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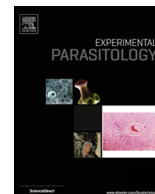
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# Experimental Parasitology

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## Further characterisation of two *Eimeria* species (*Eimeria quokka* and *Eimeria setonicis*) in quokkas (*Setonix brachyurus*)

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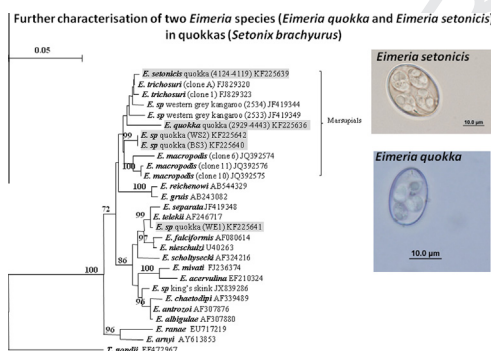
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### HIGHLIGHTS

- First study to genetically characterise *E. setonicis* and *E. quokka*.
- Characterisation at the 18S rRNA gene.
- Characterisation at the mitochondrial cytochrome oxidase gene (COI).
- Morphological characterisation.
- Insights into *Eimeria* phylogeny.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The identification and characterisation of novel *Eimeria* species has largely been based on sporulated oocyst and sporocyst morphology, the host species and the geographical range. Variation in the size and shape of *Eimeria* oocysts across their host range however, make the identification and characterisation of novel species using traditional methodologies alone problematic. The use of molecular markers and phylogenetic analysis has greatly advanced our ability to characterise *Eimeria* species and has recently been applied to understand evolutionary relationships among *Eimeria* species from Australian marsupials.

In the present study, *Eimeria* species isolated from quokkas (*Setonix brachyurus*) captured from Two Peoples Bay, Bald Island and Rottnest Island, Western Australia, were morphologically identified as *Eimeria quokka* and *Eimeria setonicis*. Both *Eimeria* species were identified as being polymorphic in nature with regards to sporulated oocyst and sporocyst morphometrics. Phylogenetic analysis using 18S rRNA and COI (cytochrome c oxidase subunit 1) genes, grouped *E. quokka* and *E. setonicis* within the *Eimeria* marsupial clade together with *Eimeria trichosuri* from brushtail possums, *Eimeria macropodis* from tammar wallabies (*Macropus eugenii*) and several unidentified macropod *Eimeria* species from western grey kangaroos (*Macropus fuliginosus*). This study is the first to characterise *E. quokka* and *E. setonicis* by molecular analysis, enabling more extensive resolution of evolutionary relationships among marsupial-derived *Eimeria* species.

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### 1. Introduction

Within the genus *Eimeria*, there are hundreds of different species, which cause disease in birds, reptiles and mammals (Barker

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et al., 1988; Cox, 1998; Power et al., 2009). Pathogenic species of *Eimeria*, which commonly cause morbidity and mortality are a major concern for the agriculture community, resulting in economical losses within the livestock and poultry industries (Aarthi et al., 2010; Barta et al., 1997; Cox, 1998).

In native mammals, over 50 *Eimeria* species have been described from a range of marsupial hosts in Australasia and the Americas including kangaroos, wallabies, wombats, quokkas, possums, bandicoots and opossums (Barker et al., 1988; Barker et al., 1989; Bennett et al., 2006; Heckscher et al., 1999; Hill et al., 2012; Mackerras, 1959; Power et al., 2009). Description of these native *Eimeria* species has largely been based on morphological characterisation by determining the size and shape of sporulated oocysts and sporocysts, as well as by various other distinguishing characteristics (Barker et al., 1989; Duszynski and Wilber, 1997; Mackerras, 1959;). The geographical location as well as the host species has also been used to aid in the classification of novel *Eimeria* species. However it has been shown that many *Eimeria* species are not strictly host-specific and can naturally have very large geographical ranges (Duszynski and Wilber, 1997).

Molecular characterisation coupled with the more traditional morphological classification methodologies results in a more robust taxonomy system and provides more information on evolutionary relationships between *Eimeria* species. The gene of choice commonly used for aiding in the evolutionary diversity of *Eimeriidae* is the 18S rRNA gene due to its conserved nature throughout the eukaryotes (Stevens et al., 1999). More recently the cytochrome c oxidase subunit 1 (COI) gene has been used because it has higher resolving power for *Eimeria* sp., especially with respect to recent speciation events (Ogedengbe et al., 2011) and is the target gene of choice for DNA bar coding of *Eimeria* spp. (Hebert et al., 2003).

There is limited information on the evolutionary relationships of *Eimeria* species isolated from Australian marsupials. To date only *Eimeria trichosuri*, a species found in brushtail possums of the genus *Trichosurus*, *Eimeria macropodis* from tammar wallabies (*Macropus eugenii*) and several unidentified macropod *Eimeria* species have been genetically characterised (Hill et al., 2012; Power et al., 2009; Yang et al., 2012). A previous study by Barker et al. (1989) morphologically described three *Eimeria* species, *Eimeria volckertzooni*, *Eimeria quokka* and *Eimeria setonicis* isolated from quokkas. However no molecular analysis was undertaken. In the present study, we characterised two *Eimeria* species from quokkas, which conformed morphologically to *E. quokka* and *E. setonicis*, at two loci (18S and COI) and determined their evolutionary relationships to other marsupial-derived *Eimeria* species to expand our knowledge of the diversity and evolution of marsupial-derived *Eimeria* species.

## 2. Materials and methods

### 2.1. Sample collection

Faecal samples from quokkas (*Setonix brachyurus*) examined in the present study were collected from Two Peoples Bay (34° 58'S, 118° 11'E) near Albany, Bald Island (34° 55'S, 118° 27'E) and Rottne Island (32° 00'S, 115° 31'E) in Western Australia. Quokkas were captured either in traps baited with peanut paste-rolled oat mix or netted by hand. Quokkas were trapped under Murdoch University animal ethics permit W2204/09 and DEC permit number SC000767. A total of 37 quokkas were captured from Two Peoples Bay, while on Bald Island and Rottne Island, a total of 34 and 41 quokkas were captured respectively. Faecal matter was collected during the time of sampling where possible (i.e. if the animal defecated during the sampling period) to identify if *Eimeria* oocysts

were present. A total of 8 faecal samples were collected from quokkas captured at Two Peoples Bay, 20 faecal samples were collected from quokkas from Bald Island, and 23 faecal samples collected from quokkas on Rottne Island and stored at 4 °C until required.

### 2.2. Screening and morphological analysis of oocysts

To detect oocysts in quokka faeces, a zinc flotation methodology was used as previously described (Dauguschies et al., 1999) combined with microscopic examination of wet mounts using a 40× objective. Oocysts were confirmed as *Eimeria* by their oval appearance, presence of four sporocysts and thick cell wall. If oocysts were identified, the faecal samples were placed into 2% potassium dichromate and left at room temperature for sporulation to take place to enable morphological identification. Sporulated oocysts were observed at 100× using Nomarski or differential interference contrast (DIC) microscopy and measured with an ocular micrometre. Images of oocysts were recorded using an Olympus DP71 Advance digital camera and Image J (Schneider et al., 2012).

### 2.3. DNA isolation and PCR amplification

Genomic DNA was extracted from 250 mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California) according to the manufacturer's instructions. Samples were screened using a nested PCR which amplified ~502 bp region of the 18S rRNA using primers as previously described (Eberhard et al., 1999). Positive samples were also amplified using a hemi nested PCR to amplify a longer fragment of the *Eimeria* 18S rRNA gene (~1300 bp) as previously described (Yang et al., 2012). Amplification of a 465 bp region of the COI locus from samples that were positive at the 18S locus was conducted as described by Ogedengbe et al. (2011) and Yang et al. (2012).

### 2.4. Sequencing and phylogenetic analysis

PCR products were purified using a QIAquick® PCR Purification kit (Qiagen) and sequenced using an ABI Prism DyeTerminator Cycle Sequencing Core kit (Applied Biosystems, USA). Sequence data was analysed by FinchTV 1.4 (<http://www.geospiza.com/Products/finchtv.shtml>), and imported into Bioedit Sequence Alignment Editor (Hall, 1999), for manipulations and alignment by CLUSTAL W (Larkin et al., 2007). Phylogenetic trees were constructed for *Eimeria* spp. at the 18S rRNA and COI genes with additional isolates from GenBank. MEGA5 (<http://www.megasoftware.net/>) was used for maximum-likelihood (ML) and Parsimony analyses (Tamura et al., 2007). 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. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

### 2.5. Statistical analysis

Prevalences were expressed as percentage of positive samples; with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rozsa et al., 2000).

## 3. Results

### 3.1. *Eimeria* prevalence

The prevalence of *Eimeria* oocysts in faecal samples isolated from quokkas captured from each of the three studied geographical

locations (Two Peoples Bay, Bald Island and Rottnest Island) by microscopy was 62.5% (5/8) (29.0–96.0, 95% CI), 45% (9/20) (23.2–66.8, 95% CI) and 43.5% (10/23) (23.2–63.7, 95% CI), respectively. The prevalence by PCR for Two Peoples Bay, Bald Island and Rottnest Island was 62.5% (5/8) (29.0–96.0, 95% CI), 85.0% (17/20) (69.4–100.6, 95% CI) and 78.3% (18/23) (61.4–95.1, 95% CI), respectively.

### 3.2. Morphological characterisation of *Eimeria* oocysts

Morphological identification of *Eimeria* species from quokkas captured at Two Peoples Bay and Bald Island were determined from sporulated oocysts incubated in potassium dichromate (Table 1). Unfortunately the *Eimeria* oocysts from the Rottnest quokkas failed to sporulate when incubated. *Eimeria* oocysts were measured from three (Q4125–4169, Q4177–4185, Q4124–4119) and five (Q4546–4547, Q2367–2332, Q4527–4578, Q4547–4546, Q2929–4443) individual quokkas from Two Peoples Bay and Bald Island respectively and two morphologically different species of *Eimeria* were identified. Based on the dimensions of the sporulated oocysts and sporocysts, along with other morphological characteristics, oocysts morphologically resembling *E. quokka* (Fig. 1) and *E. setonicis* (Fig. 2) were identified. *E. quokka* has previously been described as having ellipsoidal oocysts (13.6–21.6 µm in length and 8.8–15.2 µm in width), with a double-layered wall, outer wall smooth, clear colourless, inner wall clear; polar granule present; four ellipsoidal sporocysts (6.4–9.6 µm in length and 4.0–6.4 µm in width), with a small Stieda body, two sporozoites with granular cytoplasm, spheroidal sporozoites, refractile body and sporocyst residuum as granular clustals (Barker et al., 1988). In the present study, sporulated oocysts from both Two Peoples Bay and Bald Island conformed to the above morphological characteristics with the exception that the oocyst length, which ranged from 13.7–22.5 µm and 12.5–23.7 µm respectively, was slightly larger (Table 1) and sporocysts ranged from 5.0–10.0 µm in length and 3.7–7.5 µm in width.

*Eimeria setonicis* has previously been described as having ellipsoidal oocysts (26.4–33.6 µm in length and 16.8–19.2 µm in width) which were slightly pointed at one end with a double-layered wall, outer wall smooth, clear colourless, inner wall clear; polar granule



Fig. 1. Nomarski interference-contrast photomicrographs of an *Eimeria* oocyst isolated from quokka faeces (quokka isolate 2929–4443) from Two Peoples Bay, Western Australia, resembling *E. quokka* based on morphological characteristics.

present; four ellipsoidal sporocysts (10.4–13.6 µm in length and 7.2–8.8 µm in width) with a Stieda body, two sporozoites, spheroidal prominent sporozoites, refractile body and sporocyst residuum as granular clustals (Barker et al., 1988). Sporulated oocysts from both Two Peoples Bay and Bald Island conformed to these characteristics but ranged in length from 26.2 to 37.5 µm and 28.7 to 35 µm and in width from 17.5 to 22.5 µm and 17.5 to 25 µm, respectively and are therefore slightly larger than the previously published morphometrics for *E. setonicis* (Barker et al., 1988). Sporocysts measured 7.5–13.7 µm in length and 7.5–10 µm in width. *E. quokka* was the most prevalent *Eimeria* species detected in quokka faeces from both Two Peoples Bay and Bald Island (26% and 74%, respectively) compared to *E. setonicis* (48% and 52%, respectively). Mixed infections of both *Eimeria* species was generally observed in the faeces of individual animals. However single infections of both *E. quokka* and *E. setonicis* were detected in individual quokkas and determined by the uniformity of the oocyst. Quokka isolate Q2929–4443 was identified as having a single infection of

Table 1

Morphometric (µm) comparison of *Eimeria* oocysts from quokkas\* from Two Peoples Bay, and from quokkas• from Bald Island with previously published morphometrics for *E. quokka* and *E. setonicis* from Rottnest Island quokkas (Barker et al., 1988).

	<i>E. quokka</i> Two Peoples Bay This study	<i>E. quokka</i> Bald Island This study	<i>E. quokka</i> Barker et al. 1988	<i>E. setonicis</i> Two Peoples Bay This study	<i>E. setonicis</i> Bald Island This study	<i>E. setonicis</i> Barker et al. 1988
<b>Oocyst length</b>						
n	20	58	45	11	12	26
Mean	18.3	18.0	18.0	29.7	31.9	29.9
SD	2.3	3.2	1.7	3.2	1.8	1.71
Range	13.7–22.5	12.5–23.7	13.6–21.6	26.2–37.5	28.7–35.0	26.4–33.6
<b>Oocyst width</b>						
Mean	11.8	12.2	10.8	19.5	19.5	17.9
SD	1.24	1.4	1.1	2.6	2.2	0.6
Range	10.0–15.0	10.0–15.0	8.8–15.2	17.5–22.5	17.5–25	16.8–19.2
<b>Sporocyst length</b>						
Mean	7.0	6.4	7.7	12.1	11.0	12.0
SD	1.3	1.2	0.6	1.3	1.9	0.79
Range	5.0–10.0	5.0–8.7	6.4–9.6	8.7–13.7	7.5–13.7	10.4–13.6
<b>Sporocyst width</b>						
Mean	5.0	5.0	5.0	8.5	8.8	7.7
SD	0.7	0.8	0.5	1.0	0.8	0.4
Range	3.7–7.5	3.7–7.5	4.0–6.4	7.5–10.0	7.5–10.0	7.2–8.8

\* (Q4125–4169), (Q4177–4185), (Q4124–4119).

• (Q4546–4547), (Q2367–2332), (Q4527–4578), (Q4547–4546), (Q2929–4443).



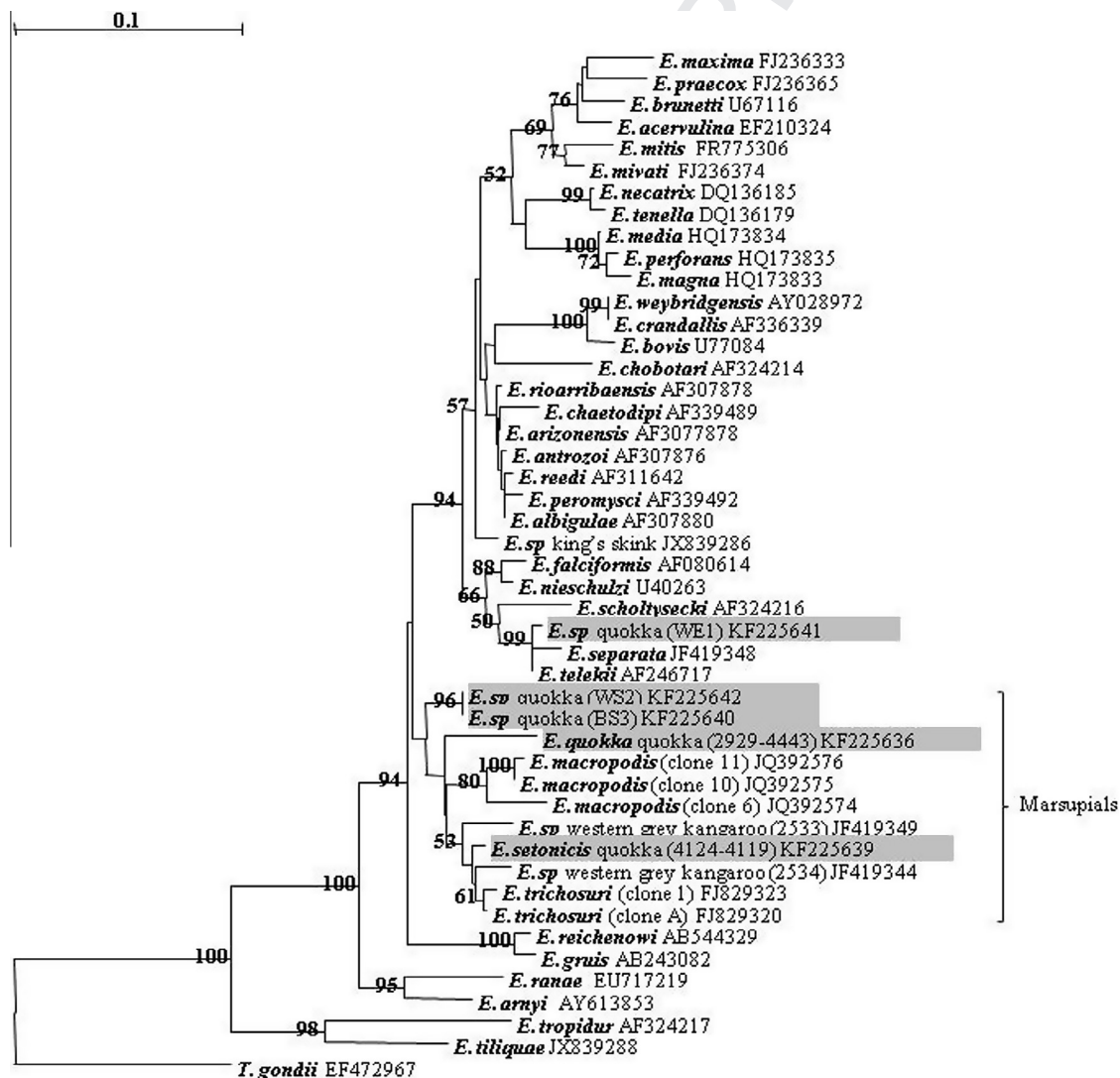


**Fig. 2.** Nomarski interference-contrast photomicrographs of an *Eimeria* oocyst isolated from quokka faeces (quokka isolate 4124–4119) from Bald Island, Western Australia, resembling *E. setonicis* based on morphological characteristics.

*E. quokka* while quokka isolate, Q4124–4119 was infected with only *E. setonicis*. DNA extracted from these two quokkas was amplified, sequenced and used for phylogenetic analysis as described above. DNA was also extracted from quokka isolates BS3, WS2 and WE1 from Rottnest Island and characterised genetically.

### 3.3. Phylogenetic analysis of *E. quokka*, *E. setonicis* and *Eimeria* sp. from the quokka at the 18S rRNA gene

Phylogenetic analysis of a partial nuclear sequence (~481 bp) of the 18S rRNA gene from isolate Q2929–4443 (positive with *E. quokka* from Two Peoples Bay) and isolate Q4124–4119 (positive with *E. setonicis* from Bald Island) using Distance, Parsimony and ML analysis produced trees with similar topographies (Fig. 3, distance tree shown). The overall tree topology was similar to previously produced phylogenetic trees for *Eimeria* (Power et al., 2009; Yang et al., 2012). Both *E. quokka* and *E. setonicis* grouped within the marsupial clade together with *E. macropodis*, *E. spp* from western grey kangaroos and *E. trichosuri*. A distance similarity matrix generated using Tajima – Nei distance (data not shown) at the 18S lo-



**Fig. 3.** Evolutionary relationships of *Eimeria quokka*, *Eimeria setonicis* and *Eimeria* sp. from the quokka (*Setonix brachyurus*) inferred by distance analysis of partial (481 bp) 18S rRNA sequences. Percentage support (>50%) from 1000 pseudoreplicates from neighbour-joining analyses is indicated at the left of the support node.

cus showed that, *E. quokka* exhibited 97.3% similarity to *E. setonicis*, 97.3% similarity with *E. trichosuri* from possums, 96.7% similarity with *E. macropodis* from the tammar wallaby and 97.3% similarity with *Eimeria* sp. from western grey kangaroos (Yang et al., 2012). *E. setonicis* exhibited 99.6% similarity to *E. trichosuri*, 98.1% similarity to *E. macropodis* (clone 10) and 99.2% similarity to *Eimeria* sp. from western grey kangaroos. Phylogenetic analysis was also conducted on the larger partial nuclear sequence (~1288 bp) of the 18S rRNA gene for isolate Q2929–4443 and Q4124–4119 using Distance, Parsimony and ML analysis and produced the same topographies as the smaller partial 18S rRNA gene sequence (data not shown).

In addition to *E. quokka* and *E. setonicis* sequences, two novel sequences were also identified. A novel sequence (referred to hereafter as novel *Eimeria* sequence 1) was identified from quokka isolates BS3 and WS2 from Rottnest. Direct sequencing of the 18S rRNA gene fragment from these two quokka isolates produced clean chromatograms, indicating that only one sequence was present. Sequences from isolates BS3 and WS2 were 100% identical to each other and grouped most closely with the marsupial clade (Fig. 3). Novel *Eimeria* sequence 1 exhibited 98.8% similarity to *E. trichosuri* (clone A), 98.8% similarity to *E. macropodis* (clone 10), 98.3% similarity to *Eimeria* sp. (2534) from western grey kangaroos, 96.7% similarity to *E. quokka* (16 single nucleotide polymorphisms, SNPs over 481 bp of sequence) and 98.3% similarity to *E. setonicis* (8 SNPs over 481 bp of sequence). A second novel sequence (novel *Eimeria* sequence 2) was identified from quokka isolate WE1 from Rottnest Island which grouped within the rodent clade and exhibited 99.8% and 99.2% similarity with *Eimeria telekii*

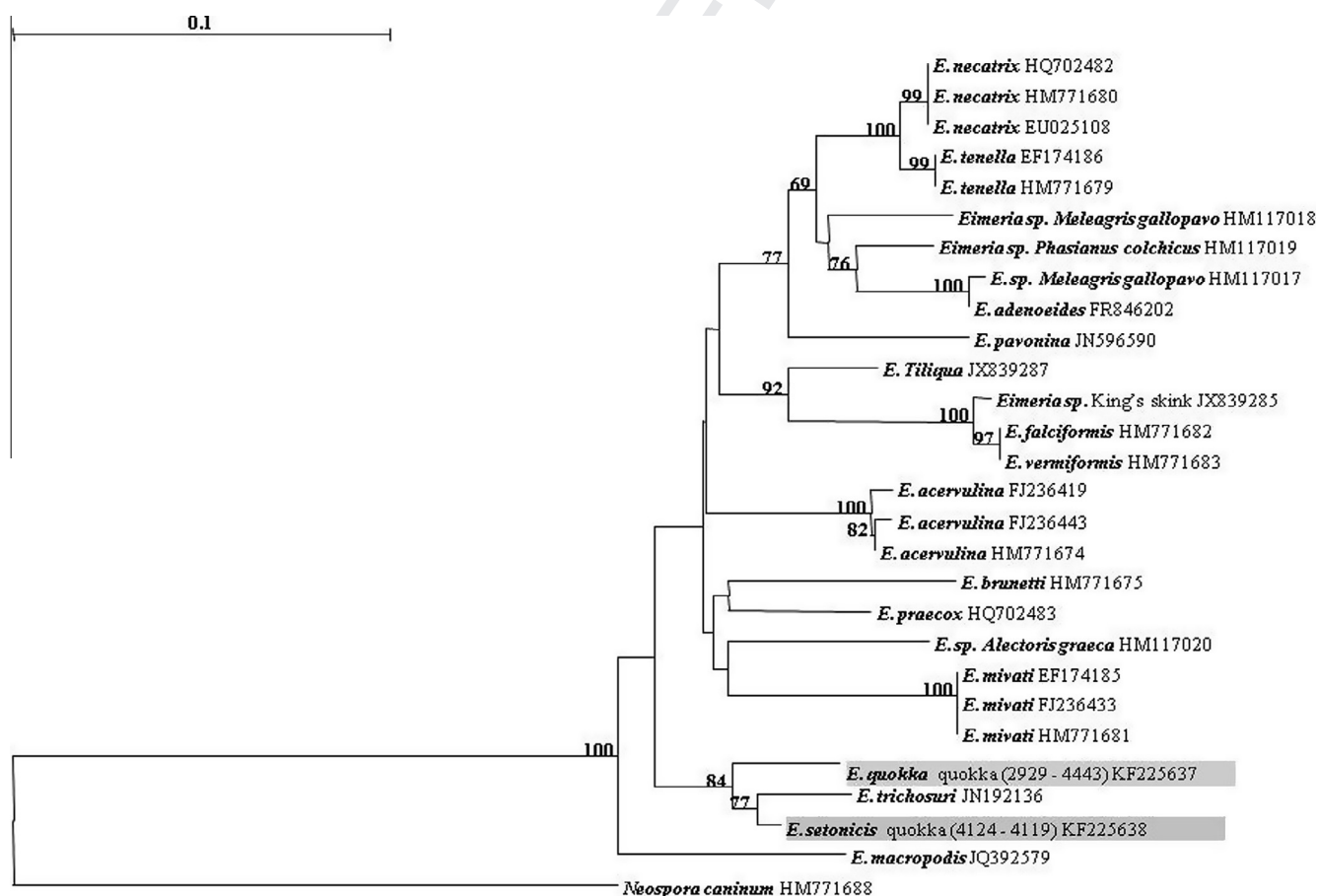
and *Eimeria separata*, respectively. The partial 18S rRNA gene sequences generated in the present study have been submitted to GenBank under Accession KF225636 and KF225639–KF225642.

### 3.4. Phylogenetic analysis of *E. quokka* and *E. setonicis* from the quokka at the COI gene

Phylogenetic analysis of the mitochondrial COI partial sequence (~426 bp) from the *E. quokka* isolate (Q2929–4443) and the *E. setonicis* isolate (Q4124–4119) produced trees of similar topography to the 18S rRNA tree and grouped both *E. quokka* and *E. setonicis* within the marsupial clade (Fig. 4, distance tree shown). A distance similarity matrix generated using Tajima – Nei distance (data not shown) at this locus showed that, *E. quokka* exhibited 96.1% similarity to *E. setonicis*, 93.8% similarity to *E. trichosuri* and 88.9% similarity to *E. macropodis*, while *E. setonicis* exhibited 96.9% similarity to *E. trichosuri* and 91.7% similarity to *E. macropodis*. Unfortunately sequences from quokka isolates BS3, WS2 and WE1 from Rottnest could not be amplified at this locus. The partial COI sequences generated in the present study have been submitted to GenBank under Accession KF225637 and KF225638.

## 4. Discussion

The quokka is a small marsupial similar in appearance to a wallaby or kangaroo and belongs to the family Macropodidae (Barker et al., 1974). The quokka is listed as vulnerable because it is endemic to the south-west of Western Australia, including Rottnest and Bald Islands and the extent of its occurrence is less than



**Fig. 4.** Evolutionary relationships of *Eimeria quokka* and *Eimeria setonicis* from the quokka (*Setonix brachyurus*) inferred by distance analysis of the mitochondrial cytochrome oxidase gene (COI) (426 bp). Percentage support (>50%) from 1000 pseudoreplicates from neighbour-joining analyses is indicated at the left of the support node.

20,000 km<sup>2</sup> (Hayward et al., 2004). *Eimeria* species have previously been isolated and described from quokkas habituating Rottneest Island only (Barker et al., 1988). Our results extend the geographical range of *Eimeria* species from quokkas to Bald Island and the mainland of Western Australia.

In the study by Barker et al. (1988), three different *Eimeria* species from quokkas were morphologically identified and described; *E. quokka*, *E. setonicis* and *E. volckertzooni*. In the present study, *Eimeria* oocysts resembling *E. quokka* and *E. setonicis* only were identified in quokka faeces collected from Two Peoples Bay, Bald Island and Rottneest Island. *E. volckertzooni* was described as having irregular blunt ellipsoid oocyst, ranging in length from 20 to 24 µm and width from 11.6 to 13.6 µm, often flattened on one side and containing a smooth clear colourless double cell wall, with the presence of a polar granule and oocyst residuum. Sporocysts were long and narrow and ranged in length from 11.2 to 15.2 µm and in width from 4.8 to 6.4 µm with an inconspicuous Steida body and two elongated sporozoites (Barker et al., 1988). Oocysts similar to this were not detected in the present study.

Microscopy analysis showed *E. quokka* was the most prevalent *Eimeria* species from Two Peoples Bay and Bald Island and was previously reported as being the most prevalent *Eimeria* species in quokkas on Rottneest Island (Barker et al., 1988). Oocyst and sporozoite length and width dimensions for the *Eimeria* species identified in the present study differed slightly from previously published measurements for *E. quokka* and *E. setonicis* but the overall oocysts characteristics were consistent with *E. quokka* and *E. setonicis* (Barker et al., 1988). Morphological variation in sporulated oocysts within individual *Eimeria* species is well documented with polymorphism reported both within and between host species from different geographical locations (Duszynski, 1971; Gardner and Duszynski, 1990). For example, variation in both oocyst and sporocyst morphometrics within *Eimeria cochabambensis* from three different marsupial host species has been previously described (Heckscher et al., 1999). However as no molecular analysis was conducted in those studies, the possibility that multiple species were present cannot be ruled out. A more recent study however, which did conduct molecular analysis, reported variation in both oocyst and sporocyst morphometrics in *E. trichosuri* oocysts from brushtail possums (Power et al., 2009). The time of infection also has been reported to play a role in morphometric differences from single *Eimeria* species from the same host (Duszynski, 1971). This is because, some members of the Eimeriidae are known to display polymorphism during patency and sporulated oocyst size can increase during this period (Duszynski, 1971).

Unfortunately the quokka faecal samples from Rottneest Island did not sporulate when incubated in potassium dichromate. This may have been largely due to time of collection and storage conditions, as samples were collected in early January during peak summer conditions with temperatures reaching 30–40 °C. These high temperatures may have inactivated the oocysts as ambient temperatures of 20–23 °C are more suited for sporulation (Duszynski and Wilber, 1997).

Molecular characterisation techniques such as sequencing of the 18S rRNA and COI loci are currently being used to characterise and detect *Eimeria* species (Hill et al., 2012; Yang et al., 2013). In the present study, molecular analysis was used to generate for the first time sequences for *E. quokka* and *E. setonicis* at two loci (18S and COI) as well as two additional novel *Eimeria* sequences at the 18S locus. Molecular detection was also more sensitive than microscopy and detected 85% vs. 45% prevalence for *Eimeria* in quokka faeces from Bald Island and 78.3% vs. 43.5% prevalence for Rottneest Island. Microscopy however is still vital for morphological characterisation of *Eimeria* oocysts from marsupial faecal

samples, as marsupials are known to have multiple *Eimeria* species within the one host and particularly as the availability of DNA sequences for marsupial *Eimeria* are limited (Power et al., 2009).

Phylogenetic analysis from both loci grouped *E. quokka* and *E. setonicis* within the marsupial clade together with *E. trichosuri* from brushtail possums and *E. macropodis* from tammar wallabies. *Eimeria* spp. from western grey kangaroos also grouped within the marsupial clade at the 18S loci. Unfortunately sequences from the *Eimeria* spp. from the western grey kangaroos were not available at the COI locus.

*E. quokka* and *E. setonicis* exhibited only 97.3% and 96.1% similarity to each other at the 18S and COI loci respectively. *E. quokka* was most closely related to *E. setonicis* at the COI gene while at the 18S gene it was shown to have the same genetic distance of 97.3% from *E. trichosuri* from brushtail possums, *Eimeria* sp. from the western grey kangaroo and from *E. setonicis* from quokkas. *E. setonicis* was most closely related to *E. trichosuri* at both loci with 99.6% and 96.9% similarity at the 18S and COI gene, respectively. The genetic differences seen in the diversity of the 18S and COI genes for the quokka isolates support those of Hill et al. (2012) and Yang et al. (2013), who highlight the need to use more than one genetic marker to fully understand the evolutionary relationship of marsupial *Eimeria* species.

Novel *Eimeria* sequence 1 (isolates WS2, BS3) from Rottneest Island was genetically distinct and formed its own clade within the marsupial group. At the 18S gene it was most similar to *E. macropodis* and *E. trichosuri* with 98.8% similarity to both of these *Eimeria* species. This novel isolate may represent a new *Eimeria* species, however morphological and genetic analysis at the COI gene needs to be conducted for this species before it can be properly validated. Novel *Eimeria* sequence 2 (isolate WE1) grouped within the rodent clade and was most closely related to *E. telekii* from a striped grass mouse (*Lemniscomys striatus*) from Kenya (Slapeta et al., 2001). To the authors' knowledge this is the first time that a rodent-like *Eimeria* species has been isolated from a marsupial host. Novel *Eimeria* sequence 2 was most likely acquired via mechanical transmission, as *Eimeria* sp. from mice are known to be host specific at the genus and species level (Hnida and Duszynski, 1999). However it has been suggested that transfers may occur among animals occurring together in the same habitat at the same site (syntopic hosts) under natural conditions (Hnida and Duszynski, 1999). This theory may account for our findings and also the recent detection of an *Eimeria* rodent isolate (genetically similar to *Eimeria falciformis* and *Eimeria vermiformis*) from a faecal sample from a King's skink (*Egernia kingii*) from Western Australia (Yang et al., 2013).

In conclusion, the present study characterised *E. quokka* and *E. setonicis* for the first time at both the 18S and COI loci and demonstrated that they are closely related to *Eimeria* from other marsupials (*E. trichosuri* and *E. macropodis*) suggesting co-evolution of marsupial *Eimeria* species regardless of the host. These findings also support the hypothesis that *Eimeria* found in marsupials diverged prior to *Eimeria* species from placental mammals (Power et al., 2009). Phylogenetic analysis has enabled the evolutionary placement of these two *Eimeria* species and has contributed to our knowledge on the genetic diversity of *Eimeria* species isolated from native marsupials.

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