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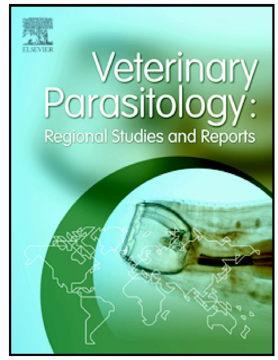


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# Occurrence of helminth parasites in the gastrointestinal tract of wild birds from Wildlife Rehabilitation and Investigation Centre of Ria Formosa in southern Portugal

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**Abstract**

This study was carried out in southern Portugal to determine the prevalence of parasitic helminths infecting wild birds. Between September and December of 2013, adult parasites were collected from the gastrointestinal tract of 22 bird specimens (belonging to 12 species) that died in Wildlife Rehabilitation and Investigation Centre of the Ria Formosa. Identified gastrointestinal parasites include the nematodes (*Contracaecum* sp., *Cosmocephalus obvelatus*, *Desportesius invaginatus*, *Dispharynx nasuta*, *Porrocaecum angusticolle* and *Synhimantus laticeps*) and cestodes (*Diplophallus* sp., *Neyraia intricata* and *Tetrabothrius* sp.). The overall level of infection was 54.5% (12/22) and the most frequent helminths present were nematodes (40.9% - 9/22), followed by cestodes (13.6% - 3/22). Helminthic richness was similar in all birds (one species or genera per bird) and the helminth species, except *Dispharynx nasuta*, exhibited a clear relationship with host diet. Five helminth species (*Cosmocephalus obvelatus*, *Desportesius invaginatus*, *Dispharynx nasuta*, *Porrocaecum angusticolle* and *Neyraia intricata*) and two genera (*Contracaecum* sp. and *Diplophallus* sp.) were reported for the first time in Portugal and the presence of *P. angusticolle* in Bonelli's eagle was recorded for the first time across all researched literature.

*Key-words*: Gastrointestinal helminths; Host-parasite associations; New records; Portugal; Wild birds.

## 1. Introduction

Helminths, commonly known as parasitic worms and separated according to their general external shape, are a large group of endoparasites of birds. Although birds may be directly infected by the ingestion of embryonated eggs or larvae from the environment, they are usually infected by consumption of intermediate or paratenic hosts, where larval forms of parasites are presents (Bowman, 2008; Cooper and Krone, 2002; Krone, 2007).

Infected birds usually grow normally and do not no exhibit clinical signs, severe disease or pathological effects (Clapham, 1957; Moore and Bell, 1983; Thomas, 1986). Lesions in the parasite-host locus of contact are the most evident pathological changes; moreover certain helminths have been described as reducing host survival and breeding success (Hudson et al., 1992; Minchella and Scott, 1991; Santoro et al., 2010). Nevertheless, these health problems usually appear when helminthic infections are combined with other factors, such as stress (Cooper and Krone, 2002).

Portugal covers an area of 92.225$km^2$ and has 106 important bird areas (IBAs), which cover a total of 2.905.586 ha (BirdLife International, 2016; Portuguese Administrative Boundaries Official Map, 2014). Furthermore, according to BirdLife International (2015) there are 307 species of birds found in Portugal. However, research on the helminthofauna in wild birds has been limited to: (I) studies focused on identification of the helminth species of specific host groups; (II) studies based on coprological analyses in which the parasite forms present species have not been identified to species level (Magalhães et al., 1998). Thus, there is a clear need for more comprehensive parasitological studies on wild birds in Portugal.

The purpose of the present study was to contribute for the establishment of the prevalence of helminth parasites infecting wild birds species in southern Portugal.

## 2. Materials and methods

### *2.1. Fieldwork*

Ria Formosa Natural Park (PNRF), with 23.296 ha, located in Olhão, Algarve, Portugal (37°2'4.55"N/7°48'46.79"O), is the most important wetland in the southern Portugal. This area offers a complex habitat, shaped by barrier islands, intertidal flat zones, salt-works, sandy coasts, lagoons and muddy freshwater shores. Furthermore, this area lies along migratory flyways of birds and comprises ten IBAs (BirdLife International, 2016; Parque Natural da Ria Formosa, 2007).

Avian population of this study was obtained from September to December 2013, at the Wildlife Rehabilitation and Investigation Centre of Ria Formosa – Association ALDEIA (RIAS/ALDEIA). This is the only wildlife animal hospital in the Algarve and admits any wild animal in need of medical care.

### 2.2. *Sampling data*

In total, 22 wild birds belonging to 7 orders, 8 families, 11 genera and 12 species were examined for helminths. Birds species were classified following Svensson et al. (2012) and each bird was classified according to age, determined by plumage features. Necropsy of the birds was performed immediately after death or in the following 48 hours; birds died from different causes at the RIAS/ALDEIA. During that time, each body was sealed in a plastic bag and stored in the refrigerator at 5ºC. Birds were dissected and the digestive tract separated and divided into esophagus, proventriculus, gizzard, small intestine, intestinal caeca, rectum and cloaca, so that the distribution of the various intestinal helminths could be ascertained. In addition, trachea, air sacs, peritoneal cavity and liver were removed, dissected and examined. The contents of the digestive tract were washed with tap water through a sieve; this washing procedure was repeated and the clean mucosal surface of the organs and the decantation product of the digestive were observed under an Olympus SZ51 stereoscopic microscope to detect parasitic helminths. All helminths found were collected and washed in physiological saline. The helminths were distended and fixed in AFA (acetic acid pure, 37% formaldehyde and 70% ethanol) (Amato and Amato, 2010). All samples were preserved separately in 70% alcohol glycerinated 2% and brought the Faculty to the Veterinary Medicine at the University of Lisbon for a specific biologic examination.

At the laboratory, helminths were identified and selected. Some specimens were cleared with lactophenol and posteriorly mounted on slides in Hoyer's Solution for morphologic and morphometric analysis with an Olympus BX40 microscope coupled to a digital camera Olympus DP10. The other samples were placed in BEEM® capsules modified according to Eisenbach (1985) and prepared for Scanning Electron Microscopy (SEM) following Amato and Amato (2010) procedure. In this preparation, specimens were first dehydrated through a graded series of ethanol solutions, ethanol-acetone solutions of increasing acetone concentration and acetone solutions. Secondly, they were dried with dioxide carbon using the critical point. Next, they were mounted on aluminum stubs with double-sided tape and sputter coated with gold in the equipment JEOL JFC-1200. Specimens were examined with a JEOL JSM-5200LV the Faculty of Sciences University of Lisbon (FCUL). Nematodes were determined to the genus level using the keys of Anderson et al. (1974-1983), whereas the keys of Khalil et al. (1994) were used for cestodes. More detailed identifications were later made (Burt, 1980; Hartwich, 1959; Hoberg et al., 1995; Li et al., 2013; Mahon, 1958; Mutafchiev et al., 2010; Şinasi et al., 2010; Wong and Anderson, 1986; Zhang et al., 2004). All specimens were stored at the

Laboratory of Parasitology of the Faculty of Veterinary Medicine of University of Lisbon.

For each helminth species, the prevalence (*P*) and the mean intensity (MI) in the represented host species was calculated.

## 3. Results

From the 22 wild birds examined, helminths were found in 12 birds (54.5%; see Table 1). Nematode infections were found in 9 (40.9%) bird species, namely in 2 species of Accipitriformes, 1 of the Charadriiformes, Pelecaniformes, Strigiformes and Suliformes. Cestodes infections were observed in 3 (13.6%) bird species, namely 2 species of Charadriiformes and 1 Bucerotiformes. No infection by Acanthocephala or Trematodes were found.

Details of the helminth species, the infection rates, site in host and geographical distribution where each association host-parasite has been identified, are summarized in Table 2.

A total of 32 helminth specimens, including 6 nematodes and 3 cestodes were found in all the birds sampled. For the phylum Nematoda, 3 families were identified: for the family Anisakidae was found *Contracaecum* sp. Railliet and Henry, 1912 (Fig. 1) in esophagus of two northern gannet; for the family Ascarididae was found *Porrocaecum angusticolle* (Molin, 1860) (Fig. 2) in small intestine of one Bonelli's eagle; and for the family Acuariidae were found *Dispharynx nasuta* (Rudolphi, 1819) (Fig. 3) in esophagus and proventriculus of two little owl, *Synhimantus laticeps* (Rudolphi, 1819) (Fig. 4) in gizzard of one black-shouldered kite, *Desportesius invaginatus* (Linstow, 1901) (Fig. 5) in gizzard of two western cattle egret and *Cosmocephalus obvelatus* (Creplin, 1825) (Fig. 6) in proventriculus of one yellow-legged gull.

For the class Cestoda, 3 helminth taxa of 2 orders were identified: for the order Cyclophyllidea were found *Neyraia intricata* (Krabbe, 1882) (Fig.7a) in small intestine of the Eurasian hoopoe and *Diplophallus* sp. Fuhrmann, 1900 (Fig. 7b-d) in small intestine of the black-winged stilt and; and for the order Tetrabothriidea was found *Tetrabothrius* sp. Rudolphi, 1819 (Fig. 7e-f) found in small intestine of the lesser black-backed gull.

## 4. Discussion

The results in this study are difficult to compare with other surveys, since the number of birds sampled by us, was very small. However, we report what is, to our knowledge, the first record of Bonelli's eagle as hosts for nematode *Porrocaecum angusticolle*. In addition, most of findings are new record for Portugal, namely *Contracaecum* sp. (in *Morus bassanus*), *Cosmocephalus obvelatus* (in *Larus michahellis*), *Desportesius invaginatus* (in *Bubulcus ibis*), *Dispharynx nasuta* (in *Athene noctua*), *P. angusticolle* (in *Aquila fasciata*), *Diplophallus* sp. (in *Himantopus himantopus*) and *Neyraia intricata* (in *Upupa epops*).

According to the laboratorial methods used only one helminth species was detected in gastrointestinal tract of each bird sampled. These results showed that each wild bird species presented specific helminth species.

In addition to the parasitized birds, others avian species were analyzed: Eurasian eagle-owl (*Bubo bubo*), Eurasian nightjar (*Caprimulgus europaeus*) and grey heron (*Ardea cinerea*), but no parasites were detected in the present study.

Most of helminth species with exception of the nematode *Dispharynx nasuta*, showed a clear relationship with birds’ diet. So, gets demonstrated the important role of intermediate and paratenic hosts, where the larval forms of parasites are presents, in the transmission these parasites. In the case of *D. nasuta*, terrestrial isopods are recognized as intermediate hosts. However Tomé et al. (2008) has not reported these invertebrates as prey of little owl in Portugal. This suggests that this nematode might use others invertebrates or/and vertebrates as intermediate and paratenic hosts, respectively, as occurs in the case of the others spirurids (Anderson, 2000).

The nematode *Contracaecum* sp. and cestodes *Diplophallus* sp. and *Tetrabothrius* sp. could not be identified to species level. In the first case, the absence of male specimens prevented the identification of the species, once this is based in the number and arrangement of their caudal papillae (Fagerholm, 1991). However, only *C. pelagicum* has been reported by Silva et al. (2005) in piscivorous birds phylogenetically close with *Morus bassanus*, namely brown booby (*Sula leucogaster*), from Brazil.

In the case of the *Diplophallus* and *Tetrabothrius* genus, the bad condition of the specimens, in part attributed to the cold of refrigerator to which the birds were exposed, prevented the identification of the species. Until the date, only *D. polymorphus* has been recorded in *Himantopus himantopus* (Burt, 1980). In turn, *T. cylindraceus* and *T. erostris* are the common species of the genus *Tetrabothrius* in gulls from north Atlantic and Mediterranean sea, being that only the later specie has been recorded in lesser black-backed gull (Alvarez et al., 2006; Bosch et al., 2000; Galkin et al., 1994; Pemberton, 1963; Sanmartín et al., 2005; Santoro et al., 2011).

Although the common paradigm present in most of these studies, where don't evaluated the diagnostic forms by helminthic infections, and so, the results are useless for staff of rehabilitation centers, this study allowed identification of helminth species, until the date, unknown in birds of Portugal.

Unfortunately, in this study were not found pathological effects linked to helminth infections. Usually, no severe disease or clinical signs are detected in *Contracaecum* and *Porrocaecum* infections (Fagerholm and Overstreet, 2008). However, Deardorff and Overstreet (1980) recorded ulcers with inflammatory infiltration in the mucosa of the proventriculus and the esophagus linked to several species of *Contracaecum*; In addition, Obendorf et al. (1980) reported acute parasitic gastric ulceration accompanied hemorrhage and occasionally the lumen of the proventriculus and gizzard, presented pale musculature and blood, in infections by *C. spiculigerum*. Little is known about the birds responses to species of *Porrocaecum*. Santoro et al. (2010) recorded cachexia, duodenal obstruction and perforation in the *Buteo buteo* and *Circus aeruginosus* parasitized by the nematode *Porrocaecum angusticolle* and *Porrocaecum* spp., respectively.

In the case of the nematode *Dispharynx nasuta*, Schulman et al. (1992) refers inflammation with multifocal petechial hemorrhages and thickening of the mucosa that leads to functional obstruction of the digestive tract. Santoro et al. (2010) reported erosions and ulcers of the gastric mucosa associated with the *Synhimantus laticeps* infection. Nothing is known about pathogenic effects of the nematodes *Desportesius invaginatus* and *Cosmocephalus obvelatus* on birds. However Obiekezie et al. (1992) reported the occurrence of stomach wall granulomas in European smelt (*Osmerus eperlanus*), an important paratenic host responsible for transmission of *C. obvelatus* to marine bird species.

Usually, wild birds infected by cestodes are asymptomatic, but when clinical signs are present, they are nonspecific (McLaughlin, 2008). *Tetrabothrius* parasites found in intestine of the lesser black-backed gull, has been associated with cachexia cases of Short-tailed Shearwaters (*Puffinus tenuirostris*) and Little Penguins (*Eudyptula minor*) (Nishigai et al., 1981; Obendorf et al., 1980). Despite the apparent malnutrition and anemia of Short-tailed Shearwaters, parasitized by *Tetrabothrius scoogi*, Nishigai et al. (1981) not identified gross and microscopic lesions. According Bosch et al. (2000), the body condition of the Yellow legged gull infected by *T. erostris* had been poorer if they were simultaneous parasitized by *Cosmocephalus obvelatus*. However, *Tetrabothrius* sp. infection, has been considered by Obendorf et al. (1980) as to be of minor significance, by the absence of lesions.

## 5. Conclusions

This study demonstrates that the common wild birds in southern Portugal are parasitized by a wide variety of helminths, being nematodes the most frequents. Helminth communities showed lower species richness for all wild birds, but might also be due to a lower sampling effort. With exception of *Dispharynx nasuta*, helminth communities showed a clear relationship with birds' diet. This is the first parasite-host association in the world for *Porrocaecum angusticolle* in Bonelli's eagle and also the first report of *Contracaecum* sp., *Cosmocephalus obvelatus*, *Desportesius invaginatus*, *Dispharynx nasuta*, *Porrocaecum angusticolle*, *Diplophallus* sp. and *Neyraia intricata* to be recorded from wild birds in Portugal.

## Conflict of interest

The authors declared that there is no conflict of interest.

## Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed. Animal manipulation in the RIAS was performed by suitably qualified professionals, according to the directive 86/609/EEC. All experimental assays, with or without the use of animals were performed in accordance with Government Veterinary Service (Direcção Geral de Veterinária/DGV).

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**Table 1**
Prevalence of infection and helminth taxa

| | Total |
|---|---|
| Hosts examined | 22 |
| Hosts infected | 12 (54.5%) |
| Infected by Nematodes | 9 (40.9%) |
| Infected by Cestodes | 3 (13.6%) |

**Table 2**
Distribution of helminths species found in the gastrointestinal tract of wild birds from RIAS (southern Portugal), including location in host, mean helminth intensity on infected hosts and references with respectively geographic location where this host-parasite has been reported.

| | Host | Common name | Location in Host | *n* | *P* (%) | MI ± SD | References – Locality where this association host-parasite has been reported |
|---|---|---|---|---|---|---|---|
| NEMATODES | | | | | | | |
| *Porrocaecum angusticolle* | *Aquila fasciata* | Bonelli's eagle | Small Intestine | 1 | 1 (100.0%) | 5.0 | – |
| *Contracaecum* sp. | *Morus bassanus* | Northern gannet | Esophagus | 6 | 2 (33.3%) | 1.5 ± 0.5 | Forrester and Spalding (2003) – United States of America |
| *Synhimantus laticeps* | *Elanus caeruleus* | Black-shouldered kite | Gizzard | 1 | 1 (100.0%) | 4.0 | Seurat (1919) – Algeria |
| *Cosmocephalus obvelatus* | *Larus michahellis* | Yellow-legged gull | Proventriculus | 3 | 1 (33.3%) | 1.0 | (Alvarez et al., 2006; Bosch et al., 2000; Parejo et al., 2015; Sanmartín et al., 2005) – Spain |
| *Desportesius invaginatus* | *Bubulcus ibis* | Western cattle egret | Gizzard | 2 | 2 (100.0%) | 2.5 ± 0.5 | Wong and Anderson (1986) – Egypt, India and Taiwan |
| *Dispharynx nasuta* | *Athene noctua* | Little owl | Esophagus and Proventriculus | 3 | 2 (66.7%) | 4.0 ± 2.0 | Gomez et al. (1993) – Spain<br>Santoro et al. (2012) – Italy |
| CESTODES | | | | | | | |
| *Tetrabothrius* sp. | *Larus fuscus* | Lesser black-backed gull | Small Intestine | 1 | 1 (100.0%) | 2.0 | Pemberton (1963) – United Kingdom<br>Galkin et al. (1994) – Russia |
| *Diplophallus* sp. | *Himantopus himantopus* | Black-winged stilt | Small Intestine | 1 | 1 (100.0%) | 3.0 | Burt (1980) – United States of America<br>Al-Awadi, (2010) – Iraq |
| *Neyraia intricata* | *Upupa epops* | Eurasian hoopoe | Small Intestine | 1 | 1 (100.0%) | 1.0 | Joyeux and Timon-David (1934) – France<br>Mahon (1958) – Egypt |

*n*: number of birds examined; *P*: prevalence (percentage of infected birds, %); MI: mean intensity (mean number of worms per infected bird); SD: Standard deviation; However, SD of mean intensity was not be calculated when the number of infected hosts was one.

**Figure Legends**

**Fig. 1.** *Contracaecum* sp. from northern gannet (*Morus bassanus*), female (a-c: Photomicrographs; d-f: Scanning electron micrographs). (a) Cephalic end, dorsal view; note lips (L), interlabia (Il) and cephalic collar conspicuous (CC). (b) Anterior part, dorsal view; note oesophagus (O), ventriculus oval (V), ventricular appendix (VA), intestinal caecum (IC) and intestine (I). (c) Region of vulva (Vu), dorsal view. (d) Anterior part of body, dorsal-lateral view. (e) Cephalic end, apical view. (f) Posterior end of body, ventral view Scale bars: a, d = 100 μm; b = 500 μm; c, f = 200 μm; e = 50 μm.

**Fig. 2**. *Porrocaecum angusticolle* from Bonelli's eagle (*Aquila fasciata*), female (a-c: Photomicrographs; d-f: Scanning electron micrographs). (a) Cephalic end, dorsal view; note lips (L) and interlabia (Il). (b) Anterior part, dorsal view; note oesophagus (O), ventriculus oblong (V), intestinal caecum (IC) and intestine (I). (c) Region of vulva (Vu), dorsal view. (d) Anterior part of body, dorsal-lateral view. (e) Denticles extending laterally along lips, almost to its base, ventral view. (f) Posterior end of body, dorsal view. Scale bars: a = 100 μm; b = 500 μm; c = 300 μm; d, f = 200 μm; e = 10 μm.

**Fig. 3**. Photomicrographs of *Dispharynx nasuta* from little owl (*Athene noctua*). (a) Anterior region, lateral view; note cordons recurrent but not anastomosed (arrows head) and cervical papillae (CP). (b) Cervical papillae tricuspid, lateral view. (c) Vulva (Vu) lies at the middle of body, lateral view. (d) Posterior end of male, lateral view; note shapes of caudal spicules; left spicule (LS) is long and slender and right spicule (RS) is short and thick. Scale Bars: a = 300 μm; b = 20 μm; c, d = 100 μm.

**Fig. 4**. Photomicrographs of *Synhimantus laticeps* from black-shouldered kite (*Elanus caeruleus*). (a) Anterior region, lateral view; note cordons recurrent and anastomosed (CA) and cervical papillae (CP). (b) Cephalic end, ventral view; note nerve ring (arrows head) located at the level of the anterior end of the muscular oesophagus. (c) Cervical papillae tricuspid, lateral view. (d) Vulva (Vu) lies at the middle of body, lateral view. Scale Bars: a = 300 µm; b = 100 µm; c = 25 µm; d = 200 µm.

**Fig. 5**. Photomicrographs of *Desportesius invaginatus* from western cattle egret (*Bubulcus ibis*). (a) Anterior region, dorsal-lateral view; note cordons recurrent and anastomosed (CA) and cervical papillae (CP). (b) Expanded posterior part of cordons, lateral view. (c) Cervical papillae tricuspid, lateral view. (d) Posterior end of male, lateral view; note shapes of caudal spicules; left spicule (LS) is long, slender and transversely striated and right spicule (RS) is long, thick and transversely striated. (e) Vulva (Vu) lies near the posterior extremity of body of the female and knob-shaped structure (arrow head), ventral view. Scale Bars: a = 300 µm; b, c = 25 µm; d = 200 µm; e = 100 µm.

**Fig. 6.** Photomicrographs of *Cosmocephalus obvelatus* from yellow-legged gull (*Larus michahellis*). (a) Anterior region, dorsal view; note cordons recurrent, anastomosed and forming loop in adjacent base of cordons (arrow head) and cervical papillae (CP). (b) Loop (black arrow head) and anastomose region (white arrow head) of the cordon, lateral view. (c) Cervical papillae bicuspid, lateral view. (d) Posterior end of male, lateral view; note shapes of caudal spicules; left spicule (LS) is long and slender and right spicule (RS) is short and thick. (e) Caudal extremity of male, ventral view; note 4

pais of precloacal pedunculate papillae (white arrow head) and 5 pairs of postcloacal pedunculate papillae (black arrow head). Scale Bars: a, d, e = 200 μm; b, c = 25 μm.

**Fig. 7**. Cestodes (a-c: Photomicrographs; d-f: Scanning electron micrographs). (a) Ripe segment of *Neyraia intricata* found in Eurasian hoopoe (*Upuppa epops*), dorsal view. (b) Scolex of *Diplophallus* sp. found in black-winged stilt (*Himantopus himantopus*), dorsal view. (c) Embryophore egg containing oncosphere of *Diplophallus* sp., dorsal view. (d) Scolex of *Diplophallus* sp. with 2 suckers visible, dorsal view. (e) Scolex of *Tetrabothrius* sp. of lesser black-backed gull (*Larus fuscus*), apical view. (f) Scolex of *Tetrabothrius* sp. showing shape of bothridia, dorsal-lateral view. Scale Bars: a, d = 200 μm; b, e, f = 100 μm; C = 20 μm.

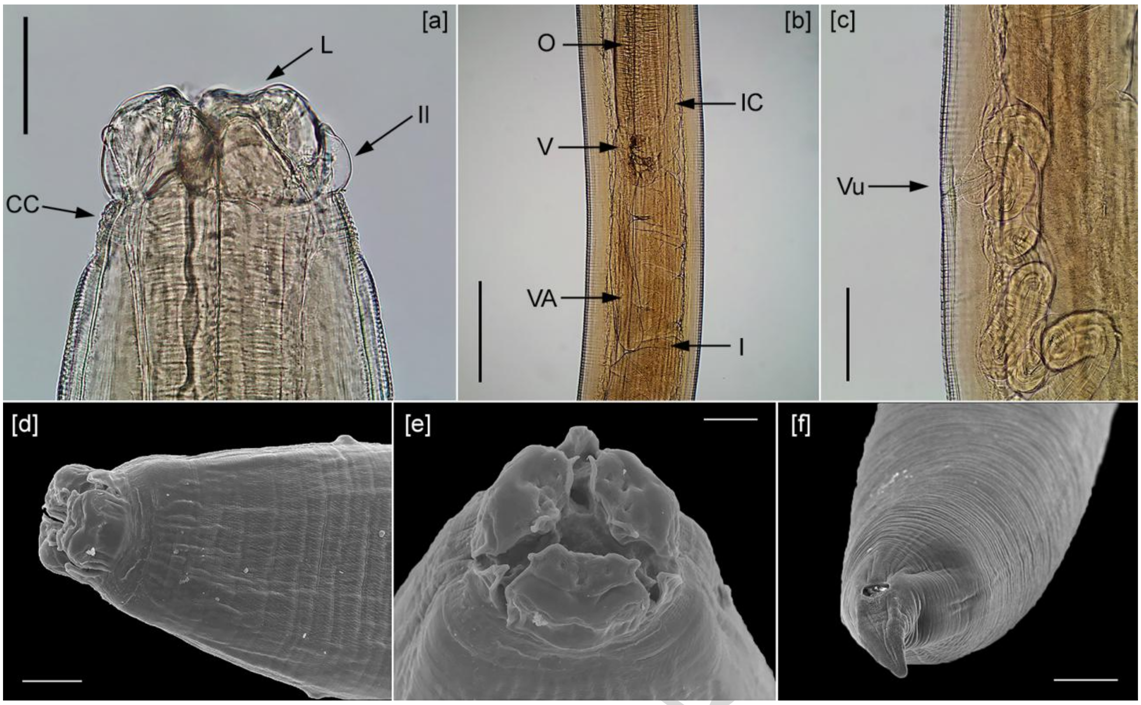
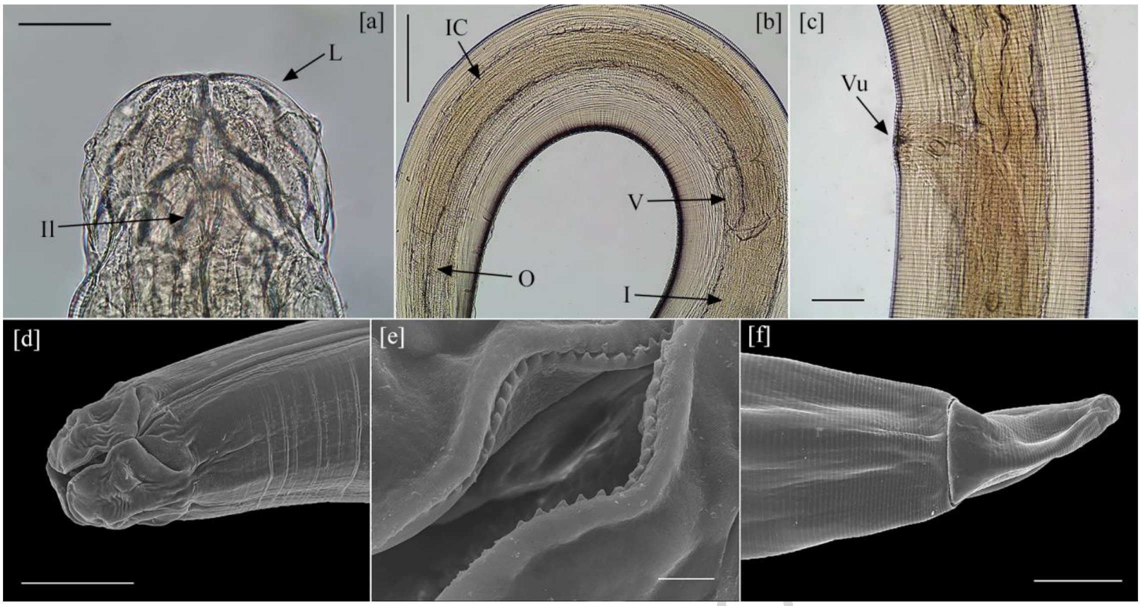



Figure 1

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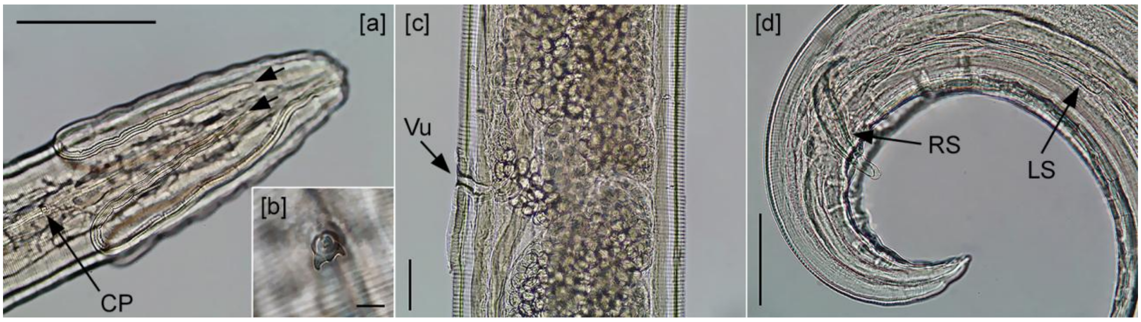


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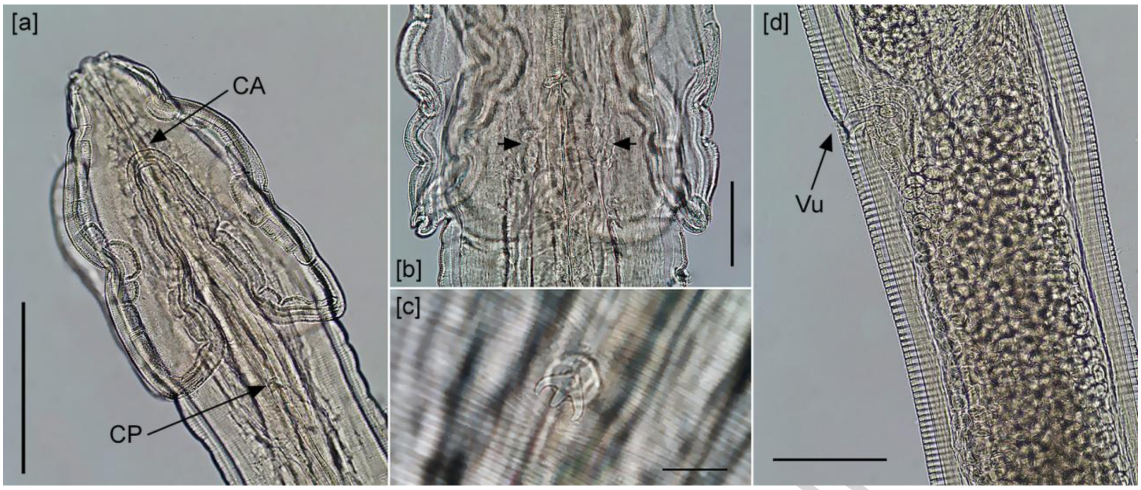



Figure 4

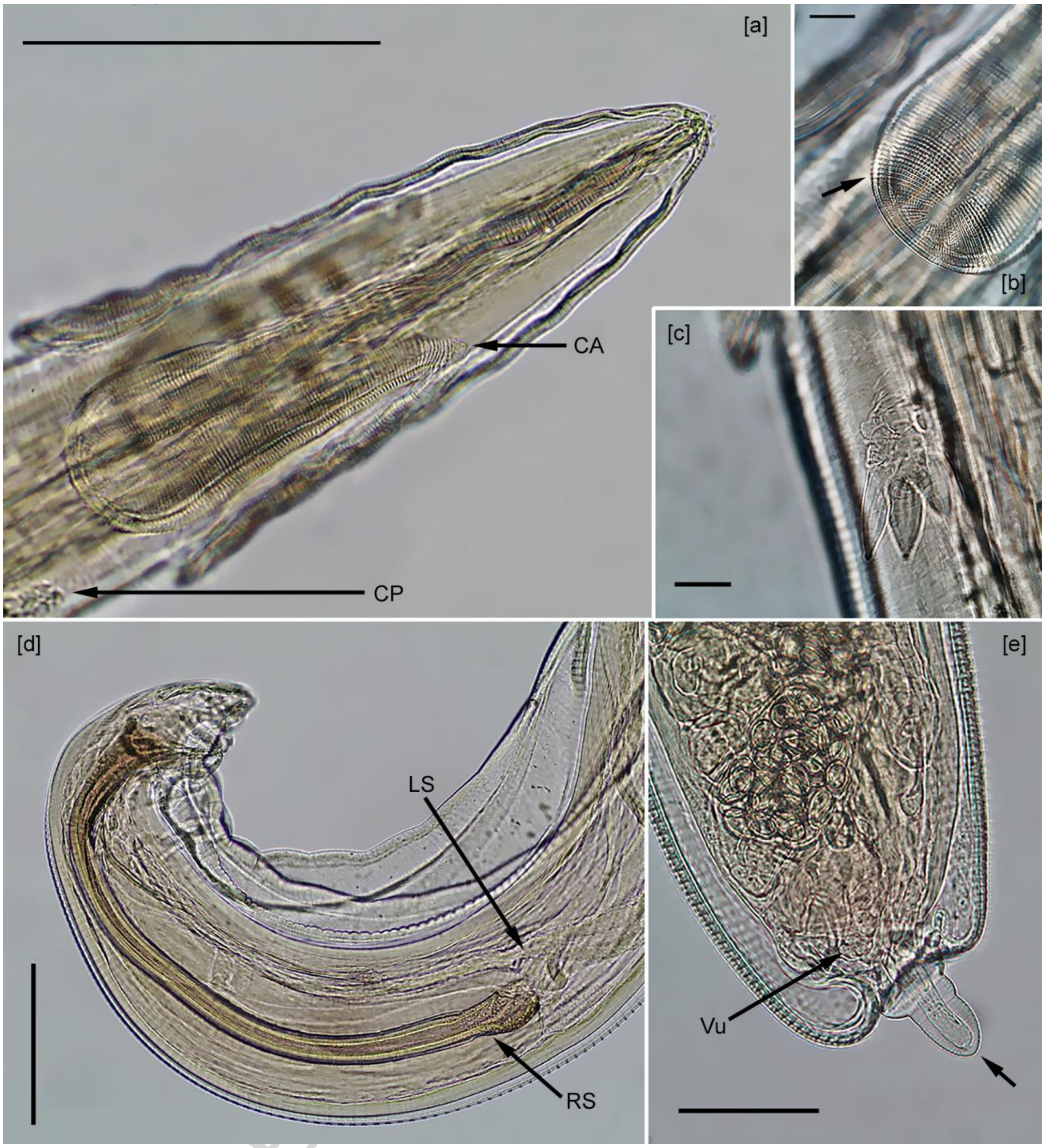



Figure 5

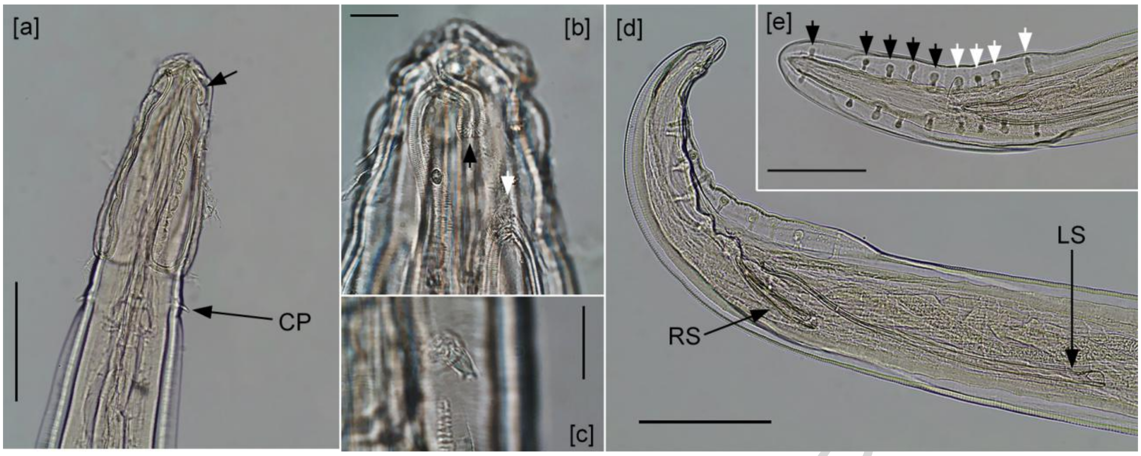
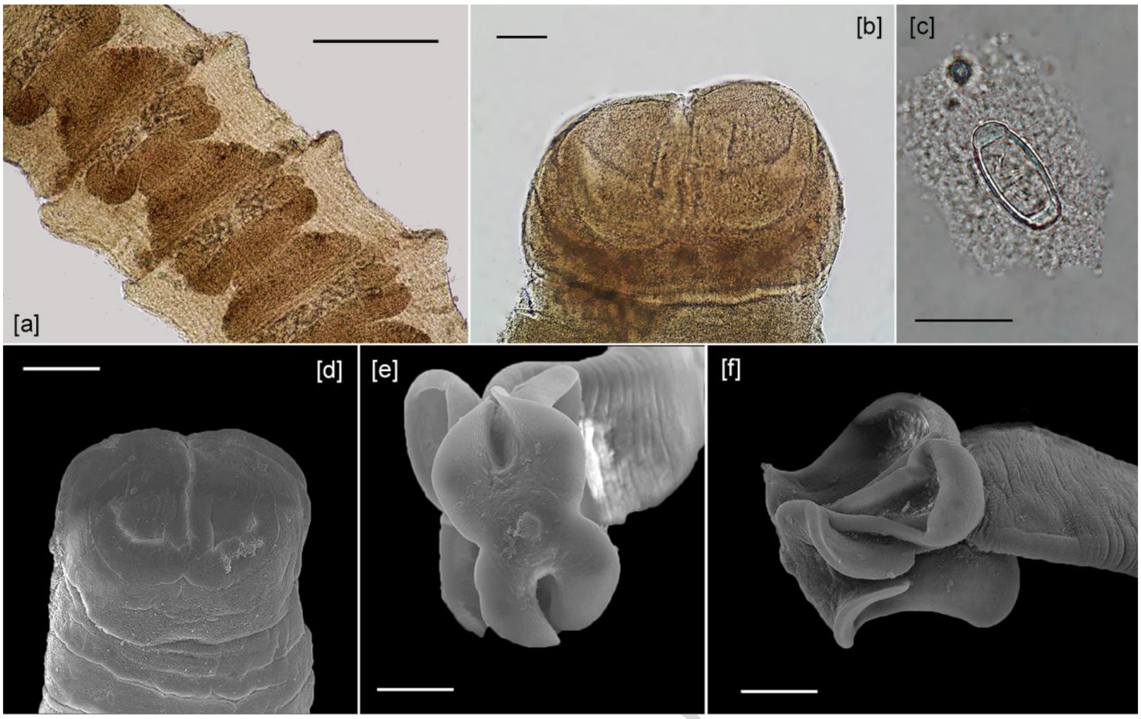



Figure 6

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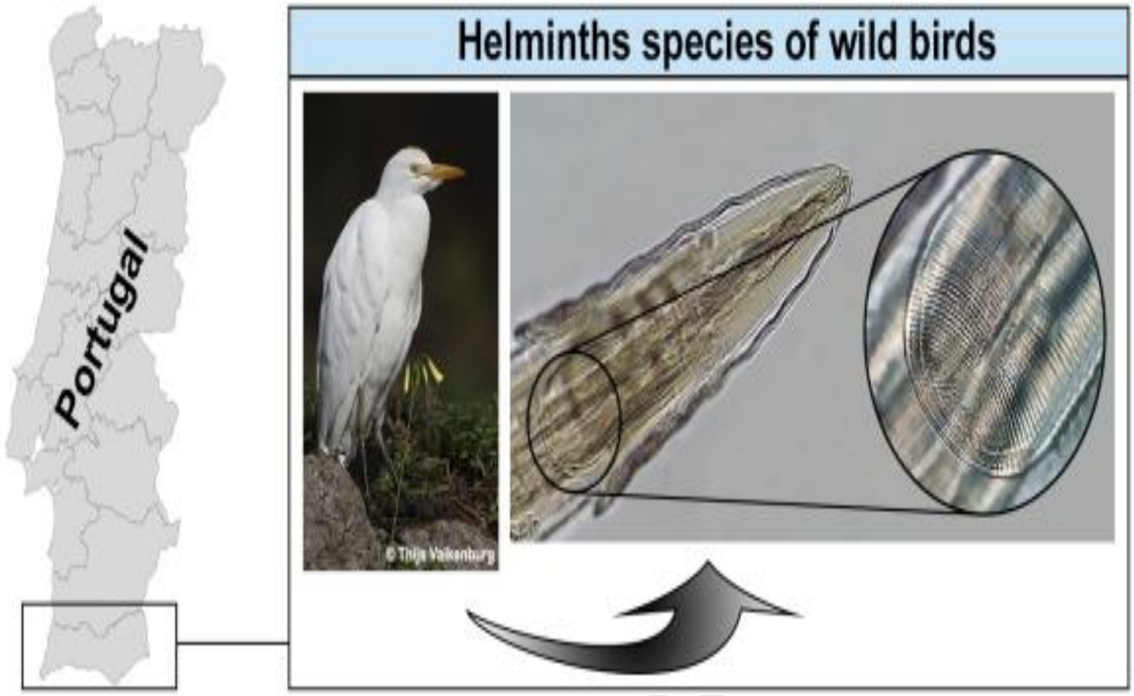


Graphical abstract

Highlights

- We investigate about helminth species of wild birds of southern Portugal.
- Helminths were found on 12 (54.5%) of the 22 wild birds examined.
- Five helminth species and two genera were reported for the first time in Portugal.
- First report in the world of *Porrocaecum angusticolle* in the Bonelli's eagle.