilities calculated would reflect average foci co-localisation.

S1: Gene sequence and RT-PCR data

S1.1: Supplemental summaries of bioinformatics analysis

Spo11

Spo11 is an evolutionarily highly conserved gene responsible for the initiation of DSBs in meiosis. The complete sequence (14 exons, 1215 bp) of the platypus *Spo11* orthologue was identified on platypus Ultra contig 516 (Ensembl platypus assembly v5.0.1). The platypus gene was shown to have conserved genomic context with the mouse *Spo11* (on chromosome 2) and thus was confirmed as an orthologue. Platypus expression of *Spo11* was determined using RT-PCR on platypus testis cDNA (Table 5) with specific intron-spanning primers for platypus *Spo11*. A product of approximately 200 bp was amplified, and DNA sequencing analysis confirmed its identity as *Spo11*. Using a multiple alignment of the protein sequence of Spo11 it was found that, over five species, there was 57.1% identical sites; furthermore, between platypus and mouse there was a 71.9% pairwise identity (Table 5). Overall Spo11 is conserved across its sequence in platypus. Protein domain prediction comparisons between mouse and platypus further confirmed the high conservation of Spo11. Each Spo11 domain listed in the databases is conserved in the platypus; indeed, the platypus protein is virtually identical to the mouse Spo11 in terms of conserved protein domains. Phylogenetic analysis of *Spo11* further supported this finding, showing a clustering of vertebrate taxa with particularly short phylogenetic distances between mammalian species, indicative of the high degree of sequence conservation. Conservation of the coding sequence and expression pattern suggests that *Spo11* has a conserved function in platypus meiotic recombination.

Rad50

Rad50, like Spo11 and Mei4 and Rec114, is involved in the initiation of crossing over; Rad50 is a part of a subcomplex of proteins that resect the newly-formed DSBs. The platypus *Rad50* (on Ultra contig 457) was found to have conserved genomic context with its orthologue on mouse chromosome 11. The expression of *Rad50* was further demonstrated in platypus adult male testis via RT-PCR (Table 5). A cDNA product approximately 400bp long was amplified using platypus specific primers. DNA sequencing confirmed its identity as *Rad50*. Over its entire sequence (1159 amino acid), the Rad50 protein is very well conserved; across chicken, platypus, opossum, mouse, and human there are 63.7% identical sites, and between platypus and mouse protein sequences there is an 83.2% pairwise identity (Table 5). Furthermore, the platypus Rad50 has all the same functional domains as the mouse and chicken. The high level of sequence conservation is reflected in the phylogenetic tree; most taxa are clustered closely together with very short branch distances. This bioinformatics profile supports the hypothesis that *Rad50* is well conserved. Given these results it is predicted that Rad50 has a conserved function in platypus meiotic recombination.

Hormad1

Hormad1 has been implicated in mediating crossover partner selection. Overall, the protein sequence of Hormad1 is not well conserved across taxa. The platypus *Hormad1* gene (located on Contig2231) has conserved genomic context with its mouse orthologue, and RT-PCR expression analysis (Table 5) using platypus adult testis cDNA amplified a cDNA product of expected size with DNA sequencing further confirming the identity as *Hormad1*. In contrast to other well-conserved genes (e.g. *Dmc1*), a multiple alignment between chicken, platypus, opossum, mouse, and human protein sequence shows only 34.7% identical sites while between mouse and platypus a 47.1% pairwise identity (Table 5). The divergence in the platypus sequence is due to three apparent deletions in the C-terminal and an insertion in the N-terminal. Despite the lack of pairwise identity, the protein domains of Hormad1 are well conserved. Only one domain was identified (the HORMA DNA-binding domain), and this same domain was also predicted to be present in chicken and platypus Hormad1.

A Bayesian phylogenetic analysis, using an amino acid alignment of *Hormad1* sequences, yielded the expected clustering of mammalian taxa. Notably, though, the platypus branch length is over eight times longer than those of other mammals. This is likely due to whole deletions in the platypus sequence. Despite the predicted changes to the Hormad1 peptide sequence, testis expression and prediction of the HORMA domain suggests that Hormad1 has a conserved role in platypus meiosis.

Rpa1

Rpa has a complex function that has been tied to the conformational changes from transition nodules (TNs) to recombination nodules (RNs).The platypus *Rpa1* gene (on Ultra contig 43) has conserved genomic context with the mouse *Rpa1* (on chromosome 11). Expression of *Rpa1* in platypus adult testis was established using RT-PCR (Table 5) which amplified a ~130bp DNA product which was confirmed as *Rpa1* by sequencing. Overall, like Rad50, Rad51, and Dmc1, the Rpa1 protein is well conserved across taxa; a multiple alignment of chicken, platypus, opossum, mouse, and human peptide sequences showed a 65.9% identical sites and an 82.5% pairwise identity between mouse and platypus (Table 5). Some notable areas of divergence include a 67 amino acid insertion near the platypus Rpa1 C-terminus. This insertion, however, does not seem to have a considerable effect on the protein domain map, as all InterPro domains present in mouse and chicken are predicted to be present in platypus, and vice-versa. Moreover, there are no additional protein domains predicted within the platypus insertion; it is possible the insertion is an error in the assembly, and PCR has not yet confirmed the presence of the insertion of the cDNA level. The *Rpa1* phylogenetic tree further illustrates this high level of conservation as all the mammalian taxa are closely clustered together and most vertebrate branch lengths are notably short. Overall, Rpa1 has undergone minimal changes across vertebrate evolution. Together, the expression and conservation data suggest that Rpa1 has a conserved function in platypus meiotic recombination.

Msh5

MutS homologues have recombinase properties and play vital roles in recombination nodules. The platypus *Msh5* (on Contig12686) does have conserved genomic context with the human *MSH5* (on chromosome 6). *Msh5* is expressed in platypus adult testis (Table 5); gene-specific primers were designed for the platypus gene and RT-PCR produced an ~100bp sequence in *Msh5* experiments. Sequencing results further confirmed the identity of *Msh5*.

The platypus *Msh5* sequence did not appear to be complete in the database, but Msh proteins derived from the partial sequence tend to be well conserved across taxa; Msh5 has a 49.8% across chicken, platypus, opossum, mouse, and human peptide sequences (Table 5). Excluding gaps in the database sequence, Msh5 has a 71.6% pairwise identity, indicating a high level of conserved in platypus (Table 5). Protein multiple alignments show that the platypus database sequence is missing N-terminal sequences for both Msh5.

The Msh5 protein has three essential protein domains: the core, the clamp, and the MutS C-terminal domain, all the same as Msh4. A domain scan (Fig 24) of the platypus Msh5 predicted the absence of the core and clamp domains, both required to form the Msh4/Msh5 sliding clamp during meiosis. Without these domains, Msh5's function would be fundamentally inhibited and, furthermore, the interaction with Msh4 which is is essential to the formation of crossovers—would be impossible. Again, an experiment was executed using designed degenerate primers (as described above), but in the case of *Msh5* PCR analysis was able to amplify a DNA product of the expected size. DNA sequencing and additional protein domain prediction confirmed the presence of the missing core and clamp domains. Therefore it appears as though the "missing" domains in platypus Msh5 were more an artefact of an incompletely assembled database. Given the expression results, the interdependence of Msh5 and Msh4, and the bioinformatics results, it is likely that Msh5 is functionally active in platypus meiosis.

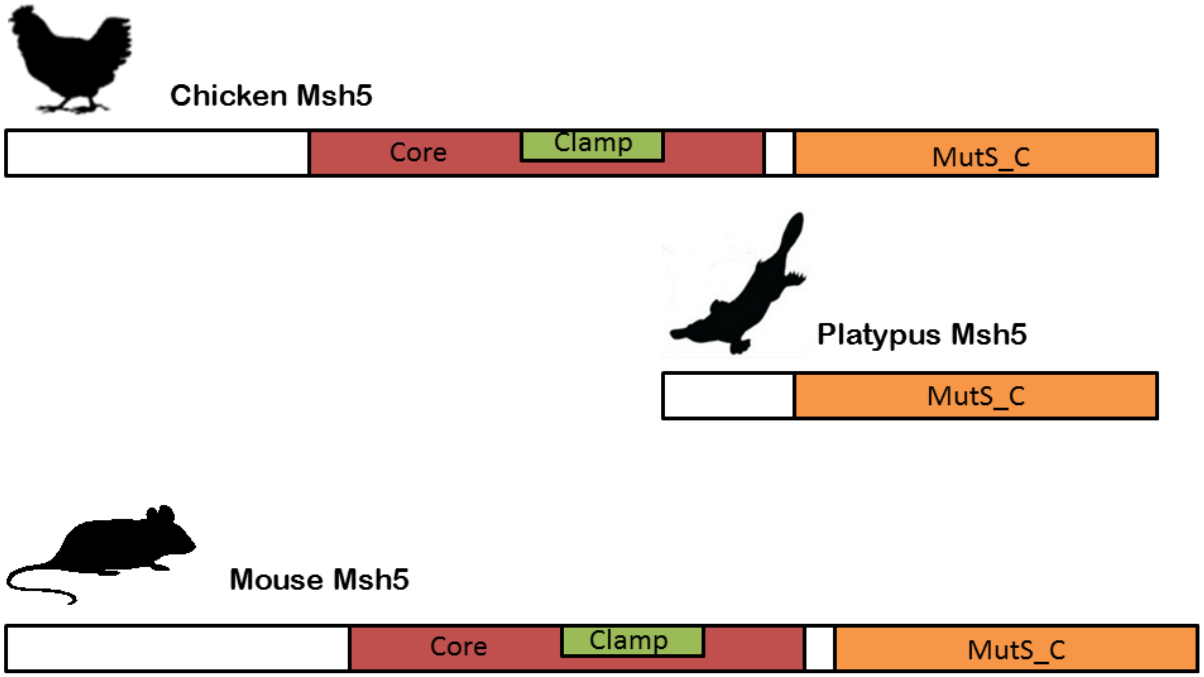

Figure S1.1: Msh5 protein domain alignment Using InterPro protein domain scans indicate that the platypus Msh5 is missing the N-terminus, including the core and the clamp domains.

Mlh1

Mlh1, due to its association with mature crossovers, has been interpreted in cytogenetic studies as a marker of chiasmata. The complete sequence of the platypus *Mlh1* gene (on Ultra contig 389) has conserved genomic context with the mouse gene (on chromosome 9). RT-PCR expression (Table 5) analysis confirmed the presence of *Mlh1* transcripts in adult platypus testis. A ~230bp PCR product was amplified from testis cDNA using *Mlh1*-specific primers which sequencing results confirmed as *Mlh1*. In an analysis of conservation, a multiple alignment comparing the peptide sequences of chicken, platypus, opossum, mouse, and human Mlh1 sequences showed 54.5% identical sites (Table 5). Mlh1, therefore, is largely conserved over much of its sequence; yet, there are several small regions of low homology in the platypus sequence. This divergence is reflected in the 65.1% pairwise identity between mouse and platypus Mlh1 (Table 5). Nonetheless, the seven functional domains predicted by domain scans in mouse are all also present in platypus and chicken, indicating an overall conservation of protein function across species. It is possible the observed sequence divergences in platypus are artefacts of errors in the assembly. This along with the expression profile indicates an overall conserved role for Mlh1 in platypus meiosis. A Bayesian phylogenetic analysis using a translated alignment of *Mlh1* sequences produced a tree that had some peculiar features, including a grouping of platypus and *Drosophila* outside of their expected evolutionary positions. While reasons for this unexpected positioning remain unclear, it is possible predicted platypus-specific deletions (while not predicted to affect protein function) may have resulted in the unexpected position of platypus. Furthermore the platypus branch length is unusually long, relative those of other mammals. Despite these unexpected results, the protein domain profile of the platypus Mlh1 is identical to that of the mouse protein.

Mlh3

Like Mlh1, Mlh3 has an important role in mature crossover formation. There was no evidence to support conserved genomic context of the platypus *Mlh3* gene (on Contig7246) with the mouse gene (on chromosome 12). An RT-PCR expression analysis using platypus testis cDNA amplified a product of the expected size and DNA sequencing confirmed the product's identity as *Mlh3*. In an analysis of conservation, a multiple alignment between chicken, platypus, opossum, mouse, and human peptide sequences was used and found 48.9% identical sites. Restricting the analysis to platypus and mouse showed only 42.8% pairwise identity (Table 5). Protein domain scan predicted that the platypus Mlh3 protein only had five of the six functional domains predicted in mouse. The missing domain, the MutL C-terminal domain (IPR014790), is essential for dimerisation of the Mlh3 protein. Further analysis using alternate domain search software (MotifScan using Prosite on ExPASy, Pfam, and InterPro databases), however, did predict the presence of the MutL C-terminal domain. To confirm the predictions of these alternative domain scans, degenerate primers were designed adjacent to the cDNA sequence coding the domain, using chicken, opossum, and mouse data to construct a consensus sequence. RT-PCR using the degenerate primers yielded a product ~350bp long in platypus testis. DNA sequencing and further domain prediction analysis confirmed the presence of sequence coding for the MutL C-terminal domain. Expression analysis using platypus specific primers (Table 5) further confirmed the presence of *Mlh3* transcripts in adult platypus testis.

Bayesian phylogenetic analysis using a translated alignment of *Mlh3* sequences yielded a tree displaying the expected clustering of mammalian taxa, though the platypus branch length was notably long, compared to other mammals, due to the high level of sequence divergence in the platypus protein. While the platypus Mlh3 seems more diverged, the conservation of functional domains and testis expression indicates an overall conserved role for Mlh3 in platypus meiosis.

Rnf212

Rnf212 has recently been shown to be associated with sex-specific differences in recombination rates. The platypus *Rnf212* gene (found on Ultra contig 544) has conserved genomic context with mouse *Rnf212* (on chromosome 5). RT-PCR expression analysis (Table 5) confirmed the presence of *Rnf212* transcripts in adult platypus testis. Using gene-specific primers a ~200bp cDNA product was amplified that sequencing results confirmed to be *Rnf212*. To estimate the level of conservation, a multiple alignment of peptide sequences from chicken, platypus, opossum, mouse, and human Rnf212 sequences was used, resulting in an estimated 40.2% identical sites across taxa (Table 5). In a two species comparison of mouse and platypus, however, a 53.8% pairwise identity was estimated (Table 5). Much of the diverged homology seems to be restricted toward the C-terminal end of the protein. Nonetheless, the two functional domains predicted by InterPro scans in mouse are all also present in platypus and chicken, indicating an overall conservation of protein function across species. This along with the bioinformatics data seems to suggest an overall conserved role for Rnf212 in platypus. Bayesian phylogenetic analysis using a translated alignment of *Rnf212* sequences displayed the expected clustering of mammalian taxa, and all mammalian branch lengths were notably short. This, along with the protein domain analysis results, seems to imply that Rnf212 is likely to be functionally conserved in platypus meiosis.

S1.2: Platypus *Rad51* gene sequence

The following full-gene sequence is partially a composite of coding sequence from Contig7495 and Contig14268. New sequence was generated following cDNA sequencing of an RT-PCR product, which was the result of an RT-PCR performed using primers spanning the two contigs.

ATGGCCATGCAGATGCAGATGGAGACCGGCGCGGACACCTCAGTGGCGGAGGAAAACTT
TGGCCCGCAGCCCATAACCCGGCTGGAGCAATGCGGCATCAGCGCCAACGACGTGAAGA
AGCTGGAGGAAGCCGGGTTTCACACGGTGGAGGCCGTGGCCTACGCCCCTAAGAAAGAG
CTGATCAACATCAAGGGCATCAGCGAAGCCAAAGCCGATAAGATCCTGACGGAGGCGGC
TAAGCTCGTCCCGATGGGCTTCACCACCGCCACCGAATTCCACCAGCGGCGGTCGGAGAT
CATCCAGATCACCACGGGCTCCAAAGAGCTGGACAAGCTGCTGCAAGGGGGCATCGAGA
CGGGCTCCATCACGGAAATGTTCGGGGAGTTCCGGACCGGAAAGACCCAGATCTGCCAC
ACCCTGGCCGTGACCTGCCAGCTCCCTATCGACCGGGGCGGCGGCGAGGGGAAGGCCAT
GTACATCGACACCGAGGGCACCTTCCGGCCCGAGCGGCTGCTGGCGGTGGCCGAGAGGT
ACGGCCTGTCGGGCAGCGACGTCCTAGACAACGTGGCGTACGCACGAGGGTTCAACACC
GACCACCAGACCCAGCTCCTCTATCAGGCCTCTGCTATGATGGTGGAGTCCAGGTATGCC
CTGCTCATCGTGGACAGCGCCACCGCCCTCTACAGAACCGACTACTCGGGCCGTGGGGAG
CTCTCCGCCCGGCAGATGCACCTGGCCCGCTTCCTGCGGATGCTGCTGCGGCTCGCAGAC
GAGGTGGGTACCCTTCTGCCGCGGCACGGAACCTCTGCCCGCATCGGCGGGCTCTGGGCC
CCCTTCACTGTCACCAAGGTCCTAGATGTAGCAGGTTTCCGCCCCGGGGCTCGGCTGGCC
AGCGCCGAGGACGGCGACCTCCTCCACGCCGGCCACCCCCTTCCCCGGCGAGCCAGAGG
GGCCTTCACATTGTCTGGGACACATCAGTGA

S1.3: Platypus *Dmc1* gene sequence

The following full-gene sequence was obtained from the Ensembl database platypus assembly, Ornithorhynchus_anatinus-5.0. The sequence below reflects a correction made to the sequence after an RT-PCR experiment uncovered an error in the 5'-end of the platypus *Dmc1* sequence coding for the Helix-hairpin-Helix domain.

ATGGAGGATCAGGTTGTGCAGGAAGAATCTGCACTTCAGGATGAGGAGGATTCTCTATTT
CAAGACATTGATCTGTTACAAAAGCATGGTATTAACATGGTTGATATCAAGAAGCTGAAA
TCGGTGGGAATCTGTACTATCAAAGGGATCCAGATGACAACAAGGAGGGCCCTATGCAA
TGTCAAAGGCCTGTCTGAAGCCAAGGTGGACAAGATTAAAGAGGCAGCCAACAAACTCA
TTGAACCAGGATTTCTGACTGCGTTTGAGTACAGCGAGAAGCGGAAAATGGTTTTCCACG
TCACCACTGGCAGCCAGGAATTTGATAAACTGCTGGGAGGGGGAATTGAAAGCATGGCA
ATCACTGAAGCTTTTGGAGAATTTCGCACAGGAAAAACCCAGCTTTCTCACACTCTCTGT
GTGACGGCTCAGCTTCCAGGAGCAGGCGGCTACCCAGGGGGGAAAATTATTTTCATCGAT
ACGGAAAATACTTTCCGTCCAGATCGCCTTCGAGACATTGCTGATCGCTTCAACTTAGATC
ACGATGCAGTTCTGGACAATGTACTCTATGCACGAGCATATACTAGTGAACATCAGATGG
AGCTACTCGATTATGTAGCAGCTAAATTCCATGAAGAGGCTGGCATCTTCAAGCTACTGA
TCATCGACTCAATAATGGCACTATTCCGTGTGGATTTCAGTGGTCGGGGAGAGCTGGCTG
AACGGCAACAGAAACTAGCTCAGATGCTGTCACGGCTCCAGAAGATCTCAGAAGAATAC
AATGTAGCTGTGTTCGTGACCAATCAGATGACTTCTGATCCAGGAGCCACCATGACCTTT
CAGGCAGACCCTAAAAAGCCCATTGGGGGCCACATCCTGGCACATGCTTCAACAACCAG
GATCAGTTTGCGGAAGGGGAGAGGAGAATTGCGCATTGCCAAGATTTATGACAGCCCTG
AGATGCCTGAAAATGAAGCCACCTTCGCAATAACTGCTGGAGGGATTGGGGATGCCAAA
GAGTAG

S1.4: Platypus *Msh5* coding sequence

The following cDNA sequence codes the Clamp (MutS IV) and Core (MutS III) domains of the platypus Msh5 protein. This sequence was obtained following cDNA sequencing of a product produced from an RT-PCR using degenerate primers designed based on corresponding consensus sequences from opossum and chicken. A UCSC BLAT further demonstrated that the following sequence was on the same contig as the platypus *Msh5* gene (Ultra 740).

AGTGAGGCCCATCCATCAGTATACAAGCTGGCCACAGGCCTGAAGGAGGGACTCAGTCT
GTTTGGACTCCTGAACAGGTGCCGCTGCAAGTGGGGAGAAAAGCAGCTCAGGCTGTGGC
TGATGCGCCCGACCCGGGACCTGAGTGAGCTGAACGCCCGGCTGGACGTGATCCAGTTCT

TTCTGCAGCCCCGGAACTTGGAGACGGCCCAGGCGCTCCATGGCCTGCTGGGAAACATCA
AGAACGTGCCCCTGATCCTCAGACGGATGACACTGTCTCACACCAAGGCCAGCGACTGGC
AGGTCCTGTACAAGACGGTGTACAGTGCTCTGGGCCTGCGGGACACTTGCCGGGCGCTGC
CTGGCTCCATCCGCCTCTTCCGAGACGTGGCCCAGGAATTCACCGACGACCTGCACCACA
TCGCCAGGCTCGTCAGTAAAGTGGTGGACTTTGAGGGCAGCTTGGCTGAAAACCGCTTCA
TCGTGAGGCCCAATGTGGATCCCGCCATTGACGAGAAGAAGAGGAAGCTGATGGGACTC
TCGGATTTCCTGACGGAGGTGGCCCGGAAGGAACTGGAGACGCTGGACAACCGGGTCCC
CTCCTGCAGCGTCCTCTACATCCCCCTGATTGCTTTCCCTTCTAAAA

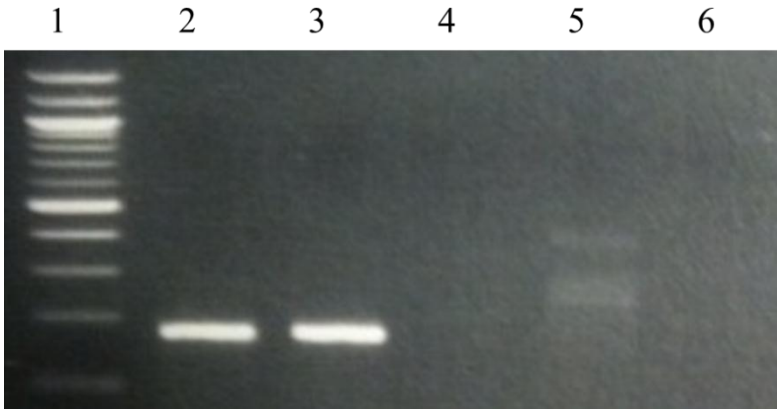

Figure S1.5: *Spo11* RT-PCR gel The results of a platypus *Spo11* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (180 bp), and DNA sequencing confirmed its identity as *Spo11*.

Figure S1.6: *Rad50* RT-PCR gel The results of a platypus *Rad50* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~400 bp), and DNA sequencing confirmed its identity as *Rad50*.

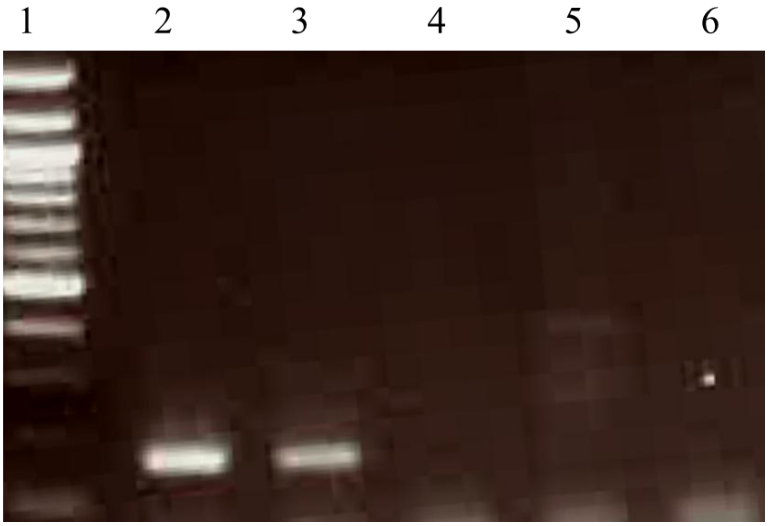

Figure S1.6: *Hormad1* RT-PCR gel The results of a platypus *Hormad1* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~170 bp), and DNA sequencing confirmed its identity as *Hormad1*.

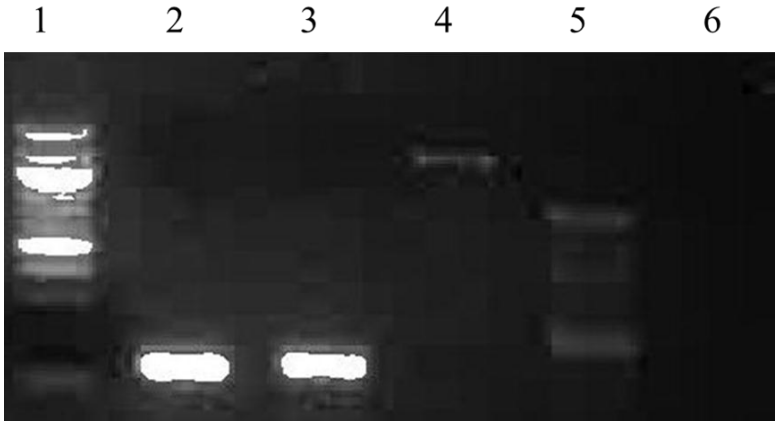

Figure S1.7: *Rpa1*RT-PCR gel The results of a platypus *Rpa1* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~130 bp), and DNA sequencing confirmed its identity as *Rpa1*.

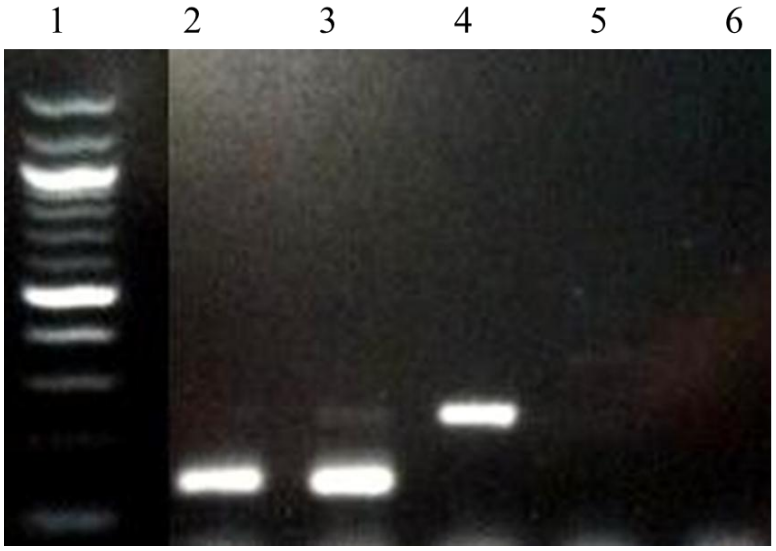

Figure S1.8: *Msh5* RT-PCR gel The results of a platypus *Msh5* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~120 bp), and DNA sequencing confirmed its identity as *Msh5*. Lane 1 here appears to be from a different gel image; however, this ladder was run on the same gel as the sample DNA. This particular experiment ran *Msh4* and *Msh5* samples together with one ladder; the *Msh4* samples have been excised from this image.

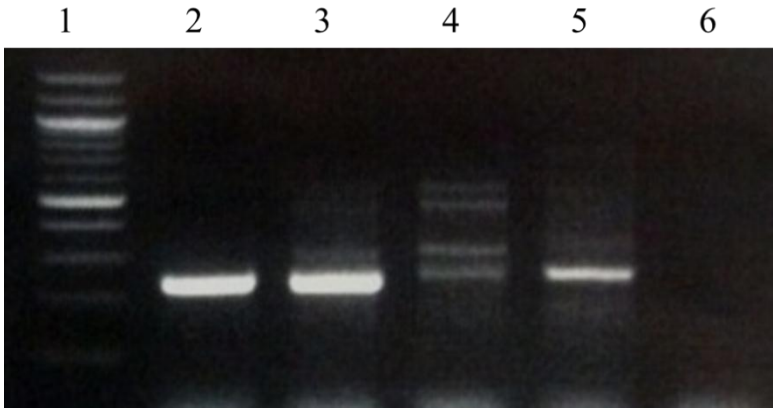

Figure S1.9: *Mlh1* RT-PCR gel The results of a platypus *Mlh1* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~240 bp), and DNA sequencing confirmed its identity as *Mlh1*.

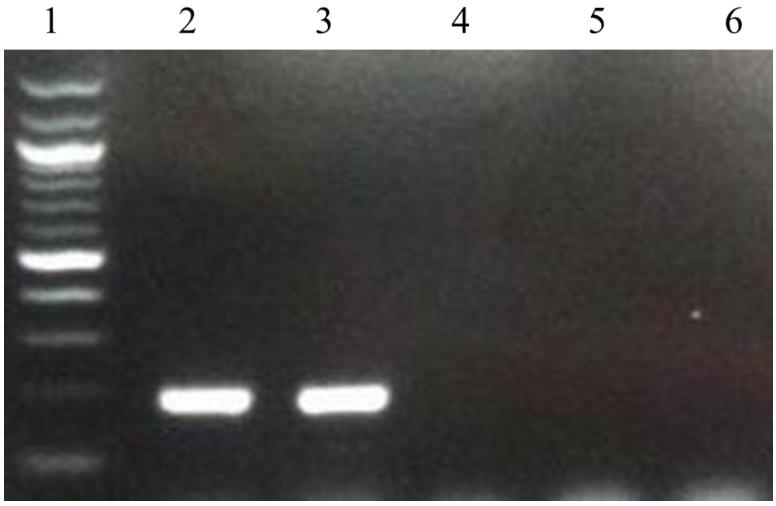

Figure S1.10: *Mlh3* RT-PCR gel The results of a platypus *Mlh3* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~200 bp), and DNA sequencing confirmed its identity as *Mlh3*.

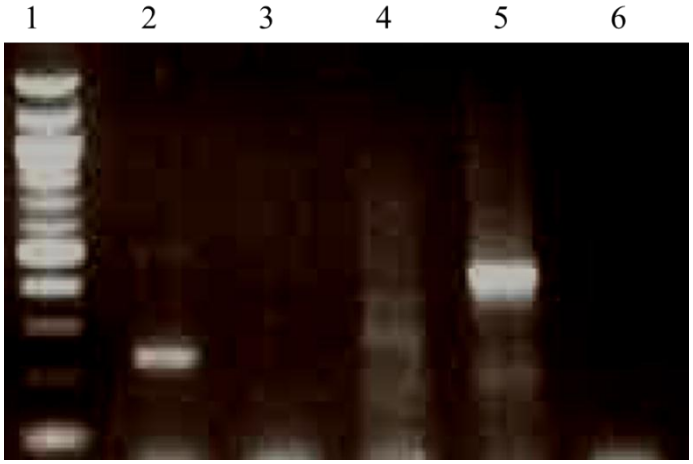

Figure S1.11: *Rnf212* RT-PCR gel The results of a platypus *Rnf212* RT-PCR experiment are shown below. In this gel, lane 1 corresponds to a standard 100bp ladder; platypus testis cDNA samples were run in lanes 2 and 3 while platypus genomic and echidna genomic DNA was run in lanes 4 and 5 respectively. Lane 6 corresponds to a blank negative control. This experiment was able to amplify the expected size product (~190 bp), and DNA sequencing confirmed its identity as *Rnf212*.

S2: Phylogenetic trees

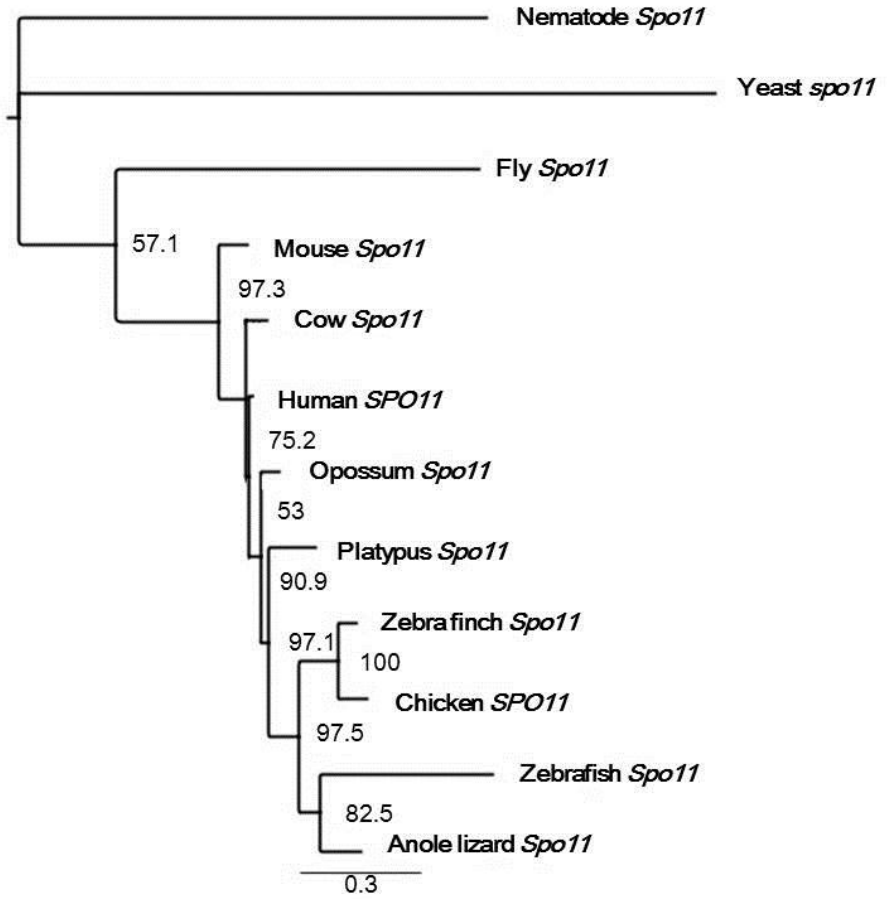

Figure S2.1: *Spo11* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.3 represents the branch length, measured in expected changes per site.

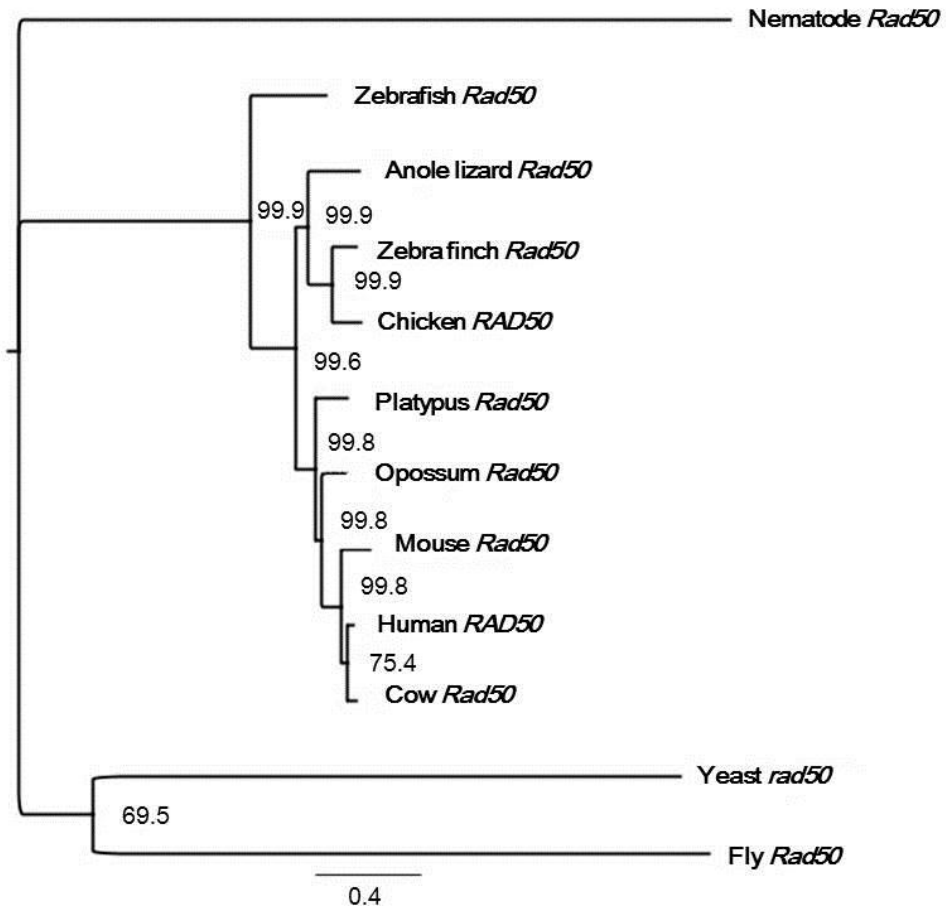

Figure S2.2: *Rad50* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.4 represents the branch length, measured in expected changes per site.

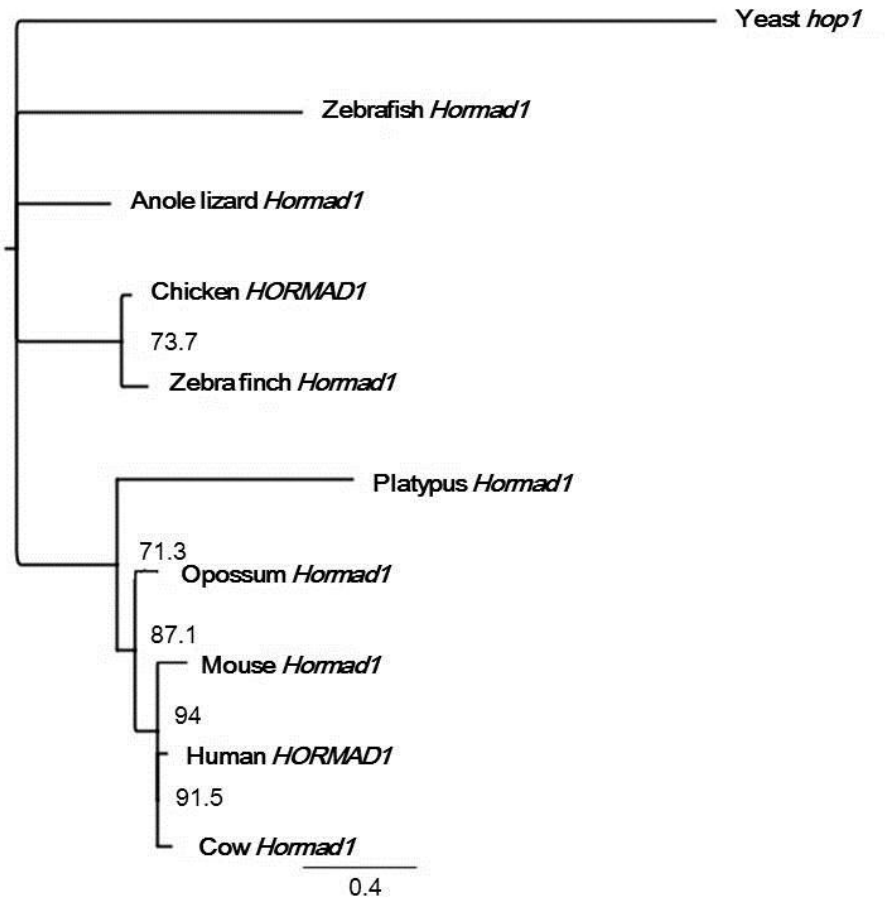

Figure S2.3: *Hormad1* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.4 represents the branch length, measured in expected changes per site.

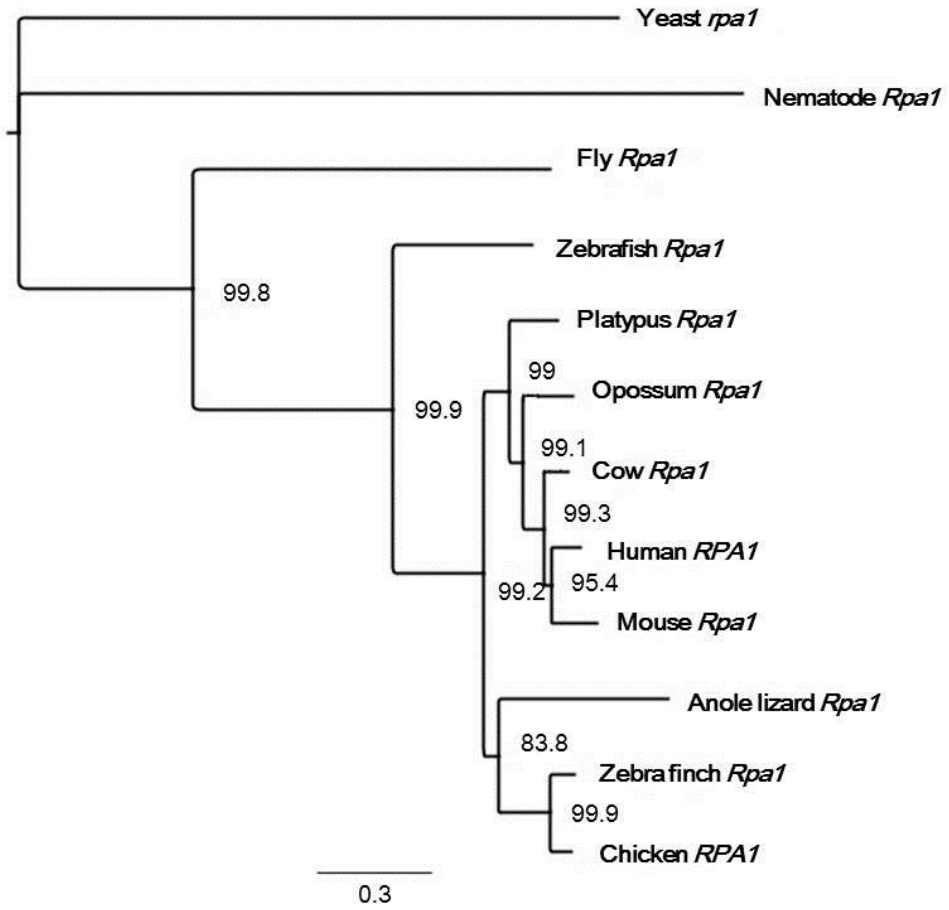

Figure S2.4: *Rpa1* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.3 represents the branch length, measured in expected changes per site.

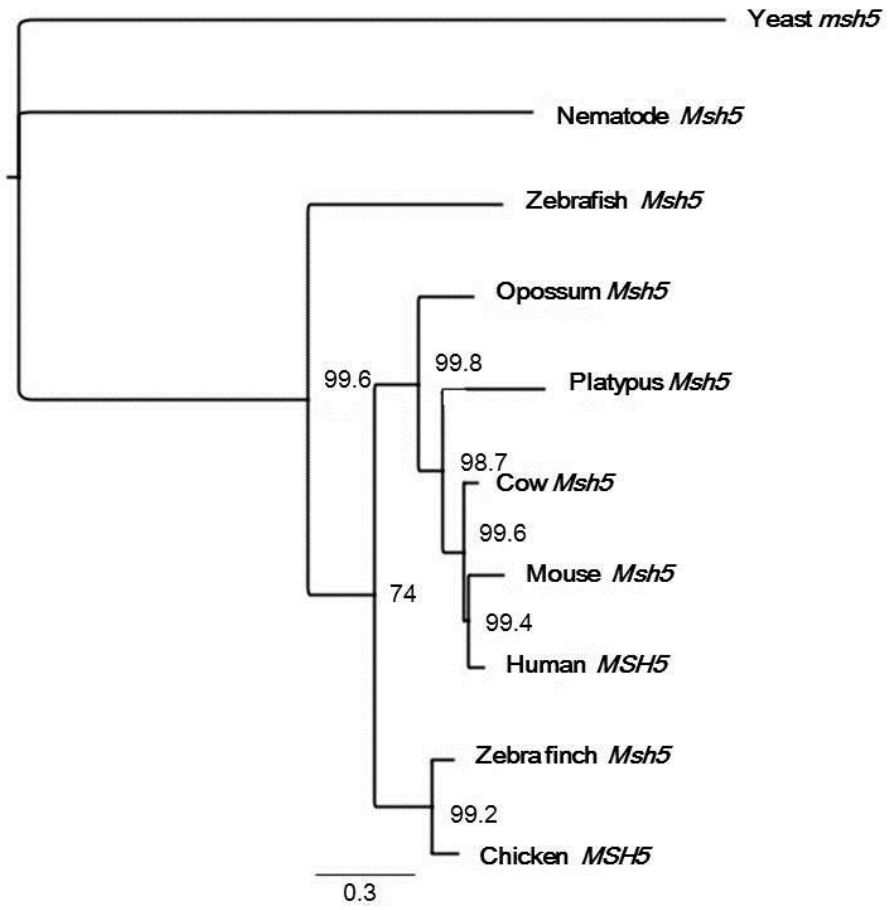

Figure S2.5: *Msh5* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.3 represents the branch length, measured in expected changes per site.

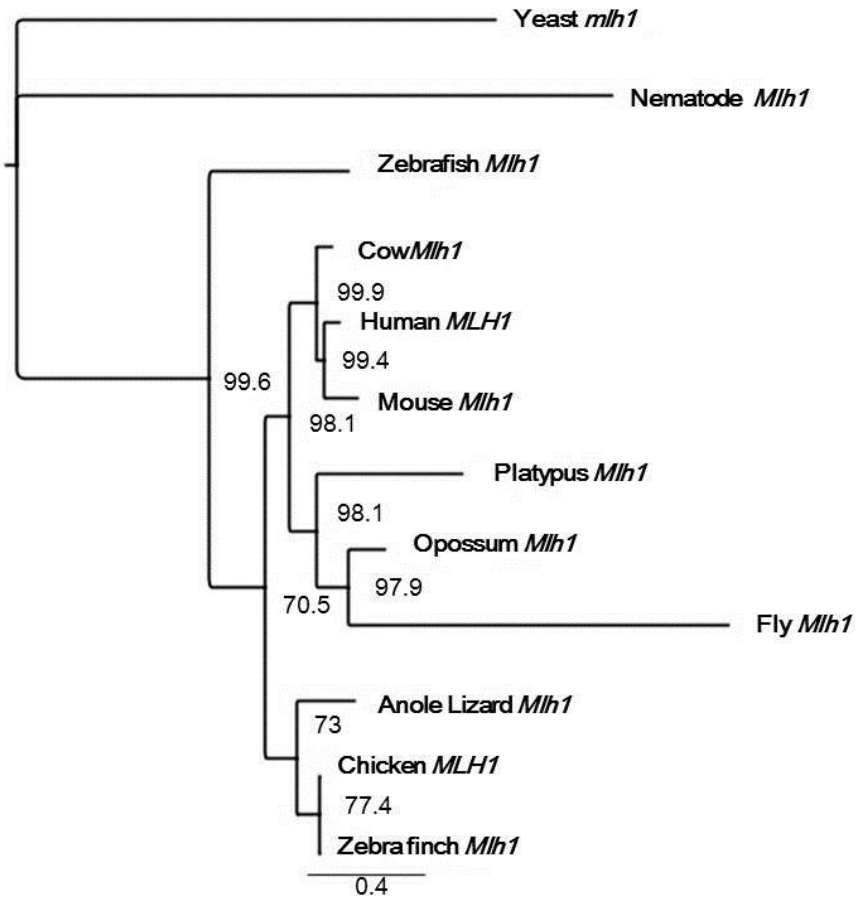

Figure S2.6: *Mlh1* phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.4 represents the branch length, measured in expected changes per site.

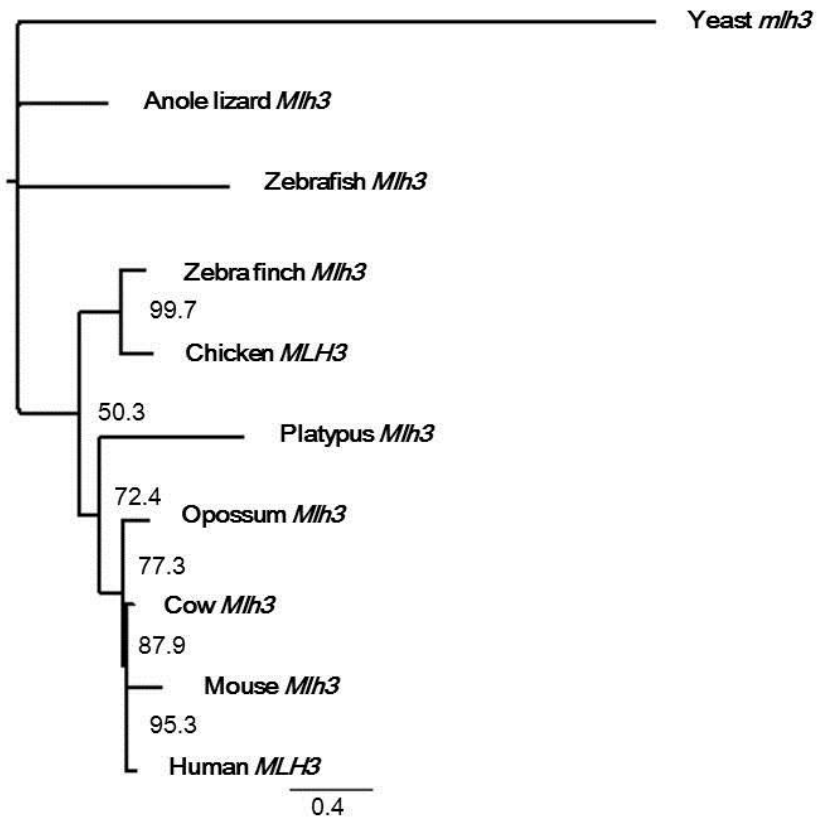

Figure S2.7: Mlh3 phylogenetic tree MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.4 represents the branch length, measured in expected changes per site.

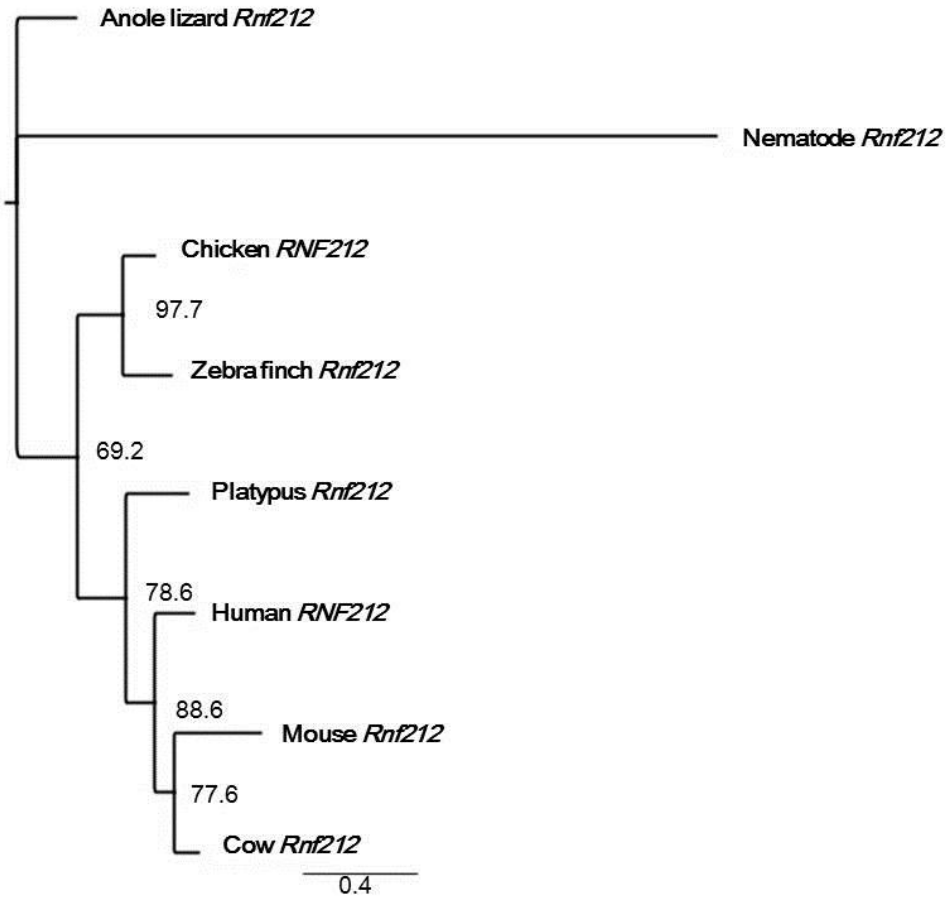

Figure S2.8 *Rnf212* phylogenetic tree: MrBayes (Ronquist and Huelsenbeck 2003) generated phylogenetic tree using a translation alignment of cDNA sequences. The numerical values marked at the nodes represent the posterior probability (the proportion of sampled trees containing the nodes). The scale bar of 0.4 represents the branch length, measured in expected changes per site.

S3: Dmc1 foci counts and surface area calculations.

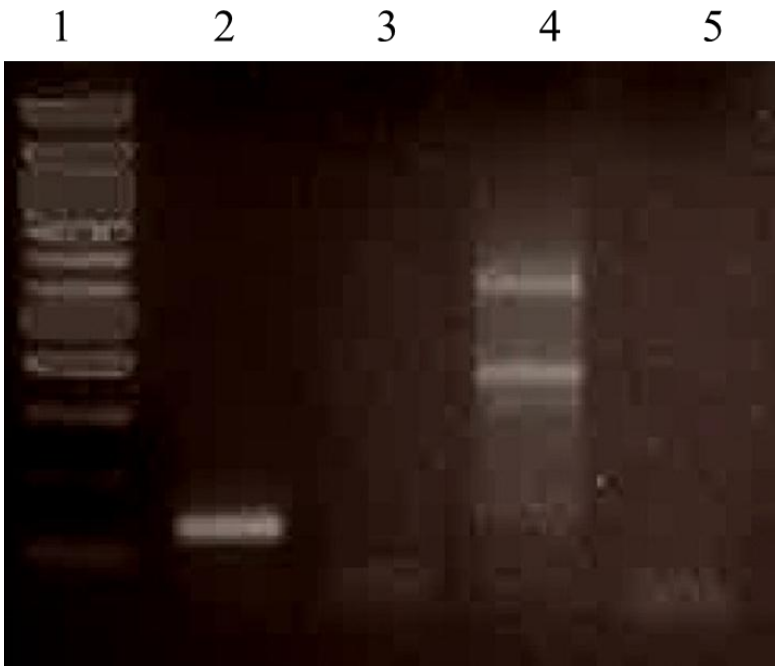

Figure S3.1: RT-PCR of platypus *Dmc1* in testis and kidney Lane 1 is a standard 100 bp marker; lane 2 is platypus testis cDNA; lane 3 is platypus kidney cDNA; lane 4 is a platypus genomic DNA positive control; and lane 5 is a blank negative control. This experiment resulted in a PCR product using *Dmc1* primers with testis cDNA but not with kidney cDNA, indicating the expression of platypus *Dmc1* is specific to meiotic tissue.

Table S3.1: Dmc1 foci counts for animal P07 P07.1-5 represent five different prophase spreads using meiotic material from platypus number P07.

P07.1		P07.2		P07.3		P07.4		P07.5	
Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci
P07.1-001	22	P07.2-001	25	P07.3-001	20	P07.4-001	21	P07.5-001	23
P07.1-002	30	P07.2-002	24	P07.3-002	27	P07.4-002	19	P07.5-002	23
P07.1-003	31	P07.2-003	20	P07.3-003	29	P07.4-003	21	P07.5-003	18
P07.1-004	20	P07.2-004	22	P07.3-004	20	P07.4-004	24	P07.5-004	24
P07.1-005	28	P07.2-005	29	P07.3-005	26	P07.4-005	29	P07.5-005	22
P07.1-006	25	P07.2-006	32	P07.3-006	26	P07.4-006	18	P07.5-006	25
P07.1-007	23	P07.2-007	35	P07.3-007	20	P07.4-007	19	P07.5-007	24
P07.1-008	23	P07.2-008	30	P07.3-008	25	P07.4-008	23	P07.5-008	22
P07.1-009	21	P07.2-009	25	P07.3-009	26	P07.4-009	36	P07.5-009	26
P07.1-010	27	P07.2-010	23	P07.3-010	21	P07.4-010	24	P07.5-010	20
P07.1-011	25	P07.2-011	16	P07.3-011	21	P07.4-011	16	P07.5-011	27
P07.1-012	24	P07.2-012	30	P07.3-012	19	P07.4-012	21	P07.5-012	20
P07.1-013	23	P07.2-013	25	P07.3-013	29	P07.4-013	20	P07.5-013	27
P07.1-014	23	P07.2-014	20	P07.3-014	22	P07.4-014	18	P07.5-014	23
P07.1-015	22	P07.2-015	34	P07.3-015	27	P07.4-015	22	P07.5-015	23
P07.1-016	33	P07.2-016	21	P07.3-016	26	P07.4-016	20	P07.5-016	20
P07.1-017	23	P07.2-017	23	P07.3-017	20	P07.4-017	27	P07.5-017	22
P07.1-018	26	P07.2-018	27	P07.3-018	23	P07.4-018	21	P07.5-018	20
P07.1-019	23	P07.2-019	24	P07.3-019	30	P07.4-019	26	P07.5-019	21
P07.1-020a	22	P07.2-020	23	P07.3-020	25	P07.4-020	26	P07.5-020	19
P07.1-020b	22	P07.2-021	24	P07.3-021	27	P07.4-021	17	P07.5-021	20
P07.1-021	23	P07.2-022	20	P07.3-022	25	P07.4-022	24	P07.5-022	18
P07.1-022	22	P07.2-023	28	P07.3-023	20	P07.4-023	21	P07.5-023	24
P07.1-023	24	P07.2-024a	19	P07.3-024	25	P07.4-024	24	P07.5-024	19
P07.1-024	23	P07.2-024b	26	P07.3-025	21	P07.4-025	29	P07.5-025	22
P07.1-025	23	P07.2-025	28	P07.3-026	26	P07.4-026	24	P07.5-026	20
P07.1-026	24	P07.2-026	25	P07.3-027	23	P07.4-027	22	P07.5-027	24
P07.1-027	19	P07.2-027	24	P07.3-028	31	P07.4-028	26	P07.5-028	24

P07.1		P07.2		P07.3		P07.4		P07.5	
Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci
P07.1-028	26	P07.2-028a	30	P07.3-029	27	P07.4-029	30	P07.5-029	19
P07.1-029	23	P07.2-028b	27	P07.3-030	29	P07.4-030	22	P07.5-030	29
P07.1-030	22	P07.2-029	30	P07.3-031	28	P07.4-031	19	P07.5-031	22
P07.1-031	25	P07.2-030	33	P07.3-032a	24	P07.4-032	22	P07.5-032	23
P07.1-032	22	P07.2-031	29	P07.2-032b	22	P07.4-033	22	P07.5-033	23
P07.1-033	28	P07.2-032	20	P07.3-033	24	P07.4-034	20	P07.5-034	25
P07.1-034	29	P07.2-033	23	P07.3-034	27	P07.4-035	30	P07.5-035	23
P07.1-035	31	P07.2-034	20	P07.3-035	23	P07.4-036	18	P07.5-036	28
P07.1-036	27	P07.2-035	24	P07.3-036	18	P07.4-037	22	P07.5-037	25
P07.1-037	35	P07.2-036	23	P07.3-037	23	P07.4-038	21	P07.5-038	18
P07.1-038	20	P07.2-037	22	P07.3-038	24	P07.4-039	35	P07.5-039	26
P07.1-039	24	P07.2-038	19	P07.3-039	23	P07.4-040	18	P07.5-040	23
P07.1-040	31	P07.2-039	25	P07.3-040	25	P07.4-041	20	P07.5-041	20
P07.1-041	24	P07.2-040	34	P07.3-041	23	P07.4-042	28	P07.5-042	25
P07.1-042	28	P07.2-041	25	P07.3-042	22	P07.4-043	26	P07.5-043	26
P07.1-043	21	P07.2-042	27	P07.3-043	18	P07.4-044	24	P07.5-044	28
P07.1-044	24	P07.2-043	32	P07.3-044	27	P07.4-045	25	P07.5-045	23
P07.1-045	27	P07.2-044	22	P07.3-045	22	P07.4-046	27	**Average**	22.80
P07.1-046	19	P07.2-045	21	P07.3-046	24	P07.4-047	22	**STDEV**	2.86
P07.1-047	22	P07.2-046	25	P07.3-047	19	P07.4-048	19		
P07.1-048	21	P07.2-047	34	P07.3-048	24	P07.4-049	22		
P07.1-049	17	P07.2-048	21	P07.3-049	20	P07.4-050	23		
P07.1-050	24	P07.2-049	29	P07.3-050	23	**Average**	23.06		
P07.1-051	21	P07.2-050	20	**Average**	**23.90**	**STDEV**	4.29		
P07.1-052	28	**Average**	**25.33**	**STDEV**	**3.23**				
P07.1-053	28	**STDEV**	**4.60**						
P07.1-054	26								
P07.1-055	27								
P07.1-056	30								

P07.1		P07.2		P07.3		P07.4		P07.5	
Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci	Cell	DMC1 foci
P07.1-059	24								
P07.1-060	26								
P07.1-061	23								
P07.1-062	20								
P07.1-063	20								
P07.1-064	21								
P07.1-065	28								
P07.1-066	20								
P07.1-067	22								
P07.1-068	23								
P07.1-069	25								
P07.1-070	20								
Average	**24.37**								
STDEV	**3.61**								

Table S3.2: Dmc1 foci counts for animal P09 P09.4, 17-21 represent six different prophase spreads using meiotic material from platypus number P09.

P09.4		P09.17		P09.18		P09.19		P09.20		P09.21	
Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci
P09.4-001	19	P09.17-001	23	P09.18-001	20	P09.19-001	23	P09.20-001	21	P09.21-001	25
P09.4-002	30	P09.17-002	23	P09.18-002	19	P09.19-002	21	P09.20-002	21	P09.21-002	29
P09.4-003	24	P09.17-003	30	P09.18-003	22	P09.19-003	23	P09.20-003	22	P09.21-003	27
P09.4-004	21	P09.17-004	33	P09.18-004	25	P09.19-004	22	P09.20-004	27	P09.21-004	26
P09.4-005	22	P09.17-005	25	P09.18-005	23	P09.19-005	24	P09.20-005	22	P09.21-005	21
P09.4-006	23	P09.17-006	26	P09.18-006	24	P09.19-006	18	P09.20-006	19	P09.21-006	24
P09.4-007	24	P09.17-007	20	P09.18-007	22	P09.19-007	19	P09.20-007	21	P09.21-007	22
P09.4-008	23	P09.17-008	25	P09.18-008	26	P09.19-008	22	P09.20-008	20	P09.21-008	21
P09.4-009	35	P09.17-009	26	P09.18-009	28	P09.19-009	23	P09.20-009	19	P09.21-009	19
P09.4-010	24	P09.17-010	23	P09.18-010	21	P09.19-010	24	P09.20-010	19	P09.21-010	31
P09.4-011	23	P09.17-011	22	P09.18-011	20	P09.19-011	22	P09.20-011	22	P09.21-011	22
P09.4-012	19	P09.17-012	23	P09.18-012	21	P09.19-012	26	P09.20-012	21	P09.21-012	18
P09.4-013	21	P09.17-013	18	P09.18-013	26	P09.19-013	24	P09.20-013	23	P09.21-013	22
P09.4-014	26	P09.17-014	27	P09.18-014	24	P09.19-014	20	P09.20-014	17	P09.21-014	25
P09.4-015	25	P09.17-015	29	P09.18-015a	21	P09.19-015a	31	P09.20-015	22	P09.21-015	24
P09.4-016	29	P09.17-016	23	P09.18-015b	21	P09.19-015b	24	P09.20-016	27	P09.21-016	23
P09.4-017	20	P09.17-017	22	P09.18-016	24	P09.19-016	24	P09.20-017	21	P09.21-017	21
P09.4-018	19	P09.17-018	20	P09.18-017	24	P09.19-017a	26	P09.20-018	18	P09.21-018	18
P09.4-019	25	P09.17-019	25	P09.18-018	23	P09.19-017b	21	P09.20-019	19	P09.21-019	22
P09.4-020	29	P09.17-020	21	P09.18-019	20	P09.19-017c	23	P09.20-020	30	P09.21-020	29
P09.4-021	33	P09.17-021	29	P09.18-020	22	P09.19-018	25	P09.20-021	25	P09.21-021	21
P09.4-022	30	P09.17-022	20	P09.18-021	24	P09.19-019	23	P09.20-022	20	P09.21-022	20
P09.4-023	26	P09.17-023	21	P09.18-022	18	P09.19-020	18	P09.20-023	29	P09.21-023	22
P09.4-024	25	P09.17-024	30	P09.18-023	21	P09.19-021	31	P09.20-024	25	P09.21-024	25
P09.4-025	27	P09.17-025	25	P09.18-024	21	P09.19-022	26	P09.20-025	16	P09.21-025	23
P09.4-026	33	P09.17-026	24	P09.18-025	22	P09.19-023	27	P09.20-026	23	P09.21-026	22
P09.4-027	23	P09.17-027	29	P09.18-026	20	P09.19-024	19	P09.20-027	22	P09.21-027	21
P09.4-028	26	P09.17-028	27	P09.18-027	21	P09.19-025	20	P09.20-028	25	P09.21-028	21

P09.4		P09.17		P09.18		P09.19		P09.20		P09.21	
Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci	Cell	DMC 1 foci
P09.4 -029	20	P09.17 -029	29	P09.18 -028	27	P09.19 -026	22	P09.2 0-029	24	P09.2 1-029	20
P09.4 -030	24	P09.17 -030	22	P09.18 -029	22	P09.19 -027	26	P09.2 0-030	20	P09.2 1-030	23
P09.4 -031	33	P09.17 -031	23	P09.18 -030	21	P09.19 -028	20	P09.2 0-031	23	P09.2 1-031	23
P09.4 -032	24	P09.17 -032	22	P09.18 -031	24	P09.19 -029	26	P09.2 0-032	23	P09.2 1-032	26
P09.4 -033	18	P09.17 -033a	24	P09.18 -032	32	P09.19 -030	22	P09.2 0-033	25	P09.2 1-033	23
P09.4 -034	23	P09.17 -033b	24	P09.18 -033	20	P09.19 -031	23	P09.2 0-034	20	P09.2 1-034	23
P09.4 -035	31	P09.17 -034	22	P09.18 -034	21	P09.19 -032	27	P09.2 0-035	23	P09.2 1-035	16
Avg.	**25.06**	P09.17 -035	23	P09.18 -035	24	P09.19 -033	23	P09.2 0-036	19	P09.2 1-036	19
STDV	**4.50**	P09.17 -036	21	P09.18 -036	24	P09.19 -034	21	P09.2 0-037	18	P09.2 1-037	23
		P09.17 -037	24	P09.18 -037	23	P09.19 -035	27	P09.2 0-038	22	P09.2 1-038	20
		P09.17 -038	22	P09.18 -038	22	P09.19 -036	22	P09.2 0-039	25	P09.2 1-039	24
		P09.17 -039	24	P09.18 -039	24	P09.19 -037	22	P09.2 0-040	26	P09.2 1-040	25
		P09.17 -040a	22	P09.18 -040	22	P09.19 -038	21	P09.2 0-041	24	P09.2 1-041	22
		P09.17 -040b	21	P09.18 -041	23	P09.19 -039	27	P09.2 0-042	20	P09.2 1-042	18
		P09.17 -041	23	P09.18 -042	26	P09.19 -040	23	P09.2 0-043	26	P09.2 1-043	21
		P09.17 -042	20	P09.18 -043	25	P09.19 -041	26	P09.2 0-044	23	P09.2 1-044	19
		P09.17 -043	27	P09.18 -044	25	P09.19 -042	27	P09.2 0-045	19	P09.2 1-045	18
		P09.17 -044	23	P09.18 -045	26	P09.19 -043	25	P09.2 0-046	19	P09.2 1-046	30
		P09.17 -045	28	P09.18 -046	27	P09.19 -044	19	P09.2 0-047	26	P09.2 1-047	20
		P09.17 -046	23	P09.18 -047	23	P09.19 -045	23	P09.2 0-048	23	P09.2 1-048	18
		P09.17 -047	19	P09.18 -048	23	P09.19 -046	23	P09.2 0-049	21	P09.2 1-049	20
		P09.17 -048	22	P09.18 -049	29	P09.19 -047	22	P09.2 0-050	24	P09.2 1-050	21
		P09.17 -049	28	P09.18 -050	24	P09.19 -048	23	**Avg.**	**22.18**	**Avg.**	**22.32**
		P09.17 -050	27	**Avg.**	**23.14**	P09.19 -049	27	**STDV**	**3.05**	**STDV**	**3.26**
		Avg.	**24.08**	**STDV**	**2.68**	P09.19 -050	32				
		STDV	**3.24**			**Avg**	**23.55**				
						STDV	**3.12**				

Table S3.3: Cell surface area measurements Surface areas for the nucleus (defined by DAPI staining), Dmc1 foci, and the six FISH signals were measured using AxioVision. If a given signal was not able to be measured, it was recorded as "N/A" and was not included in the calculation of the average surface area.

	Surface area (μm^2)							
Cell	**DAPI**	**DMC1**	**X1**	**PAR1**	**Y2**	**Y5**	**MHC**	**6**
P09.4-001	550.49	0.37	0.47	0.98	4.77	0.92	0.5	12.55
P09.4-002	868.32	0.38	0.56	0.54	5.43	0.89	0.51	18.56
P09.4-003	437.35	0.24	1.16	0.66	3.4	0.77	0.94	14.63
P09.4-004	500.67	0.37	0.69	0.54	N/A	N/A	0.52	19.54
P09.4-005	695.4	0.32	0.52	0.66	6.47	2.77	N/A	N/A
P09.4-006	384.46	0.24	0.73	0.63	3.98	1.19	0.43	15.59
P09.4-007	421.23	0.26	1.35	1.17	4.45	0.79	0.59	16.26
P09.4-008	743.76	0.27	1.26	1.71	6.06	1.83	0.52	9.26
P09.4-009	628.71	0.45	0.85	0.93	3.64	1.14	0.46	15.56
P09.4-010	437.01	0.36	0.53	0.96	2.78	1.54	0.74	19.84
P09.4-011	459.06	0.25	0.71	0.64	2.26	1.22	N/A	N/A
P09.4-012	400.48	0.32	0.51	0.74	2.3	1.1	0.48	22.88
P09.4-013	520.03	0.23	0.58	0.44	5.42	N/A	0.79	16.34
P09.4-014	442.71	0.25	N/A	N/A	N/A	N/A	N/A	N/A
P09.4-015	479.64	0.14	0.45	0.67	2.44	0.91	0.44	24.92
P09.4-016	530.28	0.16	0.66	0.76	4.27	2.23	0.71	18.58
P09.4-017	404.33	0.48	0.45	0.53	4.33	2.54	0.29	17.89
P09.4-018	442.15	0.43	0.63	0.79	6.39	0.78	0.62	14.66
P09.4-019	523.34	0.21	0.66	0.61	3.09	1.41	0.72	16.06
P09.4-020	432.83	0.24	0.48	0.82	1.46	0.89	0.37	9.7
P09.4-021	512.87	0.36	0.79	0.67	2.67	0.99	0.79	9.68
P09.4-022	375.27	0.28	N/A	N/A	N/A	N/A	N/A	N/A
P09.4-023	564.72	0.37	1.07	0.5	1.7	0.72	N/A	N/A
P09.4-024	493.27	0.42	0.69	0.79	2.83	1.06	0.85	16.92
P09.4-025	510.23	0.41	0.87	0.8	4.85	1	0.5	20.09
P09.4-026	443.43	0.31	0.58	0.39	3.42	1.22	0.36	19,13
P09.4-027	375.44	0.51	0.79	0.94	N/A	N/A	0.87	10.72
P09.4-028	439.36	0.23	0.72	0.52	2.7	1.19	0.62	18.11
P09.4-029	566.21	0.31	0.91	0.36	4.23	1.04	0.72	28.05
P09.4-030	492.13	0.54	0.77	0.57	3.2	1.03	0.45	12.27
P09.4-031	492.13	0.3	1	1.89	5.59	1.89	0.51	15.11
P09.4-032	568.07	0.37	0.99	0.89	2.91	1.78	1.03	12.53
P09.4-033	442.6	0.45	N/A	N/A	2.34	1.1	N/A	N/A
P09.4-034	449.72	0.47	0.45	0.68	3.25	1.61	0.31	20.96
P09.4-035	480.04	0.36	0.42	0.26	4.26	1.62	0.57	19.51
Average	**500.22**	**0.33**	**0.73**	**0.75**	**3.77**	**1.31**	**0.59**	**16.67**