*Survey on intestinal helminth fauna of woodcocks (*Scolopax rusticola*) in Italy*

Barbara Paoletti[1*], Angela Di Cesare[1], Raffaella Iorio[1], Domenico Tavaglione[2], Roberto Bartolini[1] & Antonio Gatti[1]

[1] *Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy.*
[2] *Veterinarian, Teramo, Italy.*

** Corresponding author at: Faculty of Veterinary Medicine, Teaching Veterinary Hospital, Località Piano D'Accio snc, 64100 Teramo, Italy. Tel.: +39 0861 266870, e-mail: bpaoletti@unite.it.*

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Keywords
Acanthocephalan, Cestoda, Helminth, Parasitological, Survey, Trematode, Woodcocks.

Summary
Every year populations of the European woodcock (*Scolopax rusticola*) migrate from Central and Northern Europe to the Mediterranean basin. South of Italy is one of the most common wintering site for this species. Given that information on parasites of woodcocks is scarce, the present study aimed at identifying the parasitic species affecting woodcocks migrating in Italy. The gastrointestinal tract of 206 woodcocks hunted in Southern Italy was removed and examined for parasites. From each animal a faecal sample was analysed by flotation test. The necropsy showed the presence of cestodes, *i.e. Paricterotaenia paradoxa* (59.4%) and *Aploparaksis filum* (49.5%), and of acanthocephalan *Prosthorhynchus scolopacidis* (22.4%). In one bird we also detected *Parastrigea robusta*, which is a trematode until now reported only in mallards (*Anas platyrhynchos*). Mixed infections (*i.e.,* polyspecific infections) were detected in 53 (27.6%) animals. The most common were those caused by *A. filum* and *P. paradoxa* (12.5%), and by *P. paradoxa* and *P. scolopacidis* (8.3%). Copromicroscopic examinations revealed the presence of eggs belonging to nematodes *Syngamus* spp. (1.94%) in 4 woodcocks and of eggs of cestodes *Aploparaksis* spp. (37.86%) in 78 woodcocks. The present results fill a gap in the knowledge on parasites affecting woodcocks.

*Fauna elmintica intestinale della beccaccia (*Scolopax rusticola*) in Italia*

Parole chiave
Acantocefali, Beccacce, Cestodi, Elminti, Indagine parassitologica, Trematodi.

Riassunto
La popolazione europea di beccacce (*Scolopax rusticola*) annualmente migra dal centro e dal nord Europa verso il bacino del Mediterraneo, in particolare in Italia meridionale, sito di svernamento ideale per questa specie. Le informazioni sui parassiti degli scolopacidi sono scarse e frammentarie, e il presente studio ha lo scopo di individuare le specie di elminti che parassitano le beccacce migrate in Italia. I ventrigli e gli intestini di 206 soggetti, abbattuti nell' Italia meridionale, sono stati esaminati per la ricerca dei parassiti, e da ciascun animale è stato prelevato un campione di feci. Dei 206 animali esaminati 192 (93,2%) sono risultati infettati da almeno una specie di elminta. Gli esami necroscopici hanno evidenziato la presenza di cestodi, *Paricterotaenia paradoxa* (59,4%) e *Aploparaksis filum* (49,5%), di acantocefali, *Prosthorhynchus scolopacidis* (22,4%), e in un unico volatile sono stati identificati dei trematodi appartenenti alla specie *Parastrigea robusta*, fino ad ora riportati solo nel germano reale (*Anas platyrhynchos*). Infezioni miste (*i.e.,* infezioni polispecifiche) sono state rilevate in 53 (27,6%) soggetti. Le infezioni più comuni sono state quelle causate da *A. filum* e *P. paradoxa* (12,5%) e *P. paradoxa* e *P. scolopacidis* (8,3%). Gli esami copromicroscopici hanno rivelato la presenza di uova appartenenti ai nematodi *Syngamus* spp. in 4 beccacce (1,94%) e ai cestodi *Aploparaksis* spp. in 78 beccacce (37.86%). I presenti risultati apportano un contributo alla conoscenza dell'elmintofauna dell'apparato digerente delle beccacce.

The presence of woodcocks (*Scolopax rusticola*) in Italy, as a nesting species, concerns only specific geographical areas (the Alpine regions, the Tuscan-Emilian Apennines, the Liguria region and the Po Valley). As a migratory species, woodcocks can be found all over the country from October to November and from February to March. Indeed, Italy, along with other countries of the Mediterranean basin, is an area of choice for the wintering of these birds. It is estimated that few million of woodcocks that spend the Winter in Southern Italy every year (ISPRA 2008). Modifications in their natural habitat, the unstoppable destruction of forests, the thinning of deciduous and coniferous forests of tall trees and hunting pressure are drivers playing a crucial role in the inexorable reduction of the population of these birds, alongside to the pathogenic role of different parasites impairing health and welfare of these birds (Combes 1996, Sorgi *et al.* 2005). Indeed, the literature on the distribution and epidemiology of parasites affecting woodcocks is scarce and fragmented (Joveux and Baer 1936, Bondarenko 1986, Bondarenko 1989, Bondarenko 1990 a, b, Bondarenko 1993, Sorgi *et al.* 2004, Sorgi *et al.* 2005). The present study was conducted to investigate the intestinal helminth fauna of woodcocks in order to enhance our knowledge of parasites affecting these scolopacid birds.

Between 2010 to 2012, 206 woodcocks were hunted in Southern Italy and delivered to the Parasitology lab of the Veterinary Medicine faculty, University of Teramo (Italy). During transportation, the woodcock carcasses were preserved at a refrigeration temperature. The gizzard and intestinal package of each animal was opened longitudinally, helminths were collected and counted according to the techniques described by Krone (Krone 2007). Worms were washed in saline solution and fixed in 70% ethanol; trematodes and cestodes were stained with Mayer's acid carmine and mounted in Canada balsam, and acanthocephalans were cleared in lactophenol on a glass slide for identification. From each terminal intestinal portion, a faecal sample was collected and stored in 5% formalin before being subjected to the copromicroscopic examination. Faeces were examined by a flotation technique using a solution of sodium nitrate and sucrose of 1,350 gravity weight.

Individual adult worms were identified morphologically and morphometrically to species according to the keys of Yamaguti (Yamaguti 1959, Yamaguti 1963), Davies (Davies 1940), Dawes (Dawes 1968), Olsen (Olsen 1970), Euzeby (Euzeby 1982), Bona (Bona 1994), Amin and colleagues (Amin *et al.* 1999), Bondarenko and Kontrimavichus (Bondarenko and Kontrimavichus 2005, Bondarenko and Kontrimavichus 2006).

At parasitological analysis, 14 specimens of trematodes were isolated from the gut of a single woodcock. After the evaluation of morphological and morphometric characteristics, the specimens were identified as *Parastrigea robusta* (Phylum Platyhelminthes, Class Trematoda Digenea, Order Strigeida, Family Strigeidae) (Dawes 1968, Euzeby 1982) (Figure 1). In particular, the specimens of *P. robusta*, were 2-2.8 mm long and broader in the anterior than in the posterior region, respective breadths 1.1 and 1.7 mm. The oral sucker was smaller than the ventral, 270 x 250 µm and 280 x 260 µm. The genital papillae were large and oviformis. Testes were fairly compact and only slightly lobed. Vitellaria was well developed in the adhesive organ; eggs large (90-110 x 60-70 µm). Fifty-five specimens of acanthocephalans have been found and collected from the intestine of 43 woodcocks. These parasites were identified as *Prosthorhynchus scolopacidis* (Phylum

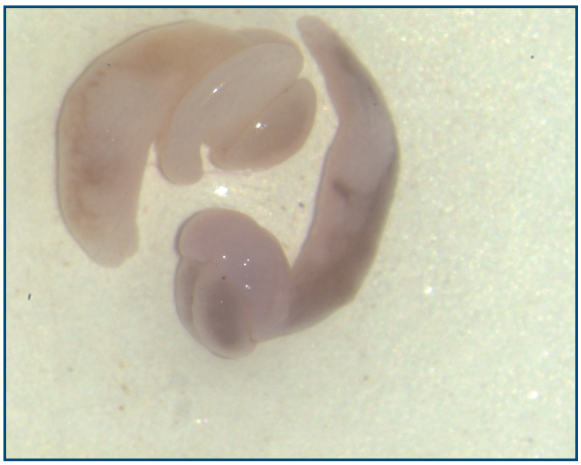

Figure 1. Parastrigea robusta*: adult specimens detected in woodcocks in Southern Italy between 2010 and 2012.*

Figure 2. Prosthorhynchus scolopacidis*: adult specimens with proboscis everted detected in woodcocks in Southern Italy between 2010 and 2012.*

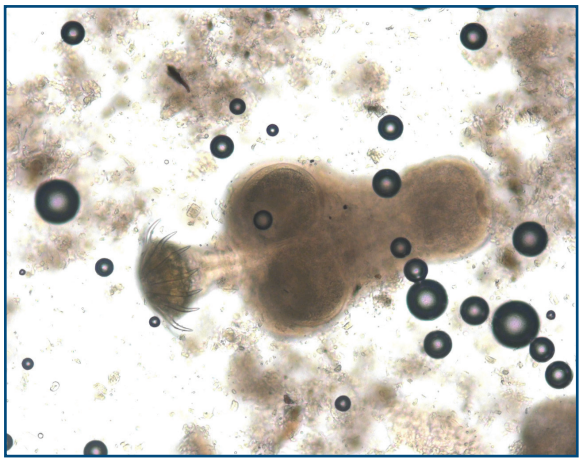

Figure 3. Paricterotaenia paradoxa*: adult specimen detected in woodcocks in Southern Italy between 2010 and 2012.*

Acanthocephales, Class Acanthocephala, Subclass Gigantorhynchinea, Order Gigantornynchidiformes, Family Prosthorhynchidae) (Yamaguti 1963, Amin *et al.* 1999) (Figure 2). Male specimens of *P. scolopacidis* were 35-50 mm long, females were 40-60 mm long, and showed cylindrical body and aspinose. Proboscis cylindrical with numerous hooks (14-30 x 7-22), the trunk was not pigmented. Proboscis with 1-3 spine-like hooks posteriorly. Eggs were short and oval shaped, always lacking of polar prolongations. The tapeworm *Paricterotaenia paradoxa* (Phylum Platyhelminthes, Class Cestoda, Subclass Nephroposticophora, Order Cyclophyllidea, Family Dilepididae) was found in the intestine of 114 woodcocks (Figure 3) (Yamaguti 1959, Bona 1994). The specimens were 0.3-2.5 mm long, with 2-6 proglottids. They had a retractable rostellum arm with a row of hooks (14-18). The proglottids were craspedote. The genital pores were usually alternated while the genital ducts ran dorsally to the excretory canals. The uterus was sac-like and highly lobulated. The testicles were few in number, pockets cirrus were undeveloped and covered with small spines. Eggs were characterized by the presence of a well-developed embryo. In addition, from the analysis of the small intestine of 95 woodcocks, 175 specimens of *Aploparaksis filum* (Phylum Platyhelminthes, Class Cestoda, Order Cyclophyllidea, Family Hymenolepididae) were identified (Davies 1940, Wilford Olsen 1970, Bondarenko and Kontrimavichus 2005, Bondarenko and Kontrimavichus 2006) (Figure 4, 5, and 6). These cestodes were 60-170 mm long with numerous proglottids, which could reach a maximum width of 1 millimeter. They had a retractable rostellum armed with 10 hooks. Strobilas were traversed by a pair of dorsal and a pair of ventral excretory vessels. The proglottids had only one testicle oval, normally placed in the middle position or slightly cranial to the genital pore, which were unilateral, marginal or submarginal. Ovaries were compact or irregularly

Figure 4. Aploparaksis filum*: scolex with protruded rostellum and with a single row of hooks isolated in woodcocks in Southern Italy between 2010 and 2012.*

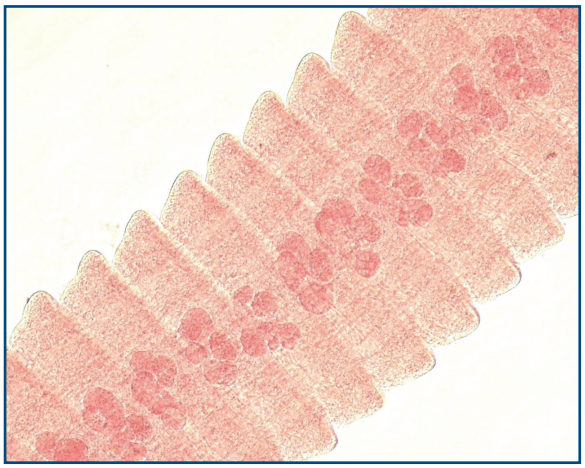

Figure 5. Aploparaksis filum*: mature hermaphroditic proglottides detected in woodocks in Southern Italy between 2010 and 2012.*

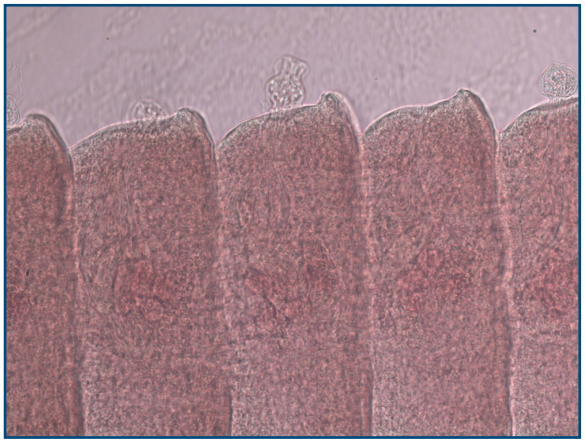

Figure 6. Aploparaksis filum*: cirrus detected in woodocks in Southern Italy between 2010 and 2012.*

Table I. *Number of woodcocks (*Scolopax rusticola*) with monospecific infection in Southern Italy between 2010 and 2012.*

Helminth species	Number of woodcocks
Paricterotaenia paradoxa	66
Aploparaksis filum	58
Parastrigea robusta	1
Prosthorhynchus scolopacidis	14

Table II. *Number of woodcocks (*Scolopax rusticola*) infested with more species of helminth (polyspecific infection) in Southern Italy between 2010 and 2012.*

Helminth species	Number of woodcocks
Paricterotaenia paradoxa, Aploparaksis filum	25
Prosthorhynchus scolopacis, Paricterotaenia paradoxa	16
Prosthorhynchus scolopacidis, Aploparaksis filum	5
Prosthorhynchus scolopacidis, Aploparaksis filum, Paricterotaenia paradoxa	8

lobed, usually in the middle position. The external vesiculars were generally visible as a large oval sac dorsal to the tip of the cirrus-sac. The interior of the cirrus-sacs was occupied by a long, coiled cirrus, and a voluminous internal vesicular seminalis. The everted cirrus reached a length of 190 µm. Uterus was at first a simple, oval, transverse sac, confined to the region between the excretory vessels. Later it becomes fairly deeply lobed. The eggs (34-32 µm) were generally oval and consisted of a hexacanth embryo surrounded by three envelopes.

Of the 206 faecal samples examined, 78 were found positive to cestodes and 4 were found positive to nematodes, *Aploparaksis* spp. (37.86%) and *Syngamus* spp. (1.94%), respectively.

Through necroscopy, it was possible to observe monospecific infection in 139 woodcocks (72.4%) (Table I) and polyspecific infection in 53 woodcocks (27.6%) (Table II); the most frequent associations were between *A. filum* and *P. paradoxa* (12.5%), and between *P. paradoxa* and *P. scolopacidis* (8.33%) (Table II). Copromicroscopic examinations revealed the presence of eggs belonging to the nematodes *Syngamus* spp. and to the cestodes *Aploparaksis* spp. Due to the specific gravity of the floating solution used in this study, acanthocephalan and trematode eggs were never highlighted (Euzeby 1982). When compared with the direct determination of helminths in the intestine, the copromicroscopic technique showed a sensitivity of 37.86% and a specificity of 100% for *Aploparaksis* spp.

In conclusion, 192 of 206 (93.20%) woodcocks herein analysed were affected by at least 1 parasite. The present investigation is one of the few studies carried out on the intestinal helminth fauna of the woodcock (*Scolopax rusticola*). Occurrence of parasites infesting this bird species is directly related to their diet, because they feed on invertebrate organisms, which could act as intermediate hosts of nematodes, acanthocephalans, cestodes, and trematodes, which recognize the woodcock as a definitive host. All cestodes and acanthocephalans identified in this study are host-specific, as demonstrated in other studies (Joyeux and Baer 1936, Sorgi *et al.* 2004, Sorgi *et al.* 2005). The lack of detection of adult nematodes of the genus *Syngamus* in birds resulted positive at copromicroscopic examination is due to the fact that these parasites are located in the respiratory tract (Taylor *et al.* 2010). The high percentage of woodcocks infested with cestodes (93.20%) corresponds to what was reported by Sorgi and colleagues (> 60%) (Sorgi *et al.* 2004, Sorgi *et al.* 2005) as well as the low sensitivity by copromicroscopic examination compared to the direct determination of cestodes in gastrointestinal tract. Unlike existing studies, in the present work *Dilepidis undula* was not found, probably due to the different presence/absence and low concentration of intermediate hosts in the geographical areas investigated. Like other countries of the Mediterranean basin, Italy is an area where the woodcock spends the Winter, while living for most of the year in Central and Northern Europe. Thus, it is likely that these birds arrive in Italy already parasitized, indeed species of parasites identified in this study have been reported in the Palearctic regions (Bodarenko 2006, Dawes 1968, Odening 1965, Yamaguti 1959, Yamaguti 1963, Scott 1965). It is worth to stress that the detection of *P. robusta* has been reported only in mallards (*Anas platyrhynchos*) so far. The metacercariae of *P. robusta* have been found in salamanders, *Tritus vulgaris* L. and *Tritus cristaus* Laur, which are intermediate hosts found in the same environments where woodcocks live (Vojtek *et al.* 1972). Further studies are needed to confirm this species as another definitive host of *P. robusta*. The pathogenic role played by the parasites found in this study has not been determined because it was difficult to differentiate the parasitic lesions from the injuries by shotguns. However, the presence of *P. scolopacidis*, which could lead to the death of the host for intestinal perforation (Yamaguti 1963, Sorgi *et al.* 2005), and of *P. robusta*, which could cause anaemia and haemorrhagic enteritis as reported in duck (Odening 1965), is worthy of note. The pathogenic role of the other species identified in this study remains unknown.

The present study fills a gap in the literature on the parasites of woodcocks. Knowledge on parasitic species harboured by woodcocks is crucial to improve the epidemiological monitoring of wild populations of origin, especially because parasites are part of the overall diversity of ecosystems and

are worth studying in their own right. It is now recognized that the influence of parasites extends beyond the individual host to the population and community level and may affect ecosystems through differential effects on host species and their effects on keystone species (McLaughlin 2001).

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