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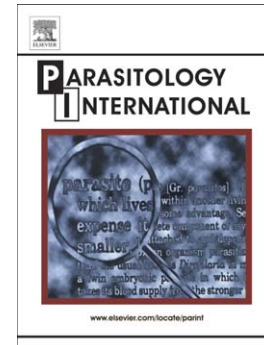
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**Chewing lice (Phthiraptera) from wild birds in southern Portugal**

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## Abstract

This study was carried out to determine chewing louse species of wild birds in the Ria Formosa Natural Park, located in southern Portugal. In addition, the hypothesis that bird age, avian migration and social behaviour have an impact on the louse prevalence was tested. Between September and December of 2013, 122 birds (belonging to 10 orders, 19 families, 31 genera and 35 species) captured in scientific ringing sessions and admitted to the Wildlife Rehabilitation and Investigation Centre of Ria Formosa were examined for lice. Twenty-six (21.3%) birds were found to be infested with at least one chewing louse species. The chewing lice identified include 18 species. Colonial birds (34.9%) and migratory birds (29.5%) had statistically significant higher prevalence than territorial birds (6.8%) and resident birds (13.1%), respectively. This paper records 17 louse species for the first time in southern Portugal: *Laemobothrion maximum*, *L. vulturis*, *Actornithophilus piceus lari*, *A. umbrinus*, *Austromenopon lutescens*, *Colpocephalum heterosoma*, *C. turbinatum*, *Eidmanniella pustulosa*, *Nosopon casteli*, *Pectinopygus bassani*, *Pseudomenopon pilosum*, *Trinoton femoratum*, *T. querquedulae*, *Craspedorrhynchus platystomus*, *Degeeriella fulva*, *Falcolipeurus quadripustulatus*, *Lunaceps schismatus*. Also a nymph of the genus *Strigiphilus* was collected from an Eurasian eagle-owl. These findings contribute to the knowledge of avian chewing lice from important birds areas in Portugal.

Keywords: Chewing louse species; Host-parasite associations; New records;

Phthiraptera; Portugal; Wild birds.

## 1. Introduction

Chewing lice (Phthiraptera: Ischnocera and Amblycera) are permanent, obligate and host-specific ectoparasites commonly found on birds. The life cycle of these insects (including egg, three nymphal and adult stages) is completed on the body of the host. Bird chewing lice feed mainly on feathers and dermal debris, although some species feed on blood [1]. Although chewing lice are relatively benign parasites, when present in large numbers, they can cause severe pruritus, plumage quality decay, small holes on feathers and an increase of feather breakage. These adverse conditions may cause changes in flight performance, thermoregulatory capacity, body mass, survival and sexual selection of the hosts [2–5].

Portugal, located in south western Europe, covers an area of 92,226 km<sup>2</sup> and has 106 important bird areas (IBAs), which cover a total of 2,905,586 hectares [6,7]. The country is divided into twenty-three NUTS 3 (Nomenclature of Territorial Units for Statistics) on the mainland and two autonomous regions (Madeira Islands and Azores Islands), each with different climates, habitats, flora and fauna [8].

To date, approximately 4,000 species of bird lice have been identified worldwide, and its geographical distribution largely overlaps the distribution of their bird hosts [1,9]. According to BirdLife International [6], there are 307 species of birds recorded from Portugal. However the number of louse species documented from birds in Portugal is limited. In the first study published in this country, Tendeiro [10] only reported species of the genus *Columbicola* Ewing, 1929. Recently, Literak et al. [11] recorded two more chewing louse species: *Brueelia tovoornikae* (Balát, 1981) and *Myrsidea sylviae* Sychra and Literak, 2008, from blackcaps (*Sylvia atricapilla*) in the Azores Islands.

Considering the scarcity of published records of lice in Portugal, additional data on the prevalence of chewing lice on wild birds is needed. Therefore, the objective of this

study was to gather new data regarding louse species from wild birds in Portugal, and in particular regarding their prevalence on their hosts, according to birds' age, phenology and social behaviour.

## **2. Materials and methods**

### **2.1. Fieldwork**

This study was conducted in the Parque Natural da Ria Formosa (PNRF), Olhão, Portugal ( $37^{\circ}2'4.55''\text{N}/7^{\circ}48'46.79''\text{O}$ ), between September and December 2013. Ria Formosa covers 23,296 ha, and is located in Algarve, the most important wetland in southern Portugal. This area offers a complex habitat, comprising barrier islands, intertidal flat zones, salt-works, sandy coasts, lagoons and muddy freshwater shores. This region is also generally fairly warm and dry due to the close proximity of the Atlantic Ocean and North Africa. Furthermore, this district lies along migratory flyways of birds and comprises ten important bird areas (IBAs) [6,12].

Data for this study were obtained from birds admitted at the Wildlife Rehabilitation and Investigation Centre of Ria Formosa – Association ALDEIA (RIAS/ALDEIA), and others captured in mist-nets during scientific ringing sessions performed in the PNRF. The RIAS/ALDEIA is the only wildlife animal hospital of the Faro District admitting wild animals in need of medical attention, delivered by competent authorities and citizens. Immediately before any medical intervention, all birds that were going to be sampled were kept in separate cages to avoid cross-contaminations.

### **2.2. Sampling data**

A total of 122 wild birds belonging to 10 orders, 19 families, 31 genera and 35 species, were examined for ectoparasites. Bird identifications were made using the field guide

by Svensson et al. [13], and each bird was classified according to: (I) age, based on plumage features; (II) social behaviour, and (III) migration status, following Catry et al. [14] and Svensson et al. [13].

Immediately following their arrival at the hospital or their capture during the scientific ringing sessions, the plumage of each bird was visually searched for chewing lice, during approximately 2 minutes. This search was followed by a standard examination involving a search of the body regions of each host following this sequence: head, nape, back, tail, wings, breast, abdomen and legs. In some cases, before the louse search, the bird's legs and wings were immobilized with the help of an assistant. During the searching period, all chewing lice seen were collected with forceps, stored in 70% ethanol and brought to the Faculty of Veterinary Medicine of University of Lisbon for examination. Animal manipulation was performed by suitably experienced professionals, minimizing the effects of the procedure.

Lice were prepared and slide-mounted according to the Canada Balsam technique [15]. Each louse was examined under a Olympus BX40 microscope coupled to a digital camera Olympus DP10, and identifications were carried out primarily following Price et al. [9]. Subsequently, were made more detailed identifications using relevant published information on the chewing lice genera involved [16–29].

The scientific names of chewing lice used in this paper follow those in the checklist published by Price et al. [9]. Slide-mounted specimens were stored in the Laboratory of Parasitology of the Faculty of Veterinary Medicine of University of Lisbon.

### **2.3. Variables**

Birds were aged according to their plumage and separated into 3 groups based on their sexual maturity: young birds ( $n=76$ ; sexually immature), adult birds ( $n=36$ ; at

reproductive age) and of indeterminate age ( $n=10$ ; their phenotype did not allow their inclusion in the other groups). Subsequently, birds were classified according to their social behaviour and migration phenology, following Catry et al. [14] and Svensson et al. [13]. Regarding social behaviour, birds were separated in two groups: colonial species ( $n=63$ ) and territorial species ( $n=59$ ). Colonial birds included *Aegyptius monachus*, *Gyps fulvus*, *Burhinus oedicnemus*, *Charadrius hiaticula*, *Chroicocephalus ridibundus*, *Larus fuscus*, *Larus michahellis*, *Himantopus himantopus*, *Calidris alpina*, *Fulica atra*, *Cyanopica cyanus*, *Bubulcus ibis*, *Phoenicopterus roseus* and *Morus bassanus*. Territorial birds were *Accipiter nisus*, *Aquila fasciata*, *Buteo buteo*, *Elanus caeruleus*, *Hieraaetus pennatus*, *Anas crecca*, *Upupa epops*, *Pluvialis squatarola*, *Scolopax rusticola*, *Certhia brachydactyla*, *Erithacus rubecula*, *Phoenicurus ochruros*, *Phylloscopus collybita*, *Sylvia atricapilla*, *Sylvia borin*, *Sylvia melanocephala*, *Turdus merula*, *Turdus philomelos*, *Ardea cinerea*, *Athene noctua* and *Bubo bubo*. According to their migration phenology, birds were divided in two groups: migratory species ( $n=61$ ; species with long-distance migration, spending the winter in Africa); non-migratory/resident species ( $n=61$ ; species that spend the winter predominantly within their breeding range or perform short-distance post-breeding movement, spending the winter in northern Africa). Migratory birds included *Accipiter nisus*, *Hieraaetus pennatus*, *Gyps fulvus*, *Anas crecca*, *Charadrius hiaticula*, *Pluvialis squatarola*, *Chroicocephalus ridibundus*, *Larus fuscus*, *Calidris alpina*, *Scolopax rusticola*, *Erithacus rubecula*, *Phylloscopus collybita*, *Sylvia borin*, *Turdus philomelos*, *Ardea cinerea*, *Phoenicopterus roseus* and *Morus bassanus*. Resident birds were *Aegyptius monachus*, *Aquila fasciata*, *Buteo buteo*, *Elanus caeruleus*, *Upupa epops*, *Burhinus oedicnemus*, *Larus michahellis*, *Himantopus himantopus*, *Fulica atra*, *Certhia*

*brachydactyla*, *Cyanopica cyanus*, *Phoenicurus ochruros*, *Sylvia atricapilla*, *Sylvia melanocephala*, *Turdus merula*, *Bubulcus ibis*, *Athene noctua* and *Bubo bubo*.

#### 2.4. Statistical analysis

Infestation prevalence and mean intensity, of each louse species from each host were evaluated for all bird species, even when the sample was one specimen. In addition, statistical associations between louse prevalence of each host order and the following variables were tested: age (birds with indeterminate age were excluded), social behaviour and migration status. The results were interpreted using Fisher's exact test with IBM® SPSS® Statistics version 20.0 (IBM Corp., Armonk, NY). Differences with  $p < 0.05$  were considered statistically significant.

### 3. Results and discussion

A total of 87 lice were collected, representing 27 males, 49 females and 11 nymphs. The specimens identified belong to suborders Amblycera and Ischnocera, distributed within 13 genera and 17 species. In addition, a nymph of *Strigiphilus* was identified to genus only.

Including the nymph of *Strigiphilus*, 12 species from eight genera and two families (Laemobothriidae and Menoponidae) belong to the suborder Amblycera, while only six species from four genera and one family (Philopteridae) belong to the suborder Ischnocera. Table 1 shows the species of lice found with their host bird species.

In the family Laemobothriidae, two species were identified: *Laemobothrion* (L.) *maximum* (Scopoli, 1763) (Fig. 1A) and *Laemobothrion* (L.) *vulturis* (Fabricius [J.C.], 1775) (Fig. 1B). In the family Menoponidae, ten species were identified: *Actornithophilus piceus lari* (Packard, 1870) (Fig. 1C), *Actornithophilus umbrinus*



(Burmeister, 1838) (Fig. 1D), *Austromenopon lutescens* (Burmeister, 1838) (Fig. 1E), *Colpocephalum heterosoma* Piaget, 1880 (Fig. 1F), *Colpocephalum turbinatum* Denny, 1842 (Fig. 1G), *Eidmanniella pustulosa* (Nitzsch [In Giebel], 1866) (Fig. 1H), *Nosopon casteli* Tendeiro, 1959 (Fig. 1I), *Pseudomenopon pilosum* (Scopoli, 1763) (Fig. 1J), *Trinoton femoratum* Piaget, 1880 (Fig. 1K) and *Trinoton querquedulae* (Linnaeus, 1758) (Fig. 1L). The five species of the family Philopteridae are: *Craspedorrhynchus platystomus* (Burmeister, 1838) (Fig. 1M), *Degeeriella fulva* (Giebel, 1874) (Fig. 1N), *Falcolipeurus quadripustulatus* (Burmeister, 1838) (Fig. 1O), *Luniceps schismatus* Gustafsson and Olsson, 2012 (Fig. 1P), *Pectinopygus bassani* (Fabricius [O.], 1780) (Fig. 1Q). In addition, one specimen of the genus *Strigiphilus* Mjöberg, 1910 (Fig. 1R) could not be identified to species due to absence of adult specimens.

Although new host-parasite associations were not found, all these 18 species of chewing lice are reported for the first time in Portugal, thus increasing their geographic distribution.

No ectoparasites were found on the remaining 25 bird species examined: azure-winged magpie (*Cyanopica cyanus*), blackcap (*Sylvia atricapilla*), black redstart (*Phoenicurus ochruros*), black-headed gull (*Chroicocephalus ridibundus*), black-shouldered kite (*Elanus caeruleus*), black-winged stilt (*Himantopus himantopus*), booted eagle (*Hieraaetus pennatus*), cinereous vulture (*Aegypius monachus*), common chiffchaff (*Phylloscopus collybita*), common ringed plover (*Charadrius hiaticula*), Eurasian blackbird (*Turdus merula*), Eurasian hoopoe (*Upupa epops*), European robin (*Erithacus rubecula*), Eurasian sparrowhawk (*Accipiter nisus*), Eurasian thick-knee (*Burhinus oedipnemos*), Eurasian woodcock (*Scolopax rusticola*), garden warbler (*Sylvia borin*), grey heron (*Ardea cinerea*), grey plover (*Pluvialis squatarola*), little owl (*Athene noctua*), lesser black-backed gull (*Larus fuscus*), sardinian warbler (*Sylvia*

*melanocephala*), short-toed tree-creeper (*Certhia brachydactyla*), song thrush (*Turdus philomelos*) and Western cattle egret (*Bubulcus ibis*).

Chewing lice were found on 26 (21.3%) of the 122 specimens of wild birds examined. Seventy-six young birds were examined, with 19 (25.0%) being infested. Among adult birds, 36 specimens were examined, with 4 (11.1%) infested. Considering social behaviour, 63 colonial birds were examined, with 22 (34.9%) infested by lice. Fifty-nine territorial birds were examined, with 4 (6.8%) infested. Sixty-one migratory birds were examined, with 18 (29.5%) infested by lice. Among resident birds, 61 specimens were examined, with 8 (13.1%) infested by lice. Chewing louse prevalence found in this study according to bird age, social behaviour and migration status are summarized in Table 2.

Infestation rates were highest in the orders Anseriformes, Phoenicopteriformes and Gruiformes (100.0%), followed by Suliformes (71.4%), Accipitriformes (61.5%), Charadriiformes (17.4%) and Strigiformes (14.3%). No lice were detected on birds belonging to the orders Passeriformes, Bucerotiformes and Pelecaniformes.

In this study, the infestation rate in charadriiform birds from this study was notably lower when compared with those from others studies, namely in Turkish shorebirds, such as the reported by Dik et al. [30], Açıci et al. [31], Dik et al. [32] and Girisgin et al. [33] with 87.8%, 66.7%, 40.0% and 28.6% of the birds parasitized, respectively. One possible explanation could be assigned to methodological differences. In our study, lice were removed by visual hand searching, without using any kind of insecticides, unlike the others studies. Therefore, it can be expected that our total number of lice found would be lower than in those studies. On the other hand, in previous studies from European countries, the infestation rate in Accipitriformes birds was notably lower when compared with this study, for example: Pérez et al. [34] reported a prevalence of

41.8% for chewing lice on Spanish raptors, Inci et al. [35] reported 42.6% on Turkish raptors, and Solt [36] 36.4% on Hungarians raptors. These differences may be due to the small size of our sample of birds of prey, comprising mainly European griffons, a highly parasitized species as reported by Pérez et al. [34].

In addition, environmental conditions such as humidity/rainfall and temperature are important factors determining seasonal variations in the infestation rate of chewing lice [1]. According to Derylo [37] high temperatures together with low humidity/rainfall are adverse factors for the viability of lice. The environmental conditions of the Faro district during our sampling was generally quite warm (temperature range: 14.6°C – 21.9°C) and dry (rainfall: 15.1mm; humidity: 66.3%) (Data refers to mean values from September to December, listed in the agro-meteorological bulletins available [38]).

Therefore, those conditions may be responsible for discrepancies of infestation rates among different collecting localities. However, this result was not consistent with that of Pérez et al. [34] where autumn was the season with the highest prevalence of chewing lice in raptors. Moreover, infestation levels can vary according to intrinsic factors, such as host body mass and health condition of the birds [11,39,40].

In the case of highly parasitized orders, Anseriformes, Phoenicopteriformes and Gruiformes, and the orders without lice, Bucerotiformes and Pelecaniformes, the sample size was too low to make any significant evaluation. On the other hand, the low prevalence or even the absence of chewing lice on Passerines, as found in this study, is quite common in the European mainland populations [11,31,32,41]. According to Rózsa [39], large-bodied bird species may harbour more lice than small passerines, i.e., small birds may provide fewer habitats enabling fewer lice to coexist and may provide fewer places of refuge for lice to evade host preening and grooming.

Although the prevalence of chewing lice was slightly higher in young birds (80.0% in Accipitriformes and 25.0% in Charadriiformes) than in adults (40.0% in Accipitriformes and 6.2% in Charadriiformes), no significant differences were observed in rate of infestation between age classes of each of these orders (Fisher's exact test,  $p > 0.05$ ). On Spanish raptors, Pérez et al. [34] also reported the absence of significant differences in rate of infestation between age classes, but in this case, adult birds had slightly higher prevalence of chewing lice. One possible explanation for the absence of significant differences may be the season when the sampling was carried out (September-December). While Spitznagel [42] reported the highest infestation during pre-breeding season, Fowler and Price [43] and Sychra et al. [41] reported that louse infestations are the lowest in the post-breeding season, which coincides with the hosts moult, an unfavourable phenomenon for louse survival. The synchronization of life cycles between some species of chewing lice and their host birds is likely to be an adaptation of these insects to maximize their opportunities to transfer from adult to chicks, especially during parental care (vertical transmission) and/or through direct contact of birds or through birds using the same nest or resting place (horizontal transmission) [1,44]. Thus, considering that sampling for this study was carried out during the post-breeding season the lack of differences in infestation rates between young and adult birds identified by us can be expected.

In this study, infestation rates of colonial Accipitriformes and Charadriiformes were particularly higher than those of territorial birds of these orders (Accipitriformes: 85.7% and 33.3%; Charadriiformes: 19.5% and 0.0% respectively). However, these differences in prevalence of chewing lice proved not to be statistically significant (Fisher's exact test,  $p > 0.05$ ). According to Rózsa et al. [45], the higher prevalence of chewing lice on gregarious birds compared to that on territorial birds is expected due to the increased

body-to-body contact among individuals, which increases the opportunities for horizontal transmissions. However, horizontal and vertical transmissions by body contact are not the only way lice spread among birds. Some ischnoceran lice are also capable of moving between hosts by phoresy on hipposboscid flies [46].

In this study, louse prevalence of migratory Accipitriformes was particularly higher than that of residents (75.0% and 40.0%, respectively). On the contrary, the infestation rate of resident Charadriiformes was slightly higher than that of migratory species (25.0% and 13.3%, respectively). In both orders, the difference between infestation rates was not statistically significant (Fisher's exact test,  $p>0.05$ ). Considering that our sampling was carried out during the post-breeding season, when the contact among birds was more effective, the absence of differences in prevalence of chewing lice between migratory and resident birds would seem obvious. However, one possible explanation for the high prevalence of chewing lice in migratory raptors is that during migration, birds may congregate at food sources and roosting places. Thus, in addition to body-to-body contact during breeding, those areas would promote further contact with possible transfer of chewing lice. The same is not observed in raptors, which tend to be necessarily territorial birds, even during migration.

Combining host factors (Table 3), 75.0% of the infested raptors were migratory and colonial birds, while the remaining were resident and territorial. The difference in infestation rates according to social behaviour and migration status of Accipitriformes was statistically significant (Fisher's exact test,  $p<0.05$ ). On the other hand, no significant differences were observed in rates of infestation according to age and migration status of raptors, as well as of Charadriiformes birds (Fisher's exact test,  $p>0.05$ ). Considering the foregoing, different infestation rates between migratory and colonial birds, and between resident and territorial birds, can be expected.

According to Brown et al. [47], the higher prevalence of chewing lice on migratory birds has substantial long-term effects on adult cliff swallows. These authors found that chewing lice together with other ectoparasites reduce individual's average annual survival probability by about 33%, suggesting that ectoparasites can cost adult cliff swallows the equivalent of an entire year in lifetime reproductive success. This may significantly influence the return rate of parasite-infested birds from nesting sites to their wintering sites.

Furthermore, chewing lice may reduce the density of plumage, leading to an increased thermal loss and a higher metabolic rate [48]. Consequently, heavily infested birds draw on fat reserves to sustain the elevated metabolic rate, leading to host body mass reduction and lower survival rates, especially during winter migration.

In summary, our study showed that colonial birds were more significantly infested than territorial birds and migratory birds were more significantly infested than residents.

Also, we record the following species of chewing lice for the first time from wild birds in Portugal: *Actornithophilus piceus lari*, *Actornithophilus umbrinus*, *Austromenopon lutescens*, *Colpocephalum heterosoma*, *Colpocephalum turbinatum*, *Craspedorrhynchus platystomus*, *Degeeriella fulva*, *Eidmanniella pustulosa*, *Falcolipeurus quadripustulatus*, *Laemobothrion (L.) maximum*, *Laemobothrion (L.) vulturis*, *Lunaceps schismatus*, *Nosopon casteli*, *Pectinopygus bassani*, *Pseudomenopon pilosum*, *Strigiphilus* sp., *Trinoton querquedulae* and *Trinoton femoratum*. This is our first contribution to knowledge of avian chewing lice associated with birds in Portugal. Judging from similar studies in neighbouring countries sharing the same species of birds, especially Spain [49,50], many louse species remain to be discovered.

### Conflict of interest

The authors declared that there is no conflict of interest.

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**Table 1**

Distribution of lice species according to their host bird species, including lice numbers and mean lice intensity on infested hosts.

n	Host scientific names	Host vernacular English names	Louse species	Louse prevalence					MI $\pm$ SD
				Ni	Male	Female	Nymph	Total	
ACCIPITRIFORMES									
Accipitridae									
1	<i>Accipiter nisus</i>	Eurasian sparrowhawk	-	-	-	-	-	-	-
1	<i>Aegypius monachus</i>	Cinereous vulture	-	-	-	-	-	-	-
1	<i>Aquila fasciata</i>	Bonelli's eagle	<i>Degeeriella fulva</i>	1	0	7	0	7	7.00
2	<i>Buteo buteo</i>	Common buzzard	<i>Degeeriella fulva</i>	1	0	1	0	1	1.00
			<i>Craspedorrhynchus platystomus</i>	1	0	2	0	2	2.00
			<i>Laemobothrion (L.) maximum</i>	1	1	1	0	2	2.00
1	<i>Elanus caeruleus</i>	Black-shouldered kite	-	-	-	-	-	-	-
1	<i>Hieraetus pennatus</i>	Booted eagle	-	-	-	-	-	-	-
6	<i>Gyps fulvus</i>	Eurasian griffon	<i>Falcolipeurus quadripustulatus</i>	6	7	6	0	13	2.17 $\pm$ 0.90
			<i>Laemobothrion (L.) vulturis</i>	4	3	2	0	5	1.25 $\pm$ 0.43
			<i>Colpocephalum turbinatum</i>	3	2	3	0	5	1.67 $\pm$ 0.47
			<i>Nosopon casteli</i>	1	0	2	0	2	2.00
ANSERIFORMES									
Anatidae									
1	<i>Anas crecca</i>	Common teal	<i>Trinoton querquedulae</i>	1	1	0	0	1	1.0
BUCEROTIFORMES									
Upupidae									
1	<i>Upupa epops</i>	Eurasian hoopoe	-	-	-	-	-	-	-
CHARADRIIFORMES									
Burhinidae									
1	<i>Burhinus oedicephalus</i>	Eurasian thick-knee	-	-	-	-	-	-	-
Charadriidae									
1	<i>Charadrius hiaticula</i>	Common ringed plover	-	-	-	-	-	-	-
2	<i>Pluvialis squatarola</i>	Grey plover	-	-	-	-	-	-	-
Laridae									
7	<i>Chroicocephalus ridibundus</i>	Black-headed gull	-	-	-	-	-	-	-
4	<i>Larus fuscus</i>	Lesser black-backed gull	-	-	-	-	-	-	-
14	<i>Larus michahellis</i>	Yellow-legged gull	<i>Actornithophilus piceus lari</i>	4	3	1	1	5	1.25 $\pm$ 0.43
Recurvirostridae									
1	<i>Himantopus himantopus</i>	Black-winged stilt	-	-	-	-	-	-	-
Scolopacidae									
14	<i>Calidris alpina</i>	Dunlin	<i>Actornithophilus umbrinus</i>	3	2	1	1	4	1.33 $\pm$ 0.47
			<i>Lunaceps schismatus</i>	2	1	2	0	3	1.50 $\pm$ 0.50
			<i>Austromenopon lutescens</i>	1	1	0	0	1	1.0

2	<i>Scolopax rusticola</i>	Eurasian woodcock	-	-	-	-	-	-	-
GRUIFORMES									
Rallidae									
1	<i>Fulica atra</i>	Eurasian coot	<i>Pseudomenopon pilosum</i>	1	0	1	0	1	1.0
PASSERIFORMES									
Certhiidae									
1	<i>Certhia brachydactyla</i>	Short-toed tree-creeper	-	-	-	-	-	-	-
Corvidae									
1	<i>Cyanopica cyanus</i>	Azure-winged magpie	-	-	-	-	-	-	-
Muscipacidae									
1	<i>Erithacus rubecula</i>	European robin	-	-	-	-	-	-	-
1	<i>Phoenicurus ochruros</i>	Black redstart	-	-	-	-	-	-	-
Phylloscopidae									
4	<i>Phylloscopus collybita</i>	Common chiffchaff	-	-	-	-	-	-	-
Sylviidae									
13	<i>Sylvia atricapilla</i>	Blackcaps	-	-	-	-	-	-	-
4	<i>Sylvia borin</i>	Garden warbler	-	-	-	-	-	-	-
2	<i>Sylvia melanocephala</i>	Sardinian warbler	-	-	-	-	-	-	-
Turdidae									
9	<i>Turdus merula</i>	Eurasian blackbird	-	-	-	-	-	-	-
3	<i>Turdus philomelos</i>	Song thrush	-	-	-	-	-	-	-
PELECANIFORMES									
Ardeidae									
1	<i>Ardea cinerea</i>	Grey heron	-	-	-	-	-	-	-
4	<i>Bubulcus ibis</i>	Western cattle egret	-	-	-	-	-	-	-
PHOENICOPTERIFORMES									
Phoenicopteridae									
2	<i>Phoenicopus roseus</i>	Greater flamingo	<i>Colpocephalum heterosoma</i>	2	0	2	0	2	1.0 ± 0.00
			<i>Trinoton femoratum</i>	1	0	1	0	1	1.0
STRIGIFORMES									
Strigidae									
5	<i>Athene noctua</i>	Little owl	-	-	-	-	-	-	-
2	<i>Bubo bubo</i>	Eurasian eagle-owl	<i>Strigiphilus</i> sp.	1	0	0	1	1	1.0
SULIFORMES									
Sulidae									
7	<i>Morus bassanus</i>	Northern gannet	<i>Pectinopygus bassani</i>	5	4	5	7	16	3.20 ± 1.17
			<i>Eidmanniella pustulosa</i>	4	2	12	1	15	3.75 ± 1.09
122	TOTALS			27	49	11	87		

**Table 2**

Prevalence (%) of chewing lice found on wild birds, according to age, social behaviour and migration status of each order of parasitized host.

	Age birds <sup>1</sup>						Social behaviour						Migration status						Total		
	Young birds			Adult birds			Territorial birds			Colonial birds			Resident birds			Migratory birds			n	Ni	%
	n	Ni	%	n	Ni	%	n	Ni	%	n	Ni	%	n	Ni	%	n	Ni	%			
Accipitriformes	5	4	80.0	5	2	40.0	6	2	33.3	7	6	85.7	5	2	40.0	8	6	75.0	13	8	61.5
	$p=0.524$						$p=0.103$						$p=0.293$								
Anseriformes	1	1	100.0	0	0	0.0	1	1	100.0	0	0	0.0	0	0	0.0	1	1	100.0	1	1	100.0
	- <sup>2</sup>						- <sup>2</sup>						- <sup>2</sup>								
Charadriiformes	28	7	25.0	16	1	6.2	5	0	0.0	41	8	19.5	16	4	25.0	30	4	13.3	46	8	17.4
	$p=0.224$						$p=0.569$						$p=0.421$								
Phoenicopteriformes	2	2	100.0	0	0	0.0	0	0	0.0	2	2	100.0	0	0	0.0	2	2	100.0	2	2	100.0
	- <sup>2</sup>						- <sup>2</sup>						- <sup>2</sup>								
Gruiformes	0	0	0.0	0	0	0.0	0	0	0.0	1	1	100.0	1	1	100.0	0	0	0.0	1	1	100.0
	-						- <sup>2</sup>						- <sup>2</sup>								
Strigiformes	1	0	0	6	1	16.7	7	1	14.3	0	0	0.0	7	1	14.3	0	0	0.0	7	1	14.3
	$p=1.000$						- <sup>2</sup>						- <sup>2</sup>								
Suliformes	7	5	71.4	0	0	0.0	0	0	0.0	7	5	71.4	0	0	0.0	7	5	71.4	7	5	71.4
	- <sup>2</sup>						- <sup>2</sup>						- <sup>2</sup>								
Total <sup>3</sup>	76	19	25.0	36	4	11.1	59	4	6.8	63	22	34.9	61	8	13.1	61	18	29.5	122	26	21.3

n: number of birds examined. Ni: number of birds infested.

<sup>1</sup>Birds with undetermined age are not included in this statistical test: three Accipitriformes, two Charadriiformes, one Gruiform and four Pelecaniformes.

<sup>2</sup>Without statistical result because the host parameter in question is a constant variable.

<sup>3</sup>All orders of birds are included, even orders not parasitized.

**Table 3**

Chewing lice prevalence (%) found in Accipitriformes and Charadriiformes parasitized, by combining different factors of the host.

	Accipitriformes parasitized				Charadriiformes parasitized			
	Resident bird		Migratory bird		Resident bird		Migratory bird	
	Ni	%	Ni	%	Ni	%	Ni	%
Social behaviour								
Territorial birds	2	25.0	0	0.0	0	0.0	0	0.0
Colonial birds	0	0.0	6	75.0	4	50.0	4	50.0
	$p=0.036$				- <sup>2</sup>			
Age birds <sup>1</sup>								
Young birds	2	33.3	2	33.3	3	37.5	4	50.0
Adult birds	0	0.0	2	33.3	1	12.5	0	0.0
	$p=0.467$				$p=1.000$			

Ni: number of birds infested. % Prevalence of birds parasitized

<sup>1</sup>Two Accipitriformes birds of undetermined age are not included in this statistical test.

<sup>2</sup>Without statistical result because the social behavior is a constant variable.

## Figure Legends

**Fig. 1.** Chewing lice identified in this study: (A) *Laemobothrion (L.) maximum*, male; (B) *Laemobothrion (L.) vulturis*, male; (C) *Actornithophilus piceus lari*, female; (D) *Actornithophilus umbrinus*, female; (E) *Austromenopon lutescens*, male; (F) *Colpocephalum heterosoma*, female; (G) *Colpocephalum turbinatum*, female; (H) *Eidmanniella pustulosa*, female; (I) *Nosopon casteli*, female; (J) *Pseudomenopon pilosum*, female; (K) *Trinoton femoratum*, male; (L) *Trinoton querquedulae*, male; (M) *Craspedorrhynchus platystomus*, female; (N) *Degeeriella fulva*, female; (O) *Falcolipeurus quadripustulatus*, male; (P) *Luniceps schismatus*, female; (Q) *Pectinopygus bassani*, female; (R) *Strigiphilus* sp., nymph.

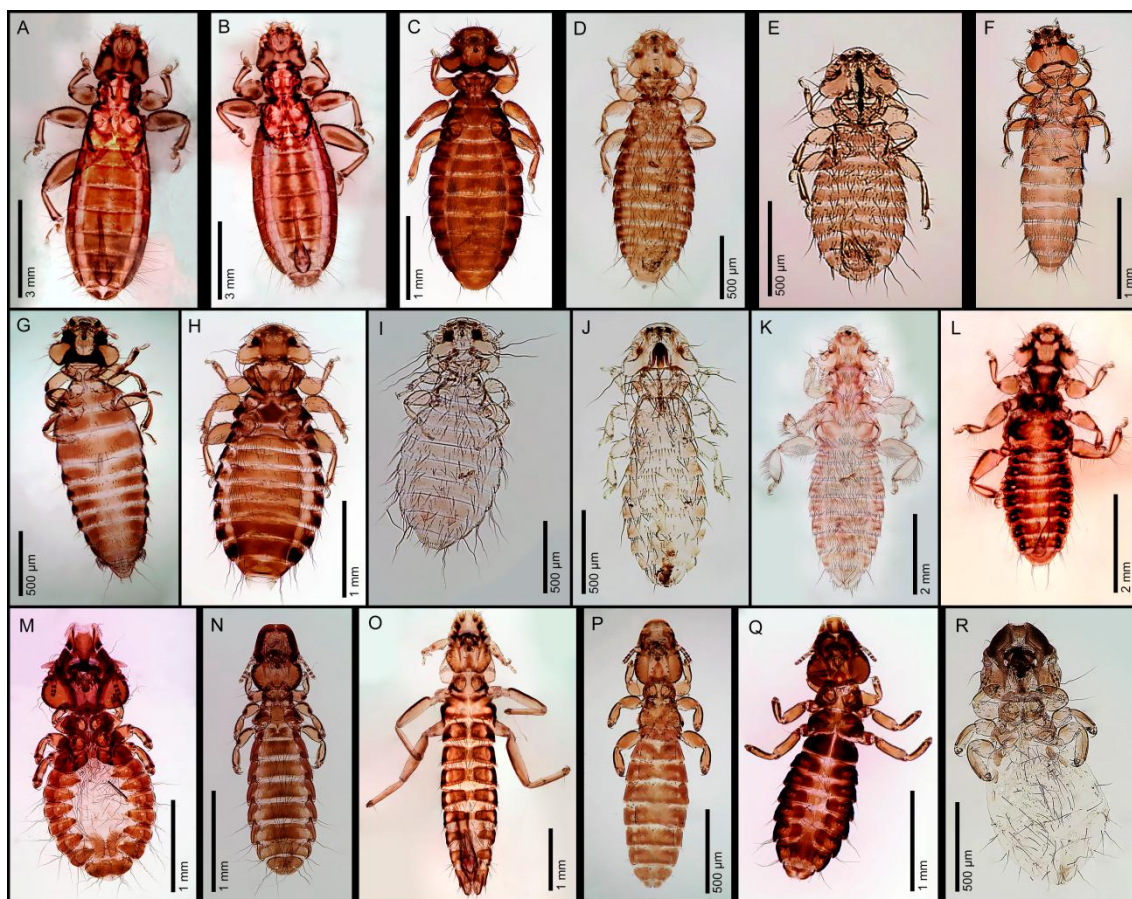
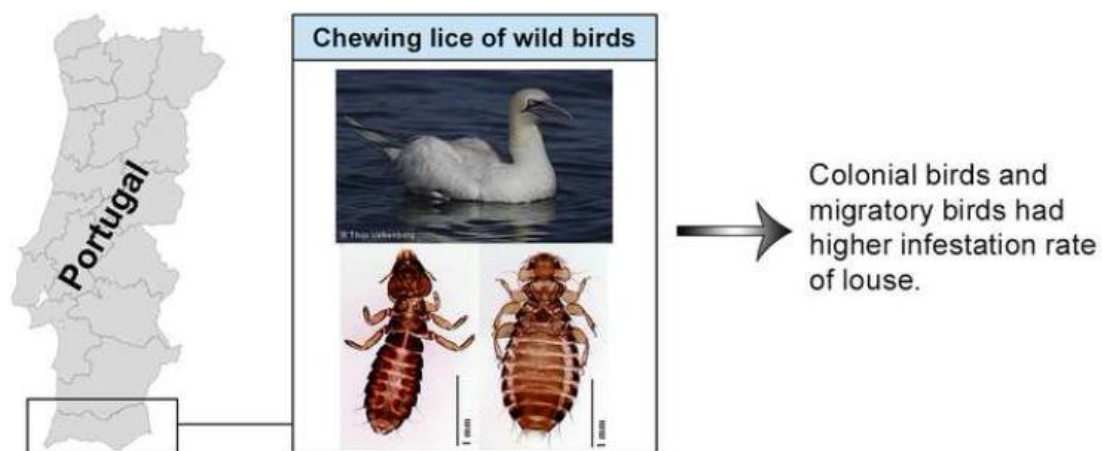


Figure 1

## Graphical abstract





### Highlights

- We investigate about chewing lice species of wild birds of Portugal.
- Chewing lice were found on 26 (21.3%) of the 122 wild birds examined.
- Colonial birds and migratory birds had higher infestation rate of louse.
- All of chewing lice, 17 species, are recorded for the first time in Portugal.