



# Identification of a novel species of *Eimeria* Schneider, 1875 from the woylie, *Bettongia penicillata* Gray (Diprotodontia: Potoroidae) and the genetic characterisation of three *Eimeria* spp. from other potoroid marsupials

Amy S. Northover · Sarah Keatley · Aileen D. Elliot · Russell P. Hobbs · Rongchang Yang · Alan J. Lymbery · Stephanie S. Godfrey · Adrian F. Wayne · R. C. Andrew Thompson

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**Abstract** Faecal samples (n = 1,093) collected from the woylie *Bettongia penicillata* Gray, in south-western Australia were examined for the presence of coccidian parasites. *Eimeria* sp. oöcysts were detected in 15.2% of samples. Faecal samples obtained from the eastern bettong *Bettongia gaimardi* (Desmarest) (n = 4) and long-nosed potoroo *Potorous tridactylus* (Kerr) (n = 12) in Tasmania, were also screened for the presence of *Eimeria* spp. (prevalence 50% and 41.7%, respectively). Morphological and genetic comparison with other known species of *Eimeria* indicates that the material identified in woylies is novel. This study aimed to (i) morphologically describe and genetically characterise *Eimeria woyliei* n. sp. found in woylies;

and (ii) genetically characterise *Eimeria gaimardi* Barker, O’Callaghan & Beveridge, 1988, *Eimeria potoroi* Barker, O’Callaghan & Beveridge, 1988, and *Eimeria mundayi* Barker, O’Callaghan & Beveridge, 1988, from other potoroid marsupials. Molecular phylogenetic analyses conducted at the 18S rDNA and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) loci revealed that *E. woyliei* n. sp. was most closely related to *Eimeria setonicis* Barker, O’Callaghan & Beveridge, 1988, at the 18S rDNA locus, and *Eimeria trichosuri* O’Callaghan & O’Donoghue, 2001, at the *cox1* locus. *Eimeria woyliei* n. sp. is the sixth species of *Eimeria* to be formally described from potoroid marsupials.

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A. S. Northover (✉) · S. Keatley · A. D. Elliot · R. P. Hobbs · R. Yang · R. C. A. Thompson  
College of Science, Health, Engineering and Education, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia  
e-mail: a.northover@live.com.au

A. J. Lymbery  
Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia

## Introduction

Coccidian parasites are known to infect potoroid marsupials, including the critically endangered woylie

S. S. Godfrey  
Department of Zoology, University of Otago, 362 Leith Street, Dunedin 9016, New Zealand

A. F. Wayne  
Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Brain Street, Manjimup, WA 6258, Australia

or brush-tailed bettong *Bettongia penicillata* Gray. Although morbidity and mortality associated with coccidial infection in free-ranging macropods is uncommon (Vogelnest & Portas, 2008), disease has been documented in macropods under stress (e.g. the eastern grey kangaroo *Macropus giganteus* Shaw; Barker et al., 1972). Given the reliance of many threatened species on interventional management practices such as translocation, a process which has been identified as a significant stressor (Hing et al., 2017), it is imperative that we gain a greater understanding of the parasite species infecting wildlife, particularly those with the potential to cause disease in their host (e.g. coccidian parasites).

To date, no coccidian parasites (Apicomplexa: Eimeriidae) have been formally described in woylies (Duszynski, 2016), though *Eimeria* sp. oöcysts have been detected in faecal samples (Northover et al., 2017). Five *Eimeria* spp. including *Eimeria potoroi* Barker, O’Callaghan & Beveridge, 1988, *Eimeria mundayi* Barker, O’Callaghan & Beveridge, 1988, *Eimeria gaimardi* Barker, O’Callaghan & Beveridge, 1988, *Eimeria aepyprymni* Barker, O’Callaghan & Beveridge, 1988, and *Eimeria burdi* Hulst, Kemp & Slapeta, 2016, have been morphologically described from potoroid marsupials (Marsupialia: Potoroidae). *Eimeria gaimardi* from the eastern bettong *Bettongia gaimardi* (Desmarest), most closely morphologically resembles *Eimeria* sp. oöcysts found in woylies. Unfortunately, genetic characterisation of potoroid *Eimeria* spp. has not been undertaken. While *Eimeria* spp. tend to be host-specific (Barker et al., 1988), members of the genus *Macropus* Shaw have been known to harbor the same *Eimeria* spp. (e.g. *Eimeria macropodis* Wenyon & Scott, 1925; see Barker et al., 1989), thus it is important that additional data (e.g. genetic characterisation) is used to support the description of new species.

During this study we aimed to (i) morphologically describe and genetically characterise *E. woyliei* n. sp. from woylies; and (ii) genetically characterise *E. gaimardi* from the eastern bettong, and *E. potoroi* and *E. mundayi* from the long-nosed potoroo *Potorous tridactylus* (Kerr).

## Materials and methods

### Sample collection

Between 2014 and 2018, woylie faecal samples (n = 1,093) were collected from various sites within south-western Australia (Table 1) as part of a collaborative project with the Department of Biodiversity, Conservation and Attractions (DBCA), Kensington, Australia. Newspaper was placed beneath each trap to collect faeces, which were stored in 70% ethanol, 10% buffered formalin and/or 2% potassium dichromate until processing.

In 2018, faecal samples collected from the eastern bettong (n = 4) were obtained from a captive population at Bonorong Wildlife Sanctuary in Brighton, Tasmania (42.71°S, 147.27°E). Samples from the long-nosed potoroo (n = 12) were acquired from wild-caught animals within the Peter Murrell reserves, south of Hobart, Tasmania (43.00°S, 147.18°E). Faecal samples from the eastern bettong and long-nosed potoroo were collected directly from traps and samples were stored in 2% potassium dichromate prior to analysis.

### Identification of coccidian oöcysts in faecal samples

For woylies, the majority of faecal samples (n = 1,073) were examined for the presence of coccidian oöcysts using simple faecal flotation with sodium nitrate (NaNO<sub>3</sub>) as described by Northover et al. (2017). These samples were formalin-fixed. To describe the morphology of *E. woyliei* n. sp., an additional 20 samples were sporulated (using potassium dichromate) and underwent faecal flotation using zinc sulphate (ZnSO<sub>4</sub>) in distilled water (SG 1.20). Briefly, faeces were placed into a 10 ml centrifuge tube (up to the 1.5–2.0 ml mark), emulsified in distilled water (tube filled to the 10-ml mark) and centrifuged (2,000 rpm for 2 min). The supernatant was removed before filling the tube with zinc sulphate solution and re-emulsifying, before final centrifugation (2,000 rpm for 2 min). A sterile wire loop was used to transfer 2–3 loops from the surface of the tube to a glass slide, and a 22 × 22 mm coverslip was placed on top. Each sample was examined at 100× magnification using an Olympus BX50 microscope. All other potoroid samples were examined using zinc sulphate. To calculate the prevalence of infection, each faecal sample was scored as positive or negative for the presence of coccidian oöcysts.

**Table 1** The number of woylies screened for coccidian parasites within each sampling site

Site	Coordinates	No. screened	No. positive	Prevalence (%)
Balban	34.10°S, 116.58°E	21	2	9.5
Boycup	34.28°S, 116.58°E	1	0	0.0
Corbal	34.10°S, 116.48°E	15	6	40.0
Dryandra	32.80°S, 116.89°E	357	10	2.8
Dudijup	34.07°S, 116.30°E	4	1	25.0
Dwalgan	34.07°S, 116.46°E	14	8	57.1
Perup Sanctuary	34.16°S, 116.56°E	151	32	21.2
Walcott	34.06°S, 116.39°E	266	56	21.1
Warrup East	34.16°S, 116.39°E	255	50	19.6
Winnejup	34.07°S, 116.35°E	9	1	11.1
Total		1,093	166	15.2

#### Morphological description of the new species

Eighty-six sporulated oocysts from a single woylie originating from Perup Sanctuary were examined at magnifications of 400–1,000 $\times$ , using an Olympus BX50 microscope with a Olympus DP71 Universal Camera with Cellsens software. Photographs of sporulated oocysts were taken using bright field microscopy. Measurements of oocyst and sporocyst length and width, and oocyst wall thickness were acquired using ImageJ software (US National Institute of Health, Bethesda, Maryland). All measurements are recorded in micrometres ( $\mu\text{m}$ ) with the range followed by the mean in parentheses. We measured a single laterally positioned sporocyst within each oocyst; if we could not identify a sporocyst in the correct position, we did not measure sporocyst length or width.

#### Morphological identification of other potorooid *Eimeria* spp.

Three distinct morphotypes of sporulated oocysts were identified from the faeces of the eastern bettong and long-nosed potoroo. Based on their size and unique oocyst and sporocyst characters as described by Barker et al. (1988) we identified *E. gaimardi* from the eastern bettong, and *E. mundayi* and *E. potoroi* from the long-nosed potoroo.

#### Genetic characterisation of *Eimeria* spp. in potorooid marsupials

For the woylie, nine faecal samples (eight ethanol- and one potassium dichromate-preserved) were used to

genetically characterise *E. woyliei* n. sp. at the 18S rDNA and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) loci. *Eimeria gaimardi*, *E. mundayi* and *E. potoroi* were genetically characterised using a single faecal sample (potassium dichromate-preserved) for each *Eimeria* species; as outlined above, morphological identification of sporulated oocysts confirmed the identity of each species prior to genetic characterisation.

#### DNA extraction and PCR amplification

Samples stored in 70% ethanol and/or 2% potassium dichromate were exposed to four freeze/thaw cycles as described by Yang et al. (2016a) in order to achieve oocyst lysis. Following the freeze/thaw step, faecal samples stored in 2% potassium dichromate were subjected to a wash step prior to lysis in order to remove the fixative, by centrifuging at 3000 rpm for 10 min and resuspending in phosphate buffered solution. DNA was isolated from 0.25 g of faecal sample using the PowerFecal DNA Isolation Kit (MolBio, Carlsbad, California) as per the manufacturer's instructions. A negative control was included to rule out contamination.

Faecal samples from the woylie were screened at the 18S rDNA locus using a nested PCR with the external *Eimeria* spp. primers EiF1 (5'-GCT TGT CTC AAA GAT TAA GCC-3') and EiR3 (5'-ATG CAT ACT CAA AAG ATT ACC-3'), and the internal *Eimeria* spp. primers EiR4 (5'-ACT CAA AAG ATT ACC TAG AC-3') and EiF4 (5'-CTA TGG CTA ATA CAT GCG CAA T-3') (Yang et al., 2016a). PCR

reactions were carried out in a total volume of 25 µl containing 12.5 µl of 2× KAPA HiFi Hotstart ReadyMix (Millennium Science Pty. Ltd, Victoria, Australia), 0.75 µl primer (10 µM) and 2 µl DNA template. All PCR reactions were performed as described by Yang et al. (2016a) consisting of a pre-PCR step of 94°C for 3 min, followed by 45 cycles of 94°C for 30 s, 55°C annealing temperature for 30 s and 72°C for 2 min, and a final extension step of 72°C for 5 min.

Faeces from the eastern bettong and long-nosed potoroo were screened using the 18S external primers EiGTF1 (5'-TTC ACT GGT CCC TCC GAT C-3') and EiGTR1 (5'-AAC CAT GGT AAT TCT ATG G-3') (Yang et al., 2016b), and the internal primers EiGTF2 (5'-TTA CGC CTA CTA GGC ATT CC-3') and EiTR2 (5'-TGA CCT ATC AGC TTT CGA CG-3'). The PCR reaction contained 10 µl 2× GoTaq PCR master mix (Promega, Alexandria NSW, Australia), 1 µl DNA (50 ng), 10 µl of each primer (10 µM stock) and 7 µl distilled water. PCR cycling conditions for the external PCR were 1 cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 2 min, and a final extension step of 72°C for 5 min. The conditions for the secondary PCR were the same except for 45 cycles instead of 35. All amplicons were visualised on a 1.5% agarose gel and bands were cut and purified using an in-house filter tip method defined by Yang et al. (2013).

For all potoroid species, amplicons were generated at the *cox1* locus using the external primers COIF1 (5'-GGT TCA GGT GTT GGT TGG AC-3') and COIF2 (5'-TAA GTA CAT CCC TAA TGT C-3') and the internal primers COIBR1 (5'-CCA AGA GAT AAT ACR AAR TGG AA-3') and COIBR2 (5'-ATA GTA TGT ATC ATG TAR WGC AA-3') (Yang et al., 2016a). The PCR reactions and conditions were the same as per the 18S PCR carried out for woylie samples.

#### Sequencing and phylogenetic analysis

Purified amplicons were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing Kit (Applied Biosystems, California, USA) according to the manufacturer's instructions. Samples were sequenced in the forward and reverse direction and the denaturation step was extended to 10 min to allow efficient primer binding. The sequences were analysed in Geneious v.8.1 (Kearse et al., 2012) and aligned

using reference libraries with the MUSCLE (Edgar, 2004) plugin for Geneious v.8.1. All novel sequences were deposited in GenBank.

Using Bayesian methods, phylogenetic analyses were conducted for both gene regions (18S and *cox1*, respectively), to determine the evolutionary lineage for *E. woyliei* n. sp. (MK182524; MK202806), *E. gaimardi* (MK182525; MK202809), *E. mundayi* (MK182526; MK202808) and *E. potoroi* (MK182527; MK202807). JModelTest (Posada, 2008) was used to determine the most appropriate nucleotide substitution method for the Bayesian analyses for each gene region, which was the GTR+I+G (general time reversible gamma proportion of invariant sites) method. The Bayesian posterior probabilities were generated using MrBayes v.3.1.2 (10,000,000 generations, sampling frequency of 1,000, 'burn in' 3,000).

## Results

The prevalence of coccidial infection in woylies is summarised by site in Table 1. It is important to note that Dryandra contains both resident woylies (endemic to the region), and translocated woylies originating from the Upper Warren region (specifically Balban, Boyicup, Corbal, Dudijup, Dwalgan and Winnejup). Likewise, Walcott and Warrup East contain both resident and translocated (originating from Perup Sanctuary) woylies. In the eastern bettong, 2 out of 4 (50%) samples were positive for *E. gaimardi*. In the long-nosed potoroo, 3 out of 12 (25.0%) samples were positive for *E. potoroi*, while 4 out of 12 (33.3%) samples were positive for *E. mundayi*; mixed infections with both coccidian parasites were identified in 2 out of 12 (16.7%) samples.

### Family Eimeriidae Minchin, 1903

#### Genus *Eimeria* Schneider, 1875

#### *Eimeria woyliei* n. sp.

*Type-host*: *Bettongia penicillata* Gray (Mammalia: Marsupialia: Potoroidae), woylie or brush-tailed bettong.

*Type-locality*: Perup Sanctuary (34.16°S, 116.56°E) in the Upper Warren region, Western Australia, Australia.

**Other localities:** Corbal (34.10°S, 116.48°E), Dwalgan (34.07°S, 116.46°E) and Winnejup (34.07°S, 116.35°E) in the Upper Warren region, Western Australia, Australia; \*Dryandra Woodland (32.80°S, 116.89°E) in the wheatbelt region, Western Australia, Australia (\*translocated host, origin Dwalgan).

**Type-material:** Oöcysts in 10% formalin and oöcyst photosyntypes have been deposited in the Western Australian museum [reference numbers WAM Z91161 (holotype) and WAM Z91162–64 (paratypes)].

**Prevalence:** 21.2% (32 out of 151 specimens of the type-host; origin Perup Sanctuary).

**Sporulation:** Unknown.

**Site of infection:** Unknown, oöcysts recovered from faeces.

**Representative DNA sequences:** DNA sequences have been deposited in the GenBank sequence database under accession numbers MK182524–MK182527 (18S rRNA gene) and MK202806–MK202809 (*cox1* gene).

**Etymology:** The name *woyliei* reflects the host species local name ‘woylie’, which is the Aboriginal name given to this animal by the Noongar people of south-western Western Australia (Abbott, 2001).

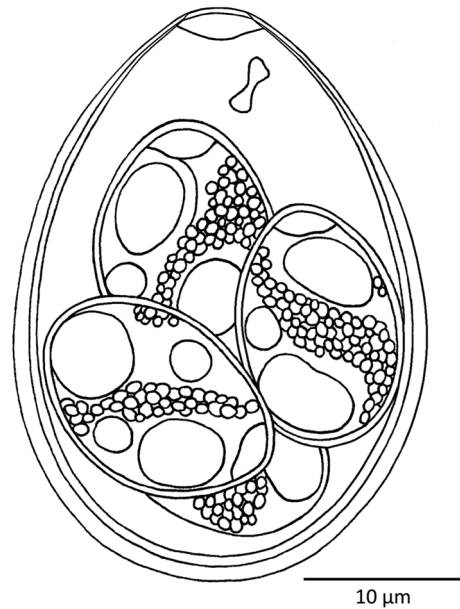
## Description (Figs. 1, 2A–C)

### *Sporulated oöcyst*

Oöcysts (n = 86) pyriform-shaped [length 31.6–40.8 (36.7), width 22.6–31.0 (26.3), length/width (L/W) ratio 1.3–1.5 (1.4)] with smooth to slightly mammillate, bi-layered oöcyst wall, 1.3–1.7 (1.6) thick. Oöcyst wall thins at apex with visible micropyle. An irregular dome-like structure (referred to here as a submicropyle body) visible beneath micropyle. Polar granule irregular-shaped, apparent, although not detected in all sporulated oöcysts. Oöcyst residuum absent.

### *Sporocyst and sporozoite*

Sporocysts (n = 76) ellipsoidal [length 13.3–17.8 (15.6), width 9.0–12.3 (10.3), L/W ratio 1.1–1.7 (1.5)]. Each sporocyst contains an indistinct dome-like Stieda body/sub-Stieda body. Sporocyst residuum present between 2 broadly elongate sporozoites. Sporozoites contain 2 distinct refractile bodies, one large (5–7 wide) and one small (up to 3 wide). Para-Stieda body absent.



**Fig. 1** Composite line drawing of a sporulated oöcyst of *Eimeria woyliei* n. sp.

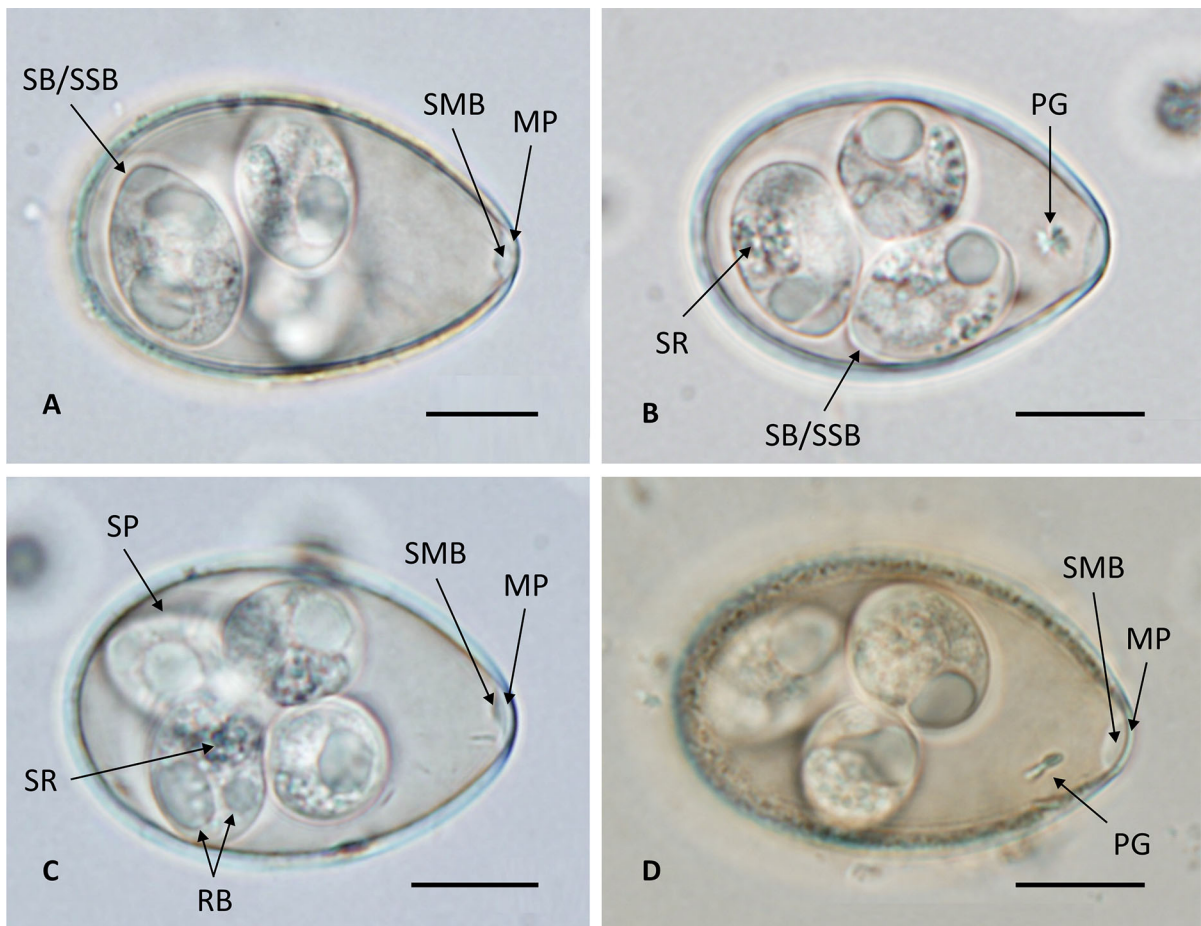
## Genetic characterisation of *Eimeria* spp. in potoroids

The phylogeny of the four potoroid *Eimeria* spp. was investigated using Bayesian analyses at two gene loci (18S and *cox1*). An alignment was generated for the 18S rDNA locus (1,158 bp, Fig. 3) and two alignments for the *cox1* locus (686 bp, Fig. 4; 211 bp, Fig. 5). All alignments contained the novel sequences belonging to the four potoroid species as well as reference sequences downloaded from GenBank including an outgroup (*Toxoplasma gondii*). Similar phylogenetic relationships were observed for both loci, although some species were not observed in the *cox1* tree due to lower availability of genetic data. A second *cox1* alignment was generated (211 bp) in order to incorporate relevant marsupial species of smaller fragment size.

### 18S rDNA locus

*Eimeria woyliei* shared 99.2% genetic similarity with *E. setonicis*, isolated from the quokka *Setonix brachyurus* (Quoy & Gaimard) (KF225639; Austen et al., 2014), and 99.1% genetic similarity with *E. trichosuri*, from the mountain brushtail possum *Trichosurus cunninghami* Lindenmayer, Dubach & Viggers (FJ8292320; Power et al., 2009). Of the potoroid





**Fig. 2** Photomicrographs of sporulated oocysts of *Eimeria woyliei* n. sp. (A–C) and a sporulated oocyst of *Eimeria gaimardi* (D). *Abbreviations:* PG, polar granule; MP, micropyle; RB, refractile body; SB/SBB, Stieda body/sub-Stieda body; SMB, submicropyle body; SP, sporocyst; SR, sporocyst residuum. *Scale-bars:* 10  $\mu$ m

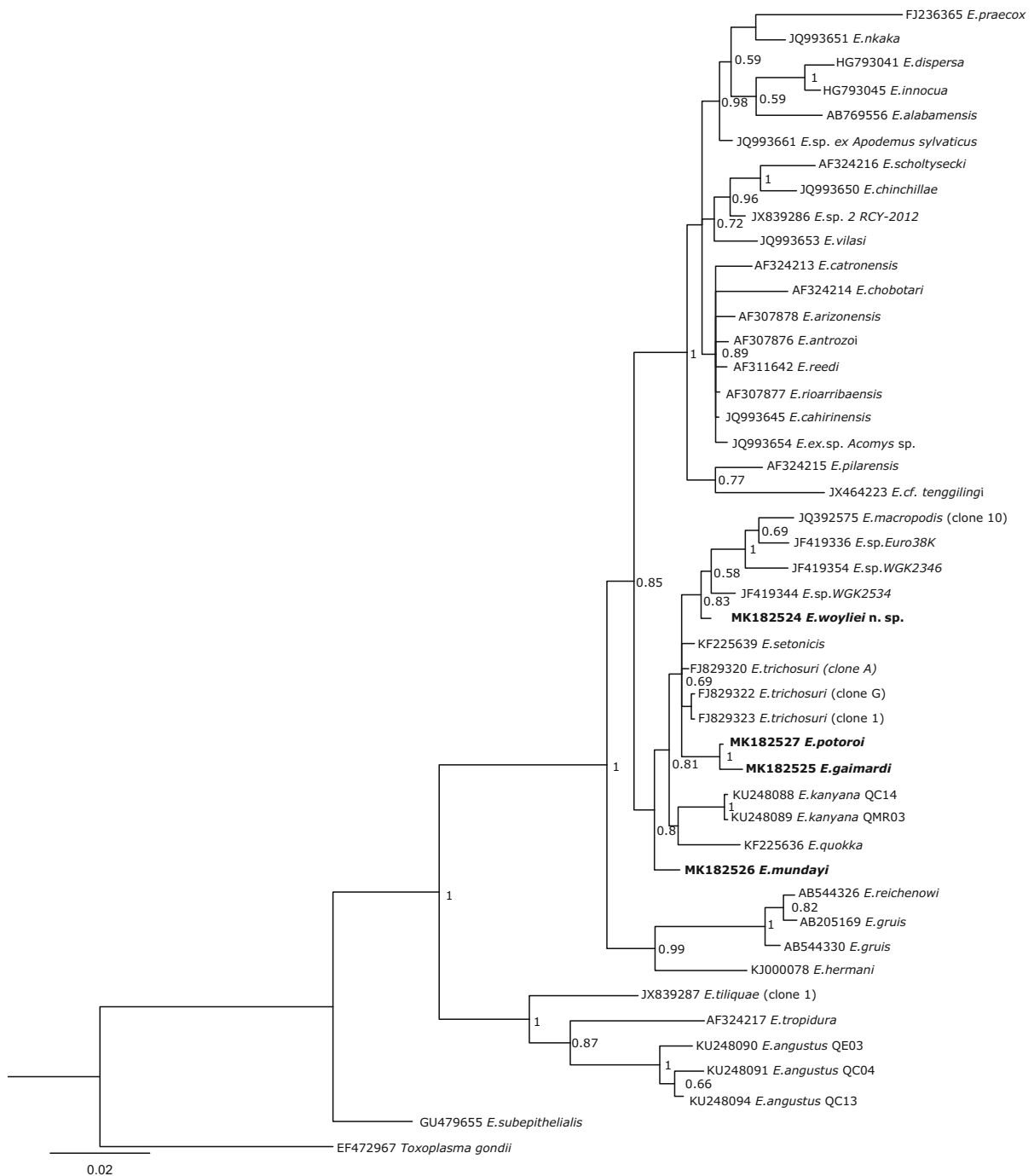
*Eimeria* spp., *E. woyliei* shared 98.8% genetic similarity with *E. mundayi* and *E. potoroi*, and 98.4% similarity with *E. gaimardi*.

#### *cox1* locus

Using the longer (686 bp) *cox1* locus, *E. woyliei* shared 97.1% similarity with *E. trichosuri* (JN192136; Ogedengbe et al., 2015), 95.7% similarity with *E. potoroi*, 95.3% similarity with *E. gaimardi* and 95.0% similarity with *E. mundayi*. Using the shorter (211 bp) *cox1* locus, *E. woyliei* shared 94.8% similarity with *Eimeria kanyana* Bennett, Woolford, O'Hara, Nicholls, Warren & Hobbs, 2006, from the quenda *Isoodon obesulus* (Shaw) (KU845563/64; Hillman, 2016), and 97.2%, 96.7% and 95.3% similarity with *E. potoroi*, *E. gaimardi* and *E. mundayi*, respectively.

#### Discussion

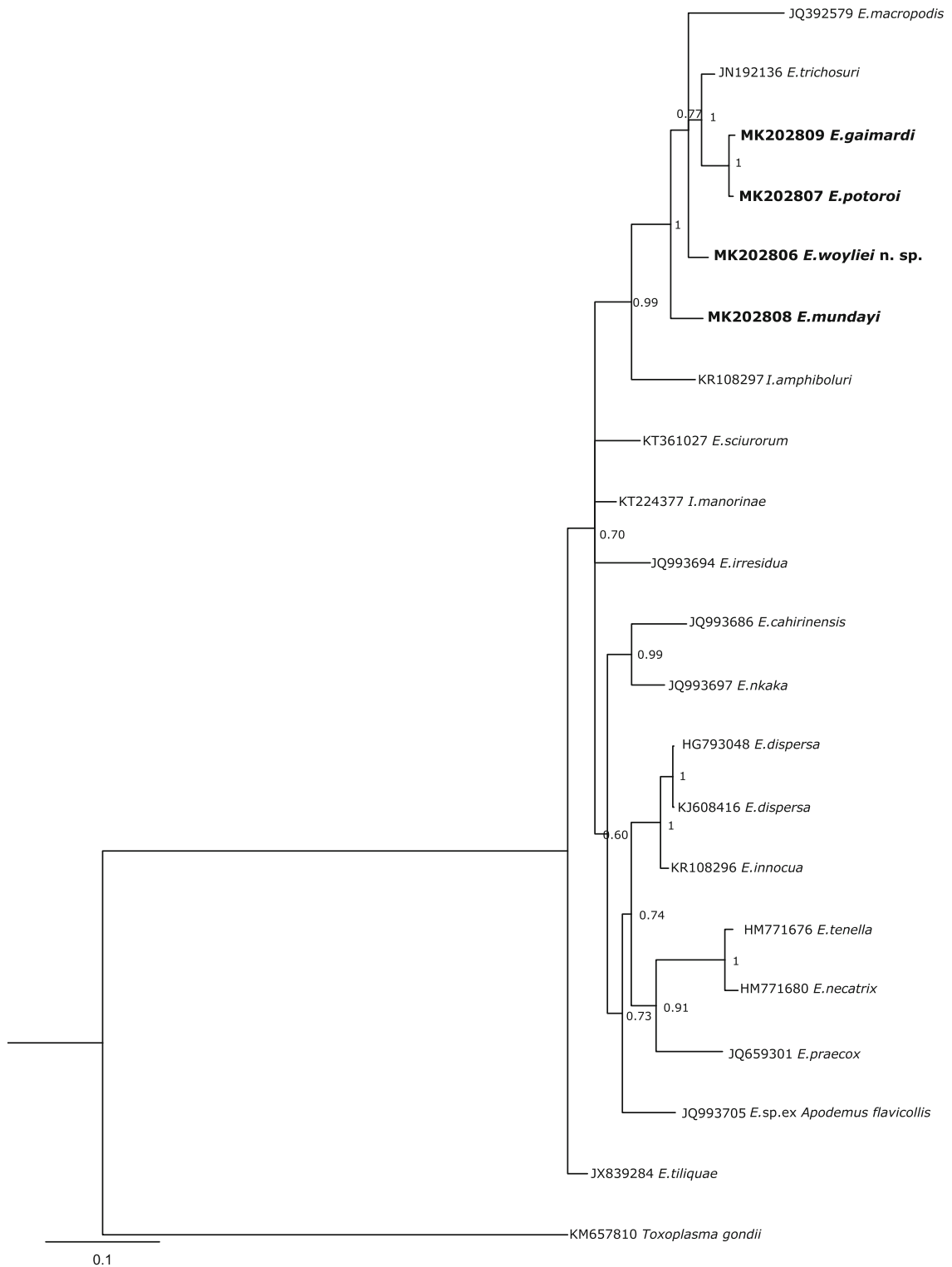
Five species of *Eimeria* have been previously described in potoroid marsupials (Barker et al., 1988; Hulst et al., 2016). Of these, *E. woyliei* oocysts most closely resemble *E. gaimardi* oocysts from the eastern bettong in shape and size (range: 31.6–40.8  $\times$  22.6–31.0 vs 32.0–39.2  $\times$  20.8–26.4  $\mu$ m; mean: 36.7  $\times$  26.3 vs 34.6  $\times$  24.3  $\mu$ m) (see Barker et al., 1988). Sporocyst size of *E. woyliei* and *E. gaimardi* is also similar (range: 13.3–17.8  $\times$  9.0–12.3 vs 13.6–16.0  $\times$  9.0–10.4  $\mu$ m; mean: 15.6  $\times$  10.3 vs 15.0  $\times$  9.6  $\mu$ m). However, oocysts and sporocysts in *E. woyliei* are slightly larger on average. *Eimeria woyliei* oocysts also contain a micropyle, submicropyle body and a polar granule; *E. gaimardi* oocysts do not according to



**Fig. 3** Phylogenetic relationships of *Eimeria woyliei* n. sp. with other *Eimeria* spp. using Bayesian analysis of an 1,158 bp fragment of the 18S rRNA gene. *Eimeria* spp. isolates obtained in this study (GenBank: MK182524–MK182527) are highlighted in bold

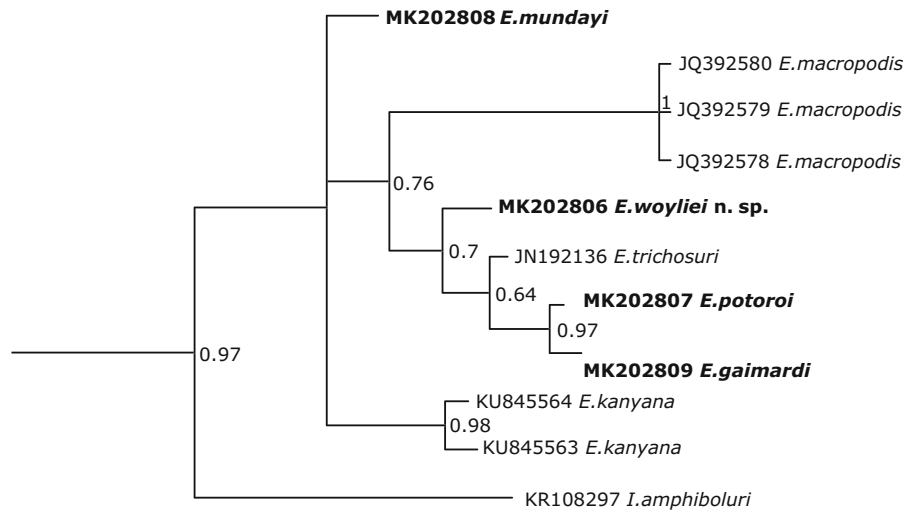
the original morphological description by Barker et al. (1988). Based on the sporulated *E. gaimardi* oöcysts examined during this study however, we propose that *E. gaimardi* oöcysts do possess a micropyle,

submicropyle body and polar granule (Fig. 2D). Thus, the major distinguishing morphological characteristic between *E. woyliei* and *E. gaimardi* is the appearance of the oöcyst wall, which for *E. gaimardi* appears



**Fig. 4** Phylogenetic relationships of *Eimeria woyliei* n. sp. with other *Eimeria* spp. using Bayesian analysis of the large 686 bp fragment of the *cox1* gene. *Eimeria* spp. isolates obtained in this study (GenBank: MK202806–MK202809) are highlighted in bold





**Fig. 5** Phylogenetic relationships of *Eimeria woyliei* n. sp. with other *Eimeria* spp. using Bayesian analysis of the smaller 211 bp fragment of the *cox1* gene. *Eimeria* spp. isolates obtained in this study (GenBank: MK202806–MK202809) are highlighted in bold

mammilated and more robust (Fig. 2D). The oöcyst wall of *E. woyliei* in comparison is smooth to slightly mammilate.

*Eimeria woyliei* oöcysts are noticeably larger than *E. potoroï* oöcysts [24.0–29.6 × 16.8–22.4 (26.2 × 18.5) µm] (Barker et al., 1988) and *E. mundayi* oöcysts [13.6–20.8 × 13.6–19.2 (16.9 × 16.2) µm] from the long-nosed potoroo (Barker et al., 1988). *Eimeria woyliei* oöcysts are comparable in size to *E. aepyprymni* oöcysts [32.0–42.8 × 18.4–25.2 (36.7 × 21.9) µm] from the rufous bettong *Aepyprymnus rufescens* (Gray) (Barker et al., 1988); however, *E. aepyprymni* oöcysts are ovoid rather than pyriform. While similar in shape (pyriform), *E. woyliei* oöcysts are considerably larger than *E. burdi* oöcysts [21.0–24.0 × 14.0–16.0 (22.6 × 14.9) µm] from the burrowing bettong *Bettongia lesueur* (Quoy & Gaimard) (Hulst et al., 2016).

Using the 18S rDNA locus, *E. woyliei* was grouped within the marsupial clade and was most similar to *E. setonicis* from the quokka. *Eimeria woyliei* oöcysts can be morphologically distinguished from oöcysts of *E. setonicis* by shape (pyriform vs ellipsoidal) (Austen et al., 2014). Despite the morphological similarity between oöcysts of *E. woyliei* and *E. gaimardi*, *E. woyliei* was more genetically similar to *E. mundayi* and *E. potoroï*, rather than *E. gaimardi*, though this difference was minimal.

Using the *cox1* locus, *E. woyliei* was most similar to *E. trichosuri* from the mountain brushtail possum and

grouped within the same clade as *E. macropodis* from the tammar wallaby *Macropus eugenii* (Desmarest) (Hill et al., 2012), and *E. kanyana* from the quenda (Hillman et al., 2016). *Eimeria woyliei* oöcysts can be differentiated from oöcysts of *E. trichosuri* by shape (pyriform vs ellipsoidal; Power et al., 2009); *E. trichosuri* oöcysts are also larger [34.4–49.2 × 18.4–27.8 (41.4 × 22.7) µm] (O’Callaghan & O’Donoghue, 2001). Unexpectedly, *Isospora amphiboluri* Canon, 1967, from the central bearded dragon *Pogona vitticeps* (Ahl) was also present within the same phylogenetic clade (GenBank: KR108297). Although *cox1* sequences are deemed superior to nuclear 18S rDNA sequences for delimiting species (Ogedengbe et al., 2011), *cox1* sequences on GenBank are limited. Nonetheless the supposed relationship between *E. woyliei* and *I. amphiboluri* remains unclear.

Within Australia various coccidial species (*Eimeria* and less commonly *Isospora*) have been recorded in captive and free-ranging macropods (Mykytowycz, 1964; Barker et al., 1989; Yang et al., 2012; Duszynski, 2016). Traditional morphological criteria have been useful for identifying coccidial species infecting wildlife. However, the implementation of genetic characterisation combining both 18S rDNA and *cox1* loci helps to discriminate between morphologically similar species and provides an accurate measure of the evolutionary relationship between coccidial species

(Power et al., 2009; Yang et al., 2012). As some *Eimeria* spp. are capable of infecting more than one marsupial host (Barker et al., 1989) and marsupials tend to harbour multiple *Eimeria* spp. (Power et al., 2009), this knowledge may be useful for predicting potential avenues of disease spread during the management of threatened populations (e.g. during fauna translocation). This study contributes toward our knowledge of *Eimeria* spp. infecting potoroid marsupials. *Eimeria woyliei* parasitising woylies is the sixth *Eimeria* spp. to be formally described from potoroid marsupials and we have genetically characterised four of the six known potoroid *Eimeria* species.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Samples from the woylie were collected under DBCA Scientific Licenses (Regulation 4: written notice of lawful authority; and 17: licence to take fauna for scientific purposes) and with approval from the Murdoch University Animal Ethics Committee (RW2659/14). Samples from the eastern bettong were collected with permission from Bonorong Wildlife Sanctuary. Samples from the long-nosed potoroo were collected under authorities and permits issued to the Department of Primary Industries, Parks, Water and Environment (DPIPWE) staff to live-trap wildlife on reserved land in Tasmania, following the Standard Operating Procedures for *Live-trapping and Handling of Wild Tasmanian Mammals 2013* by the DPIPWE.

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