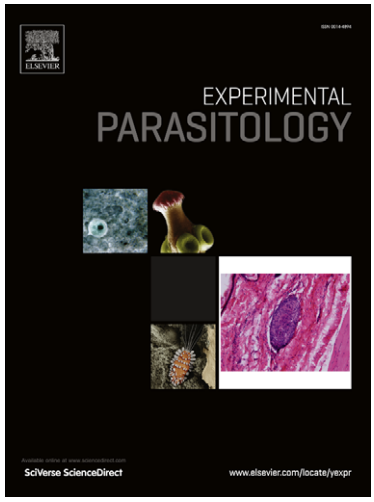

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Molecular characterisation of *Eimeria* species in Macropods.

Rongchang Yang[a] , Stan Fenwick[a], Abbey Potter[a], Aileen Elliot[a], Michelle Power[b], Ian Beveridge[c] and Una Ryan[a,*]

[a]*Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, 6150.*

[b]*Department of Biological Sciences, Macquarie University, North Ryde, Sydney NSW 2109, Australia.*

Corresponding author. Mailing address: Division of Health Sciences, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, Australia 6150. Phone: 61 89360 2482. Fax: 61 89310 414. E-mail: Una.Ryan@murdoch.edu.au

ABSTRACT

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

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

1. Introduction

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

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

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

2. Materials and methods

2.1 Sample collection

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

2.2 DNA isolation

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

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

2.3 PCR amplification and sequencing

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

PCR reactions were performed in a 25 µl volume that contained approximately 15 ng DNA, 1 x PCR buffer (FisherBiotech, Perth, WA), 0.2 mM deoxynucleoside triphosphates, 2.5 mM $MgCl_2$, 5% (wt/vol) dimethyl sulfoxide, 0.2 µMol each primer and 1 unit of Tth+ DNA polymerase (FisherBiotech, Perth, WA).

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

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

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

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

2.4 Phylogenetic analysis

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

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

3.2 Morphological analysis of oocysts

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

3.2 Phylogenetic analysis of Eimeria species and genotypes in marsupials

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

4. Discussion

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

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

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

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

The morphological similarity of oocysts, the broad host specificity of some *Eimeria* species and the diversity of *Eimeria* within one host compound species delimitation (Tenter et al., 2002). Molecular data is therefore essential to accurately 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

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362

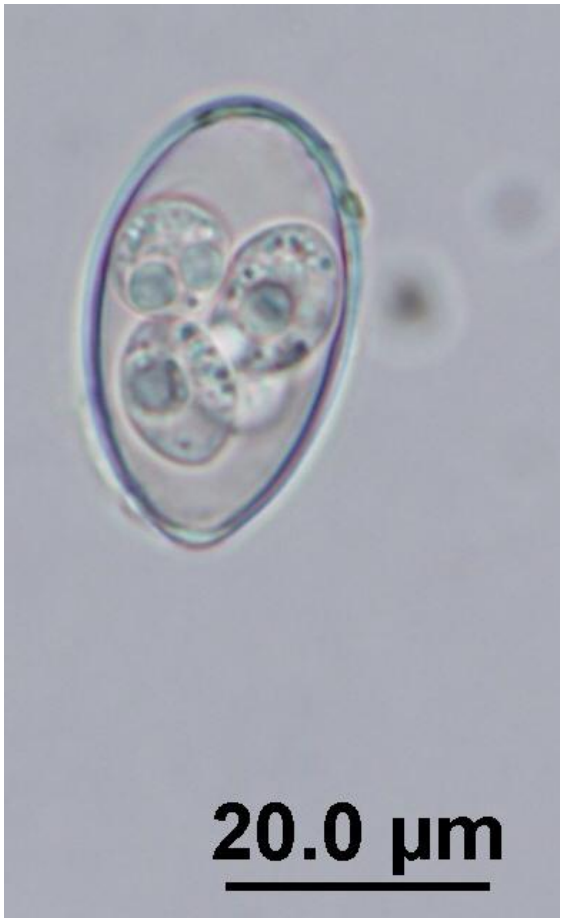
363 **Fig. 1.** Sporulated oocyst from a red kangaroo resembling *E. wilcanniensis*.

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.

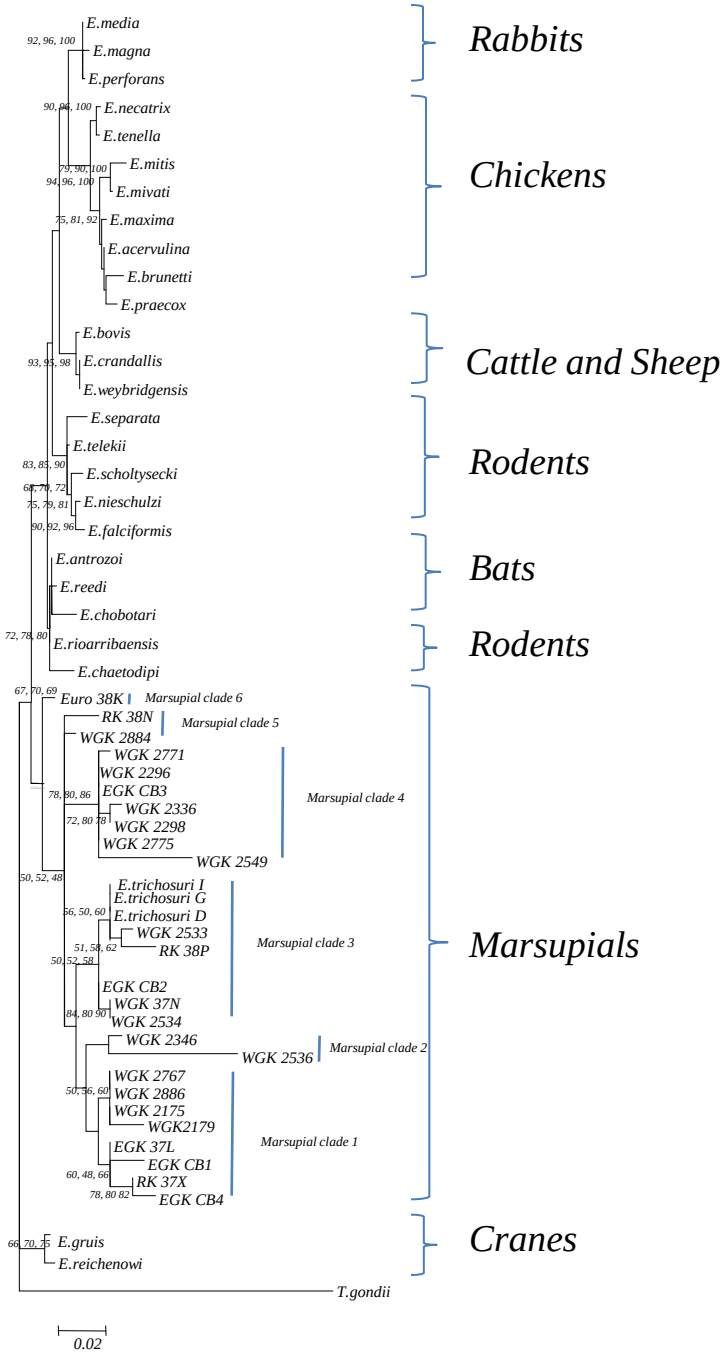
368

369

Figure



Figure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E.weybridgensis
Cattle and Sheep
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E.telekii
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90, 92, 96
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Rodents
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Bats
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E.chaetodipi
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Euro 38K
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RK 38N
Marsupial clade 5
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EGK CB3
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WGK 2336
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WGK 2775
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E.trichosuri G
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E.trichosuri D
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51, 58, 62
RK 38P
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EGK CB2
WGK 37N
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WGK 2346
WGK 2536
Marsupial clade 2
WGK 2767
50, 56, 60
WGK 2886
WGK 2175
WGK2179
EGK 37L
Marsupial clade 1
EGK CB1
60, 48, 66
RK 37X
78, 80 82
EGK CB4
Marsupials
66, 70, 75
E.gruis
E.reichenowi
Cranes
T.gondii
0.02

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
WGK's from WA					
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)
Euro's from WA					
Karratha and Roeburne	12	4	8	1	8.3 (0-24)
Red kangaroos from WA					
Roeburne and Fortescue	10	4	6	3	30 (1.6-48.4)
EGK's from Vic					
Brimpaen and Cape Bridgewater	10	8	2	5	50 (19-81)
WGK's from Vic					
Brimpaen	1	0	1	1	100 (100-100)
Total	**597**	**256**	**341**	**145**	**24.3 (20.8-27.7)**

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

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Tables

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	*E. trichosuri*
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	
E. trichosuri	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

Research Highlights

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

***Graphical Abstract**