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FIRST RECORD OF ECTOPARASITIC INSECTS ON THE CANARIAN HOUBARA BUSTARD (GRUIFORMES: OTIDIDAE)

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Abstract.— Data on an infestation of ectoparasites on a Canarian Houbara Bustard *Chlamydotis undulata fuerteventurae* Rothschild et Hartert, 1894 are presented for the first time. Two insect groups were discovered: *Icosta* (*Rhyponotum*) *pilosa* (Macquart, 1843) (Hippoboscidae, Diptera) and a possibly unnamed species of Philopteridae (Phthiraptera) closely allied to *Otidoecus houbarae* (Barthélemy, 1836). For the latter, a DNA-barcode is presented. The philopterid was found in a phoretic relationship with the hippoboscid.

Key words.— Canarian Houbara Bustard, Hippoboscidae, Philopteridae, host-parasite association, phoresy.

INTRODUCTION

Ectoparasites of birds are known from six arthropod groups: Acarina (mites), Ixodidae (ticks), and among the insect orders Diptera (true flies), Heteroptera (true bugs), Phthiraptera (lice), and Siphonaptera (fleas). Mites and lice feed directly on the feathers or on debris that flakes off the skin, and also take up eye-fluid as has been observed for some species of lice in the suborders Amblycera and Ischnocera (Mey 1978). True bugs, ticks, fleas and flies, pierce the skin to suck blood or other fluids. Ectoparasites rarely kill adult birds. However, depending on the level of infestation, they reduce the overall fitness of the host. Heavy infestation can cause serious blood loss and damage of the functionality of plumage and skin, making the host more vulnerable against predators and diseases. Apart from that, especially blood sucking parasites act as vectors for various diseases (Proctor and Lynch 1993).

Depending on the parasite group, information on host-parasite relationship is often rather scarce and general but the data available implicate that host specificity can be significant between certain groups of parasites and their avian or mammalian hosts, leading to cospeciation in Phthiraptera for example (Hafner and Nadler 1988).

In the following, we report on an infestation of the Canarian Houbara Bustard *Chlamydotis undulata fuerteventurae* Rothschild et Hartert, 1894 with a species of louse fly (Diptera: Hippoboscidae) and a chewing louse (Phthiraptera: Philopteridae), respectively. Until now, data on parasitic infestation on Canarian Houbara Bustards were limited to endoparasites, with Foronda (2002) recording three cestode species when examining five deceased specimens of *C. u. fuerteventurae*.

The Canarian Houbara is endemic to the eastern islands of the Canary Archipelago. According to

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Idaghdour *et al.* (2004), the presence of Houbara Bustards on the Canary Islands can be dated back 130–170,000 years. A second colonisation from mainland Africa followed 19–30,000 years ago, with subsequent isolation. A genetic analysis pinpoints the separation of *C. u. fuerteventurae* from the nominate subspecies at around 20–25,000 years ago (Idaghdour *et al.* 2004). The population size of this largest native and non-migratory member of the Canarian avifauna has repeatedly been surveyed during the past 15 years, with varying results. Whereas Heredia (1995) estimated a number of 700–750 birds (Fuerteventura and Lobos: 300–350; Lanzarote and La Graciosa: 400), the final report of an EU funded five year conservation project stated a population size of approximately 1,100 (La Graciosa: < 20; Lanzarote: > 700; Fuerteventura: ~ 400) (SEO 2007). A more recent study lessens the number of Houbara Bustards on Fuerteventura to 177 individuals (Carrascal *et al.* 2008).

METHODS

The Hippoboscidae was collected from the dead Canarian Houbara by hand. The Philopteridae were discovered under the stereoscope when identifying the fly. All material is currently preserved in ethanol and deposited in the senior author's collection except one philopterid which was donated to Vince Smith (Natural History Museum London — NHM).

The hippoboscid specimen was identified using the keys in Maa (1963, 1969). In addition, five male and five female specimens of *I. pilosa* were taken on loan from the NHM London for comparison. This material was partly included in the work of Maa (1969) who revised the most specious hippoboscid genus *Icosta* Speiser, 1905 and established the monotypic subgenus *Rhyponotum* Maa, 1969. No taxonomic or nomenclatorial act has been proposed since then, leaving *Icosta* with 52 species plus 4 additional subspecies, making up 24% of the currently described Hippoboscidae world fauna of 213 taxa (Dick 2006).

DNA-sequencing was conducted on the chewing louse in order to generate molecular evidence that acts as a surrogate voucher, ensuring that future nomenclatural changes to taxonomy will be easy to track. Prior to molecular work, the air-dried Hippoboscidae and Philopteridae were rehydrated in 1xTE buffer for 24 hours so that two lice could be cautiously detached from the fly's abdomen. Genomic DNA was extracted from one philopterid specimen (DNA voucher CK600), whose abdomen was lacerated, using the innuPREP DNA Mini Kit of Analytik Jena AG (Jena, Germany). We amplified a 379 base pair fragment of the mitochondrial CO1 gene, using the forward primer L6625 (5'-CCGGATCCTTYTGRTTYTTYGGNCAYCC-3') and the reverse primer H7005 (5'-CCGGATCCACANCR TARTANGTRTCRTG-3') of Hafner *et al.* (1994). PCR was performed with 5 μl of DNA extraction in a 20 μl volume (1 μl of each primer at 10 pmol, 1 μl of dNTP-mix at 10 pmol, and 1 unit of Taq polymerase (Bioron DFS Taq, Ludwigshafen, Germany), 2 μl PCR buffer 10× incl. $MgCl_2$, ultra pure H_2O). PCR conditions were an initial denaturation of 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s, and a final elongation of 72°C for 10 min. Cleaning of PCR and sequencing-PCR products were performed by salt-ethanol precipitation using 4M NH_4Ac (PCR) or 3M NaAc (PCR-sequencing). For sequencing-PCR the same forward and reverse primers were used in a total reaction volume of 10 μl (2 μl sequencing buffer, 1 μl premix, 1 μl primer at 5pmol concentration, 3 μl of DNA template, ultra pure H_2O) with 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min using the ABI PRISM Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was done on an ABI 3130XL Genetic Analyser. PCR products were visualised on a 1% agarose gel. The resulting CO1 barcode was compared to the GenBank database using the BLAST algorithm in order to validate the morphological identification. Uncorrected pairwise genetic distances (p-distance) between sequences were calculated with the software MEGA5 (Tamura *et al.* 2011).

RESULTS AND DISCUSSION

The ectoparasite specimens of this study were collected from a dead specimen of Canarian Houbara Bustard *Chlamydotis undulata fuerteventurae* Rothschild et Hartert, 1894 (Fig. 1) found on the cultivated landscape of La Rosa de los Negrines, north of La Oliva on Fuerteventura, in the early morning of the 27th October 1996. They comprise one female specimen of the hippoboscid *Icosta* (*Rhyponotum*) *pilosa* (Macquart, 1843) (Fig. 2) and four specimens of a possibly unnamed philopterid addressed as *Otidoecus* aff. *houbarae* (Barthélemy, 1836) (Fig. 3). Both ectoparasite species are recorded for the first time for the fauna of the Canary Islands.

Judging by its good state and the fact that it was still invested by Hippoboscidae, the *Chlamydotis* carcass must have deceased overnight. All Mallophaga specimens were attached to the abdomen of the hippoboscid, three posterolaterally and one anteroventrally. When hosts are dying or dead, lice use this means of transport to reach a new host (Rothschild and Clay 1952). The normal way of dispersal is physical contact during mating of the host birds. This finding is another example of the long known phoretic relationship between lice and hippoboscid flies (for a summary see Keirans 1975), although approximately 90% of known louse phoretic

Figure 1. Dead specimen of *Chlamydotis undulata fuerteventurae* Rothschild et Hartert, 1894 found on Fuerteventura, Canary Islands, Spain. This figure is available in colour in the online edition of the paper (http://www.ingentaconnect.com, http://www.bioone.org).

associations with hippoboscids usually involve species of *Brueelia* Kéler, 1936 (Vince Smith in litt.).

The known hosts of *I. pilosa* comprise Galliformes (Phasianidae: *Francolinus*), Gruiformes (Otididae: *Afrotis*, *Chorions*, *Eupodotis*, *Lissotis*, *Neotis*) and Pterocliformes (Pteroclidae: *Pterocles*), whereas breeding is apparently limited to Otididae (Maa 1969). The same author characterised the distribution of *I. pilosa* as widespread over Eastern and Southern African: Sudan, Ethiopia, Kenya, Uganda, Tanzania (as Tanganyika), Zimbabwe (as Rhodesia), Namibia (as SW Africa), Botswana (as Bechuanaland) and South Africa (as Transvaal, Orange State and Zululand). Additional records from Morocco and Congo were considered by him as *"seasonal or strays"* and a citing from Reunion Island by Bigot (1862) is *"apparently erroneous"* (see also Bequaert 1945). The new finding of *I. pilosa* occurring on an endemic, non-migratory bird of the Canary Islands not only represents a new host record and a range expansion of this species, but also rehabilitates the historic citing from Morocco by Bequaert (1945) (Morocco, Tiznit, about 370 km NE of the Canary Archipelago, one male, two females on *Pterocles orientalis* (Linnaeus, 1758)). Therefore it seems likely that *I. pilosa* is also a well established

Figure 2. Sampled female of *Icosta* (*Rhyponotum*) *pilosa* (Macquart, 1843) from left anterolateral view. Figured are head and part of thorax with its prominent fore femur. This figure is available in colour in the online edition of the paper (http://www.ingentaconnect.com, http://www.bioone.org).

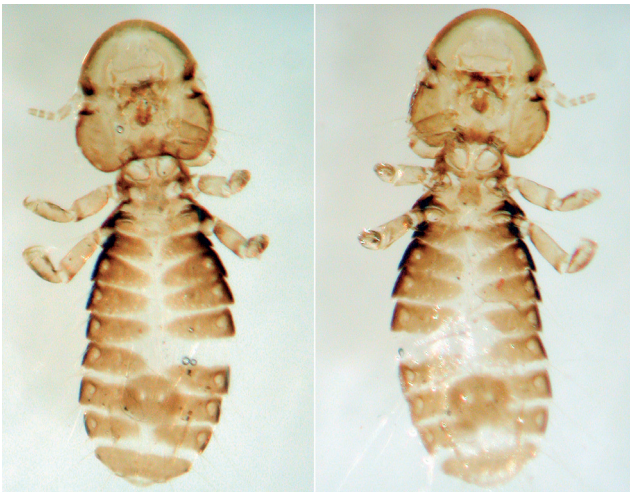

Figure 3. Female *Otidoecus* aff. *houbarae* (Barthélemy, 1836) with its abdomen lacerated for DNA extraction. Left fore leg and right antenna missing. Dorsal view on left hand side. Ventral view on right hand side. This figure is available in colour in the online edition of the paper (http://www.ingentaconnect.com, http://www.bioone.org).

ectoparasite on the African Houbara Bustard *C. undulata undulata* (Jacquin, 1784) and present in large parts of its distributional range, especially in northwestern Africa.

Based on the morphology of the head, host record and locality, the recorded philopterid species closely resembles *Otidoecus houbarae* (Tomáš Najer in litt.). Although the taxonomic knowledge on the genus *Otidoecus* Bedford, 1931 is rather poor, it would have been positively attributed to this taxon if it was not for the DNA-barcode. When searching the GenBank database, our query sequence (GenBank accession number HE863672) was closest to *O. houbarae* from Chund Bharwana, Punjab Province, Pakistan, taken from a *Chlamydotis undulata*, and originally published by Johnson *et al.* (2003) (no locality data is given in their paper but see GenBank entry under accession number AF545738). Their voucher specimen was presumably identified by Vince Smith and is in the collection of the University of Glasgow (see Cruickshank *et al.* 2001, table 1 and under GenBank #AF320435).

However, the uncorrected pairwise genetic distance between these sequences was 18.2% using all codon positions. Such large genetic distances in a mitochondrial gene have been documented for lice and discussed by Johnson *et al.* (2003), pointing towards a substantially increased substitution rate in this group. When third codon positions are excluded, p-distance is lowered to 4.0%. And when translated into amino acids, the two specimens differ by 2.4% (3 out of 126 amino acids). Much more sampling of *O. houbarae* over its geographical distribution range is necessary to investigate whether the name actually harbours a complex of species. Phthiraptera exhibit a high degree of host specificity, and true *O. houbarae* could only be recorded from *C. undulata undulata* on a few occasions until now (Bailey 2008, Hopkins and Clay 1952, Price *et al*. 2003). Also, hardly any data are available on the geographical distribution of this species, known records are restricted to Tunisia (locus typicus: Sfax) (Barthélemy 1836), the United Arab Emirates (Bailey 2008) and Pakistan (Johnson *et al.* 2003).

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