Murdoch
UNIVERSITY

MURDOCH RESEARCH REPOSITORY

This is the author's final version of the work, as accepted for publication following peer review but without the publisher's layout or pagination. The definitive version is available at
http://dx.doi.org/10.1016/j.exppara.2016.09.012

Hillman, A.E., Yang, R., Lymbery, A.J. and Thompson, R.C.A. (2016) Eimeria spp. infecting quenda (Isoodon obesulus) in the greater Perth region, Western Australia. Experimental Parasitology, 170 . pp. 148-155.

http://researchrepository.murdoch.edu.au/34149/

Copyright: © 2016 Elsevier Inc.

Accepted Manuscript

Eimeria spp. infecting quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia

Alison E. Hillman, Rongchang Yang, Alan J. Lymbery, R.C. Andrew Thompson

PII: S0014-4894(16)30205-3

DOI: 10.1016/j.exppara.2016.09.012

Reference: YEXPR 7303

To appear in: *Experimental Parasitology*

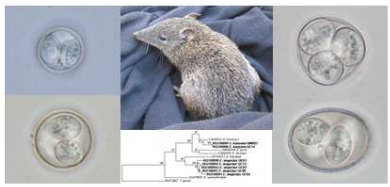
Received Date: 20 May 2016

Revised Date: 22 September 2016

Accepted Date: 27 September 2016

Please cite this article as: Hillman, A.E., Yang, R., Lymbery, A.J., Thompson, R.C.A., *Eimeria* spp. infecting quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia, *Experimental Parasitology* (2016), doi: 10.1016/j.exppara.2016.09.012.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Eimeria spp. infecting quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia

Alison E Hillman[1]; Rongchang Yang[2]; Alan J Lymbery[1,3]; R. C. Andrew Thompson[1]

[1] Parasitology Group, Murdoch University

[2] Wheat Research Group, Murdoch University

[3] Freshwater Fish Group and Fish Health Unit, Murdoch University

[1,2,3] c/o School of Veterinary and Life Sciences, Murdoch University

90 South St, Murdoch

Perth, WA

Australia 6150

Alison Hillman (corresponding author): a.hillman@murdoch.edu.au; (ph) 0061 8 9360 2690

Rongchang Yang: R.Yang@murdoch.edu.au

Alan Lymbery: A.Lymbery@murdoch.edu.au

Andrew Thompson: A.Thompson@murdoch.edu.au

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ABSTRACT

Parasites of wildlife inhabiting urbanised and peri-urban environments are of interest regarding wildlife population health, and also veterinary public health in the case of parasites that can also infect humans and domestic animals. This study aimed to: identify, and estimate the prevalence of, species of *Eimeria* parasitic in quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia; 2) morphologically describe and genetically characterise a novel observed species of *Eimeria* as *E. angustus;* and 3) genetically characterise *E. kanyana*. *Eimeria* spp. prevalence was 76.1% (95% CI 64.9 – 84.5%), and four putative species of *Eimeria* were identified. *Eimeria kanyana* was identified infecting quenda for the first time, with a prevalence of 54.9% (43.4 - 66.0%). *Eimeria quenda* was less prevalent, at 7.0% (3.1 – 15.5%). The novel species *E. angustus* was present in 45.1% of sampled quenda (34.0 – 56.6%). A second novel morphotype of *Eimeria* was present in 2.8% of sampled quenda (0.9 - 9.7%). Mixed *Eimeria* spp. infections were present in 21/71 quenda (29.6%, 95% CI 20.2 – 41.1%). Molecular phylogenetic analyses of *E. kanyana* and *E. angustus* were conducted at the 18S rRNA and mitochondrial cytochrome oxidase loci. At both loci, two isolates identified as *E. kanyana* grouped in a phylogenetic clade with *E. trichosuri*. Five isolates identified as the novel *E. angustus* were most closely related to *E. tropidura* at the 18S locus. At the COI locus, no sequence data were available for *E. tropidura*; isolates of *E. angustus* grouped with *E. sciurorum*.

KEY WORDS

Bandicoot, coccidiosis, marsupial, urban, wildlife disease.

ACCEPTED MANUSCRIPT

1. INTRODUCTION

Parasites of wildlife inhabiting urbanised or peri-urbanised environments are of importance regarding their potential impact on population health (Thompson et al., 2009). The role of parasites as a density-dependent regulator of host population size may be of particular significance in this case, as urbanisation can be associated with abnormally increased population densities of wildlife species that adapt to living in urban areas (Bradley and Altizer, 2007). Additionally, zoonotic parasites present in wildlife in urban or peri-urban areas are of public health significance (Mackenstedt et al., 2015).

Though urbanisation is associated with habitat loss for wildlife in Australia and worldwide (McKinney, 2002; Garden et al., 2006), some wildlife species are able to adapt and survive in urbanised environments. Quenda (syn. southern brown bandicoots, *Isoodon obesulus*) are a small, terrestrial peramelid marsupial, and have survived in many urbanised areas of Perth, Western Australia (Howard et al., 2014). Published documentation of parasites infecting quenda in this region are restricted to small samples of quenda tested for *Giardia* spp. (Thompson et al., 2010) and *Eimeria* spp. (Bennett and Hobbs, 2011).

Eimeria is a genus of apicomplexan parasites, species of which have been recorded in a wide range of vertebrates (including mammals, birds, reptiles and fish). Two species of *Eimeria* have been documented parasitising peramelid marsupial hosts: *E. kanyana*, parasitic in western barred bandicoots (*Perameles bougainville*) (Bennett et al., 2006), and *E. quenda*, parasitic in quenda (Bennett and Hobbs, 2011). *Eimeria* spp. have also been described, morphologically and in some cases genetically, from a range of other Australian marsupial species (Mykytowycz, 1964; Barker et al., 1988a, 1988b,1988c, 1989; O'Callaghan and O'Donoghue, 2001; Power et al., 2009; Hill, et al., 2012; Austen et al., 2014). Although

70 *Eimeria* species are typically host-specific (Joyner, 1982), there are a number of examples of infection of
71 multiple host species within the same marsupial genus (*Macropus* spp. - Barker et al., 1988a, 1989;
72 *Trichosurus* spp.- O'Callaghan and O'Donoghue, 2001; Power et al., 2009).
73
74 *Eimeria* spp. are an important cause of gastrointestinal illness in livestock (Chapman et al., 2013).
75 However, though morbidity and mortality attributable to coccidiosis can occur in various Australian
76 marsupial species (e.g. Winter, 1959; Barker et al., 1972), no studies have specifically investigated how
77 common morbidity or mortality are following *Eimeria* sp. infection in these hosts. Previous research has
78 suggested that the pathogenicity of various *Eimeria* sp. in certain marsupial hosts may be mild in
79 otherwise healthy individuals (Mykytowycz, 1964; Bennett et al., 2006).
80
81 We aimed to: 1) identify, and estimate the prevalence of, species of *Eimeria* parasitic in quenda in the
82 greater Perth region, Western Australia; 2) morphologically describe and genetically characterise a novel
83 observed species of *Eimeria* as *Eimeria angustus;* and 3) genetically characterise the previously
84 described species *Eimeria kanyana*, for the purposes of distinguishing it genetically from *E. angustus*.
85
86 **2. MATERIALS AND METHODS**
87 The target host population was free-ranging quenda in the Statistical Division of Perth. Quenda were
88 trapped using Sheffield (cage) traps. Trapping was undertaken on 29 bushland sites and 35 urbanised
89 sites (7 private non-residential properties and 28 private residential properties), from March 2013 to
90 July 2015. A subset of trapped quenda were included in this study. Inclusion was based on the
91 availability of fresh faeces from the animal. Trapping and sampling was undertaken under a Murdoch

University Animal Ethics Permit (R2530/12), and Department of Parks and Wildlife Regulation 17 (SF009640) and Regulation 4 (CE004287) permits.

2.1 Identifying species of *Eimeria* present in Perth quenda

Faeces were collected directly from the traps after removal of the quenda, and traps were cleaned and disinfected between animals. Faecal samples may have been passed from the animal up to approximately 10 hours before collection from the trap. Faecal samples were stored in an insulated field box until processed, which was no later than 6 hours after collection.

One millilitre of faeces was preserved for genetic characterisation of coccidia by mixing thoroughly into 4 mL 70% ethanol. Preserved samples were stored at 4˚C until analysis. The rest of the fresh faeces were mixed 1:4 into 2% potassium dichromate solution ($K_2Cr_2O_7$). This faecal mixture was poured into small petri dishes, and left in a dark cupboard at room temperature to facilitate sporulation of coccidian oocysts. The faecal mixture was checked intermittently for sporulated coccidian oocysts, by concentrating oocysts present in a small portion of the sample by zinc sulphate flotation, and examining the sample microscopically. Briefly, a portion of faecal mixture was centrifuged at 850 G for 2 minutes, with supernatant discarded, and then resuspended in distilled water and re-centrifuged twice, to remove potassium dichromate. Samples were then mixed thoroughly with zinc sulphate solution (SG 1.18) at a ratio of 1:4, and centrifuged at 850 G for 2 minutes. A flamed wire loop was used to transfer surface material (containing coccidian oocysts) on to a slide. Sporulated oocysts were examined at 400x to 1000x magnification, using an Olympus BX50 microscope. Photographs of sporulated oocysts were taken using bright field and Nomarski differential interference microscopy.

115 **2.2 Prevalence estimates**

116 Only oocysts that sporulated and could be unequivocally assigned to species based on morphology were
117 included in estimates of prevalence. Ninety-five per cent confidence intervals were calculated using
118 Jeffrey's method (Brown et al., 2001).

119

120 **2.3 Morphological description of *Eimeria angustus***

121 One hundred and fifteen sporulated *Eimeria* sp. oocysts of a consistent, novel morphology (obtained
122 from 21 quenda hosts) were examined and photographed at 1000x magnification, using bright field and
123 Nomarski differential interference microscopy (Olympus BX50 microscope). Images were analysed using
124 ImageJ software (US National Institute of Health, Bethesda, Maryland), to obtain measurements of
125 oocyst length and width, oocyst wall thickness and sporocyst length and width. Due to the compacted
126 nature of this species of *Eimeria,* measurements were only taken from one sporocyst per oocyst- the
127 sporocyst that was subjectively identified as being positioned laterally. Where no sporocysts could be
128 manipulated into lateral position within the oocyst, sporocyst length measurement was not taken.

129

130 **2.4 Genetic characterisation of *Eimeria kanyana* and *Eimeria angustus***

131 Ethanol-preserved faecal samples from two quenda were used to characterise *E. kanyana*, and ethanol-
132 preserved faecal samples from five quenda were used to characterise *E. angustus*, at the nuclear 18S
133 rRNA and mitochondrial cytochrome oxidase (COI) loci. *Eimeria quenda* was not characterised as part of
134 this study, due to logistical limitations.

135

136 DNA was extracted using the Power Soil DNA Kit (MolBio, Carlsbad, California), as described in Yang et
137 al. (2016). A nested PCR protocol was employed to amplify a 1285 bp product of the 18s rRNA locus,

using methods described in Yang et al. (2016). A nested PCR protocol was also used to amplify sequences in subunit I of the COI gene, using methods described in Dolnik et al. (2009). Amplified products were purified, and sequenced using forward and reverse primers, as described in Yang et al. (2016).

Phylogenetic analyses of sequences were undertaken using MEGA6 (Tamura et al., 2013). Maximum likelihood (ML), neighbour-joining (NJ) and maximum parsimony (MP) analyses were all conducted, using additional isolates retrieved from GenBank®. These additional isolates comprised published sequences representing all possible host families (Supplementary Tables 1 and 2). The robustness of nodes within the resulting trees was inferred from 1000 cycles of bootstrap resampling.

3. RESULTS

3.1 *Eimeria* spp. identified in Perth quenda

Seventy-one quenda were included in this study. These comprised 60 quenda from 12 bushland sites, nine quenda from two private residential properties, and two quenda from one private non-residential property.

Coccidian oocysts (Family Eimeriidae) were identified in 65 / 71 faecal samples. Sporulated coccidian oocysts were identified in 54 of these 65 samples: all sporulated oocysts were *Eimeria* spp., giving a prevalence of 76.1% (95% CI 64.9 – 84.5%). Sporulated oocysts in five faecal samples represented *Eimeria quenda* infection (7.0%, 95% CI 3.1 – 15.5%) (Figure 1), and in 39 faecal samples represented *Eimeria kanyana* infection (54.9%, 95% CI 43.4- 66.0%) (Figure 2). Sporulated oocysts representing one

161 novel species of *Eimeria*, formally described in this paper as *Eimeria angustus*, were identified in 32
162 samples (45.1%, 95% CI 34.0 - 56.6%) (Figures 3,4 and 5). Sporulated oocysts representing a second
163 novel morphotype of *Eimeria* were sporulated in two samples (2.8%, 95% CI 0.9 - 9.7%) (Figure 6). Mixed
164 infections were observed in 21 / 71 faecal samples (29.6%, 95% CI 20.2 – 41.1%) - 20 samples had two
165 species of *Eimeria* present and one sample had three species of *Eimeria* present.
166
167 **3.2 Morphological description of *Eimeria angustus***
168 Sporulated oocysts are small and spheroidal to subspheroidal. They have a smooth, bilaminate oocyst
169 wall, without a micropyle. Oocysts contain 4 oval sporocysts, which are distinctly compacted within the
170 oocyst. Oocysts do not contain residuum or polar granules. Within each sporocyst, two elongate
171 sporozoites are wrapped around a distinct mass of residuum. Sporocyst surfaces do not have sporopodia
172 or adhering membranes; sporocysts do not have a Stieda body, parastieda body or a substieda body.
173 Each sporozoite contains two refractile bodies; the larger of the two tends to be readily visible, while the
174 smaller refractile body can be difficult to visualise under light microscopy due to the compactness of the
175 cyst obscuring features. Morphological measurements are described in Table 1.
176
177 **3.3 Genetic characterisation of *Eimeria angustus* and *Eimeria kanyana* at the 18S rRNA locus**
178 A 1,216 bp PCR product of *E. kanyana* was successfully amplified and sequenced from the faeces of two
179 quenda - quenda QC14 (isolate KU248088) and QMR13 (KU248089). A 1222 bp PCR product of *E.*
180 *angustus* was successfully amplified and sequenced from five quenda - quenda QE03 (isolate
181 KU248090), QC04 (KU248091), QC07 (KU248092), QC09 (KU248093) and QC13 (KU248094).
182 Phylogenetic analyses at the 18S rRNA locus using distance, parsimony and maximum likelihood
183 methods produced similar results (Figure 7, maximum likelihood tree shown). *Eimeria kanyana* isolates

KU248088 and KU248089 shared 98.9% and 99.3% similarity with *E. trichosuri* (FJ829322), respectively. They were grouped in a clade with *E. trichosuri* (FJ829322), which was identified from mountain brushtail possums (*Trichosurus cunninghami*) in Australia (Power et al., 2009). The five isolates of *E. angustus* (KU248090, KU248091, KU248092, KU248093 and KU248094) grouped in a clade with *E. tropidura* (AF324217), which was identified from Hood Island lizards (*Tropidurus delanonis*) in the Galápagos Archipelago (Aquino-Shuster et al., 1990) and they shared genetic similarities with *E. tropidura* at 96.5%, 96.4%, 96.6%, 96.7% and 96.6%, respectively (Figure 7).

3.4 Genetic characterisation of *Eimeria angustus* and *Eimeria kanyana* at the COI locus

A 228 bp PCR product of *Eimeria kanyana* was successfully amplified and sequenced from the faeces of two quenda - quenda QMR03 (isolate KU845563) and QC14 (KU845564), while the same size product of *E. angustus* was successfully amplified and sequenced from the faeces of five quenda - quenda QE03 (isolate KU845565), QC04 (KU845566), QC07 (KU845567), QC09 (KU845568) and QC13 (KU845569). Similar to the results from the phylogenetic analysis at the 18S rRNA locus, the two isolates of *E. kanyana* (KU845563 and KU845564) were most closely related to *E. trichosuri* (JN192136) with the similarities at 93.9% and 93.4%, respectively (Figure 8). There was no COI sequence from *E. tropidura* in GenBank, and the five *E. angustus* isolates (KU845565, KU845566, KU845567, KU845568 and KU845569) were most closely related to *E. sciurorum* (KT361027), which was originally identified from squirrels (Family Sciuridae) from the Czech Republic, with similarities of 95.2%, 95.2%, 95.6%, 95.2% and 96.1%, respectively (Figure 8). At the amino acid level, the two COI sequences from *E. kanyana* were identical, as were the five sequences from *E. angustus*, and they differed from each other by only one amino acid (data not shown).

ACCEPTED MANUSCRIPT

4. DISCUSSION

This study suggests that *Eimeria* spp. infections are highly prevalent in Perth quenda. Indeed, prevalences may have been underestimated, as not all coccidian oocysts consistent with *Eimeria* spp. sporulated in all samples. Although we cannot exclude bias due to the use of non proportionate sampling methodology, which was utilised out of practical necessity, these findings concur with the prevalence of unsporulated coccidian oocysts (consistent with *Eimeria* spp.) observed in a survey of quenda carcasses obtained opportunistically from the same region, suggesting that the use of traps is unlikely to have been a source of selection bias (Hillman et al., unpublished results). Consistent with these findings, moderate to high prevalences of *Eimeria* spp. have been recorded in other free-ranging Australian marsupial populations (Mykytowycz, 1964; Barker et al., 1988a, 1988c, 1989; O'Callaghan et al., 1998; Bennett et al., 2006; Yang et al., 2012, Austen et al., 2014).

Four putative species of *Eimeria* were identified in quenda in this study, with only two of these species having been previously described. To our knowledge, this is the first time that *E. kanyana* has been documented in quenda, expanding the known host range of this parasite to two genera of peramelids. This is an unusual finding, given the typical host specificity of *Eimeria* spp. (Joyner, 1982). *Eimeria* spp. have been previously documented parasitising multiple host species within the same marsupial genus (Barker et al., 1988a, 1989; O'Callaghan and O'Donoghue, 2001; Power et al., 2009), but not multiple marsupial hosts spanning different genera. The documentation of two undescribed species of *Eimeria* in quenda is not unexpected, based on findings of Bennett and Hobbs (2011), who observed a novel *Eimeria* sp. when describing *E. quenda*, and reported unpublished archives of two morphologically distinct *Eimeria* sp. oocyst types isolated from quenda hosts. We have described the most prevalent undescribed species in this study as *E. angustus*.

Our data suggest that concurrent infection with multiple species of *Eimeria* is relatively common in quenda. Furthermore, we may have underestimated the prevalence of mixed infections, as in some samples there were unsporulated oocysts of mixed morphology, but oocysts of one or both morphotypes failed to sporulate. The relatively common occurrence of mixed infections concurs with findings from studies of *Eimeria* spp. infections in the Australian marsupial hosts Pearson Island rock wallabies (*Petrogale lateralis pearsoni*) (O'Callaghan et al., 1998) and quokka (*Setonix brachyurus*) (Austen et al., 2014). Additionally, though mixed infections were not specifically discussed, the findings of Mykytowycz (1964) and Barker et al. (1988a) indicated the presence of mixed *Eimeria* spp. infections in various species of macropods. In contrast, a study of Bolivian marsupials rarely identified mixed *Eimeria* spp. infections (Heckscher et al., 1999). The implications of mixed *Eimeria* spp. infections on infection pathogenicity in quenda is uncertain. Studies in other host species have variably identified synergistic, antagonistic or no effects on pathogenicity from mixed *Eimeria* spp. infections (e.g. Hein, 1976; Cornelissen et al., 2009; Moreno et al., 2013). It has been suggested that this may depend on whether or not the sites of infection for the various *Eimeria* species in the small intestine overlap (Catchpole and Norton, 1979), which is not known regarding *Eimeria* spp. in quenda.

This study did not identify any parasites of the Family Eimeriidae that are of known anthropozoonotic significance in Perth quenda.

4.1 *Eimeria angustus* description- taxonomic summary:

Host type: *Isoodon obesulus* - quenda (syn. southern brown bandicoot). *Eimeria angustus* was obtained from both subadult and adult quenda.

Type locality: *Eimeria angustus* was sporulated from quenda faecal samples obtained from eleven sites throughout the greater Perth region, Western Australia.

Sporulation time: unknown

Site of Infection: unknown

Prepatent and patent periods: unknown

Material deposited: formalin preserved and ethanol preserved sporulated oocysts, and oocyst photosyntypes, have been deposited at the Western Australian Museum (specimen registration no. WAM Z68786)

Etymology: The specific epithet *angustus* is from the Latin, meaning "contracted, small, not spacious" – a distinctive feature of this species of *Eimeria.*

4.2 *Eimeria angustus* morphology, as compared to other known *Eimeria* spp. of bandicoots

Eimeria angustus is substantially smaller than *E. quenda,* and has a distinct spherical mass of residuum within the sporocysts, unlike *E. quenda*. *Eimeria angustus* tends to be smaller than *E. kanyana*, though the size ranges of these species do overlap. Unlike *E. kanyana, E. angustus* lacks Stieda bodies and polar granules, and has a distinct spherical mass of residuum within the sporocysts. There is another novel *Eimeria* sp. in quenda, observed previously (A. Elliot, Murdoch University - pers. comm.) and as part of the current study. This *Eimeria* sp. is substantially larger and distinctly ovoid, compared to *E. angustus*. Stieda bodies are present in this novel species, which are not found in *E. angustus*.

4.3 Genetic characterisation of *Eimeria kanyana* and *Eimeria angustus*

Earlier phylogenetic analyses of different marsupial *Eimeria* spp. at the 18S rRNA locus indicated that they form a monophyletic group, separate from species found in eutherians and birds (Power et al.,

2009; Yang et al., 2012). These studies also suggested that more detailed phylogenetic inferences, within the clade found in marsupial hosts, would be assisted by analyses at multiple loci, hence the use of both the 18S rRNA and COI loci in this study.

Across both loci tested, *E. kanyana* was most closely related to *E. trichosuri,* parasitic in mountain brushtail possums (*Trichosurus cunninghami),* another marsupial species endemic to Australia (Power et al., 2009). The phylogenetic placement of *E. angustus* was not so clear. As there are limited COI sequences in GenBank®, and no other *Eimeria* sp. parasitic in hosts of the Order Peramelemorphia had been genetically characterised prior to this study, it is not possible at this stage to confirm the phylogenetic placement of *E. angustus*. However, it seems that *E. angustus* is not closely related to *E. kanyana,* also parasitic in peramelid hosts. This is unexpected, given previous studies have suggested monophyletic origin for *Eimeria* spp. parasitising marsupials (Power et al., 2009; Yang et al., 2012). The putative relationship with *E. tropidura* seems especially unusual, as this species was isolated from a reptile host. As lizards and other reptiles are not known to be a typical component of *I. obesulus* diets (Heinsohn, 1966; Quin, 1998), and *E. angustus* was highly prevalent, it seems unlikely that *E. angustus* was being shed in quenda faeces after consumption of a reptilian host. Additionally, the large number of oocysts observed in many samples, and the fact that all were passed unsporulated, suggests that quenda were amplifying the infection rather than acting as a vector.

5. CONCLUSIONS

Quenda in the greater Perth region are commonly parasitised by one or more species of *Eimeria*, of which *E. kanyana* and *E. angustus* are the most prevalent. *Eimeria kanyana* groups in a phylogenetic clade with *E. trichosuri* at the 18S rRNA and COI loci. *Eimeria angustus* does not seem to be closely

299 related to *E. kanyana,* however its phylogenetic placement is uncertain in light of limited available
300 comparative data.
301
302 **ACKNOWLEDGEMENTS**
303 The authors gratefully acknowledge: the expert advice and technical support provided by Russ Hobbs
304 and Aileen Elliot; the Department of Parks and Wildlife; WWF-Australia; John Forrest National Park,
305 Botanic Parks and Gardens Authority, Whiteman Park, Perth Airport, and the Cities of Joondalup,
306 Cockburn and Armadale; collaborating property managers, staff and owners; volunteer field assistants;
307 and Kanyana Wildlife Rehabilitation Centre. This project was supported by The Holsworth Wildlife
308 Research Endowment- Equity Trustees Charitable Foundation.
309
310 **REFERENCES**
311 Aquino-Shuster, A.L., Duszynski, D.W., Snell, H. L., 1990. Three new coccidia (Apicomplexa) from the
312 Hood Island lizard, *Tropidurus delanonis*, from the Galapagos archipelago. J. Parasitol. 76, 313-318.
313 Austen, J., Friend, J., Yang, R., Ryan, U., 2014. Further characterisation of two *Eimeria* species (*Eimeria*
314 *quokka* and *Eimeria setonicis*) in quokkas (*Setonix brachyurus*). Exp. Parasitol. 138, 48-54.
315 Barker, I. K., Harrigan, K. E., Dempster, J. K., 1972. Coccidiosis in wild grey kangaroos. Int. J. Parasitol. 2,
316 187-192.
317 Barker, I. K., O'Callaghan, M. G., Beveridge, I., Close, R. L., 1988a. Host-parasite associations of *Eimeria*
318 spp. (Apicomplexa: Eimeriidae) in rock wallabies, *Petrogale* spp. (Marsupialia: Macropodidae). Int. J.
319 Parasitol. 18, 353-363.

ACCEPTED MANUSCRIPT

320 Barker, I. K., O'Callaghan, M. G., Beveridge, I., 1988b. *Eimeria* spp. (Apicomplexa: Eimeriidae) parasitic in
321 the rat-kangaroos *Hypsiprymnodon moschatus, Potorous tridactylus, Aepypymnus rufescens* and
322 *Bettongia gaimardi* (Marsupialia: Potoroidae). Int. J. Parasitol. 18, 947-953.
323 Barker, I. K., O'Callaghan, M. G., Beveridge, I., 1988c. *Eimeria* spp. (Apicomplexa:Eimeriidae) parasitic in
324 wallabies and kangaroos of the genera *Setonix*, *Thylogale*, *Wallabia*, *Lagorchestes* and *Dendrolagus*
325 (Marsupialia:Macropodidae). Int. J. Parasitol. 18, 955-962.
326 Barker, I. K., O'Callaghan, M. G., Beveridge, I., 1989. Host-parasite associations of *Eimeria* spp.
327 (Apicomplexa: Eimeriidae) in kangaroos and wallabies of the genus *Macropus* (Marsupialia:
328 Macropodidae). Int. J. Parasitol. 19, 241-263.
329 Bennett, M., Hobbs, R., 2011. A new *Eimeria* species parasitic In *Isoodon obesulus* (Marsupialia:
330 Peramelidae) in Western Australia. J. Parasitol. 97, 1129-1131.
331 Bennett, M., Woolford, L., O'Hara, A., Nicholls, P., Warren, K., Hobbs R., 2006. A new *Eimeria* species
332 parasitic in western barred bandicoots, *Perameles bougainville* (Marsupialia: Peramelidae), in Western
333 Australia. J. Parasitol. 92, 1292-1294.
334 Bradley, C. A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends. Ecol. Evolut.
335 22, 95-102
336 Brown, L. D., Cat, T. T., DasGupta, A., 2001. Interval estimation for a proportion. Stat.Sci. 16, 101-133.
337 Catchpole, J., Norton, C. C., 1979. The species of *Eimeria* in rabbits for meat production in
338 Britain. Parasitology. 79, 249-257.
339 Chapman, H. D., Barta, J. R., Blake, D., Gruber, A., Jenkins, M., Smith, N. C., Suo, X., Tomley, F. M., 2013.
340 A selective review of advances in coccidiosis research. Adv. Parasitol 83, 93-171.

ACCEPTED MANUSCRIPT

341 Cornelissen, J. B., Swinkels, W. J. C., Boersma, W. A., Rebel, J. M. J., 2009. Host response to simultaneous
342 infections with *Eimeria acervulina*, *maxima* and *tenella*: a cumulation of single responses. Vet. Parasitol.
343 162, 58-66.
344 Dolnik, O. V., Palinauskas, V., Bensch, S., 2009. Individual oocysts of *Isospora* (Apicomplexa: Coccidia)
345 parasites from avian faeces: from photo to sequence. J. Parasitol. 95, 169-174.
346 Garden, J., Mcalpine, C., Peterson, A. N. N., Jones, D., Possingham, H., 2006. Review of the ecology of
347 Australian urban fauna: a focus on spatially explicit processes. Austral Ecol. 31, 126-148.
348 Heckscher, S. K., Wickesberg, B. A., Duszynski, D. W., Gardner, S. L., 1999. Three new species of *Eimeria*
349 from Bolivian marsupials. Int. J. Parasitol. 29, 275-284.
350 Hein, H. E., 1976. *Eimeria acervvlina*, *E. brunetti*, and *E. maxima*: pathogenic effects of single or mixed
351 infections with low doses of oocysts in chickens. Exp. Parasitol. 39, 415-421.
352 Heinsohn, G. E., 1966. Ecology and reproduction of the Tasmanian bandicoots (*Perameles gunni* and
353 *Isoodon obesulus*). University of California Publications in Zoology 80, 1-96.
354 Hill, N. J., Richter, C., Power, M. L., 2012. Pinning down a polymorphic parasite: new genetic and
355 morphological descriptions of *Eimeria macropodis* from the Tammar wallaby (*Macropus eugenii*).
356 Parasitol. Int. 61, 461-465.
357 Howard, K., Barrett, G., Ramalho, C. E., Friend, J. A., Boyland, R. J., Hudson, J., Wilson, B., 2014.
358 Community Quenda Survey 2012. WWF- Australia and the Department of Parks and Wildlife. Perth,
359 Australia.
360 Joyner, L. P., 1982. Host and site specificity. In *The Biology of the Coccidia.* (ed. Long, P. L.), pp 35–62.
361 University Park Press, Baltimore, USA.
362 Mackenstedt, U., Jenkins, D., Romig, T., 2015. The role of wildlife in the transmission of parasitic
363 zoonoses in peri-urban and urban areas. Int J Parasitol Parasites Wildl. 4, 71-79.

McKinney, M. L., 2002. Urbanization, biodiversity, and conservation. BioScience 52, 883-890.

Moreno, P. G., Eberhardt, M. A. T., Lamattina, D., Previtali, M. A. and Beldomenico, P. M., 2013. Intra-phylum and inter-phyla associations among gastrointestinal parasites in two wild mammal species. Parasitol. Res. 112, 3295-3304.

Mykytowycz, R., 1964. Coccidia in wild populations of the red kangaroo, *Megaleia rufa* (Desmarest), and the grey kangaroo, *Macropus canguru* (Müller). Parasitology. 54, 105-115.

O'Callaghan, M. G., Barker, I. K., Beveridge, I., Hornsby, P., 1998. *Eimeria* species in the Pearson Island Rock Wallaby, *Petrogale lateralis pearsoni*. *International Journal for Parasitology* 28, 1889-1892.

O'Callaghan, M.J., O'Donoghue, P. J., 2001. A new species of *Eimeria* (Apicomplexa: Eimeriidae) from the brushtail possum, *Trichosurus vulpecula*. T. Roy. Soc. South Aust. 125, 129-132.

Power, M., Richter, C., Emery, S., Hufschmid, J., Gillings, M., 2009. *Eimeria trichosuri*: phylogenetic position of a marsupial coccidium, based on 18S rDNA sequences. Exp. Parasitol. 122, 165-168.

Quin, D. G., 1988. Observations on the diet of the southern brown bandicoot, *Isoodon obesulus* (Marsupialia: Peramelidae), in southern Tasmania. Aust. Mammal. 11, 15-25.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30, 2725-2729.

Thompson, R. C. A, Kutz, S. J., Smith, A., 2009. Parasite zoonoses and wildlife: emerging issues. Int. J. Environ. Res. Public Health. 6, 678-693.

Thompson, R. C. A., Smith, A., Lymbery, A. J., Averis, S., Morris, K. D., Wayne, A. F., 2010. *Giardia* in Western Australian wildlife. Vet. Parasitol. 170, 207-11.

Winter, H., 1959. Coccidiosis in kangaroos. Aust. Vet. J. 35, 301-303.

Yang, R., Fenwick, S., Potter, A., Elliot, A., Power, M., Beveridge, I., Ryan, U., 2012. Molecular characterization of *Eimeria* species in macropods. Exp. Parasitol. 132, 216-221.

Yang, R., Brice, B., Ryan, U., 2016. Morphological and molecular characterization of *Choleoeimeria pogonae* n. sp. coccidian parasite (Apicomplexa:Eimeriidae, 1989, Paperna and Landsberg) in a western bearded dragon (*Pogona minor minor*). Exp. Parasitol. 160, 11-16.

LEGENDS TO FIGURES

Figure 1: Photomicrograph of sporulated oocysts of *Eimeria quenda.*

Figure 2: Photomicrograph of sporulated oocyst of *Eimeria kanyana*.

Figure 3: Photomicrograph of sporulated oocyst of *Eimeria angustus*. Spherical mass of residuum (◅); large refractile body (◄).

Figure 4: Photomicrograph of sporulated oocyst of *Eimeria angustus.* Large refractile body (◄); sporozoites (⟵).

Figure 5: Photomicrograph of sporulated oocyst of *Eimeria angustus*. Bilaminate cyst wall (⇦).

Figure 6: Photomicrograph of sporulated oocyst of novel *Eimeria* sp. morphotype.

Figure 7: Phylogenetic relationships of *E. angustus* and *E. kanyana* isolates obtained in this study, compared to other species of *Eimeria* found in Australia and overseas. Evolutionary history inferred using maximum likelihood analysis of a 1216 bp (*E. kanyana*) or 1222 bp (*E. angustus*) sequence of the

18S rRNA gene. Numbers at nodes indicate % support from 1000 bootstrap replications. *Toxoplasma gondii* is used as the out group.

Figure 8: Phylogenetic relationships of *E. angustus* and *E. kanyana* isolates obtained in this study, compared to other species of *Eimeria* found in Australia and overseas. Evolutionary history inferred using maximum likelihood analysis of a 228 bp sequence of the COI gene. Numbers at nodes indicate % support from 1000 bootstrap replications. *Toxoplasma gondii* is used as the out group.

VITAE

Alison Hillman:

Alison has a clinical veterinary background, an MSc in Veterinary Epidemiology (University of London) and Membership of the Australian and New Zealand College of Veterinary Scientists (Epidemiology Chapter). Alison recently completed a PhD at Murdoch University, investigating the epidemiology of parasitic infections in marsupials in the greater Perth region, Australia.

Rongchang Yang:

Dr Yang was awarded his PhD degree from Murdoch University in 1999. He was employed as a research scientist for Grain Biotech Australia from sep.1999 to Jan. 2007. Dr Yang joined the Molecular Epidemiology Group in Feb. 2007 and has been on a range of projects in molecular parasitology, mainly focus on the pathogens of *Cryptosporidium*, *Giardia* and *Coccidia* in livestock and wild animals. Dr Yang is currently working on a project for improving Australian wheat grain quality in Murdoch University. Dr Yang has over 60 peer reviewed journal articles and 5 patent applications.

Alan Lymbery:

Alan Lymbery is Associate Professor of Parasitology at Murdoch University. Alan obtained a BSc and PhD in Zoology from the University of Western Australia and, after some years as an Australian Research Council Postdoctoral Fellow, worked as a Quantitative Geneticist for the Western Australian Department of Agriculture. He joined Murdoch University in 1999, where he teaches parasitology and animal breeding. Alan's research interests are primarily in disease ecology and wildlife conservation.

Andrew Thompson:

Professor Andrew Thompson Heads the Parasitology Section in the School of Veterinary and Life Sciences, Murdoch University. He is a recent past President of the Australian Society for Parasitology and has over 30 years experience in basic and applied parasitology, with over 350 publications in books and refereed journals. His research covers the biology, taxonomy and life-cycles of parasites of wildlife and zoonoses including *Echinococcus* and other helminths, *Trypanosoma*, *Giardia*, *Cryptosporidium*, and he is a lead investigator of a major research programme of drug discovery against vector-borne neglected tropical diseases.

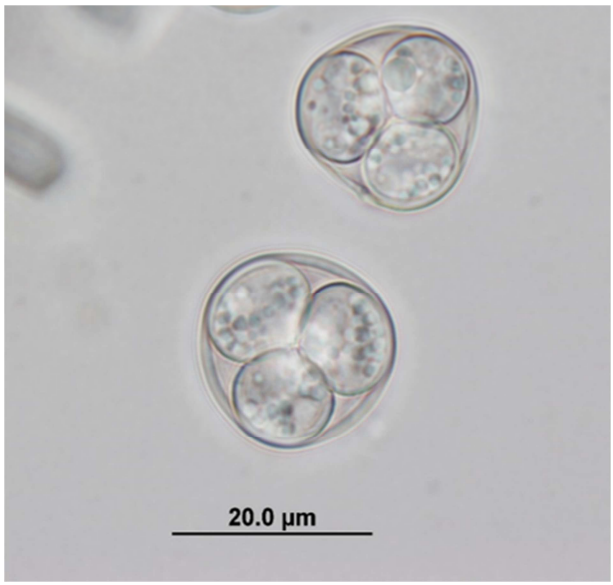
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

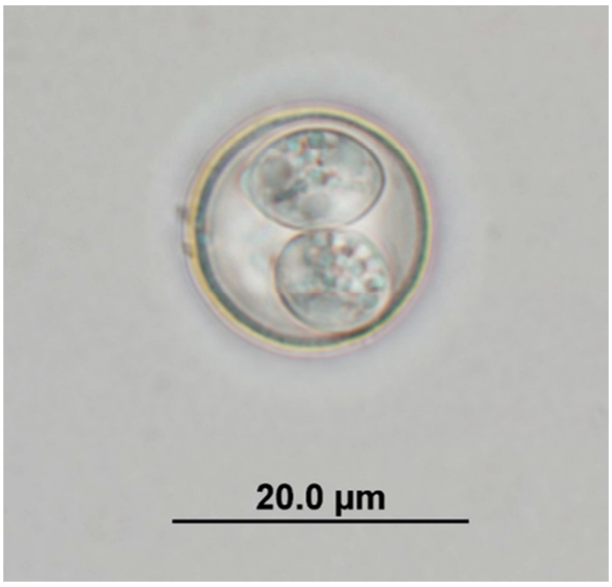
Table 1: *Eimeria angustus* morphological measurements

Feature	Mean (µm)	Standard deviation (µm)	Range (µm)
Oocyst wall width	1.02	0.12	0.7 - 1.33
Oocyst length	16.01	1.43	13.07 - 18.52
Oocyst width	15.62	1.31	12.96 - 18.35
Oocyst length: width ratio	1.03	0.02	0.99 - 1.14
Sporocyst length	8.10	0.68	6.20 - 9.74
Sporocyst width	6.03	0.56	4.66 – 7.55
Sporocyst length: width ratio	1.35	0.07	1.11 – 1.52

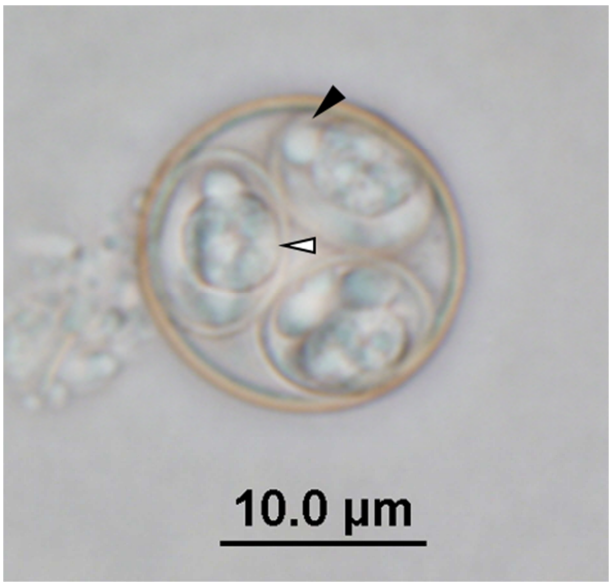
ACCEPTED MANUSCRIPT



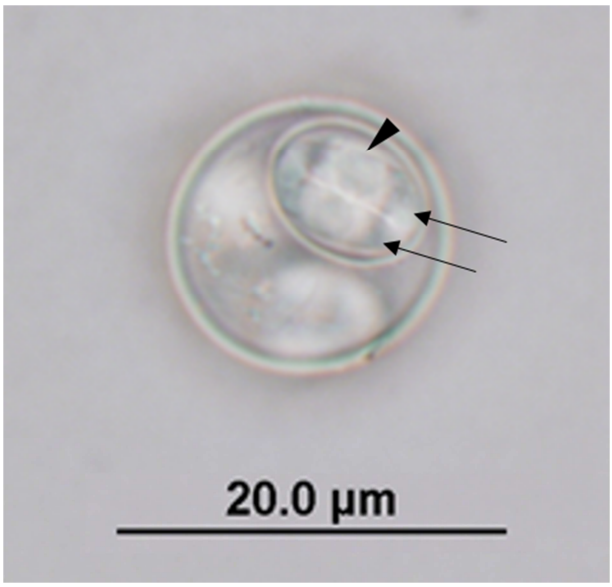
ACCEPTED MANUSCRIPT



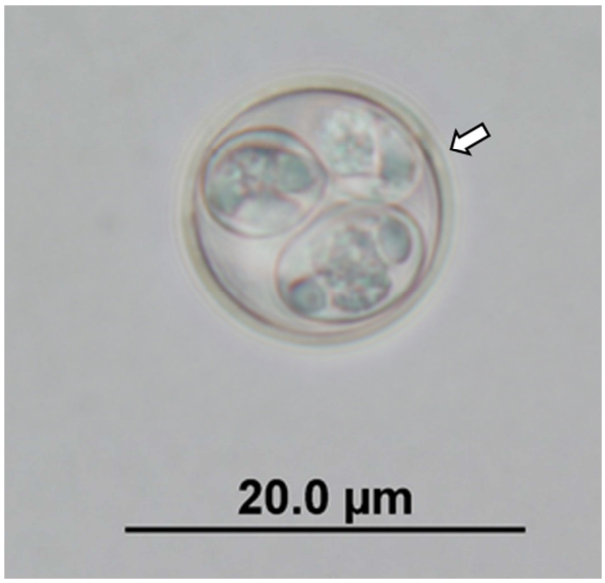
ACCEPTED MANUSCRIPT



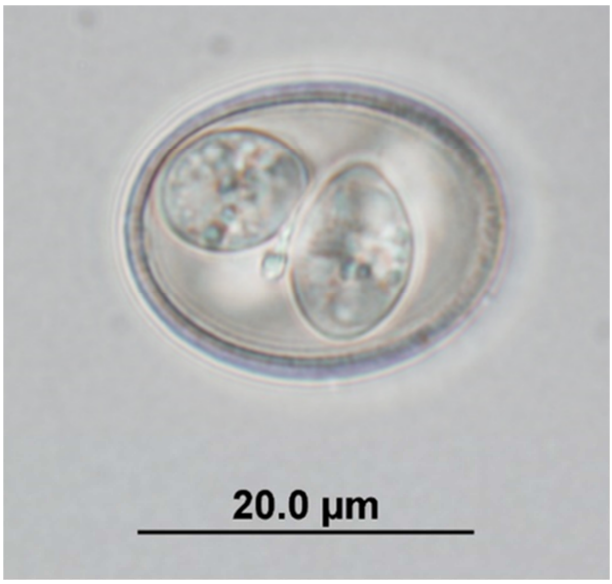
ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

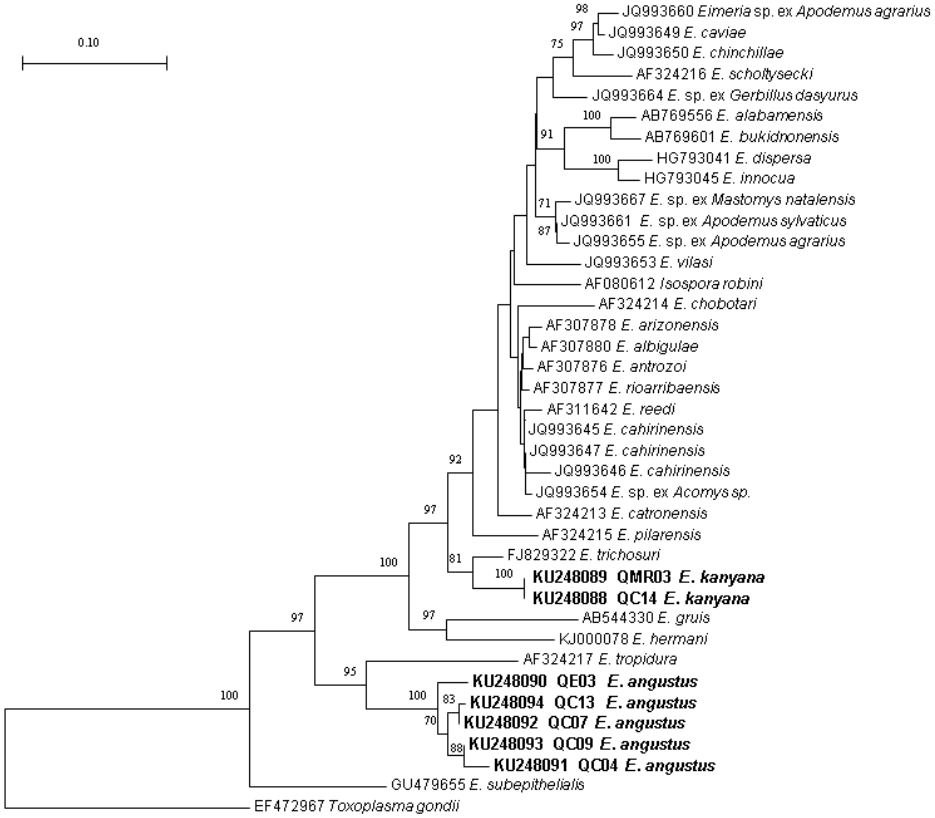


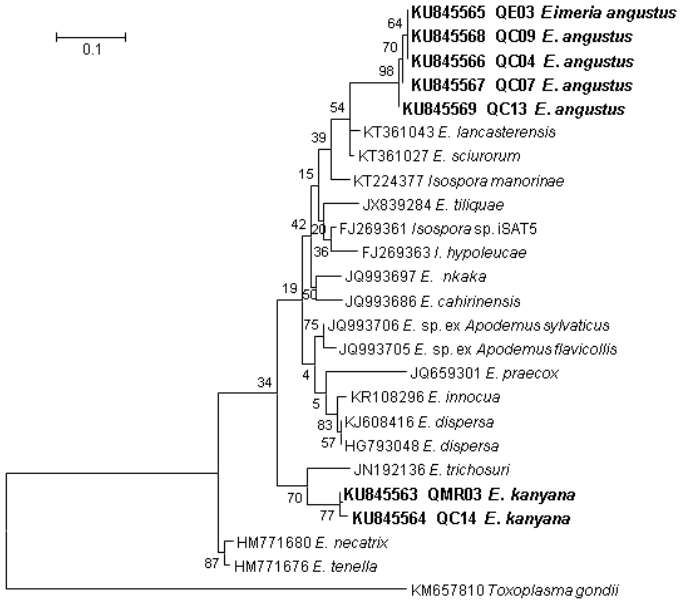
ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT





ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

HIGHLIGHTS

1. *Eimeria* sp. infections are highly prevalent in free-ranging quenda in Perth
2. Concurrent infection with multiple *Eimeria* sp. is common in Perth quenda
3. *Eimeria angustus*, a novel species of *Eimeria* parasitic in the quenda, is described
4. *Eimeria kanyana* is documented infecting quenda for the first time
5. *Eimeria angustus* and *Eimeria kanyana* are genetically characterised

ACCEPTED MANUSCRIPT