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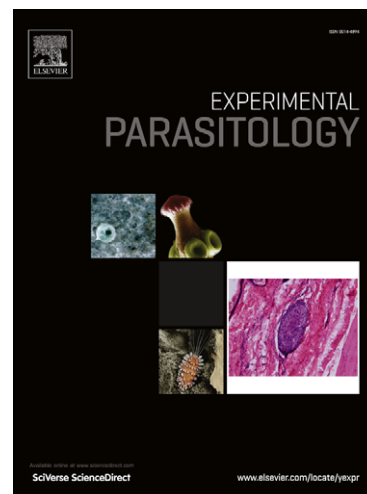
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1 **Molecular characterisation of *Eimeria* species in Macropods.**

2

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16

17 ABSTRACT

18 A total of 597 faecal samples were collected from western grey kangaroos
19 (*Macropus fuliginosus*), Euros (*M. robustus*), red kangaroos (*M. rufus*) in Western
20 Australia and Eastern Grey Kangaroos (*M. giganteus*) from Victoria and screened for
21 the presence of *Eimeria* by PCR at the 18S ribosomal RNA (rRNA) locus. The overall
22 prevalence was 24.3% (145/597). At the 18S rRNA locus, sequences were obtained
23 for 25 of the 145 positives. Phylogenetic analysis indicated that all the macropod-
24 derived *Eimeria* species grouped in a separate marsupial clade that included *Eimeria*
25 *trichosuri* from brushtail possums. At least 6 different clades were identified within
26 the marsupial isolates and many of the genotypes identified are likely to be valid
27 species, however morphological and biological data need to be collected to match
28 sequences to previously characterized *Eimeria* species or identify if they are new
29 species.

30

31 *Keywords:* *Eimeria*; genetic characterization; phylogeny; western grey kangaroos;
32 Eastern grey kangaroos; red kangaroos; euros.

33

34 **1. Introduction**

35 *Eimeria* spp. are enteric coccidian parasites that infect a wide range of
36 vertebrate hosts (McDonald and Shirley, 2009). There are several pathogenic eimerian
37 species that cause severe clinical disease and economic loss in poultry and production
38 animals (Aarthi et al., 2010; Fitzgerald, 1980; Taubert et al., 2010). Traditionally,
39 identification of *Eimeria* species has been based largely on oocyst morphology but
40 also host specificity, pathology and geographic distribution (Duszynski and Wilber,
41 1997; Tenter et al., 2002). However, some species of *Eimeria* are morphologically
42 identical and occur in several hosts and it is now recognized that molecular data is
43 essential to accurately delimit species and infer phylogenetic relationships among
44 *Eimeria* (Tenter et al., 2002).

45 More than forty-two *Eimeria* species have been described from a range of
46 marsupial hosts in Australasia and the Americas including kangaroos and wallabies,
47 wombats, possums, bandicoots and opossums (Mykytowycz, 1964; Joseph, 1974;
48 Barker et al., 1988a; Barker et al., 1988b; Barker et al., 1988c; Barker et al., 1989;
49 Bennett et al., 2006; Heckscher et al., 1999; O'Callaghan et al., 1998; O'Callaghan
50 and O'Donoghue, 2001; Teixeira et al., 2007). However, to date only *Eimeria*
51 *trichosuri*, a species found in brushtail-possums of the genus *Trichosurus* has been
52 genetically characterized (Power et al., 2009). That analysis placed *E. trichosuri*
53 clones in a clade that diverged before the major clade comprising species from
54 placental mammals, which was consistent with host phylogeny where marsupials
55 represent an ancient evolutionary line that predates the eutherian lineage (Power et al.,
56 2009). In the present study, we characterized *Eimeria* DNA from western grey
57 kangaroos (*Macropus fuliginosus*), Euros (*M. robustus*), red kangaroos (*M. rufus*) in
58 Western Australia (WA) and Eastern Grey Kangaroos (*M. giganteus*) from Victoria

59 (Vic) at the 18S rRNA locus and examined their phylogenetic relationship to other
60 eimerian species.

61

62 **2. Materials and methods**

63

64 *2.1 Sample collection*

65

66 A total of 564 faecal samples were collected from wild western grey
67 kangaroos (WKG's) (*M. fuliginosus*) from five kangaroo harvesting centres in WA
68 between December 2007 and January 2009 (Table 1). Faecal samples were also
69 collected from wild Euros or Common Wallaroo (*M. robustus*) (n = 12) from Karratha
70 and Roeburne in WA, red kangaroos (RK) (*M. rufus*) (n=10) from Roeburne and
71 Fortescue in WA, Eastern Grey Kangaroos (EGK's) (*M. giganteus*) (n = 10) and 1
72 western grey kangaroo from Brimpaen and Cape Bridgewater in Victoria (Table 1).
73 Unfortunately at the time of collection, the majority of samples were not stored in
74 potassium dichromate but were stored frozen. Attempts were made to sporulate all
75 PCR positives by incubating samples in 2% (w/v) potassium dichromate at room
76 temperature.

77

78 *2.2 DNA isolation*

79 Genomic DNA was extracted from 200mg of each faecal sample using a QIAamp
80 DNA Mini Stool Kit (Qiagen, Hilden, Germany) or from 250mg of each faecal sample

81 using a Power Soil DNA Kit (MolBio, Carlsbad, California). A negative control (no
82 faecal sample) was used in each extraction group.

83

84 2.3 PCR amplification and sequencing

85 Samples were initially screened at the 18S rRNA locus for *Eimeria* using
86 primers and conditions described by Power et al., (2009). However, as this resulted in
87 non-specific amplifications, a genus specific hemi-nested PCR was re-designed using
88 the forward primer EIF1 5' - GCT TGT CTC AAA GAT TAA GCC described by
89 Power et al., (2009) and the reverse primer EIR3 5' – ATG CAT ACT CAA AAG
90 ATT ACC (this study). Diluted amplicons (1:10) of the first PCR were used as
91 template for a second amplification with the forward primer EIF3 5' - CTA TGG CTA
92 ATA CAT GCG CAA TC (this study) and the reverse primer EIR3 to produce a
93 1,399-1,407 bp product. Primers were designed with the assistance of GeneTool Lite
94 software (www.biotoools.com). Primer specificity was confirmed by BLAST searches
95 in GenBank and amplification testing on human, bacterial (*Salmonella*,
96 *Campylobacter* and *Yersinia*) and *Cryptosporidium*, *Giardia* and *Eimeria* DNA.

97 PCR reactions were performed in a 25 µl volume that contained approximately
98 15 ng DNA, 1 x PCR buffer (FisherBiotech, Perth, WA), 0.2 mM deoxynucleoside
99 triphosphates, 2.5 mM MgCl₂, 5% (wt/vol) dimethyl sulfoxide, 0.2 µMol each primer
100 and 1 unit of Tth+ DNA polymerase (FisherBiotech, Perth, WA).

101 Cycling conditions consisted of 94°C for 3 min and then 35/45 cycles of 94°C
102 for 30 s, 60°C for 30 s, and 72°C for 90 sec. The final extension was 72°C for 7 min.
103 The cycle number was 35 for the first round PCR and 45 for the second one.

104 PCR contamination controls were used including negative controls and
105 separation of preparation and amplification areas. A spike analysis (addition of 0.5 µL

106 of positive control DNA into each sample) was conducted on randomly selected
107 negative samples from each group of DNA extractions to determine if negative results
108 were due to PCR inhibition.

109 The amplified DNA fragments from the secondary PCR product were
110 separated by gel electrophoresis and purified using the freeze-squeeze method (Ng et
111 al., 2006). Purified PCR products were sequenced using an ABI Prism™ Dye
112 Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California)
113 according to the manufacturer's instructions with the exception that the annealing
114 temperature was raised to 58 °C. The results of the sequencing reactions were
115 analysed and edited using Chromas lite version 2.0
116 (<http://www.technelysium.com.au>), compared to existing *Eimeria* 18S rDNA
117 sequences on GenBank using BLAST searches and aligned with reference genotypes
118 from GenBank using Clustal W (<http://www.clustalw.genome.jp>).

119

120 *2.4 Phylogenetic analysis*

121 Phylogenetic trees were constructed for *Eimeria* at the 18S locus with additional
122 isolates from GenBank. Distance estimation was conducted using TREECON (Van de
123 Peer and De Wachter, 1994), based on evolutionary distances calculated with the
124 Tamura-Nei model and grouped using Neighbour-Joining. Parsimony analyses were
125 conducted using MEGA version 3.1 (MEGA3.1: Molecular Evolutionary Genetics
126 Analysis software, Arizona State University, Tempe, Arizona, USA). Bootstrap
127 analyses were conducted using 1,000 replicates to assess the reliability of inferred tree
128 topologies. Maximum Likelihood (ML) analyses were conducted using the program
129 PhyML (Dereeper et al., 2008) and the reliability of the inferred trees was assessed by
130 the approximate likelihood ratio test (aLRT) (Anisimova and Gascuel, 2006).

131 2.5 *Statistical Analysis*

132 Prevalences were expressed as percentage of positive samples, with 95%
133 confidence intervals calculated assuming a binomial distribution, using the software
134 Quantitative Parasitology 3.0 (Rozsa et al., 2000). Statistical analysis was performed
135 using SPSS 17.0 (Statistical Package for the Social Sciences) for Macintosh OS X
136 (SPSS inc. Chicago, USA) to determine if there was any association between the
137 prevalence of *Eimeria* and factors such as host gender, location, seasonality/rainfall
138 and temperature.

139

140 **3. Results**

141 3.1 *Prevalence of Eimeria in marsupials*

142 *Eimeria* was detected in 145 of 597 samples screened, an overall prevalence of
143 24.3% (20.8-27.7% CI) (Table 1). The prevalence in the different sampling locations
144 ranged from 8.3 to 100% (only 1 sample) (Table 1). The majority of isolates were
145 obtained from adult kangaroos, subjectively aged based on the animal's size and
146 apparent sexual maturity (583/597). Adults were considered to be those animals 3
147 years of age and older. Only 5 samples were collected from pouch young and 9 from
148 sub-adults. No positives were detected in pouch young and only 1/9 sub-adults from
149 Capel were positive for *Eimeria*. The remaining 144 positives were detected in adults.

150 There were 256 females and 341 males. There was no significant difference in
151 the prevalence of *Eimeria* between males and females ($p=0.313$). In the WGK
152 samples from WA, the prevalence in different seasons ranged from 17.2% to 36.6%
153 and this difference was significant ($p<0.001$). The highest rate was from isolates

154 collected during the 07/08 and 08/09 summer period (36.6%), followed by the 08
155 autumn sampling period (26%). The lowest rate was detected during the 08 winter
156 sampling period (17.2%) and the second lowest rate was from the 08 spring period
157 (19.2%).

158

159 3.2 Morphological analysis of oocysts

160 Unfortunately, only one oocyst from a red kangaroo (isolate RK 38N) near
161 Fortescue roadhouse in WA was successfully sporulated. The oocyst was ellipsoidal
162 and slightly pointed at one end and measured 35.1 x 20.4 μm and contained 4
163 ellipsoidal sporocysts measuring 12.2-8.8 μm (Fig. 1). There was no clear-cut Stieda
164 body and morphologically the oocyst appeared to match most closely with *E.*
165 *wilcanniensis* previously described in red kangaroos, EGK's, WGK's and Euros
166 (Mykytowycz, 1964; Barker et al., 1989). *Eimeria wilcanniensis* is separated from
167 oocysts with overlapping size ranges on the basis of the absence of a Stieda body,
168 oocyst shape and sporocyst size (Barker et al., 1989).

169

170

171 3.2 Phylogenetic analysis of *Eimeria* species and genotypes in marsupials

172

173 At the 18S rRNA locus, sequences were obtained for 25 of the 145 positives
174 (Table 2). Phylogenetic analyses of the partial nucleotide sequences (~1,290 bp) from
175 marsupials including four sequences from *E. trichosuri* (Power et al., 2009) and a
176 range of *Eimeria* species from birds, bats, production animals and rodents at the 18S
177 locus using Distance, Parsimony and ML analyses produced similar results (Fig. 2
178 ML tree shown). The overall tree topology was similar to previously produced

179 phylogenetic trees for *Eimeria* (Power et al., 2009). Species from single host groups
180 formed monophyletic branches except for the two species from bats, which were
181 placed within the rodent clade. The nodes for monophyletic clades representing single
182 host groups were supported by high bootstrap values. All the marsupial-derived
183 *Eimeria* sequences generated as part of the present study, grouped in a clade with *E.*
184 *trichosuri*.

185 Seven broad clades were observed within the marsupial group. Clade 1
186 consisted of isolates WKG 2767, WGK 2886, WKG 2175, from WGK's which were
187 100% identical and grouped with WGK 2179, EGK CB1, EGK CB4, EGK 37L, RK
188 37X. The genetic similarities within this clade ranged from 100-99.5%. Clade 2
189 consisted of isolates WGK 2346 and WGK 2536, which only shared 98.9% genetic
190 similarity. Clade 3 consisted of *E. trichosuri*, isolates WKG 2533, RK 38P, WGK
191 2534, EGK CB2 and WGK 37N.

192 None of the *Eimeria* sequences derived from kangaroos were 100% identical to
193 *E. trichosuri*. Isolate WGK 2533 from WA shared the highest similarity with *E.*
194 *trichosuri* (99.8%). Clade 4 consisted of isolates WGK 2771, WGK 2296, WGK
195 2775, WGK 2549, WGK 2336, WGK 2298 from WGK's from WA and isolate EGK
196 CB3 from Vic and shared 99.8-99.3% genetic similarity. Clade 5 consisted of isolates
197 WGK 2884 and RK 38N (the *E. wilcanniensis*-like isolate), which shared 99.6%
198 similarity. Clade 6 consisted of a single isolate (RK 38K), which was genetically
199 distinct and was most closely related to isolate WGK 2884 from clade 5 and WGK
200 2767 from Clade 1 (99.6% similarity). Sequences generated in the present study have
201 been submitted to GenBank under accession numbers JF419336 to JF419360.

202

203 **4. Discussion**

204

205 In the present study, the overall prevalence of *Eimeria* in macropods was
206 24.3%. Previous studies have reported a prevalence of 6 to 14% in populations of red
207 kangaroos and from 26 to 70% in EGK's (Mykytowycz, 1964). No clinical cases of
208 coccidiosis were reported (Mykytowycz, 1964). Other studies have reported a
209 prevalence of 2 – 100% in kangaroos and wallabies (Barker et al., 1989). In the
210 present study, the prevalence of *Eimeria* in EGK's was 50% and 30% in red
211 kangaroos. Clinical information on the kangaroos was not available.

212 Attempts were made to sporulate oocysts to identify species morphologically,
213 however, due to the age of the faecal samples at the time of analysis (up to 12
214 months), we were only successful in sporulating one oocyst from a red kangaroo
215 (isolate 38N) from WA which morphologically appeared closest to *E. wilcanniensis*
216 previously described in red kangaroos (Mykytowycz, 1964). *Eimeria wilcanniensis*
217 Mykytowycz, 1964 was subsequently redescribed from red kangaroos, and the host
218 range was extended to EGK's, WGK's and Euros (Barker et al., 1989). As oocysts
219 were unavailable from previous studies, it was not possible to obtain genetic
220 sequences from these holotypes and match genetic sequences obtained with species
221 identifications. However, preliminary evidence suggests that isolate RK 38N from a
222 red kangaroo may be *E. wilcanniensis* and this therefore may be the first genetic
223 sequence available for this species which expands our knowledge of the molecular
224 phylogeny of *Eimeria*. However, it should be noted that as only one sporulated oocyst
225 was available, we cannot be certain what species was present and more sporulated
226 oocysts would need to be examined before a definitive diagnosis could be made.

227 A total of 25 new *Eimeria* sequences from macropods representing potentially 6
228 or more species of *Eimeria* were generated in this study. Sequence data has only been
229 previously available for *E. trichosuri* (Power et al., 2009). Phylogenetic analysis of *E.*
230 *trichosuri* suggested that *Eimeria* found in marsupials diverged prior to *Eimeria* from
231 eutherians. This is consistent with mammalian evolution and the geographic isolation
232 and radiation of marsupials (Power et al., 2009). The present study also supports this
233 hypothesis.

234 There are a number of major limitations in undertaking DNA analyses on
235 species of *Eimeria*, one of which is the lack of availability of holotypes due to the
236 difficulties in preserving oocyst material (Duszynski and Wilber, 1997). Another
237 difficulty is the fact that multiple *Eimeria* species have been reported from the one
238 marsupial host (Mykytowycz, 1964; Heckscher et al., 1999; O'Callaghan et al., 1998;
239 Barker et al., 1989). In the present study, clean genetic sequences could only be
240 obtained from 25 of the 145 positives indicating potentially mixed infections as
241 evidenced by clean sections of chromatograms followed by multiple overlapping
242 peaks. Accurate correlation of genetic sequences and morphology would therefore
243 require micromanipulation to sort individual sporulated oocysts for genetic typing. As
244 a result of these difficulties, a previous study chose *E. trichosuri* for genetic analysis
245 (Power et al., 2009) as *E. trichosuri* is the only species recorded in brushtail possums.
246 In addition, *E. trichosuri* has uniform oocyst characteristics in isolates from different
247 individuals and from different localities (O'Callaghan and O'Donoghue, 2001).

248 The morphological similarity of oocysts, the broad host specificity of some
249 *Eimeria* species and the diversity of *Eimeria* within one host compound species
250 delimitation (Tenter et al., 2002). Molecular data is therefore essential to accurately
251 delimit species. Though the importance of *Eimeria* morphology should not be

252 discounted, these difficulties are not unique to *Eimeria* and several species of
253 protozoan parasites have been described based on molecular data because of the
254 limitations of the respective morphological characteristics. For example, for the
255 genera *Theileria* and *Babesia*, a genetic distance 0.7% and 3.4% respectively at the
256 18S rRNA locus is sufficient to be classified as a distinct species (Schnittger et al.,
257 2003). Similarly, for *Cryptosporidium*, if the genetic distance at two unlinked loci is
258 equal to or greater than currently accepted species, then this is strongly supportive of
259 species status (Xiao et al., 2004).

260 In the present study, the genetic similarities within the marsupial clade ranged
261 from 98.7-100% and between the marsupial isolates and other *Eimeria* species ranged
262 from 98.2-96.4% similarity. The *E. wilcanniensis*-like isolate from a red kangaroo
263 (RK 38N) shared 99.6% similarity with isolate WGK 2884 and 99.5% genetic
264 similarity with *E. trichosuri*. The genetic differences between different marsupial
265 genotypes (Table 3), are similar to the genetic differences between accepted species
266 of *Eimeria*. For example, the genetic similarity between *E. tenella* and *E. necatrix* and
267 between *E. bovis* and *E. crandallis* is 99.7 and 99.6% respectively. By these criteria,
268 many of the marsupial-derived *Eimeria* genotypes are likely to be separate species,
269 however morphological and biological data need to be collected for these genotypes
270 before they can be properly validated.

271 In the present study, novel *Eimeria* sequences from a range of macropod hosts
272 were obtained and a molecular phylogeny of marsupial-derived *Eimeria* constructed.
273 The present data supports the hypothesis that *Eimeria* found in marsupials diverged
274 prior to *Eimeria* from placental mammals (Power et al., 2009). Future studies need to
275 concentrate on obtaining morphologically characterized *Eimeria* species derived from
276 macropods and generating sequence data that is directly related to described species.

277 This in turn will enable the sequences generated in the present study to be placed into
278 context of *Eimeria* taxonomy. Analyzing the isolates at multiple loci will also provide
279 a more in-depth analysis of the evolution of marsupial-derived *Eimeria*.

280

281

ACCEPTED MANUSCRIPT

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362

363 **Fig. 1.** Sporulated oocyst from a red kangaroo resembling *E. wilcanniensis*.

364 **Fig. 2.** Evolutionary relationships of *Eimeria* marsupial-derived isolates inferred by
365 ML analysis of 18S rRNA sequences. Percentage support (>50%) from 1000
366 pseudoreplicates from ML, neighbor-joining and parsimony analyses is indicated at
367 the left of the supported node.

368

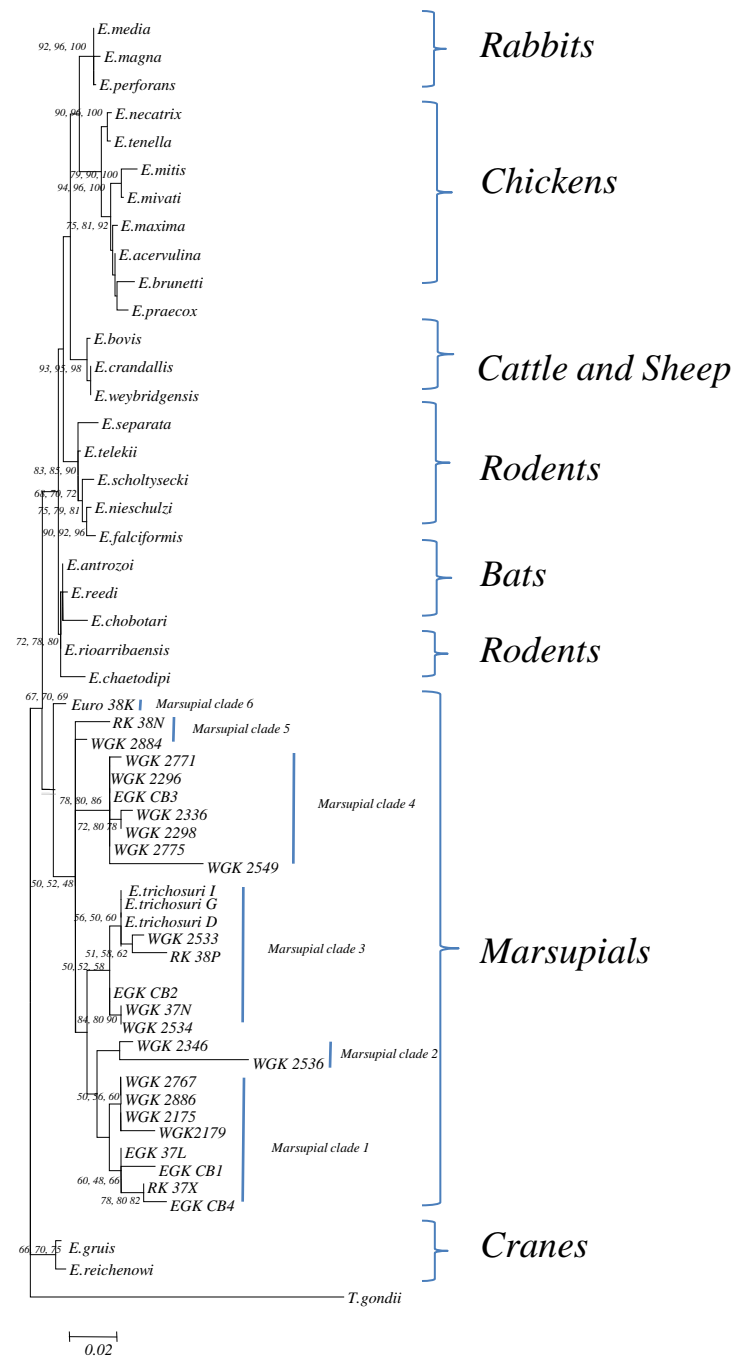
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Figure



Figure



370 Table 1. The prevalence of *Eimeria* in western grey kangaroos (WGK's), Euros and
 371 Eastern grey Kangaroos (EGK's) in the different sampling locations. 95% confidence
 372 intervals are listed in parenthesis.

Locations	No. of samples	Female	Male	PCR positives	% prevalence
<u>WGK's from WA</u>					
Capel	129	45	84	25	19.4 (12.6-26.2)
Badgingarra	110	45	65	29	26.4 (18.1-24.6)
Preston Beach	47	29	18	7	14.9 (4.7-25.1)
Eneabba	74	43	31	24	32.4 (21.8-43.1)
Manjimup	204	78	126	50	24.5 (18.6-30.2)
<u>Euro's from WA</u>					
Karratha and Roeburne	12	4	8	1	8.3 (0-24)
<u>Red kangaroos from WA</u>					
Roeburne and Fortescue	10	4	6	3	30 (1.6-48.4)
<u>EGK's from Vic</u>					
Brimpaen and Cape Bridgewater	10	8	2	5	50 (19-81)
<u>WGK's from Vic</u>					
Brimpaen	1	0	1	1	100 (100-100)
Total	597	256	341	145	24.3 (20.8-27.7)

373

374

375 Table 2. Marsupial species positive for *Eimeria* for which an 18S sequence was
 376 obtained.

Sample ID	Host name	Common name	Locality	Sex
EGK CB1	<i>Macropus giganteus</i>	Eastern Grey Kangaroo	Cape Bridgewater, Vic	F
EGK CB2	<i>Macropus giganteus</i>	Eastern Grey Kangaroo	Cape Bridgewater, Vic	F
EGK CB3	<i>Macropus giganteus</i>	Eastern Grey Kangaroo	Cape Bridgewater, Vic	M
EGK CB4	<i>Macropus giganteus</i>	Eastern Grey Kangaroo	Cape Bridgewater, Vic	F
EGK 37L	<i>Macropus giganteus</i>	Eastern Grey Kangaroo	Brimpaen, Vic	F
WGK 37N	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Brimpaen, Vic	M
Euro 38K	<i>Macropus robustus</i>	Euro or Common Wallaroo	6 km N of Fortescue Roadhouse, WA	M
RK 38N	<i>Macropus rufus</i>	Red Kangaroo	65 km N of Fortescue Roadhouse, WA	M
RK 38P	<i>Macropus rufus</i>	Red Kangaroo	50 km N of Fortescue Roadhouse, WA	F
RK 37X	<i>Macropus rufus</i>	Red Kangaroo	21 km N of Roeburne, WA	M
WGK 2175	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Manjimup, WA	M
WGK 2179	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Manjimup, WA	M
WGK 2296	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Preston Beach, WA	F
WGK 2298	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Preston Beach, WA	F
WGK 2336	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Manjimup, WA	M
WGK 2346	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Manjimup, WA	M
WGK 2533	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Badgingarra, WA	M
WGK 2536	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Badgingarra, WA	M
WGK 2549	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Manjimup, WA	M
WGK 2767	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Capel, WA	M
WGK 2771	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Eneabba, WA	M
WGK 2775	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Eneabba, WA	M
WGK 2884	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Eneabba, WA	M
WGK 2886	<i>Macropus fuliginosus</i>	Western Grey Kangaroo	Eneabba, WA	M

377

378

Table 3: Percentage sequence similarity at the 18S rRNA locus between *Eimeria* sequences found in marsupials in this study and *E. trichosuri* calculated using Tamura-Nei.

	EGK CB1	EGK CB2	EGK CB3	EGK CB4	EGK 37L	WGK 37N	Euro 38K	RK 38N	RK 38P	RK 37X	WGK 2175	WGK 2179	WGK 2298	WGK 2336	WGK 2346	WGK 2533	WGK 2536	WGK 2549	WGK 2767	WGK 2771	WGK 2775	WGK 2884	<i>E.</i> <i>trichosuri</i>	
EGK CB1	100																							
EGK CB2	99.3	100																						
EGK CB3	99.1	99.5	100																					
EGK CB4	99.4	99.2	99.0	100																				
EGK 37L	99.7	99.6	99.4	99.7	100																			
WGK 37N	99.4	99.9	99.4	99.3	99.6	100																		
Euro 38K	99.3	99.5	99.5	99.2	99.6	99.4	100																	
RK 38N	99.1	99.6	99.5	99.0	99.4	99.4	99.5	100																
RK 38P	98.8	99.6	99.1	98.8	99.1	99.5	99.1	99.1	100															
RK 37X	99.6	99.4	99.2	99.8	99.8	99.5	99.4	99.2	99.0	100														
WGK 2175	99.6	99.7	99.4	99.5	99.8	99.6	99.6	99.4	99.3	99.6	100													
WGK 2179	99.3	99.5	99.1	99.2	99.6	99.4	99.1	99.1	99.0	99.4	99.7	100												
WGK 2298	99.0	99.4	99.8	99.0	99.3	99.3	99.4	99.4	99.0	99.1	99.3	99.0	100											
WGK 2336	99.0	99.3	99.8	99.0	99.2	99.2	99.3	99.3	99.0	99.0	99.2	99.0	99.9	100										
WGK 2346	99.3	99.7	99.3	99.4	99.6	99.4	99.3	99.3	99.0	99.4	99.5	99.3	99.2	99.1	100									
WGK 2533	99.2	99.7	99.3	99.1	99.5	99.6	99.5	99.3	99.6	99.3	99.7	99.4	99.2	99.1	99.3	100								
WGK 2536	98.4	98.8	99.0	98.5	99.6	98.7	98.8	98.9	98.4	99.0	98.7	98.4	99.0	98.9	98.9	98.3	100							
WGK 2549	98.4	98.8	99.3	98.3	98.7	98.7	98.8	98.8	98.4	98.5	98.7	98.4	99.2	99.1	98.8	98.6	98.3	100						
WGK 2767	99.6	99.7	99.4	99.5	99.8	99.6	99.6	99.4	99.3	99.6	100	99.7	99.3	99.2	99.6	99.6	98.7	98.7	100					
WGK 2771	99.2	99.4	99.9	99.0	99.3	99.3	99.4	99.4	99.0	99.1	99.3	99.0	99.8	99.7	99.3	99.2	98.9	98.1	99.3	100				
WGK 2775	99.1	99.4	100	99.0	99.4	99.4	99.5	99.5	99.1	99.2	99.4	99.1	99.9	99.8	99.3	99.3	99.0	99.3	99.4	99.9	100			
WGK 2884	99.3	99.6	99.0	99.2	99.6	99.6	99.6	99.6	99.3	99.4	99.6	99.1	99.6	99.5	99.5	99.5	99.1	98.8	99.6	99.6	99.6	100		
<i>E.</i> <i>trichosuri</i>	99.2	99.2	99.5	99.1	99.5	99.8	99.5	99.5	99.6	99.3	99.6	99.4	99.4	99.3	99.4	99.8	98.8	98.8	99.6	99.6	99.4	99.6	100	

379 **Research Highlights**

380

- 381 • First study to genetically characterize *Eimeria* sequences from a range of
382 macropod hosts
- 383
- 384 • A total of 25 new *Eimeria* sequences from macropods representing potentially
385 6 or more species of *Eimeria* were generated
- 386
- 387 • A molecular phylogeny of marsupial-derived *Eimeria* constructed
- 388
- 389 • Supports the hypothesis that *Eimeria* found in marsupials diverged prior to
390 *Eimeria* from placental mammals

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*Graphical Abstract

