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Remarks on *Eimeria* spp. (Apicomplexa: Eimeriidae) from *Kobus* spp. (Bovidae: Reduncini), with supplementary morphological data of *Eimeria congolensis* Ricci-Bitti et al., 1973 from a new host subspecies, the common waterbuck *Kobus ellipsiprymnus ellipsiprymnus* (Ogilbyi, 1833)

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ABSTRACT

Reduncin bovids of *Kobus* spp. (Bovidae: Reduncini) are natively distributed in sub-Saharan Africa, although some populations have been introduced into parks and zoos around the world. The majority of the species has declining populations, being categorized as threatened by the International Union for Conservation of Nature and Natural Resources; therefore, protective measures for the conservation of *Kobus* spp. are necessary, including the study of their parasites, such as the eimeriid coccidians (Apicomplexa: Eimeriidae). In this context, the aim of the current study was to brings together the taxonomic data from the descriptions and reports of *Eimeria* spp. from reduncin bovids, based on the detailed morphological identification of *Eimeria congolensis* Ricci-Bitti, Pampiglione & Kabala, 1973 from a new host subspecies, the common waterbuck *Kobus ellipsiprymnus ellipsiprymnus* (Ogilbyi, 1833), in a safari park of Portugal. Five *Eimeria* spp. are recorded from reduncin bovids, in addition to six more reports identified generically as *Eimeria* sp., which were compared and taxonomically rearranged. The oocysts identified as *E. congolensis* in the current study were compatible with the original description and were supplemented for some taxonomic characters not originally included, such as: Stieda body flattened to nipplelike, sub-Stieda body rounded to trapezoidal, sporocyst residuum granular and membrane-bound, in addition to greater details of the micropyle, among others. Finally, the current study highlights the importance of studying the coccidians of reduncin bovids for the conservation of *Kobus* spp. due to the possibility of these *Eimeria* spp. are extra-intestinal parasites, which can potentially cause severe coccidiosis associated with increased morbidity and mortality in certain threatened populations of *Kobus* spp.

1. Introduction

Infections caused by parasites can affect the behaviour and survival of wild vertebrate populations and act as a contributory cause of threat to wildlife conservation. In captive animals in zoological parks, it may pose an increased risk due to the change in the natural environment in which parasitism is generally achieved due to a biological balance in the parasite-host relationship. In zoos, the influence of the artificial environment or stress can upset the balance and, as a result, the development and severity of disease (Berto and Lopes, 2020).

Coccidiosis is a gastrointestinal disorder caused by parasites of the genus *Eimeria* Schneider, 1875, among other genera. These chromist parasites can affect the development, behavior and survival of wild animals under natural conditions. In captive animals, coccidiosis can occur much more frequently due to the confined space, ideal development conditions and greater susceptibility to reinfections (Panayotova-Pencheva, 2013; Berto and Lopes, 2020). Therefore, monitoring the prevalence and density of coccidian parasites in animal populations

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in the wild, and especially in captivity, is essential for preventing epizootics with increased morbidity and mortality. This monitoring fundamentally involves the quantification and morphological identification of coccidian oocysts in feces (Berto and Lopes, 2020).

The aim of the current study was to bring together the taxonomic data from the descriptions and reports of *Eimeria* spp. from reduncin bovids, which are scarce in scientific literature despite the near threat of extinction of some *Kobus* spp. in the wild (IUCN, 2023), based on the detailed morphological identification of *Eimeria congolensis* Ricci-Bitti et al., 1973 from a new host subspecies, the common waterbuck *Kobus ellipsiprymnus ellipsiprymnus* (Ogilbyi, 1833), in a safari park of Portugal.

2. Materials and methods

2.1. Sample collection

A fieldwork was carried out to collect fecal samples from a common waterbuck herd of about nine animals, in the Badoca Safari Park. This park (38°02′29.3″S, 8°44′40.0″W) is home to several wild animals, such as giraffes, zebras, elands, wildebeests, buffaloes, primates, reptiles, ostriches and birds of prey. The common waterbuck herd was observed from a distance until defecations were seen, which were then sought and found. Depending on the type of surface that the feces were found, they were discarded, giving preference to feces shed on leaves, rocks, or other surfaces less susceptible to contamination. Each fecal sample was placed into plastic vials containing 2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) solution in a 6:1 ratio.

2.2. Morphological analyses

All samples were incubated at room temperature (25 °C) for seven days. Oocysts were isolated by flotation in Sheather's sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations at 1000x magnification, line drawings, photomicrographs and measurements were made using a Leica ICC50 W binocular microscope (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) equipped with a Leica Software LAS X. Line drawings and photomicrographs were edited using two software applications (Corel DRAW and Corel PHOTO-PAINT) from CorelDRAW® (Corel Draw Graphics Suite, Version, 2020; Corel Corporation, Canada). All measurements are in micrometres and are given as the range followed by the mean in parentheses.

3. Results

3.1. Prevalence and morphology

Two fecal samples were collected from the common waterbucks of the Badoca Safari Park, and these two were positive for coccidian oocysts. These oocysts were initially unsporulated, but 70% had sporulated by day seven. Despite the polymorphism observed in some taxonomic characters of the oocysts, a single morphotype was identified, which was classified in the genus *Eimeria*. After reviewing the taxonomic literature of coccidian species recorded from reduncin bovids (Table 1), this morphotype identified was minimally compatible in the main taxonomic characters with *E. congolensis* (Ricci-Bitti et al., 1973). Therefore, the morphology of these oocysts is presented below, supplementing the original description of *E. congolensis*.

Eimeria congolensis Ricci-Bitti et al., 1973 (Figs. 1 and 2)
Kingdom: Chromista Cavalier-Smith, 1981
Phylum: Miozoa Cavalier-Smith, 1987
Infraphylum: Apicomplexa Levine, 1970
Class: Coccidiomorphea Doflein, 1901
Subclass: Coccidia Leuckart, 1879
Family: Eimeriidae Minchin, 1903
Genus: *Eimeria* Schneider, 1875

Oocysts (n = 30) ovoidal to pear-shaped, 33–40 × 25–31 (36.0 × 27.8); length/width (L/W) ratio 1.2–1.5 (1.30). Wall bi-layered, 2.9–3.1 (3.0) thick, outer layer slightly to medium rough, c.2/3 of total thickness. Micropyle present, polymorphic in width and prominence, 4.0–8.8 (6.8) wide; occasionally with an invagination of the inner layer. Oocyst residuum absent, but one or two small polar granules are present. Sporocysts (n = 30) ovoidal to ellipsoidal, 14–18 × 8–10 (16.0 × 9.0); L/W ratio 1.5–2.0 (1.78). Stieda body present, flattened to nipplelike, 0.6–1.0 × 1.5–2.0 (0.7 × 1.8); sub-Stieda body present, rounded to trapezoidal, 0.7–1.6 × 1.8–3.0 (1.1 × 2.4); para-Stieda body absent; sporocyst residuum present, composed of granules, in variable quantities, that appear to be membrane-bound, 2.2–5.6 × 1.5–4.9 (3.7 × 3.0). Sporozoites vermiform, with a robust posterior refractile body, 5.7–7.2 × 4.2–5.4 (6.3 × 4.9), and a noticeable nucleus.

3.2. Diagnosis

Eimeria congolensis was the second species described by Ricci-Bitti et al. (1973) from defassa waterbucks *Kobus ellipsiprymnus defassa* (Rüppell, 1835) captured in different areas of the Albert National Park in the Democratic Republic of the Congo. Although the oocysts of this species are similar in measurements to *Eimeria macieli* Yakimoff and Matschoulsky (1938), *Eimeria kobi* Ricci-Bitti et al., 1973 and *Eimeria katangensis* Ricci-Bitti et al., 1973, it can be differentiated by shape and measurements of the sporocysts, which are typically more ovoidal, as they have a shorter length in relation to width (Table 1) (Yakimoff and Matschoulsky, 1938; Ricci-Bitti et al., 1973). Added to this, the current study added some taxonomic characters not included in the original description of Ricci-Bitti et al. (1973), although these were observed in the original photomicrographs shown, such as: Stieda body flattened to nipplelike, sub-Stieda body rounded to trapezoidal, sporocyst residuum granular and membrane-bound, in addition to greater details of the micropyle and other taxonomic characters. Thus, these new characteristics presented in the current study easily differentiate and typify *E. congolensis* in comparison with other *Eimeria* spp. of reduncin bovids *Kobus* spp. (Table 1).

3.3. Taxonomic summary

Type host: *Kobus ellipsiprymnus defassa* (Rüppell, 1835) (Mammalia: Cetartiodactyla: Bovidae: Hippotraginae: Reduncini), defassa waterbuck (Ricci-Bitti et al., 1973).

Other host: *Kobus ellipsiprymnus ellipsiprymnus* (Ogilbyi, 1833) (Mammalia: Cetartiodactyla: Bovidae: Hippotraginae: Reduncini), common waterbuck (current study).

Type locality: National Park Albert, Kivu and North Katanga, Democratic Republic of the Congo (Ricci-Bitti et al., 1973).

Other locality: Badoca Safari Park (38°02′29.3″S, 8°44′40.0″W), Portugal (current study).

Type-material: Not stated in the original description of Ricci-Bitti et al. (1973).

Representative specimens (current study): Photomicrographs, line drawing and oocysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al., 2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number 135/2023. Photographs of the host specimens are deposited in the same collection.

ZooBank registration: The data relating to this species, which was supplemented in its description and deposited in taxonomic collection in the current work, were submitted to ZooBank (ICZN, 2012). The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:9525E798-C449-4CFE-8C0A-252E4D19F3BB.

Site of infection: Unknown, oocysts were recovered from feces.

Table 1
Comparative morphology of *Eimeria* spp. recorded from reduncin bovids (Bovidae: Reduncini).

Coccidia	Hosts	References	Oocysts								Sporocysts						
			Shape	Length (μm)	Width (μm)	L/W ratio	Wall (μm)	Micropyle (μm)	Polar granule	Residuum	Shape	Length (μm)	Width (μm)	L/W ratio	Stieda Body (μm)	Substieda body	Residuum (μm)
Eimeria macieli Yakimoff and Matschoulsky (1938)	*Kobus ellipsiprymnus ellipsiprymnus* (Ogilbyi, 1833)	Yakimoff and Matschoulsky (1938)	ovoidal	24–34 (29.7)	20–24 (21.2)	1.2–1.6 (1.39)	yellow, with radial strips, (1.5)	present	absent	absent	ovoidal	10–14	4–6	–	–	–	present
Eimeria kobi Ricci-Bitti et al. (1973)[a]	*Kobus ellipsiprymnus defassa* (Rüppell, 1835)	Ricci-Bitti et al. (1973)	ellipsoidal	34–41 (37.8)	26–30 (27.9)	1.2–1.5 (1.36)	brown, rough, (2.0)	present	absent	absent	ellipsoidal	(21.0)	(7.7)	–	present	–	present, granules along the sporozoites
Eimeria congolensis Ricci-Bitti et al. (1973)[a]	*K. e. defassa*	Ricci-Bitti et al. (1973)	ovoidal	27–33 (29.7)	19–24 (22)	(1.35)	brown, rough, (1.5)	present	absent	absent	ellipsoidal	(14.2)	(7.1)	–	present, very small and hardly visible	–	present
	K. e. ellipsiprymnus	**current study**	**ovoidal to pear-shaped**	**33–40 (36.0)**	**25–31 (27.8)**	**1.2–1.5 (1.30)**	**slightly to medium rough, 2.9–3.1 (3.0)**	**present, quite noticeable, 4.0–8.8 (6.8)**	**present, 1 or 2**	**absent**	**ovoidal to ellipsoidal**	**14–18 (16.0)**	**8–10 (9.0)**	**1.5–2.0 (1.78)**	**present, flattened to nipplelike, 0.6–1.0 × 1.5–2.0 (0.7 × 1.8)**	**present, rounded to trapezoidal, 0.7–1.6 × 1.8–3.0 (1.1 × 2.4)**	**present, granules in variable quantities, membrane-bound**
Eimeria katangensis Ricci-Bitti et al., 1973	*K. e. defassa*	Ricci-Bitti et al. (1973)	ovoidal	34–44 (41.0)	22–26 (24.6)	1.3–1.8 (1.66)	brown, smooth, (2.0)	present	absent	absent	ellipsoidal with pointed ends	(19.7)	(7.5)	–	present, little	–	present, granules grouped into irregular heap
Eimeria sp.[b]	*K. e. defassa*	Ricci-Bitti et al. (1973)	subspherical	16–20 (17.8)	14–18 (16.0)	1.0–1.3 (1.11)	colourless, smooth, (1.0)	indistinct	absent	present, several scattered refractile granules	ellipsoidal	(9.7)	(4.9)	–	indistinct	–	indistinct
Eimeria dathei Tscherner (1976)[c]	*K. e. ellipsiprymnus*	Tscherner (1976)	ovoidal	52–59	35–39	–	dark brown, rough, (3.0)	present, (10)	absent	absent	spindle-shaped	(28)	(10)	–	present	–	granular, scattered
Eimeria sp. (type 1)[c]	*Kobus leche leche* Gray, 1850	Flach et al. (1991)	ovoidal	(55.9)	(35.0)	–	dark brown, rough, (4.0)	present	–	–	–	(24.5)	(11.7)	–	–	–	–
Eimeria sp. (type 2a)[a]	*K. l. leche*	Flach et al. (1991)	ovoidal	(40.1)	(25.6)	–	dark, smooth	present	–	–	–	(18.9)	(8.5)	–	–	–	–
Eimeria sp. (type 2b)[a]	*K. l. leche*	Flach et al. (1991)	ovoidal	(34.9)	(22.2)	–	brown, rough, (2.0)	present	–	–	–	(16.7)	(8.5)	–	–	–	–
Eimeria sp. (type 2c)[a]	*K. l. leche*	Flach et al. (1991)	ovoidal	(30.7)	(19.6)	–	smooth	present	–	–	–	(14.9)	(7.2)	–	–	–	–
Eimeria sp. (type 3)[b]	*K. l. leche*	Flach et al. (1991)	ovoidal	(21.3)	(14.5)		smooth	indistinct	–	–	–	–	–	–	–	–	–

[a] *Eimeria* sp. (type 2a-c) of Flach et al. (1991) are probably *Eimeria kobi* and/or *Eimeria congolensis*.
[b] *Eimeria* sp. of Ricci-Bitti et al. (1973) and *Eimeria* sp. (type 3) of Flach et al. (1991) probably are the same species.
[c] *Eimeria* sp. (type 1) of Flach et al. (1991) is probably *Eimeria dathei*.

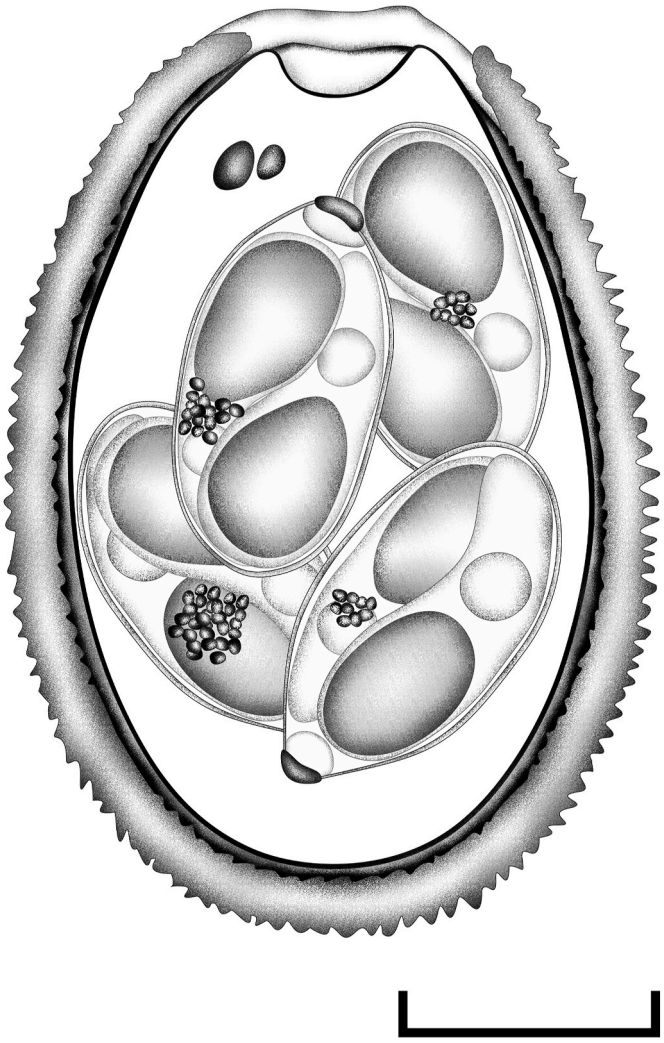

Fig. 1. Composite line drawing of the sporulated oocyst of *Eimeria congolensis* from common waterbucks *Kobus ellipsiprymnus ellipsiprymnus* in a safari park of Portugal. Scale-bar: 10 μm.

Prevalence: 50% (2/4) in the original description from *K. e. defassa* (Ricci-Bitti et al., 1973); 100% (2/2) in the current study from *K. e. ellipsiprymnus*.

4. Discussion

Genus *Kobus* Smith, 1840 brings together 5 species and 10 subspecies (IUCN, 2023). All of these species are natively distributed in sub-Saharan Africa, although reduncin bovids have been introduced into several parks and zoos around the world (Jeffery et al., 1989; IUCN, 2023). The five species and six of the ten subspecies have declining populations in recent years, being categorized by the International Union for Conservation of Nature and Natural Resources as threatened at different levels from 'near threatened' to 'critically endangered' (IUCN, 2023). One of the subspecies, the Roberts' lechwe *Kobus leche robertsi* (W. Rothschild, 1907), originally distributed in Zambia, is listed as extinct as there are no surviving populations either in the wild or in captivity (Jeffery et al., 1989). This conservation status of *Kobus* spp. justifies various protective measures for its different species and subspecies, including the study of its parasites, such as *Eimeria* spp. that have host specificity to the genus *Kobus* or tribe Reduncini (Jeffery et al., 1989; Duszynski and Wilber, 1997; Berto and Lopes, 2020; Duszynski, 2021).

The first report of an eimeriid coccidian parasitizing *Kobus* spp. was made by Yakimoff and Matschoulsky (1938) for a species described as *E. macieli* from common waterbucks *K. e. ellipsiprymnus* (Ogilbyi, 1833) in a zoo in Leningrad (present-day Saint Petersburg) in Russia. After this study, only in the 1970s, Ricci-Bitti et al. (1973) described 4 new *Eimeria* spp., plus one unnamed *Eimeria* sp., from defassa waterbucks *K. l. defassa* captured in different areas of the Albert National Park in Zaire (present-day Democratic Republic of Congo).

Tscherner (1976) described *Eimeria dathei* Tscherner (1976) from common waterbucks *K. e. ellipsiprymnus* in captivity at Berlin Zoo, Germany; apparently, unaware of the study by Ricci-Bitti et al. (1973) and with a very low level of detail in the morphological description of oocysts. In any case, the oocysts of *E. dathei* are significantly larger than those of other *Eimeria* spp. of *Kobus* spp., being, therefore, potentially a valid species.

Flach et al. (1991) reported different *Eimeria* spp. from the red lechwe *Kobus leche leche* Gray, 1850 in Edinburgh Zoo, Scotland. Oocysts with different morphotypes were observed, which were identified as types 1, 2a-c, 3 and 4, but none of these types were specifically described or named. Type 1 is morphometrically compatible with *E. dathei* and, therefore, they are most likely the same species (Table 1). Types 2a, 2b and 2c had subtle morphological differences, even though the taxonomic morphological study in Flach et al. (1991) was insufficient. These types were differentiated basically by measurements; however, the total range of measurements for types 2a-c overlaps the range of measurements for *E. congolensis* and *Eimeria kobi* Ricci-Bitti et al., 1973; Ricci-Bitti et al. (1973). These two *Eimeria* spp. of Ricci-Bitti et al. (1973) are mainly differentiated by sporocysts, which were minimally detailed by Flach et al. (1991). This same remark was concisely noted in the database 'Coccidia of the World' of Duszynski et al. (2001). In the current study, some small variations in the morphology and morphometry of oocysts were observed, similar to the variation of types 2a-c by Flach et al. (1991). These small variations could support the description of new species; however, these variations potentially represent intra-specific differences of a polymorphic species, as highlighted by Gardner and Duszynski (1990) and, more recently, by Ortúzar-Ferreira et al. (2024). The main variations were observed in the roughness and thickness of the oocyst wall, wide and prominence of the micropyle and measurements and shape of the oocysts; however, sporocysts were typical and constant in all taxonomic characters. Therefore, it was decided to identify only a single species, *E. congolensis*, being reasonably polymorphic, instead of describing new species based on minimal morphological and/or morphometrical details (Table 1). Type 3 was morphologically compatible with the unnamed *Eimeria* sp. reported by Ricci-Bitti et al. (1973), which is an easily distinguishable morphotype and, therefore, a valid species, but not yet named/described for the taxonomy of coccidia of reduncin bovids (Table 1). Type 4 was seen sporadically in samples and was not adequately defined, although Flach et al. (1991) reported that these oocysts were even smaller than type 3.

The most recent report of eimeriid coccidians parasitizing reduncin bovids was made by Wessels et al. (2011). These authors reported two cases of hepatic coccidiosis in 12- to 18-monthold red lechwes *K. l. leche* which were submitted to Animal Health and Veterinary Laboratories Agency of Preston, England, for postmortem examination with a brief history of diarrhoea before death. In this report, endogenous coccidial stages were observed in the bile duct, in addition to oocysts in bile and feces. These oocysts were identified as morphologically similar to those reported by Flach et al. (1991); however, no morphological or morphometrical study of oocysts was presented by Wessels et al. (2011). In this context, *Eimeria* spp. of reduncin bovids has greater relevance for the conservation of *Kobus* spp. due to the possibility of these *Eimeria* spp. are parasites of the bile ducts of the liver. *Eimeria* spp. with extra-intestinal cycles are generally more pathogenic (Berto and Lopes, 2020), such as *Eimeria stiedae* (Lindemann, 1865) which similarly parasitizes bile ducts of rabbits (Barriga and Arnoni, 1979). Therefore, hepatic coccidiosis caused by *Eimeria* spp. could potentially cause

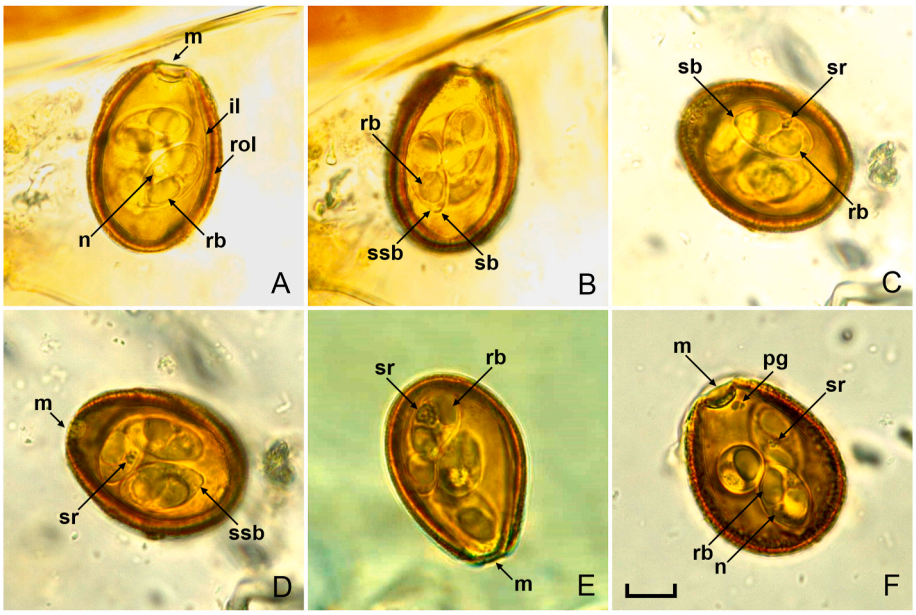

Fig. 2. Photomicrographs of sporulated oocysts of *Eimeria congolensis* from common waterbucks *Kobus ellipsiprymnus ellipsiprymnus* in a safari park of Portugal. Note the inner layer (il) and rough outer layer (rol) of the oocyst wall, micropyle (m), nucleous (n), polar granule (pg), refractile body (rb), sporocyst residuum (sr), Stieda (sb) and sub-stieda (ssb) bodies. Scale bar: 10 μm.

increased morbidity and mortality in certain populations of *Kobus* spp., as reported in these two cases by Wessels et al. (2011) that died.

Finally, based on the taxonomic characters of *Eimeria* spp. recorded from reduncin bovids, which were compared with each other and with the oocysts analyzed in the current study, taxonomic rearrangements are suggested and *E. congolensis* is identified and supplemented in its description, documenting a new host subspecies, the common waterbuck *K. e. ellipsiprymnus* (Ogilbyi, 1833), in a safari park of Portugal.

Ethics approval

All procedures were reviewed and approved by zoo and safari park authorities before conducting the research. The samples in this study were collected opportunistically in the territories of the common waterbuck herd. Non-invasive method was used during the collection of fecal samples.

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CRediT authorship contribution statement

Ana Maria Duque de Araujo Munhoz: Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Ema Albuquerque Fernandes:** Investigation. **João David Molarinho Marques:** Investigation. **Bruno Pereira Berto:** Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Data curation.

Declaration of competing interest

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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