Chewing lice (Insecta: Phthiraptera) found on Griffon Vultures (*Gyps fulvus*) from a wild breeding colony in central Spain.

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Abstract

The Griffon Vulture (*Gyps fulvus*) is a social species which breeds in colonies and feeds in groups. This can facilitate horizontal transmission of ectoparasites between conspecifics. In 2015, in the province of Guadalajara, Spain, 28 vultures from a wild population were examined for ectoparasites, 24 of which were parasitized by chewing lice (Phthiraptera). Three species of lice were identified: *Laemobothrion (Laemobothrion) vulturis, Colpocephalum turbinatum* and *Falcolipeurus quadripustulatus*. Data on the prevalence and richness of louse species in the vulture population are also presented.

Introduction

Chewing lice (Insecta: Phthiraptera) are obligate and permanent ectoparasites of birds. They can produce health problems in their hosts, such as dermatological conditions and can be vectors of bacteria, fungi or filarial worms, causing a deterioration in the health status of avian populations (Martín-Mateo 2002). Sedentary and colonial birds have a higher prevalence of chewing lice than migrating and territorial birds (Sychra *et al.* 2011, Diakou *et al.* 2017, Bernal *et al.* 2022). Colonial behaviour facilitates horizontal transmission of lice between individuals in the same population (Rózsa *et al*. 1996, Darolova *et al*. 2001).

Vultures are one of the groups of birds with the highest close contact behaviour between individuals, especially in the act of feeding on carrion and roosting (García-Ripollés *et al*. 2004). Griffon Vultures (*Gyps fulvus*) usually breed in large colonies, so the transmission of parasites between individuals can be potentially high. In addition, their communal feeding and roosting behaviour can also increase transmission. Studies regarding the parasitological diversity of Griffon Vultures in Europe are broader in terms of the community of endoparasites and bacteria (Kocijan *et al*. 2009, Salvador 2016, Chakarov & Blanco 2021) than studies on ectoparasites, which could be considered an indicator of the health status of that population. Several authors have recorded species of lice in Griffon Vultures, such as *Aegypoecus trigonoceps* and *Falcolipeurus quadripustulatus* (Ischnocera); and *Colpocephalum gypsi, Colpocephalum turbinatum, Cuculiphilus* (*Aegypiphilus*) *gypsi, Laemobothrion* (*Laemobothrion*) *vulturis, Laemobothrion* (*L*) *maximun* and *Nosopon casteli* (Amblycera) (Martín-Mateo 2002, 2009, Ilieva 2009, Salvador 2016, Vas *et al*. 2012).

Most of the research carried out on the ecology of chewing lice collected from Griffon Vultures in the Iberian Peninsula, corresponds to vultures from wildlife recovery centres (Pérez *et al*. 1996, Tomás *et al*. 2016). These individuals may have some pathology or health problems that incapacitate them for their reincorporation into the natural

environment, which can favour the transmission of pathogens and parasites between them. The health conditions of these birds can provide data on parasitology which could be very different from those that occur in the wild. Therefore, this study aimed to assess the prevalence and richness of chewing lice in a wild population of Griffon Vultures in Spain.

Methods

The area of study was in the province of Guadalajara, Spain (41°11'19.9"N, 3°11'44.2"W). The surrounding habitat mainly consisted of heathland (*Erica arborea, Erica australis* and *Calluna vulgaris*), Scots Pine forest (*Pinus sylvestris*) and oaks (*Quercus* spp.). This area includes a complex of cliffs and forests where a significant population of Griffon Vultures lives and breeds (Del Moral 2009).

The study was carried out in accordance with the permits from Spanish Bird Ringing Centre and the government of Castilla-La Mancha. Vultures were captured and handled by authorized personnel, minimizing handling time to reduce stress. After handling, the vultures were released in good condition at the same location. Vultures were captured using a large cage (5x8x2 m) placed in a regularly used feeding place and baited with livestock carcasses. Captures were done between 10 October and 4 December 2015. Each vulture was fitted with leg rings and measured with rulers and digital callipers, and weighed with balances. Vultures were aged following the methods of Forsman (2007), Duriez *et al*. (2011) and Zuberigoitia *et al*. (2013) which recommended feathers moults as the most reliable sign of vulture age. Individuals were assigned to three age classes: adults (birds ≥5.5 years old), subadults (birds between their first moult and 5.5 years old) and juveniles (birds in their first year). Subadults included second, third and fourth calendar year.

Chewing lice were collected directly from the vultures' feathers following the method of Martín-Mateo (1994, 2002) and Clayton & Drown (2001). This method involves meticulously searching among the feathers as different parasite species show a preference for certain parts of the body to avoid interspecific competition (Martín-Mateo 2002). Lice were stored in capped tubes containing 70% ethanol and were cleaned in 10% KOH, mounted in DMHF on slides, and identified to species level under a light-microscope and stereo-microscope (Martín-Mateo 1994). To avoid biases in these determinations, all specimens were identified and classified by the same researchers (C. Talabante & I. Bernal) and deposited in the authors' collections. The nomenclature of lice follows Martín-Mateo (2002, 2009).

The prevalence of chewing lice species was computed using Quantitative Parasitology on the Web (Reiczigel *et al*. 2019), while confidence intervals (CI) were calculated according to Sterne´s method (Klaschka & Reiczigel 2021).

Results

Out of 28 Griffon Vultures that were captured and inspected, 24 individuals were hosts to chewing lice (prevalence= 85.7%; CI = 68.1-95.0) (Table 1). Of the different age classes, 75% of adults (n = 16), 100% of subadults (n = 7) and 100% of juveniles (n = 5) were parasitized by lice. Three species of lice were identified: *Laemobothrion (Laemobothrion) vulturis* (Figure 1), *Colpocephalum turbinatum* (Figure 2) and *Falcolipeurus quadripustulatus* (Figure 3). Two vultures hosted all three identified species of lice. One vulture hosted *L. vulturis* and *F. quadripustulatus*; two vultures hosted *L. vulturis* and *C. turbinatum*; and six vultures hosted *C. turbinatum* and *F. quadripustulatus*. No louse flies (Hippoboscidae), ticks (Ixodidae-Argasidae) or mites (Acarina) were found.

Table 1: Diversity and prevalence of chewing lice detected on 28 Griffon Vultures from a breeding colony in central Spain.

Suborder	Family	Species	Prevalence (n)
Amblycera	Laemobothriidae	*Laemobothrion (Laemobothrion) vulturis*	25% (7)
	Menoponidae	*Colpocephalum turbinatum*	46% (13)
Ischnocera	Philopteridae	*Falcolipeurus quadripustulatus*	61% (17)

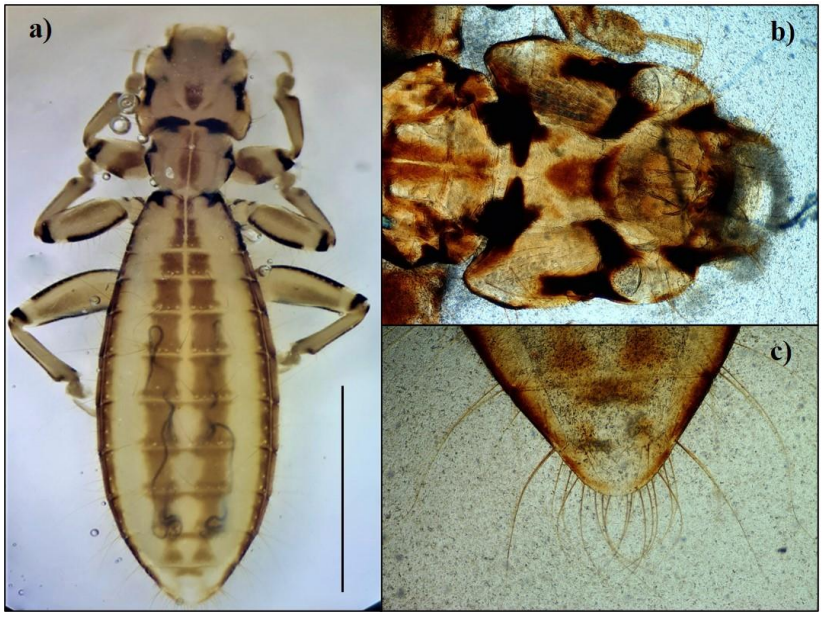

Figure 1: a) *Laemobothrion (Laemobothrion) vulturis*, female; b) view of head; c) view of terminal segments. Unit scale: 3 mm.

Figure 2: a) *Colpocephalum turbinatum*, male; b) view of head; c) view of genitalia. Unit scale: 500 µm.

Figure 3: a) *Falcolipeurus quadripustulatus*, female; b) view of head, male; c) view of terminal segments and genitalia; d) view of head, female; e) view of terminal segments, female. Unit scale: 1 mm.

Discussion

Of the three chewing lice species found on Griffon Vultures in this study, *L. vulturis* has been found on Secretary Bird (*Sagittarius serpentarius*) and raptor species (Accipitridae); *C. turbinatum* parasitizes different families of birds (Accipitridae, Ciconiidae, Columbidae, Corvidae, Falconidae, Pandionidae, Strigidae); and *F. quadripustulatus* only parasitizes birds of the Accipitridae family (Martín-Mateo 2002, Price *et al*. 2003, Martín-Mateo 2009). *L. vulturis*, due its haematophagous behaviour, can be considered a species of veterinary interest (Pérez *et al*. 1996).

L. vulturis and *C. turbinatum* favour the belly and vent areas, while *F. quadripustulatus* parasitizes areas of the wing (Pérez *et al*. 1996). The coexistence of two species of lice in the same body area can cause interspecific competition that can influence the structure of lice communities (Bush & Malenke 2008). Our results show that *C. turbinatum* and *F. quadripustulatus* have high prevalence. These species do not parasitize the same body area, so this lack of interspecific competition can facilitate transmission, since lice can colonize areas of the body free of parasite load.

Our results show that the lice prevalence obtained in this study are similar to previous studies with the same sampling method. Pérez *et al.* (1996) sampled 17 Griffon Vultures, of which 12 were parasitized (70%). In addition, the prevalence of each species of louse was similar to our results (*L. vulturis* (23.5%), *C. turbinatum* (52.9%) and *F. quadripustulatus* (52.9%)). On the other hand, Tomás *et al*. (2016) obtained a prevalence of 100% in six Griffon Vultures. Both studies were carried out with specimens from wildlife recovery centres. This origin can be a determinant on the prevalence of ectoparasites, since injured or sick specimens may be sampled. Although our results from a wild population show a prevalence (85.7%) similar to those obtained with specimens from recovery centres, for future studies it is necessary to take into account the origin and health status of sampled birds, so that prevalence results can be correctly interpreted.

In general, the number and diversity of lice recorded per individual tends to be higher in Griffon Vultures than other raptor species (Pérez *et al*. 1996). This can be attributed to various hypotheses, one of which is that larger birds have a larger body surface area which could host a greater

number of ectoparasite species and individuals (Rózsa 1997, Durkin *et al*. 2015). Another hypothesis is that colonial birds have high numbers and diversity of ectoparasites due to horizontal transmissions, through body-to-body contact (Rózsa *et al*. 1996, Darolova *et al*. 2001). Pérez *et al*. (1996) detected high prevalence of lice in large or gregarious birds of prey, such as Griffon Vulture (70.6%), Red Kite *Milvus milvus* (50.0%), Black Kite *M. migrans* (47.9%), Common Kestrel *Falco tinnunculus* (47.4%) or Short-toed Snake Eagle *Circaetus gallicus* (53.8%), while small or solitary species had a lower prevalence (Common Buzzard *Buteo buteo* (40.2%) and Booted Eagle *Hieraaetus pennatus* (14.8%)).

Due to the complexity of sampling chewing lice on wild vultures, the abundance of lice or intensity of parasitisation on each vulture was not calculated. Therefore, it would be interesting in future work to measure ecological parameters (e.g. analysis of environmental contaminants and toxins in vulture tissues) to better understand the dynamics of the lice community and its forms of transmission. Similarly, assessing these ecological parameters in relation to biometric measurements (e.g. of body areas favoured by lice; bill morphology) and different behaviours (e.g. preening) that can affect the lice population would be of interest.

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