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Morphological and molecular characterization of *Eimeria purpureicephali* n. sp. (Apicomplexa:Eimeriidae) in a red-capped parrot (*Purpureicephalus spurius*, Kuhl, 1820) in Western Australia

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ABSTRACT

A new *Eimeria* species is described from a red-capped parrot (*Purpureicephalus spurius*). Sporulated oocysts (n = 31) were spherical to subspherical, with a rough bilayered oocyst wall 0.8 μm thick. Oocysts measured 24.0 × 22.8 (20.4–26.4 × 18.3–25.9) μm, oocyst length/width ratio, 1.10. Oocyst residuum, polar granule and micropyle were absent. Sporocysts are elongate-ovoid, 11.0 × 7.3 (12.7–9.2 × 7.9–6.6) μm, sporocyst length/width ratio, 1.51 (1.33–1.71). The thin convex Stieda body and indistinct substieda bodies were present and the sporocyst residuum was composed of numerous small granules less than 1.0 μm in diameter dispersed randomly. Each sporocyst contained 2 sausage-shaped sporozoites in head-to-tail arrangement. The sporozoite nuclei were located centrally surrounded by refractile bodies. Molecular analysis was conducted at two loci; the 18S ribosomal RNA gene and the cytochrome c oxidase subunit I gene. At the18S locus, the new isolate shared 99.0% genetic similarity with *Eimeria dispersa* and *Eimeria innocua* from the turkey. At the cytochrome c oxidase subunit I gene locus, this new isolate was most closely related to *E. dispersa and E. innocua*, presented 99.0% and 98.0% genetic similarity, respectively. This new isolate and *E. dispersa* grouped together in the same clade. Based on the morphological and molecular data, this isolate is a new species of coccidian parasite, which is named *Eimeria purpureicephali* n. sp. after its host, the red-capped parrot (*Purpureicephalus spurius*).

1. Introduction

The red-capped parrot (*Purpureicephalus spurius*), also called the pileated parakeet (Alderton, 2003) and king parrot locally in Western Australia (Lendon, 1973), is an Australian species of broad-tailed parrot which is related to the rosellas. The colourful red-capped parrot has a specialized long beak, which helps them to remove seeds from gumnuts of marri (*Eucalyptus calophylla*) as well as seeds from other eucalypts and native plants. These parrots live in eucalypt forests, woodlands, timbered watercourses, parks, orchards and gardens. Red-capped parrots are endemic to the south west of Western Australia. (Pizzey and Knight, 2007).

Eimeria (Coccidia: Eimeriidae), is a genus of apicomplexan parasites that includes various species and is known as the enteric monoxenous coccidian parasite. In birds, pathogenic *Eimeria* causes enteric disease and major economic losses in the global poultry industry (McDougald and Reid, 1997). *Eimeria* usually invade the intestinal tract, but some invade other organs, such as the liver and kidney. In recent years, more *Eimeria* species have been identified from free-range birds globally (Hofstatter and Guaraldo, 2011; Yang et al., 2014).

A total of four *Eimeria* species have been identified and recorded in the coccidian database (Duszynski et al., 2000) from the family Psittaciformes including *E. aratinga* (Upton and Wright, 1994), *Eimeria dunsingi* (Farr, 1960), *Eimeria haematodi* (Varghese, 1977) and *E. psittacina* (Gottschalk, 1972). Recently, a new *Eimeria* species, *Eimeria ararae* n. sp., from the blue-and-yellow macaw *Ara ararauna* (Linnaeus) in Brazil was added in the family Psittaciformes. With the exception of *E. haematodi*, which was molecularly characterized by Yang et al. (2015), the other four *Eimeria* species were identified by their oocyst morphological features only. To date there have been no reported cases of *Eimeria* species identified from the red-capped parrot (*Purpureicephalus spurius*, Kuhl, 1820). This is the first study to characterize *Eimeria purpureicephali* n. sp. in a red-

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capped parrot in Western Australia, using both morphological data and molecular techniques.

2. Materials and methods

2.1. Sample collection and examination

A juvenile red-capped parrot came into care at the Kanyana Wildlife Rehabilitation Centre (KWRC), Perth in November, 2014. On admission it was observed that this bird had labored breathing. Radiographs revealed multiple fractures of the keel bone and congestion of the air sacs. Reduced breast muscle mass was also noted. No clinical signs of coccidiosis were observed. Treatment was implemented but a decision was made to euthanize the bird a few days later. A faecal sample was taken soon after admission to look for evidence of avian gastric yeast (AGY). Microscopic examination of the faeces found no AGY in the sample, however unsporulated coccidian oocysts were seen.

Faecal flotation was conducted using a saturated sodium chloride and 50% sucrose (w/v) solution. A portion of faeces was placed in 2% (w/v) potassium dichromate solution ($K_2Cr_2O_7$), mixed well and poured into petri dishes to a depth of less than 1 cm and kept at room temperature in the dark to facilitate sporulation. Sporulated oocysts were observed using an Olympus DP71 digital micro-imaging camera and images were taken using Nomarski contrast with a 100× oil immersion objective. Faecal samples from another 23 red-capped parrots were screened for AGY (by wet mount) during the period January to December 2014. None of these 23 samples were found to be positive for coccidia.

A 3 axis hydraulic micromanipulator (MO-102, Nirashige, Japan) was used to isolate four separate single oocysts for DNA extraction and PCR.

2.2. DNA isolation

Oocyst DNA extraction was as described by Yang et al. (2014). Briefly, isolated single oocysts were placed on a slide and checked under the microscope (Olympus DP71 digital micro-imaging camera). Once the existence of a single oocyst on the cover slip was confirmed, photographs were recorded for morphological identification. The coverslip was then transferred into a PCR tube containing 10 μl of lysis buffer (0.005% SDS in TE solution). After a brief centrifugation, the tube was frozen in liquid nitrogen and thawed in a 95 °C water bath for four rounds to disrupt the oocyst wall. After the addition of 0.5 μl proteinase K (20 mM), the tube was incubated at 56 °C for 2 h and then at 95 °C for 15 min. The entire lysate from the single oocyst was used for three separate PCRs as described below.

2.3. PCR amplification and sequencing

A nested PCR with the primers EiGTF1 and EIGTR1 was used for the external amplification of the 18S rRNA gene. The expected PCR product was ~1510 bp. The primers EiGTF2 and EiGTR2 (Yang et al., 2015) were used for the internal reaction.

A partial COI gene sequence (723 bp) was amplified using a nested PCR with the following primers COIF1 (Ogedengbe et al., 2011) and COXR1 (Dolnik et al., 2009) for the external reaction and COIF2 (Yang et al., 2013a) and COXR2 (Dolnik et al., 2009) for the internal reaction.

The amplicons from the second round PCRs were gel purified using an in house filter tip method as previously described (Yang et al., 2013b). All the PCR products were sequenced using forward and reverse primers in duplicate using amplicons from different PCR runs. An ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) was used for Sanger sequencing according to the manufacturer's instructions.

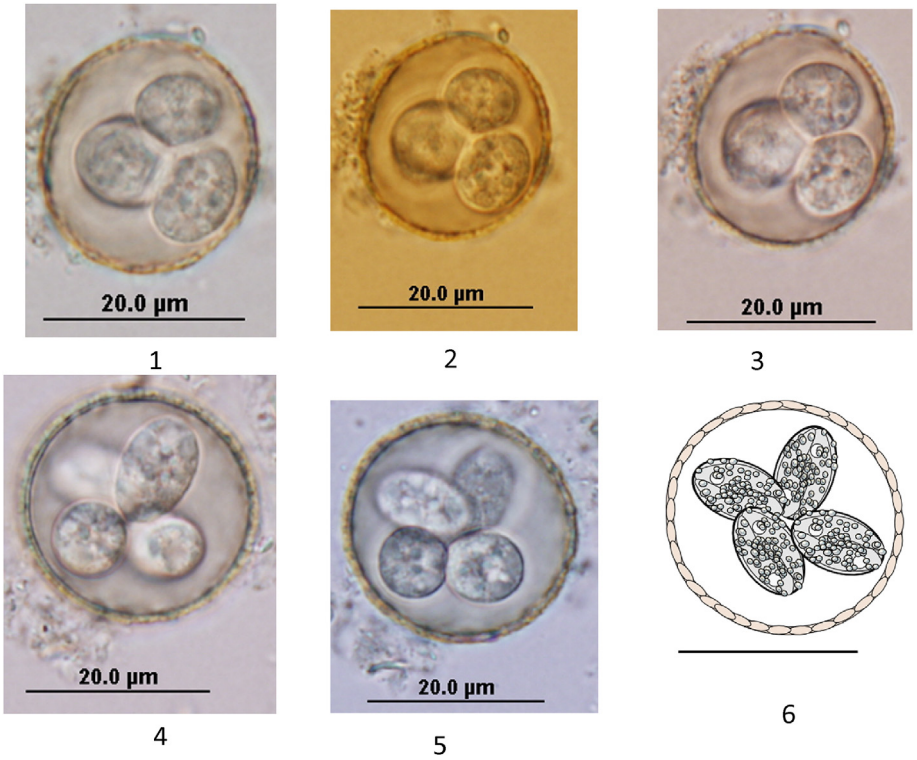

Fig. 1. Nomarski interference-contrast photomicrographs of *E. purpureicephali* n. sp. oocysts showing spheroidal to subspheroidal sporocysts (scale bar = 20 μm) (1–5) and line drawing of the sporulated oocyst of *E. purpureicephali* n. sp. Scale bar = 20 μm (6).