Theileria gilberti n. sp. (Apicomplexa: Theileriidae) in the Gilbert’s Potoroo (Potorous gilbertii)

JEREMY Y. LEE,1 UNA M. RYAN,2 RYAN JEFFERIES,1 LINDA M. MCINNES,3 DAVID FORSHAW,1 J. ANTHONY FRIEND2 and PETER J. IRWIN1

1Division of Health Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia, and 2School of Biological Sciences, University of Bristol, Bristol BS8 1UG, United Kingdom, and 3Department of Agriculture and Food, Albany, Western Australia 6330, Australia

ABSTRACT. The morphology and genetic characterisation of a new species of piroplasm identified in the blood of the Gilbert’s potoroo (Potorous gilbertii) from the Two Peoples Bay Nature Reserve near Albany, Western Australia, is described from blood and tissue samples from 16 Gilbert’s potoroos. Microscopy of blood showed these parasites are highly pleomorphic with a mean length of 1.8 μm and mean width of 0.85 μm. Phylogenetic analysis of 18S rRNA sequence data identified the piroplasm as a new species of Theileria that is closely related to other Australian marsupial piroplasm species. Based on biological and molecular data, it is proposed that the parasite from Gilbert’s potoroos be given the name Theileria gilberti n. sp.

Key Words. Australia, haemoparasite, marsupial isolate, piroplasm, potoroo, Theileria gilberti.

The term ‘piroplasm’ is a collective name for protozoan parasites of similar phenotype that infect mammalian erythrocytes in their life cycle and encompass two main genera: Theileria and Babesia. Information on the piroplasms of native Australian wildlife is sparse and most records are confined to individual case reports and incidental findings (O’Donoghue 1997). The first official documentation of a piroplasm in a native Australian animal was made by Priestley (1915) who observed and named Theileria tachyglossi in the short-beaked echidna, a monotreme, and since then a variety of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1). Most recently three varieties of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1). Most recently three varieties of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1). Most recently three varieties of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1). Most recently three varieties of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1). Most recently three varieties of piroplasms has been recorded from 10 marsupial and eutherian species of two monotreme species in Australia (Fig. 1).

Gilbert’s potoroos (Potorous gilbertii) is a critically endangered small marsupial (Order: Diproodontia, Family: Potoroidea) that was rediscovered in November 1994 after being presumed to be extinct for at least 100 yr (Sinclair, Danks, and Wayne 1996). It is now known to inhabit only a small area of Two Peoples Bay Nature Reserve near Albany, Western Australia (WA) (Courtenay and Friend 2004). The present study describes the morphological and genetic characterisation of a small piroplasm identified in the blood of Gilbert’s potoroos and its relationship with a morphologically similar parasite of the long-nosed potoroo (Potorous tridactylus) and with other piroplasm species of the Macropodoidea. We consider that the piroplasm infecting Gilbert’s potoroos is a new species and propose the name Theileria gilberti n. sp.

MATERIALS AND METHODS

Isolates.

Gilbert’s potoroos. Sixteen blood and seven mixed tissue samples were collected from 16 trapped or deceased Gilbert’s potoroos of various ages from Two Peoples Bay Nature Reserve near Albany, WA (Table 1). Tissue samples were 1 cm³ and were preserved in 2 ml 70% ethanol while blood samples were stored in 1.3 ml potassium EDTA microtubes (SARSTEDT, Hildesheim, Germany) to prevent clumping. Tissue and blood samples were stored at room temperature and —20 °C, respectively.

Long-nosed potoroos. Liver samples were collected from five wild-caught, now deceased, long-nosed potoroos from Victoria, eastern Australia, identified previously as being piroplasm-positive by microscopy (Table 2).

Ectoparasites. Seven adult ticks, two nymphal ticks, and one adult flea, concurrently collected with blood samples from live Gilbert’s potoroos, were identified and screened for the presence of the piroplasm using polymerase chain reaction (PCR) analysis of the 18S rDNA gene (Table 3).

Microscopy. A single drop of peripheral blood obtained from the lateral caudal vein of P. gilbertii was used to make thin blood smears, which were stained with a modified Wright’s stain using an Ames Hema-Tek® slide stainer (Bayer, Leverkusen, Germany). Fifteen blood smears were made from four wild Gilbert’s potoroos (Table 1). Smears were examined for intra-erythrocytic parasites in the peripheral regions of the film and 1,000 erythrocytes were counted using a 100X objective and the number of infected cells noted for the calculation of parasitemia in each blood smear (% of 1,000 erythrocytes infected).

DNA extraction. DNA was isolated from ticks and 200 μl of whole blood using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) and QIAamp® DNA Mini Kit (Qiagen), respectively, according to the manufacturer’s instructions.

PCR amplification. A nested set of universal piroplasm primers was used to amplify an 850-bp fragment of the 18S rDNA gene as described by Jefferies, Ryan, and Irwin (2007). For the primers BTF-1 and BTR-1, 1 μl of extracted DNA was added to a 24-μl reaction mixture comprising 0.6875 U of 7th Plus DNA Polymerase (Fisher Biotech, West Perth, Australia), 200 μM of each dNTP (Fisher Biotech), 12.5 pmol of each primer (Invitrogen, Carlsbad, CA), 2.5 μl 10imes PCR buffer (Fisher Biotech), and 1.5 μl of 25 mM MgCl₂ (Fisher Biotech). Amplification was performed using a GeneAmp PCR thermal cycler (Perkin Elmer, Foster City, CA) beginning with an activation step of 94 °C for 3 min, 58 °C for 1 min, and 72 °C for 2 min followed by 45 cycles of amplification (94 °C for 30 s, 58 °C for 20 s, and 72 °C for 30 s), and a final extension phase at 72 °C for 7 min. Polymerase chain reaction reagent concentrations, and cycling conditions for BTF-2 and BTR-2 were similar to that described above except 1 μl of primary phase PCR product was used as template in the secondary reaction and the annealing temperature was increased to 62 °C. Amplified DNA was electrophoresed at 80 V for 60 min in a 1.5% agarose
gel (Promega Corporation, Madison, WI) stained with 10 mg/ml ethidium bromide (Amresco, Solon, OH).

**DNA sequencing.** DNA was sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing Kit (Applied Biosystems [ABI], Foster City, CA) according to the manufacturer’s instructions.

**DNA sequence and phylogenetic analysis.** The sequenced products were analysed using the computer program SeqEd v.1.0.3 (ABI) and were aligned using the software package CLUSTAL W (Chenna et al. 2003). Phylogenetic analyses were conducted on the sequences obtained from Gilbert’s potoroos from Western Australia, long-nosed potoroos from Victoria, ectoparasites collected from Gilbert’s potoroos, and a number of additional sequences obtained from GenBank including those of *T. brachyuri* (DQ437684, DQ437685), *T. fuliginosa* (DQ437686), and *T. penicillata* (DQ437687). Sequences obtained during this study have been deposited in GenBank under the Accession numbers EF554394 (Gilbert’s potoroo) and EF554395 (long-nosed potoroo).

**jModelTest 0.1.1** (David Posata—http://www.darwin.uvigo.es/) was used to select an appropriate evolutionary model. Distance analyses were conducted using TREECON v1.3b (Van de Peer and De Wachter 1993) using Kimura’s two-parameter distance and parsimony analysis was carried out using MEGA v2.1 (Kumar et al. 2001). *Plasmodium falciparum* (Accession number M19172) was used as an outgroup. Maximum Likelihood (ML) analyses

---

**Fig. 1.** Geographic distribution of recorded marsupial and monotreme hosts and piroplasms. 1. Northeast drainage, 2. Southeast drainage, 3. Tasmanian drainage, 4. Murray-Darling drainage, 5. South Australian drainage, 6. Southwest drainage, 7. Far west drainage, 8. Northwest drainage, 9. Carpentarian drainage, 10. Lake Eyre drainage, 11. Bulloo-Bancannia drainage, and 12. Western plateau drainage (modified from O’Donoghue and Adlard 2000). Scale bar: 500km.

---

| Symbol | Host Species | Piroplasm sp. | References |
|--------|--------------|---------------|------------|
| ☐      | *Ornithorhynchus anatinus* (Platypus) | *Theileria ornithorhynchi* | Mackerras (1958) |
| ▣      | *Tachyglossus aculeatus* (Short-beaked echidna) | *Theileria tachyglossi* | Mackerras (1959); Priestley (1915); Seddon (1952); Seddon and Albiston (1966) |
| ▣      | *Isodon obesulus* (Southern brown bandicoot) | *Theileria peramelis* | Mackerras (1959); Munday (1978, 1988) |
| ▣      | *Perameles nasuta* (Long-nosed bandicoot) | *Theileria peramelis* | Mackerras (1959) |
| ▣      | *Potorous tridentatus* (Long-nosed potoroo) | *Theileria peramelis* | Mackerras (1959) |
| ☐      | *Tachyglossus aculeatus* (Short-beaked echidna) | *Babesia tachyglossi* | Backhouse and Bolliger (1959); Ristic and Lewis (1977) |
| ☐      | *Isodon obesulus* (Southern brown bandicoot) | *Babesia thylacis* | Mackerras (1959) |
| ☐      | *Ornithorhynchus anatinus* (Platypus) | *Theileria sp.* | Mackerras (1958) |
| ☐      | *Perameles nasuta* (Long-nosed bandicoot) | *Theileria sp.* | Mackerras (1958); Munday, 1978, 1988 |
| ☐      | *Isodon macrourus* (Northern brown bandicoot) | *Theileria sp.* | Seddon and Albiston (1966) |
| ☐      | *Potorous tridentatus* (Long-nosed potoroo) | *Theileria sp.* | Mackerras et al. (1953); Mackerras (1958); Munday 1978; Speare et al. (1989) |
| ☐      | *Tachyglossus aculeatus* (Short-beaked echidna) | *Babesia sp.* | Backhouse and Bolliger (1957); Mackerras (1959) |
| ☐      | *Antechinus stuartii* (Brown antechinus) | *Babesia sp.* | Arundel, Barker and Beveridge (1977) |
| ☐      | *Petrogale perpsephone* (Proserpine rock wallaby) | *Babesia sp.* | O’Donoghue (1997) |
| ☐      | *Setonix brachyurus* (Quokka) | *Theileria brachyuri* | Clark & Spencer (2007) |
| ☐      | *Macropus fuliginosus* (Western Grey Kangaroo) | *Theileria fuliginosa* | Clark & Spencer (2007) |
| ☐      | *Bettongia penicillata* (Brush-tailed bettong) | *Theileria penicillata* | Clark & Spencer (2007) |
| ☐      | *Potorous gilbertii* (Gilbert’s potoroo) | *Theileria gilberti n. sp.* | This study |
were used to validate the phylogenetic relationship inferred by the neighbour joining (NJ) analyses. Maximum Likelihood analysis was performed by PAUP* (Version 4.0b2) using an heuristic search, conducted using the following settings: K80 model settings with two substitution types; transition/transversion ratio estimated by ML; empirical base frequencies used; starting branch lengths obtained using Rogers–Swofford method; branch-length optimisation by one dimensional Newton–Raphson with pass limit = 20; starting trees obtained by step wise addition; addition sequence = as-is; branch swapping algorithm = TBR. Bootstrap analyses for distance and parsimony methods were conducted using 1,000 replicates or 108 replicates (ML analysis) to assess the reliability of inferred tree topologies.

RESULTS

Morphology. *Theileria gilberti* n. sp. appeared as dark, basophilic bodies surrounded by pale cytoplasm with a fine limiting membrane. The organisms were highly pleomorphic and occurred mostly singly within an erythrocyte, but occasional pairs and multiple parasites were noted (Fig 2–5). The piroplasms ranged in size from 1 to 2.5 m, with a mean of 1.8 ± 0.51 m, and in width from 0.5 to 1.2 m with a mean of 0.85 ± 0.21 m (n = 100). A parasitaemia of approximately 10–15% was observed in each of the blood smears examined.

Genetic analysis. All 16 blood and seven tissue samples from the 16 Gilbert’s potoroos were positive for *T. gilberti* n. sp. by PCR. Genetic sequences from all these samples and the ectoparasites tested in this study were sequenced and showed 100% similarity to the sequences obtained from the Gilbert’s potoroo and the long-nosed potoroo is genetically very closely related to *T. penicillata*, exhibiting only 0.2% genetic distance from it (Table 4).

| Potoroo ID | Health | Origin | Sample type | Blood smears | Microscopy |
|-----------|--------|--------|-------------|--------------|------------|
| 6         | Deceased | Captive | Blood       | NA           | NA         |
| 7         | Deceased | Captive | Blood       | NA           | NA         |
| 10        | Deceased | Captive | Liver       | NA           | NA         |
| 11        | Deceased | Captive | Blood       | NA           | NA         |
| 19        | Deceased | Captive | Blood       | NA           | NA         |
| 27        | Deceased | Captive | Liver       | NA           | NA         |
| 32        | Deceased | Captive | Liver       | NA           | NA         |
| 50        | Live     | Wild trapped | Blood    | 3 P+        |            |
| 66        | Live     | Wild trapped | Blood   | NA           | NA         |
| 68        | Live     | Wild trapped | Blood   | 3 P+        |            |
| 83        | Live     | Wild trapped | Blood   | NA           | NA         |
| 89        | Live     | Wild trapped | Blood   | 3 P+        |            |
| 92        | Live     | Wild trapped | Blood   | 3 P+        |            |
| 94        | Live     | Wild trapped | Blood   | NA           | NA         |
| X         | Deceased | Wild trapped | Liver   | NA           | NA         |

NA, not available; P+, piroplasm positive.

Discussion

We have described the morphology and genetic characterisation of a new species of *Theileria*—*T. gilberti* n. sp. infecting Gilbert’s potoroo. The small size of the organism initially indicated that it was most likely a member of the order Piroplasmida, within the phylum Apicomplexa, and could be either a ‘‘small’’ *Babesia* or a species of *Theileria* or *Cytauxzoon*. It is similar in size to *T. brachyuri* (0.6–3.1 × 0.6–1.9 m) (Clark and Spencer 2007). Piroplasms are morphologically similar with sizes typically ranging from 1 to 2.5 m (Mahoney 1977) and 1 to 2 m (Mehlhorn and Shein 1984). Therefore, genetic analysis is required to more accurately identify the species. Sequence and phylogenetic analysis using distance, parsimony, and ML analysis showed that *T. gilberti* n. sp. is genetically distinct but closely related to *T. brachyuri* isolate PSC5. It has been proposed that the genetic distance at the 18S rRNA locus required for a piroplasm to be classified as a distinct species is 0.7% and 3.4% for the genera *Theileria* and *Babesia*, respectively (Schmittger et al. 2003). As the genetic distance reported here between *T. gilberti* n. sp. and *T. brachyuri* isolate PSC5 is 0.7%, this indicates that they are separate species. Similarly as the genetic distance between *T. brachyuri* isolates PSC5 and PSC12 is 3.4%, we suggest that these two isolates actually represent two genetically distinct species of *Theileria* and not the one species (*T. brachyuri*) as described by...
Clark and Spencer (2007). No discussion is made of the genetic differences between *T. brachyuri* isolates PSC5 and PSC12 by these authors although the two isolates are clearly different in the phylogenetic tree provided (Clark and Spencer 2007). Furthermore, because *T. penicillata* and our isolate from the long-nosed potoroo exhibit only 0.2% genetic distance from each other, then using the 0.7% criterion for species differentiation (Schnittger et al. 2003), the isolate from the long-nosed potoroo described here should be considered conspecific with *T. penicillata*.

We chose the 18S rRNA gene for the analyses reported here because it has been shown to be highly conserved (Appels and Honeycutt 1986; Hillis and Dixon 1991; Mindell and Honeycutt 1990). *Theileria gilberti* n. sp. and the isolates from the long-nosed potoroo reported, together with the three other *Theileria* species recently identified (Clark and Spencer 2007), represent the first genetically classified piroplasm species of Australian marsupials.

The original description of a piroplasm in the long-nosed potoroo was made by Mackerras, Mackerras, and Sanders (1953) in samples collected from the Mount Nebo area, near Brisbane, South QL. Despite its being morphologically different, this isolate was named *Theileria peramelis* because it was thought to be the same species of piroplasm that Mackerras et al. (1953) had been previously identified in the long-nosed bandicoot, *Perameles nasuta*. In order to establish the true identity of the parasite in the long-nosed potoroo, the organism would need to be genetically characterised from both the original long-nosed potoroo and long-nosed bandicoot, a procedure that is probably not possible unless original archival material still exists from the animals studied by Mackerras et al. (1953).

Microscopy and PCR were equally effective in the detection of *Theileria* in our samples. These results differ from a number of reports in which it was concluded that PCR was much more effective in detecting piroplasms than microscopy (Almeria et al. 2001; Figueroa et al. 1996; Homer et al. 2000). In situations where circulating parasite levels are extremely low it can be very difficult to visually detect piroplasms in erythrocytes. Diagnosis can be particularly problematic with the “small Babesia” and *Theileria* species. We assume that microscopy was so effective in the detection of *Theileria* infection in our study of the Gilbert’s potoroo because the marsupials had relatively high parasitaemias.

Before this report of *T. gilberti* n. sp. in Gilbert’s potoroo, piroplasm infections in native Australian mammals were documented in 11 host species: two species of monotreme—the short-beaked echidna and platypus; and nine species of marsupial—the long-nosed potoroo, Proserpine rock-wallaby, southern and northern brown bandicoots, long-nosed bandicoot, brown antechinus,

### Table 4. Genetic distances based on Kimura’s distance between marsupial-derived *Theileria* isolates.

|                     | *Theileria brachyuri* PSC5 | *Theileria brachyuri* PSC12 | *Theileria gilberti* | *Theileria penicillata* | *Theileria fuliginosa* | Long-nosed Potoroo | *Theileria bicornis* |
|---------------------|---------------------------|-----------------------------|---------------------|------------------------|-----------------------|-------------------|---------------------|
| *Theileria brachyuri* PSC5 | 0                         | 3.4                         | 0.7                 | 1.3                    | 6.2                   | 1.1               | 5.2                 |
| *Theileria brachyuri* PSC12 | 3.4                       | 0                           | 3.8                 | 2.6                    | 4.6                   | 2.4               | 6.2                 |
| *Theileria gilberti*       | 0.7                       | 0                           | 0                   | 1.7                    | 6.4                   | 4.8               | 5.2                 |
| *Theileria penicillata*    | 1.3                       | 2.6                         | 1.7                 | 0                      | 5.2                   | 1.9               | 5.0                 |
| *Theileria fuliginosa*     | 6.2                       | 4.6                         | 6.4                 | 5.2                    | 0                     | 0.2               | 5.0                 |
| Long-nosed Potoroo        | 1.1                       | 2.4                         | 1.9                 | 0.2                    | 5.0                   | 0.2               | 5.4                 |
| *Theileria bicornis*      | 5.2                       | 6.2                         | 4.8                 | 5.2                    | 7.2                   | 5.4               | 0                   |

Fig. 2–5. Infected erythrocytes from *Potorous gilbertii*. Arrows indicate the typical appearance of intraerythrocytic *Theileria gilberti* n. sp.
wowy, western grey kangaroo, and the quokka (O’Donoghue and Adlard 2000). As some native Australian mammals may be endangered, parasite–host interactions should be studied to determine the role that piroplasms might play in influencing population dynamics.

Finally, the presence of *T. gilberti* n. sp. observed in the blood from all of the Gilbert’s potoroo reported here indicates that theileriosis is possibly endemic in the species in the Two Peoples Bay area. However, its significance at the individual and population levels is unknown at the present time.

The genetic and biological data discussed above indicate that the differences between the new species of *Theileria* infecting Gilbert’s potoroo and other *Theileria* spp. are comparable to those between established species. Therefore, we recommend naming the *Theileria* from Gilbert’s potoroo *T. gilberti* n. sp. after the host in which it was found.

**Phylum** Apicomplexa  
**Order** Piroplasmida  
**Family** Theileriidae  
*Theileria gilberti* n. sp. (Fig 2–5)  
**Description.** *Theileria gilberti* n. sp. appear as dark, basophilic bodies surrounded by pale cytoplasm with a fine limiting membrane. The organisms are highly pleomorphic and occur mostly singly within an erythrocyte, but occasional pairs and multiple parasites are observed. This piroplasm ranges in length from 1 to 2.5 μm with a mean of 1.8 ± 0.51 μm, and in width from 0.5 to 1.2 μm with a mean of 0.85 ± 0.21 μm (n = 100).

**Type host.** Gilbert’s Potoroo (*Potorous gilbertii*).  

**Other hosts.** Unknown.  
**Type locality.** Two Peoples Bay Nature Reserve near Albany, WA (latitude 34.9856° S, longitude 118.1792° E).  
**Other localities.** Unknown.  
**Location in host.** Intraerythrocytic.  
**Prepatent period.** Unknown.  
**Patent period.** Unknown.  
**Etymology.** This species is named *Theileria gilberti* n. sp. to reflect its host species.

**ACKNOWLEDGMENTS**

The authors wish to thank the Department of Environment and Conservation, Albany, WA for permission to conduct this study and Mr. Russell Hobbs of Murdoch University for identifying the ectoparasites collected during the course of this study. Dr. Phil Clark provided comments on the manuscript. We also thank Dr. Peter Spencer for providing the long-nosed potoroo samples that were used in this study.

**LITERATURE CITED**

Almeria, S., Castella, J., Ferrer, D., Ortuño, A., Estrada-Pena, A. & Gutiérrez, J. F. 2001. Bovine piroplasms in Minorca (Balearic Islands, Spain): a comparison of PCR-based and light microscopy detection. *Vet. Parasitol.*, 99:249–259.

Appels, R. & Honeycutt, R. L. 1986. rDNA evolution over a billion years. *Vet. Parasitol.*, 20:24–25.

Arundel, J. H., Barker, I. K. & Beveridge, I. 1977. Diseases of marsupials. *In: Stonehouse, B. & Gillett, D. (ed.), The Biology of Marsupials*. Macmillan Press, Sydney. p. 141–154.

Backhouse, T. C. & Bolliger, A. 1957. A piroplasm of the echidna (*Tachyglossus aculeatus*). *Aust. J. Sci.*, 20:320–322.

Clark, P. & Spencer, P. B. S. 2007. Description of three new species of *Theileria* Bettencourt, Franca & Borges, 1907 from Macropodoidea in Western Australia. *Trans. R. Soc. South Aust.*, 131:100–106.

Coutenay, J. & Friend, J. A. 2004. Gilbert’s Potoroo Recovery Plan. Department of Conservation and Land Management, Perth, WA.

Figueroa, J. V., Alvarez, J. A., Canto, G. J., Ramos, J. A., Mosqueda, J. J. & Buening, G. M. 1996. Comparative sensitivity of two tests for the diagnosis of multiple hemoparasite infection of cattle. *Ann. NY Acad. Sci.*, 791:117–127.

Hillis, D. M. & Dixon, M. T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quart. Rev. Biol.*, 66:411–453.

Homer, M. J., Aguilar-Delfin, I., Telford III, S. R., Krause, P. J. & Persing, D. H. 2000. Babesiosis. *Clin. Micro. Rev.*, 13:451–469.

Jefferys, R., Ryan, U. M. & Irwin, P. J. 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper-based technologies. *Vet. Parasitol.*, 144:20–27.

Maier, D. P. & Honeycutt, R. L. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. *Annu. Rev. Ecol. Syst.*, 21:541–566.
Munday, B. L. 1978. Marsupial diseases. In: Fauna Part B. Post Graduate Committee in Veterinary Science: University of Sydney, p. 335–385.

Munday, B. L. 1988. Marsupial diseases. In: The John Keep Refresher Course for Veterinarians. Post Graduate Committee in Veterinary Science: University of Sydney, p. 299–365.

O’Donoghue, P. J. 1997. Protozoan parasites of wildlife in south-east Queensland. In: Tribe, A. (ed.), Proceedings of the 1997 Conference of the Australian Association of Veterinary Conservation Biologists. Australian Veterinary Association, Brisbane. p. 119–136.

O’Donoghue, P. J. & Adlard, R. D. 2000. Catalogue of protozoan parasites recorded in Australia. Mem. Qld. Museum, 45:1–163.

Priestley, H. 1915. *Theileria tachyglossi* (n. sp.). A blood parasite of *Tachyglossus aculeatus*. Ann. Trop. Med. Parasitol., 9:233–238.

Ristic, M. & Lewis, G. E. 1977. *Babesia* in man and wild and laboratory-adapted mammals. In: Kreier, J. P. (ed.), Parasitic Protozoa. IV. Academic Press, New York. p. 53–76.

Schnittger, L., Yin, H., Gubbels, M. J., Beyer, D., Niemann, S., Jongejan, F. & Ahmed, J. S. 2003. Phylogeny of sheep and goat *Theileria* and *Babesia* parasites. Parasitol. Res., 91:398–406.

Seddon, H. R. 1952. Diseases of Domestic Animals in Australia. Part 4. Protozoan and Viral Diseases. Department of Health Services, Publisher, Division, Vet. Hyg. No. 8, Canberra.

Seddon, H. R. & Albiston, H. E. 1966. Diseases of Domestic Animals in Australia. Part 4. Protozoan and Viral Diseases. 2nd ed. Department of Health. Services, Publisher,Division, Vet. Hyg. No. 8, Canberra, p. 34.

Sinclair, E. A., Dunks, A. & Wayne, A. F. 1996. Rediscovery of Gilbert’s potoroo, *Potorous tridactylus*, in Western Australia. Aust. Mamm., 19:69–72.

Speare, R., Donovan, J. A., Thomas, A. D. & Speare, P. J. 1989. Diseases of free-ranging Macropodoidea. In: Grigg, G., Jarman, P. & Hume, I. (ed.), Kangaroos, Wallabies and Rat-Kangaroos. Surrey Beatty & Sons, Sydney. p. 705–734.

Van de Peer, Y. & De Wachter, R. 1993. TREECON: a software package for the construction and drawing of evolutionary trees. Comput. Appl. Biosci., 9:177–182.