

Avispora mochogalegoi n. sp. (Apicomplexa: Sarcocystidae) in the little owl, *Athene noctua* (Strigiformes: Strigidae), in mainland Portugal

Avispora mochogalegoi n. sp. (Apicomplexa: Eimeriidae) no mocho-galego, *Athene noctua* (Strigiformes: Strigidae), em Portugal Continental

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Abstract

The little owl *Athene noctua* (Scopoli, 1769) is a small raptor that is widely distributed from northern to southern Portugal and several other countries in Europe, Asia and North Africa, and which has been introduced into New Zealand. In the current study, 18 fecal samples were collected from little owls kept at the Lisbon Center for Wild Animal Recovery, which is located in Monsanto Forest Park, Lisbon, Portugal. Twelve (67%) of them were found to be passing an undescribed species of *Avispora* in their feces. The oocysts of *Avispora mochogalegoi* n. sp. were ellipsoidal with a bilayered wall and measured $38.9 \times 32.9 \mu\text{m}$, with a shape index of 1.18. No micropyle, oocyst residuum or polar granule was present. The sporocysts were subspherical, measuring $21.1 \times 20.1 \mu\text{m}$. Stieda, sub-Stieda and para-Stieda bodies were absent. The sporocyst residuum was composed of a compact subspherical mass of granules. This is the fourth species of *Avispora* reported in Strigiformes.

Keywords: Coccidia, *Avispora*, *Caryospora*, oocysts, taxonomy, raptors.

Resumo

O mocho-galego *Athene noctua* (Scopoli, 1769) é uma pequena ave de rapina amplamente distribuída de norte a sul de Portugal, em vários países da Europa, Ásia e norte da África, e foi introduzida na Nova Zelândia. No presente trabalho, 18 amostras de fezes foram coletadas de mochos-galegos mantidos no Centro de Recuperação de Animais Silvestres de Lisboa, localizado no Parque Florestal de Monsanto, Lisboa, Portugal. Doze (67%) deles eliminaram uma espécie não descrita de *Avispora* em suas fezes. Os oocistos de *Avispora mochogalegoi* n. sp. foram elipsóides, com parede de dupla camada, medindo $38,9 \times 32,9 \mu\text{m}$, e índice morfométrico de 1,18. A micrópila, resíduo do oocisto e grânulo polar foram ausentes. Os esporocistos foram subesféricos, medindo $21,1 \times 20,1 \mu\text{m}$. Corpos de Stieda, substieda e parastieda foram ausentes. O resíduo do esporocisto foi composto de uma massa subesférica compacta de grânulos. Esta é a quarta espécie *Avispora* relatada em Strigiformes.

Palavras-chave: Coccidia, *Avispora*, *Caryospora*, oocistos, taxonomia, aves de rapina.

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Introduction

The little owl *Athene noctua* (Scopoli, 1769) is a small raptor that is often observed in Portugal because of its diurnal and nocturnal habit. It is widely distributed from northern to southern Portugal and in several other countries in Europe, Asia and North Africa, and it has also been introduced into New Zealand. It is common on agricultural land with a few scattered trees and in olive groves. It is often seen on ruins or heaps of stones, which it uses to nest in. It is absent from high-altitude areas and densely forested areas (IUCN, 2016; ELIAS, 2017).

The genus *Caryospora* Leger, 1904, is traditionally recognized as one of the main genera of coccidian parasites of both raptors and reptiles (UPTON et al., 1990; BERTO et al., 2014). Hence, the species of *Caryospora* that have been recorded in raptors were recently taxonomically transferred to a new genus, termed *Avispora* Schuster, Woo, Poon, Lau, Sivakumar, Kinne, 2016. This reclassification was based on the morphological, biological and, finally, molecular differences of *Caryospora* spp. found in raptors and reptiles (BERTO et al., 2014; SCHUSTER et al., 2016).

In this context, the current study describes a new species of *Avispora* found in little owls (*A. noctua*) that were being kept for rehabilitation and reintroduction into the wild at the Lisbon Center for Wild Animal Recovery (Centro de Recuperação de Animais Silvestres de Lisboa, LxCRAS), in Monsanto Forest Park, Lisbon, Portugal.

Materials and Methods

Eighteen fecal samples were collected from little owls (*A. noctua*) that were being kept in individual cages on the premises of the Lisbon Center for Wild Animal Recovery (LxCRAS), which is located in Monsanto Forest Park, Lisbon, Portugal. The samples were collected immediately after defecation and were placed in plastic vials containing 2.5% potassium dichromate ($K_2Cr_2O_7$) solution

at 1:6 (v/v). In the laboratory, the samples were incubated at room temperature for 10 days or until day three when around 70% of the oocysts had sporulated. The oocysts were recovered by means of flotation in Sheather's sugar solution (specific gravity: 1.20). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX40 microscope equipped with a digital camera (Olympus DP10). This processing for identification and description was made a few days after sporulation of the oocysts to avoid morphological alterations in the oocysts derived from the long storage time. The line drawings were edited using two software applications in CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), specifically Corel DRAW and Corel PHOTO-PAINT. All measurements were made in micrometers and are given as the range followed by the mean in parentheses. The descriptions of oocysts and sporocysts followed the guidelines of Duszynski & Wilber (1997) and Berto et al. (2014), using the following: oocyst (O) length (L) and width (W) and their ranges and ratios (L/W); micropyle (M), oocyst residuum (OR), polar granule (PG) and sporocyst (SP) length (L) and width (W) and their ranges and ratios (L/W); and Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), sporocyst residuum (SR), sporozoite (SZ), refractile body (RB) and nucleus (N).

Results

Avispora mochogalegoi n. sp.

(Figures 1A-E)

Description of sporulated oocyst: Oocyst shape: ellipsoidal; oocyst wall: bilayered, ~1.5 thick; outer layer smooth; $L \times W$ ($n = 15$) 38.9×32.9 ($37-43 \times 31-37$); L/W 1.18 (1.15–1.23). M, OR, PG: all absent. Distinctive features of oocysts: large oocyst, ellipsoidal, lacking M, OR and PG.

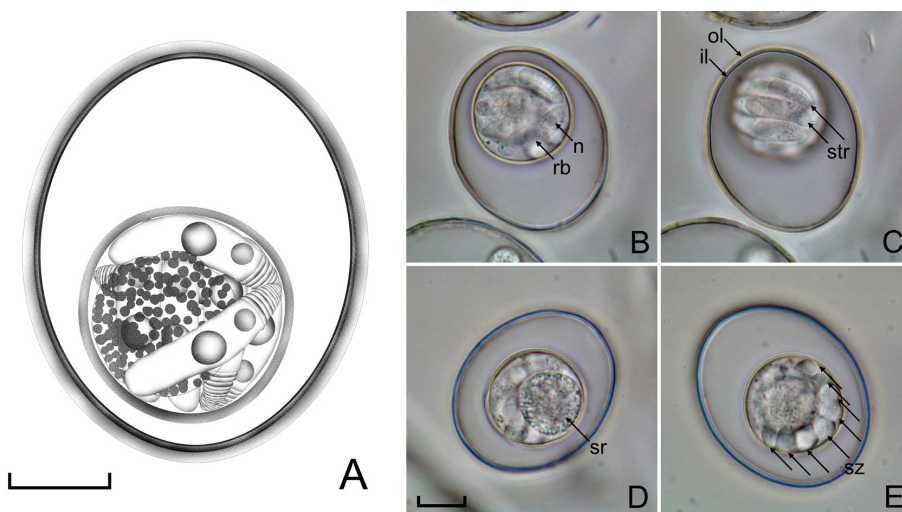


Figure 1. Sporulated oocysts of *Avispora mochogalegoi*, a new coccidium species recovered from the little owl *Athene noctua*. (A) Composite line drawing. (B–E) Photomicrographs. Note the inner (il) and outer layer (ol) of the oocyst wall; the sporocyst residuum (sr); and the nucleus (n), refractile body (rb) and striations (str) of the sporozoites (sz). Bars: 10 μ m.

Description of sporocyst and sporozoites: Sporocyst shape: subspherical; sporocyst wall: single-layered, ~1.0 thick, smooth; L × W (n = 15) 21.1 × 20.1 (20–24 × 19–23); L/W 1.02 (1.00–1.07); SB, SSB, PSB: all absent; SR: present; SR characteristics: usually as a distinctly irregular-sub spherical body consisting of numerous granules that appear to be membrane-bounded, L × W (n = 15) 13.6 × 13.1 (11–16 × 11–16). SZ: stout, L × W (n = 5) 16.6 × 4.7 (15–18 × 4–5) in situ, arranged parallel to one another in SP. Each SZ with striations, one spherical PRB, ~3.0 wide, and a robust N in midpoint of body. Distinctive features of sporocyst: subspherical shape, smooth wall, presence of an irregular-sub spherical body consisting of numerous granules that appear to be membrane-bounded.

Taxonomic summary

Type host: little owl *Athene noctua* (Scopoli, 1769) (Aves: Strigiformes: Strigidae).
Other hosts: None.
Type specimens: Phototypes and line drawings have been deposited and are available in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at Universidade Federal Rural do Rio de Janeiro, in the municipality of Seropédica, Rio de Janeiro, Brazil (LABICOC, 2017). Photographs of the host specimens have been deposited in the same collection. The repository number is P-78/2017.
Type locality: Lisbon Center for Wild Animal Recovery (LxCRAS), Lisbon, Portugal (38°44'22.9" N; 9°11'02.3" W).
Prevalence: 12 out of 18 specimens (67%).
Sporulation: Exogenous. All oocysts were passed in the feces unsporulated and became fully sporulated by day 7 in K₂Cr₂O₇ solution at room temperature.
Prepatent and patent periods: Unknown.
Site of infection, definitive host: Unknown. Oocysts recovered from feces.

Site of infection, secondary host: Unknown.
Endogenous stages, definitive host: Unknown.
Endogenous stages, secondary host: Unknown.
Cross-transmission: None to date.
Pathology, definitive host: Unknown.
Pathology, secondary host: Unknown.
Etymology: The specific epitaph is derived from the common local name for the host, which is ‘mocho-galego’ (= galician owl).

Remarks

Table 1 shows some characteristic features of *Caryospora* spp., described from Strigiformes. Among the *Avispora* species listed in Table 1, only *Avispora bubonis* (CAWTHORN & STOCKDALE, 1981) and *Avispora henryae* (YAKIMOFF & MATIKASCHWILI, 1932) have oocyst measurements similar to those of *A. mochogalegoi*. The main distinctive feature of *A. mochogalegoi* is the irregular-sub spherical body consisting of numerous granules that appear to be membrane-bounded. In contrast, *A. bubonis* and *A. henryae* have scattered sporocyst residuum. In fact, as emphasized by Upton et al. (1986), *A. henryae* was described from several hosts of different forms and, therefore, it is possible that more than one species may be involved; however, in any case, none of these descriptions of *A. henryae* contain sub-spherical sporocysts with an irregular-sub spherical body consisting of numerous granules that appear to be membrane-bounded (UPTON et al., 1986; 1990).

Discussion

The order Strigiformes Wagler, 1830, comprises 241 species of owls, owlets and boobooks distributed in the families Strigidae Vigors, 1825, and Tytonidae Mathews, 1912. The family Tytonidae comprises only 16 species distributed in 2 genera, *Phodilus* Saint-Hilaire, 1830, and *Tyto* Billberg, 1828; whereas the

Table 1. Comparative data of *Avispora* spp. recorded from Strigiformes.

<i>Avispora</i> spp.	Host	Locality	Reference	Oocyst		Sporocyst		
				Shape	Size (µm)	Shape	Size (µm)	Residuum
<i>A. bubonis</i> (Cawthorn, Stockdale, 1981)	<i>Bubo virginianus</i> (Gmelin, 1788)	Canada	Cawthorn and Stockdale, (1981); Upton et al. (1986; 1990)	subspherical	43.9 × 40.2 (38.0–52.0 × 33.0–7.0)	subspherical	26.6 × 25.6 (20–33 × 20–32)	granular and diffuse
<i>A. henryae</i> (Yakimoff, Matikaschwili, 1932)	<i>Bubo bubo</i> (Linnaeus, 1758)	Russia	Upton et al. (1986; 1990)	subspherical or ovoidal	41.0 × 37.0 (39.6–43.2 × 36.0–39.6)	subspherical	21.6–25.2 × 19.8–21.6	granular and diffuse
<i>A. strigis</i> (Gottschalk, 1972)	<i>Tyto alba</i> (Scopoli, 1769)	Europe	Upton et al. (1986; 1990)	ovoidal	13.8 × 10.9 (11.9–15.0 × 10.3–12.5)	no data	no data	granular and diffuse
<i>A. mochogalegoi</i>	<i>Athene noctua</i> (Scopoli, 1769)	Portugal	Current work	ellipsoidal	38.9 × 32.9 (37–43 × 31–37)	subspherical	21.1 × 20.1 (20–24 × 19–23)	compact, consisting of numerous granules that appear to be membrane-bounded

family Strigidae comprises 225 species distributed in 28 genera. The genus *Athene* Boie, 1822, comprises 4 species: *Athene brama* (TEMMINCK, 1821), which occurs in Asia; *Athene cunicularia* (MOLINA, 1782), which occurs in North, Central and South America; *Athene superciliaris* (VIEILLOT, 1817), which is endemic to Madagascar; and *A. noctua*, which is distributed in Europe, Asia and North Africa (IUCN, 2016; BRANDS, 2017).

Thus, the type-host of *A. mochogalegoi* recorded in this study is only sympatric with *A. brama* in Asia. Therefore, *A. brama* becomes a potential host for this coccidium, since it is congeneric with *A. noctua*. At the same time, there is a well-established consensus of intra-familial specificity for coccidia of Aves, which was suggested by Duszynski & Wilber (1997) and has been reaffirmed in several subsequent studies on coccidians in birds (BERTO et al., 2011). In this context, more than 100 species of Strigidae distributed in the Old World would be susceptible to *A. mochogalegoi* (IUCN, 2016).

Avispora henryae, which was originally described from Eurasian eagle-owls *Bubo bubo* (LINNAEUS, 1758), was subsequently reported in several raptors of the orders Accipitriformes and Falconiformes (HOARE, 1933). However, Upton et al. (1986) contested this transmission between different orders of Aves, taking the view that these subsequent reports should be of other coccidian species. *Bubo bubo* is sympatric with *A. noctua* in the Russian region (KONTORSHIKOV et al., 1996); therefore, it would be possible for these owls to share the same coccidian species, but as mentioned above, the morphology identified in the oocysts of the current work is incompatible with all descriptions of *A. henryae*.

The genus *Avispora* was recently introduced after observation of the wide phylogenetic distance between species of the genus *Caryospora* in raptors and species of *Caryospora* in reptiles, through comparison of DNA sequencing of the *cox1*, *18S* ribosomal ribonucleic acid (rRNA) and *28S* rRNA genes (SCHUSTER et al., 2016). In fact, previous studies had already highlighted the evident morphological and biological differences of *Caryospora* spp. found in raptors and reptiles, such as the presence of a Stieda body and developmental details in the intermediate host, especially regarding caryocysts, along with the evident host specificity (CAWTHORN & STOCKDALE, 1981; WACHA & CHRISTIANSEN, 1982; UPTON et al., 1986; BERTO et al., 2014).

The molecular phylogenetic analysis provided conclusive evidence for transferring the species of the genus *Caryospora* in raptors to the new genus *Avispora*. Moreover, it placed these species closer to another coccidian family: Sarcocystidae, which comprises the cyst-forming coccidians. This inclusion in Sarcocystidae is consistent with the morphological characteristics of the oocysts, given that like *Avispora*, sarcocystids do not have a Stieda body.

Therefore, based on the morphological features described above, *A. mochogalegoi* is considered to be new to science and to be the fourth species reported in Strigiformes.

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