Eimeria spp. and Tyzzeria perniciosa Allen, 1936 (Apicomplexa: Eimeriidae) from a Pacific black duck, Anas superciliosa Gmelin (Aves: Anseriformes), in western Australia

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A B S T R A C T

Four species of the Eimeriidae, Eimeria anatis Scholtyseck, 1955, Eimeria aythyae Farr, 1965, Eimeria krylovi Svanbaev & Rakhmatullina, 1967 and Tyzzeria perniciosa Allen, 1936, were morphologically identified from oocysts recovered from a Pacific black duck, Anas superciliosa Gmelin. Additionally, genotypic characterization of E. anatis is provided via sequencing of the mitochondrial cytochrome c oxidase subunit 1 (cox1) and the small subunit ribosomal RNA (18S) genes. The four species are redescribed, providing additional morphological details. The validity of genera and coccidian species parasitizing birds of the order Anseriformes such as Wenyenola Hoare, 1933 and some Tyzzeria spp. are discussed. Molecular phylogenetic analyses for the cox1 and 18S rRNA genes resulted in monophilies of Eimeria spp. from Anseriformes which included the sequences obtained from E. anatis oocysts.

1. Introduction

The Pacific black duck Anas superciliosa Gmelin (Anseriformes: Anatidae) is a dabbling duck commonly seen in waterways, swamps, streams and ponds in Australia, New Zealand, Indonesia through to Papua New Guinea, Polynesia, the islands of the West Pacific and the sub-Antarctic islands (Pizzey and Knight, 2007).

Both domestic and wild ducks are commonly infected with gastrointestinal parasites including coccidia, which are obligate intracellular protozoans of the Apicomplexa (Gajadhar et al., 1983). Species of Eimeria Schneider, 1875 (Eimeriidae) are the most common coccidia found in birds, including ducks, with mixed infections being common (Leibovitz, 1968). Eimeria anatis Scholtyseck, 1955 infects the mallard Anas platyrhynchos (L.) (Duszynski et al., 2001) and Eimeria aythyae Farr, 1965 infects the lesser scaup Aythya affinis (Etyon) (Gajadhar et al., 1983; Duszynski et al., 2001). Windingstad et al. (1980) reported recurring epizootic infection in A. affinis resulting from infection with E. aythyae. The host range of Eimeria krylovi Svanbaev & Rakhmatullina, 1967 includes the green-winged teal Anas carolinensis Gmelin, the northern shoveler Spatula clypeata (L.), the European wigeon Mareca penelope (L.), the gadwall Mareca strepera (L.) and the garganey Spatula querquedula (L.) (Svanbaev & Rakhmatullina, 1967).

Coccidia of the genus Tyzzeria Allen, 1936, have also been described predominantly from ducks (Gajadhar et al., 1983). This genus is made up of coccidia whose oocysts lack sporocysts (Duszynski et al., 1998). Cole & Friend (1999) reported that Tyzzeria spp. were less commonly seen in ducks than Eimeria spp. Tyzzeria perniciosa Allen, 1936 is an important pathogenic coccidian in ducks and is especially pathogenic for ducklings (Baker, 2007). Reported duck hosts of T. perniciosa are the northern pintail Anas acuta L., the lesser scaup A. affinis, the common shelduck Tadorna tadorna (L.), the tufted duck Aythya fuligula (L.), the mallard A. platyrhynchos and the white-headed duck Oxyura leucocephala (Scopoli) (Duszynski et al., 1998).

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A study from China reported outbreaks of coccidiosis due to *T. verrucosa* and *Wenyonella philippinevi* Leibovitz, 1968, amongst farmed ducklings (Peiyun et al., 1982). A study in Iraq detected *E. anatis* in 17% and *T. verrucosa* in 11% of domesticated ducks screened (n = 80) (Abdullah, 2010) while another study on domestic ducks in Iran found a variety of protozoan parasites including Cryptosporidium spp., *Tyzzeria* spp., *W. philippinevi*, *Isospora mandari* Bhatia, Chauhan, Arora & Agrawal, 1971 as well as other coccidian species (Larki et al., 2018). The coccidia infecting ducks are similar in size and have very similar morphologies. This makes identification difficult using morphology alone (Leibovitz, 1968; Gajadhar et al., 1983). Those coccidia infecting wild ducks have not been well studied. In this study, we morphologically identified *E. anatis*, *E. aythyae*, *E. krylovi* and *T. verrucosa* from a Pacific black duck. Additionally, we provided genotypic characterization via sequencing of the mitochondrial cytochrome c oxidase subunit 1 (cox1) and the small subunit ribosomal RNA (18S) genes for *E. anatis*.

2. Materials and methods

2.1. Sample collection and examination

A wild, juvenile Pacific black duck was admitted to the Kanyana Wildlife Rehabilitation Centre (KWRC), Perth, Australia, in January 2021, after it was struck by a motor vehicle. Physical examination on admission revealed no external injuries; however, the duck was extremely quiet, reluctant to walk and was leaning to one side. It had a body condition score of 2/5. The duck was given supportive treatment of fluids and pain relief medication before being sent to a veterinarian for further assessment. A preliminary diagnosis of concussion and possible internal injuries was made. A faecal sample was collected on admission to KWRC. Initial direct light microscopy revealed a heavy, mixed parasitic load including large numbers of unsporulated coccidian oocysts of various sizes as well as trophozoites of *Trichomonas* Donné, 1836, eggs of *Capillaria* Zeder, 1800 and tapeworm eggs. The duck was treated for the worm infection with praziquantel and moxidectin (20 mg/kg and 1 mg/kg of each ingredient respectively), per os (PO), once daily (OD), which was repeated after 14 days. The coccidia were treated with toltrazuril (15 mg/kg, PO, OD) for three consecutive days and then again 7 days later. Metronidazole (50 mg/kg, PO, OD) was given for 7 days for the *Trichomonas* infection. The duck made a full recovery and was released near the found location 4 weeks later.

A portion of faeces was placed in 2% (w/v) K₂Cr₂O₇, mixed well and placed in a refrigerator, until transport to Murdoch University (within 48 h) for further investigation. On arrival at the Murdoch University laboratory, the faecal sample was poured into a Petri dish (to a depth of less than 1 cm). The Petri dish was stored in a dark environment and kept at room temperature (22 °C), to facilitate sporulation. The sample was checked daily for oocyst sporulation using an Olympus DP71 digital microimaging camera. Sporulated oocysts were observed using the 100× oil immersion objective. Images were taken using Nomarski contrast with a 100× oil immersion objective. Line drawings were edited using two software applications of CorelDRAW® (Corel Draw Graphics Suite, Version, 2020; Corel Corporation, Canada), i.e. Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

2.2. Oocyst isolation, DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Five morphologically similar oocysts were isolated for a bulk DNA extraction with the method described by Yang et al. (2015). The DNA extraction, PCR amplification of the 18S rDNA and cox1 genes and sequencing were conducted according to the protocols described by Yang et al. (2013, 2016).

Phylogenetic trees were constructed for *E. anatis* using partial 18S rDNA and partial cox1 sequences aligned with additional species/isolates from GenBank using ClustalW (http://www.phylogeny.fr/one_task.cgi?task_type=clustalw). Distance analyses and phylogenies were conducted using MEGA X (Kumar et al., 2018) as described in detail by Yang et al. (2021) with the most appropriate nucleotide substitution models (TN93 + G + I for 18S and TN93 + G for the cox1 gene). Bootstrap support was estimated from 1000 pseudoreplicates.

3. Results

Based on the morphological analysis of the coccidian oocysts in the faecal sample, four species were identified: *E. anatis*, *E. aythyae*, *E. krylovi* and *T. verrucosa*. The newly collected material is described below.

3.1. *Eimeria anatis* Scholtyseck, 1955

[Description based on 20 oocysts and 40 sporocysts; Fig. 1.] Oocysts ellipsoidal, 17–19 × 11–13 (17.6 × 11.9); length/width (L/W) ratio 1.4–1.6 (1.5). Oocyst wall bi-layered, 0.9–1.3 (1.0) thick; outer layer smooth to slightly rough, c.2/3 of total thickness. Micropyle cap absent. Micropyle present, generally with invagination of inner layer. Oocyst residuum absent, but 1–2 polar granules present. Sporocysts 4, ellipsoidal, 7–9 × 5–6 (7.9 × 5.9); L/W ratio 1.3–1.4 (1.3). Stieda body present, flattened; sub-Stieda absent or indiscernible; para-Stieda body absent. Sporocyst residuum present, composed of small, randomly dispersed granules. Sporozoites 2, with robust anterior and posterior refractile bodies and indiscernible nucleus.

3.2. *Eimeria aythyae* Farr, 1965

[Description based on 20 oocysts and 40 sporocysts; Fig. 2.] Oocysts ellipsoidal, 20–23 × 14–16 (21.3 × 15.2); L/W ratio 1.3–1.5 (1.4). Oocyst wall bi-layered, 1.0–1.4 (1.2) thick; outer layer smooth, c.2/3 of total thickness. Micropyle cap present as a translucent, delicate, curved protrusion. Micropyle present with no invagination of inner layer. Oocyst residuum and polar granule absent. Sporocysts 4, ellipsoidal, 9–11 × 7–8 (10.5 × 7.7); L/W ratio 1.2–1.4 (1.4). Stieda body present, flattened; sub-Stieda present, but delicate or indiscernible in some sporocysts; para-Stieda body absent. Sporocyst residuum present, composed of large, randomly dispersed granules. Sporozoites 2, with robust anterior and posterior refractile bodies and centrally located nucleus.

3.3. *Eimeria krylovi* Svanbaev & Rakhmatullina, 1967

[Description based on 25 oocysts and 50 sporocysts; Fig. 3.] Oocysts ellipsoidal, 20–23 × 16–17 (21.7 × 16.1); L/W ratio 1.3–1.4 (1.3). Oocyst wall bi-layered, 1.0–1.4 (1.2) thick; outer layer smooth, c.2/3 of total thickness. Micropyle cap present as a dense cover. Micropyle present with no invagination of inner layer. Oocyst residuum and polar granule absent. Sporocysts 4, sub spherical to ellipsoidal, 8–10 × 7–8 (8.8 × 7.7); L/W ratio 1.1–1.2 (1.1). Stieda body flattened, barely or not discernible; sub-Stieda absent; para-Stieda body absent. Sporocyst residuum present, composed of many large and dense granules which are widely diffused within the sporocyst. Sporozoites 2, with anterior and posterior refractile bodies and indiscernible nucleus.

3.4. *Tyzzeria verrucosa* Allen, 1936

[Description based on 25 oocysts; Fig. 4.] Oocysts ellipsoidal, 10–11 × 7–8 (10.7 × 7.4); L/W ratio 1.4–1.5 (1.4). Oocyst wall bi-layered, 0.4–0.7 (0.6) thick; outer layer smooth, c.2/3 of total wall
Fig. 1 Composite line drawing (A) and photomicrographs (B, C) of sporulated oocysts of *Eimeria anatis* from the Pacific black duck *Anas superciliosa*. Note the anterior (arb) and posterior (prb) refractile bodies; micropyle (m); polar granule (pg); rough oocyst wall (row); Stieda body (sb); and sporocyst residuum (sr). Scale-bars: 10 μm.

Fig. 2 Composite line drawing (A) and photomicrographs (B, C) of sporulated oocysts of *Eimeria aythyae* from the Pacific black duck *Anas superciliosa*. Note the micropyre (m); micropyre cap (mc); nucleus (n); Stieda body (sb); sporocyst residuum (sr); and refractile body (rb). Scale-bars: 10 μm.
Fig. 3 Composite line drawing (A) and photomicrographs (B, C) of sporulated oocysts of *Eimeria krylovi* from the Pacific black duck *Anas superciliosa*. Note the micropyle (m); micropyle cap (mc); sporocyst residuum (sr); and refractile body (rb). Scale-bars: 10 μm.

Fig. 4 Composite line drawing (A) and photomicrographs (B, C) of sporulated oocysts of *Tyzzeria perniciosa* from the Pacific black duck *Anas superciliosa*. Note the inner (il) and outer (ol) layers of the oocyst wall; oocyst residuum (or); refractile body (rb); and sporozoite (sz). Scale-bars: 10 μm.
thickness. Oocyst residuum present as granules of different sizes usually clustered at one end of oocyst, measuring 2.5. Sporozoites curved and tapered at anterior end, measuring 6.9/2.5-1.8, with robust, prominent posterior refractile body and without discernible nucleus.

3.5. Molecular identification

PCR amplification for the 18S rRNA and cox1 genes from oocyst DNA of the four coccidian species were conducted; unfortunately, PCR amplicons were successfully obtained only for E. anatis oocysts.

3.5.1. Phylogenetic analyses of the 18S rRNA gene

A 1209 bp 18S rDNA sequence with clean sequencing chromatography was obtained from the five morphological similar oocysts of E. anatis isolated from the faecal samples of Anas superciliosa; this was aligned with 37 sequences for Eimeria spp., 5 for Cyclospora spp. and 2 for Isospora spp. based on the NCBI BLAST similarities. The alignment covered all available Eimeria spp. sequences. A 18S rRNA gene sequence (GenBank: L24381) of Toxoplasma gondii was used as the outgroup. Eimeria anatis showed 97.6% and 96.6% similarity with Eimeria stigmosa Klimes, 1963 (GenBank: KP789181) and Eimeria anseris Kotlan, 1932 (GenBank: KJ000077), respectively, both of which were obtained from Anser anser (L.) in China (sequences published in GenBank only). Eimeria anatis also shared a genetic similarity of 94.6% with both Eimeria gruis Yakimoff & Matschoulsky, 1935 (GenBank: AB544336) and Eimeria reichenowi Yakimoff & Matschoulsky, 1935 (GenBank: AB544314), both identified from Grus monacha Temminck in Japan and reported by the same group (Honma et al., 2011). In addition, E. anatis shared 93.0% similarity with Eimeria paludosa (Leger & Hesse, 1922) (GenBank: KJ761877) from Gallinula tenebrosa Gould in Western Australia (Yang et al., 2014). As shown in Fig. 5A, E. anatis was placed in a separate strongly supported clade with E. stigmosa and E. anseris, closely associated with a sister clade composed of E. gruis, E. reichenowi and E. paludosa.

Eimeria anatis is often related to the coccidian species W. philiplevini; however, there is no 18S DNA sequence from W. philiplevini available, only a 422-bp 18S sequence presented in the paper by Wu et al. (2013). The 18S sub-tree generated from a shortened alignment including both E. anatis and W. philiplevini showed that E. anatis belongs to the same clade as that of the 18S phylogenetic tree based on the long alignment (Fig. 5A), whereas W. philiplevini was positioned close to T. gondii, outside of the Eimeria spp. clades (Fig. 5B). The genetic similarity between E. anatis and W. philiplevini was 86.5%.

3.5.2. Phylogenetic analyses of the cox1 gene

The cox1 gene was amplified from E. anatis oocyst DNA and a 650-bp sequence was successfully obtained and aligned with 21 sequences for Eimeria spp. from different animal species, 4 for Isospora spp. and one for Caryospora sp. All cox1 reference sequences were selected based on the NCBI BLAST similarities and covered all Eimeria spp. in the database. A sequence for T. gondii (GenBank: HM771690) was used as the outgroup. Eimeria anatis showed the highest genetic similarity (91.9%) with an unnamed Eimeria sp. isolated from the pink-footed goose Anser brachyrhynchus Baillon (GenBank: MT833388) (Myšková et al., 2021), and grouped with this Eimeria sp. in the same clade in the phylogenetic tree (Fig. 6).

Fig. 5 Evolutionary relationships of Eimeria anatis inferred by maximum likelihood analysis (ML) of 18S rDNA sequences (A, alignment length 1209 bp; B, alignment length 424 bp). Percentage support (> 70%) from 1000 pseudoreplicates from the ML analysis is indicated at the nodes.
4. Discussion

_Eimeria anatis_, _E. aythya_, _E. krylovi_ and _T. perniciosa_ have all been previously reported to infect ducks. The Pacific black duck in this study was found to be infected with all three of these _Eimeria_ spp. simultaneously as well as _T. perniciosa_. This is not unusual as co-infections with _Eimeria_ spp. are regularly observed in birds.

The oocysts of the three _Eimeria_ spp. identified in this study were morphologically compatible with their respective original descriptions (Tables 1 and 2). However, it is noteworthy that in the present study some adjustments for some characteristic features were added to the descriptions of these species. For example, a Stieda body and a sub-Stieda body were observed in _E. anatis_ and _E. aythya_, respectively, which were not identified in the original descriptions (Scholtyseck, 1955; Farr, 1965) or in later reports (Gajadhar et al., 1983). _Eimeria krylovi_ was not originally described with a Stieda body, and indeed this structure was hardly observed and photomicrographed in this study, being reported here as “barely or not discernible”. In this context, it is important to highlight that a Stieda body is a synapomorphy facilitating and enabling reliable identification of these species in further studies.

Morphologically, _E. anatis_ is easily confused with _W. philipleviini_ due to the difficulty of distinguishing the sporocyst residuum and the number of sporozoites in their sporocysts. In this context, Duszynski et al. (2000) considered that the descriptions, photomicrographs and line drawings of _Wenyonella_ spp. were inadequate; additionally, many species have been described and named from degenerate oocysts. Thus, Duszynski et al. (2000) suggested that all species identified as _Wenyonella_ should be viewed dubiously and considered _species inquirendae_. Specifically for _W. philipleviini_, Duszynski et al. (2000) considered that both the line drawing and photomicrograph suggest that the refractile bodies and/or sporozoites were all confused in the original description of Leibovitz (1968) and other studies reviewed by Gajadhar et al. (1983). In the oocysts identified as _E. anatis_ in the present study, two sporozoites were clearly observed with their anterior and posterior refractile bodies in each sporocyst, justifying that the material belongs to the genus _Eimeria_. Furthermore, the results of the phylogenetic analysis including the newly generated 18S sequence for _E. anatis_ showed its inclusion into a clade of _Eimeria_ spp. from ducks, while being distant from the only partial 18S sequence from oocysts identified as _W. philipleviini_ by Wu et al. (2013).

_Tyzeria perniciosa_ is the type-species of the genus _Tyzeria_, which consists of coccidia with oocysts containing eight sporozoites without sporozoites. All consensually valid species are recorded from birds of the order Anseriformes. Descriptions in hosts of other vertebrate classes were published, although they must be misidentified. As this species was described after _E. anatis_, it is a junior synonym of _Anser albinus_. 

Similarly, _Tyzzeria chalcides_ (Gajadhar et al., 2007) described from the red-tailed boa _Boa constrictor_ L. and _Tyzeria chalcides_ Prohert, Roberts & Wilson, 1988 was described from the ocellated skink _Chalcides ocellatus_ (Forskål), which potentially represent a species of _Klossiella_ and a species of _Choleoeimeria_, respectively misidentified from oocysts that sporulated abnormally (Duszynski et al., 1998).

The most frequently reported species in the literature are _T. perniciosa_ from teals, mallards and other ducks (Anatinae) and _Tyzzeria parvula_ (Kotlan, 1933) from geese (Anserinae) (Berto et al., 2007). Although the oocysts of these species are morphologically very similar, they are specialised for parasitism at the subfamily level, i.e. _T. parvula_ does not infect teals, mallards and ducks, just as _T. perniciosa_ does not infect geese, even in experimental infections (Berto et al., 2007).

In addition to _T. perniciosa_, _Tyzzeria pellerdyi_ Bhatia & Pande, 1966 was described and reported from _Anas_ spp. in some studies in the 1960s, 1970s and 1980s. However, there is no morphological or biological differentiation that so far justifies and fundamentally defines _T. pellerdyi_. As this species was described after _T. perniciosa_, it is likely that _T. pellerdyi_ is a junior synonym of _T. perniciosa_ (Gajadhar et al., 1983; Duszynski et al., 1998) (Table 3). Similarly, two species, i.e. _Tyzzeria allena_ Chakravarty & Basu, 1946 and _Tyzzeria chemicusae_ Ray & Sarkar, 1967, were described from the cotton pygmy-goose _Nettapus boae_ Lainson & Paperna, 1983 and _N. hispaniolensis_ (Table 3).
| Species             | Host                     | Shape                        | Size (μm) | Shape index | Polar granule | Wall (μm) | Micropyle (μm) | Reference                                      |
|---------------------|--------------------------|------------------------------|-----------|-------------|---------------|-----------|---------------|------------------------------------------------|
| *Eimeria abramovi*  | *Anas platyrhynchos* (L.)| Ovoidal to ellipsoidal       | 21–22 × 16–17 | –           | Absent        | Smooth, (1.4) thick | Present, (2.4) wide, with micropyle cap       | Svanbaev & Rakhmatullina, 1967               |
| *Eimeria anatis*     | *A. platyrhynchos*       | Ovoidal                      | 14–19 × 11–16 (16.8 × 14.1) | –          | –             | Smooth, 0.7–1.0 thick | Present, closed by a plug-like mass           | Scholtyseck (1955)                          |
| *Eimeria superciliosa* | *Anas superciliosa* Gmelin| Elongate-ovoidal             | 17–19 × 11–13 (17.6 × 11.9) | 1.4–1.6 (1.5) | Present, 1–2 | Smooth to slightly rough, 0.9–1.3 (1.0) thick | Present, with an invagination of the inner layer, without micropyle cap | Present study                                |
| *Eimeria aythyae*    | *Aythya affinis* (Eyton) | Broadly ellipsoidal to a round-bottomed urn with shoulder | 15–24 × 10–18 (20.1 × 15.5) | –          | Absent        | Smooth or lightly sculptured, 0.6–0.8 thick | Present, (3.6) wide                          | Farr (1965)                                 |
| *Eimeria bactakhi*   | *A. platyrhynchos*       | Subspherical to ovoidal      | 19–24 × 16–21 (21.0 × 18.0) | 1.1–1.2     | Present, 1   | Smooth, 1.0–2.0 thick | Present, without invagination of the inner layer; micropyle cap as a translucent and delicate curved protrusion | Dubey & Pande (1963)                        |
| *Eimeria boschadis*  | *A. platyrhynchos*       | Bottle-shaped                 | 18–27 × 12–13 (23.9 × 12.7) | –          |  –            | Finely granulated | Present, 2–3 wide                             | Walden (1961)                               |
| *Eimeria bucephalae* | *Bucephala clangula* (L.)| Elongate-ovoidal             | 25–39 × 13–20 (30.3 × 15.6) | –          | –             | –                      | Present, narrow                             | Christiansen & Madsen (1948)                |
| *Eimeria c. Grafner* | *Anas platyrhynchos*     | Ovoidal                      | 19–23 × 11–15 (18.7 × 12.5) | –          |  –            | 0.6–1.0 thick | Present                     | Grafner et al. (1965)                        |
| *Eimeria coganes*    | *Spatula querquedula* (L.)| Ovoidal or ellipsoidal       | 21–25 × 13–21 (21.5 × 16.1) | –          | Absent        | 0.8–1.0 thick | Present, (5.0) wide                             | Present study                                |
| *Eimeria krylovi*    | *Anas carolinensis Gmelin; Spatula clypeata* (L.); *S. querquedula*; *Mareca penelope* (L.); *Mareca strepera* (L.) | Subspherical | 15–21 × 13–17 | –          | Present, 1   | Smooth, 1.2 thick | Present at the flattened end, 4.0–6.0 wide, covered by a (4.0) wide and (2.0) high micropyle cap | Present study                                |
| *Eimeria nyroca*     | *Aythya nyroca* (Güldenstädt) | Ovoidal                      | 21–40 × 17–19 (25.4 × 17.7) | –          | Absent        | Smooth, 1.0–2.0 thick | Present, surrounded by collars, 4.0–6.0 wide, with 2.0–3.0 high micropyle cap | Present study                                |
| *Eimeria somateriae* | *Aythya nyroca* (Güldenstädt) | Ovoidal                      | 21–40 × 17–19 (25.4 × 17.7) | –          | Absent        | Smooth, 1.0–2.0 thick | Present, surrounded by collars, 4.0–6.0 wide, with 2.0–3.0 high micropyle cap | Present study                                |

*Note: E. boschadis and E. somateriae are kidney parasites, while the remaining species are intestinal parasites.*

*a Range (Mean).*
### Table 2
Comparative morphology of the sporocysts of *Eimeria* spp. recorded from ducks (Anseriformes: Anatidae: Anatinae)

| Species                  | Host                      | Shape                        | Size (μm)*  | Shape index | Stieda body | Sub-Stieda body | Sporocyst residuum | Reference                  |
|--------------------------|---------------------------|------------------------------|-------------|-------------|-------------|-----------------|---------------------|---------------------------|
| *Eimeria abramovi*       | *Anas platyrhynchos* (L.) | Ovoidal                      | 7–9 × 5     | –           | –           | –               | Present, small granules | Svanbaev & Rakhmatullina, 1967 |
| *Eimeria anatis*         | *A. platyrhynchos*        | Ovoidal                      | –           | –           | –           | –               | Present, few central granules | Scholtyseck (1955) |
|                          | *Anas superciliosa* Gmelin | Ellipsoidal                   | 7–9 × 5–6 (7.9 × 5.9) | 1.3–1.4 (1.3) | Present, flattened | Present, small | Absent or indiscernible | Present study |
| *Eimeria aythyae*        | *Aythya affinis* (Eyton)  | –                            | –           | –           | –           | –               | Present, compact residual mass | Farr (1965) |
| *Eimeria battakhi*       | *A. platyrhynchos*        | Ovoidal                      | 11–13 × 6–8 | –           | –           | Present, small | Present, delicate or indiscernible | Present study |
| *Eimeria boschadis*      | *A. platyrhynchos*        | –                            | –           | –           | –           | –               | Present, large granules randomly dispersed | Present study |
| *Eimeria bucephalae*     | *Bucephala clangula* (L.) | –                            | –           | –           | –           | –               | Present, delicate or indiscernible | Present study |
| *Eimeria danailovi*      | *Anas platyrhynchos* (L.) | Ovoidal                      | 9–11 × 7–8 (10.5 × 7.7) | 1.2–1.4 (1.4) | Present, flattened | Present, small | Present, large granules randomly dispersed | Present study |
| *Eimeria koganae*        | *Spatula querquedula* (L.) | Subspherical to ovoidal      | 9–11 × 8–10 | –           | –           | –               | Present, clear globules irregularly spaced | Svanbaev & Rakhmatullina, 1967 |
| *Eimeria krylovi*        | *Anas carolinensis* Gmelin; *Spatula clypeata* (L.); *S. querquedula*; *Mareca penelope* (L.); *M. strepera* (L.) | Subspherical or ovoidal      | 8 × 6–8     | –           | –           | –               | Absent | Svanbaev & Rakhmatullina, 1967 |
|                          | *Anas superciliosa* Gmelin | Subspherical to ovoidal      | 8–10 × 7–8 (8.8 × 7.7) | 1.1–1.2 (1.1) | Barely or not discernible | Absent | Present, large, dense granules diffused | Present study |
| *Eimeria krylovi*        | *A. nyroca* (Güldenstädte) | Ovoidal                      | 11–13 × 8–11 | –           | –           | –               | Present, granular | Svanbaev & Rakhmatullina, 1967 |
| *Eimeria saitamae*       | *A. platyrhynchos*        | –                            | –           | –           | –           | –               | Present, small, granular | Musaev et al. (1966) |
| *Eimeria somateriae*     | *Clangula hyemalis* (L.)  | –                            | (11 × 6)    | –           | –           | –               | Absent | Christiansen (1952) |

Note: *E. boschadis* and *E. somateriae* are kidney parasites, while the remaining species are intestinal parasites.

* Range (Mean).
| Species                      | Host                                           | Oocyst Shape | Size (μm)a | Shape index | Residuum (μm)b | Wall (μm)c | Sporozoite Shape | Size (μm)b | Refractile body | Nucleus                  | Reference                  |
|------------------------------|-----------------------------------------------|--------------|------------|-------------|---------------|------------|------------------|------------|-----------------|--------------------------|---------------------------|
| Tyzzeria perniciosa          | Anas platyrhynchos                             | Ellipsoidal  | 10–13 × 9–11 | –           | Present, large, composed of variously sized granules | Relatively thick | Curved, with one end more rounded and broader | (10.0 × 3.5) | –               | –                        | Allen (1936)               |
|                              | Anas superciliosa                              | Ellipsoidal  | 10–11 × 7–8  | 1.4–1.5 (1.4) | Present, granules of different sizes usually clustered at one end of the oocyst, c.2.5 | Smooth, 0.4–0.7 (0.6) | Curved and tapered at anterior end | 6–8 × 1.2            | Robust, prominent | Not discernible           | Present study              |
| Tyzzeria alleni              | Nettapus comorandelianus                       | Ovoidal      | 14–17 × 10–12| –           | Present, coarsely granular, c.6.4                   | –          | Tapered at one end | 5.3–6.5               | –               | –                        | Chakravarty & Basu (1946) |
| Bhatia & Pande, 1966         | Mareca strepera (L.); Aythya nyroca (Güldenstätt); Spatula clypeata (L.); Anas carolinensis Gmelin; A. platyrhynchos | Subspherical to ovoidal | 11–16 × 8–11 | 13.0 × 10.0 | Present, c.4.0–5.0 | Smooth, 0.5–0.7 | Banana-shaped | (8.5 × 2.0)            | Prominent | Present, central | Bhatia & Pande (1966); Bristol et al. (1981) |
| Tyzzeria chenicaseae         | N. comorandelianus                             | Broad and cylindrical | 20–28 × 14–20 | (1.5)      | Large, compact, at one pole of the oocyst | (1.4)     | Club-shaped | (13.2 × 4.2)          | Present at the broader end | –                        | Ray & Sarkar (1967) |

Note: All species are intestinal parasites.

*a Range (Mean).
**Declaration of competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Berto, B.P., Teixeira, M., Lopes, C.W.G., 2007. The newly generated sequences for E. anatis in the Pacific black duck both at the 18S rRNA and cox1 loci were most close to those from domestic goose (GenBank: KP789171, KJ000077 and MT833388). As this is, to the best of our knowledge, the first study using molecular tools to the identification of duck coccidia, further similar studies on additional species of coccidia parasitic in ducks would be beneficial to the taxonomy of duck coccidia and assessment of their relationships with coccidian species parasitic in other host groups.

This study has revealed that, besides infecting the mallard A. platyrhynchos, E. anatis also infects the Pacific black duck. Hybridisation (interbreeding) between the introduced mallard and the Pacific black duck in Australia occurs at a rate of around 1.5% (Tayson, 2016), so it is likely that these two species of duck share some of their coccidian species as well.

**5. Conclusion**

In conclusion, the coccidia E. anatis, E. aythyae, E. krylovi and T. perniciosa are redescribed with supplementary morphological data, in order to ensure and facilitate their future identification from A. superciliosa or from other duck species. In addition, a genotypic characterization of E. anatis and taxonomic remarks on species and genera of dubious validity reported from Anseriformes are provided, aiming to contribute to the knowledge of coccidian species of ducks.

**CRediT author statement**

Bruno P. Berto: morphological identification of the species, preparation of line drawings, writing - review & editing. Belinda Brice: coccidian primary screening and identification, writing - original draft and paper reviewing. Gwyneth Thomas: sample collection and coccidian primary screening, writing - review & editing. Aileen Elliot: oocyst imaging, morphological identification of the species, writing - review & editing. Alireza Zahedi: oocyst isolation, DNA extraction, PCR, sequencing, writing - review & editing. Rongchang Yang: overseeing and coordinating this study, phylogenetic analysis, writing - review & editing. All authors read and approved the final manuscript.

**Data availability**

The newly generated sequences for E. anatis are deposited in the GenBank database under the accession numbers OL604501 (18S rDNA) and OL656104 (cox1). Photomicrographs and line drawings of the oocysts are deposited and available (http://r1.ufrj.br/labicoc/coleciao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRJ, under repository numbers 120/2021 (E. anatis), 121/2021 (E. aythyae), 122/2021 (E. krylovi) and 123/2021 (T. perniciosa), along with the photovouchers of the A. superciliosa specimen.

**Ethical approval**

Not applicable.

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