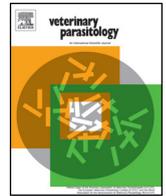




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Short communication

Molecular characterization of bot flies, *Oestrus* spp., (Diptera, Oestridae), from domestic and wild Bovidae hosts

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ABSTRACT

The identification of *Oestrus* spp. larvae from Bovidae hosts is a difficult task due to the great morphological similarity between species. The lack of unambiguous identification criteria could have also serious epidemiological implications since domestic and wild hosts are sympatric in many natural areas. In order to accurately identify the *Oestrus* parasitizing hosts, we characterized two different genetic markers, 28S (rRNA) and COI, in larvae collected from domestic sheep and goats, European mouflon and Iberian ibex. Our sequence analyses demonstrate that all samples, except those from Iberian ibex, greatly resembles *O. ovis* and so we conclude that the species parasitizing this ibex is not *O. ovis*. Further studies will be needed to confirm whether it is in fact *O. caucasicus*, as previously suggested, or even a new species.

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1. Introduction

Oestrus spp. are obligate mesoparasites as their larvae develop within the nasal cavities and frontal sinuses of domestic and wild hosts (Zumpt, 1965). Myiasis affects animal's welfare, causing mild to severe effects in individuals and economic losses in the livestock industry (Otranto and Stevens, 2002).

Currently, four *Oestrus* species are recognized: *O. ovis*, *O. variolosus*, *O. aureoargentatus* and *O. caucasicus*. The identity of *O. bassoni* and *O. macdonaldi* is still under discussion (Colwell et al., 2006). *O. ovis*, parasitizes sheep and domestic goats worldwide and has been reported to parasitize Asiatic ibex, argali, bighorn sheep, European mouflon, Alpine ibex, Nubian ibex and aoudad (Grunin, 1957; Moreno et al., 1999; Colwell et al., 2006). On the other hand, *O. caucasicus* larvae have been collected from Caucasian tur, Asiatic ibex and Iberian ibex (Grunin, 1957; Pérez et al., 1996).

O. caucasicus differs from *O. ovis* by its strong regular dorsal spinulation on segments III–V of the third-instar larvae and by the black

wing veins in adult flies. The description of *O. caucasicus* was completed with a scanning electron microscopy study of larval instars collected from Iberian ibex and imagoes (Guitton et al., 2001). These two species, *O. ovis* and *O. caucasicus*, are morphologically and biologically quite similar (Pérez et al., 2006).

In light of this similarity and the fact that Iberian ibex and domestic sheep and goats are sympatric in many mountainous areas, we decided to study whether or not the morphological differences mentioned above are enough to separate these *Oestrus* species or whether, on the contrary, they merely reflect phenotypic variability within a single taxon.

Molecular markers have been used in species identification, host-specificity and the distribution of members of dipteran families. Among the most frequently used markers are the mitochondrial gene encoding for the cytochrome c oxidase subunit I (COI) and the nuclear 28S (rDNA), which also provide useful information on population genetics and for phylogenetic analyses (Otranto et al., 2003a,b, 2005a,b; Otranto and Stevens, 2002; Grisez-Durantón et al., 2002; Nelson et al., 2012).

In this paper we describe a molecular characterization of the COI and 28S (rDNA) sequences of *Oestrus* spp. larvae collected from different wild and domestic hosts. Our goal was to address the

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Table 1
Host species, number of larvae and sequences analyzed.

Host species	Location	Numbers of individuals	Reference	Studied larvae	COI	28 S 1 Kb	28 S 1.5 Kb	
Domestic sheep (<i>Ovis aries</i>)	Llucmajor (Mallorca Island)	1	D. sheep Ma	1	9	2		
	Cáceres (Extremadura, Central-West Spain)	2	D. sheep Ex 1	1	5			
			D. sheep Ex 2	1	1			
	Murcia (Southeastern Spain)	2	D. sheep Mu 1	1	8	3	4	
			D. sheep Mu 2	1	8			
	Republic of Iran	4	D. sheep Ir 1	1	2			
			D. sheep Ir 2	1	2			
			D. sheep Ir 3	1	1			
			D. sheep Ir 4	1	1			
			D. goat Gr (F)1	1	2			
	Domestic goat (<i>Capra hircus</i>)	Fondón (Granada, Southern Spain)	2	D. goat Gr (F)2	1	2	2	
		Berja (Granada, Southern Spain)	4	D. goat Gr (B)1	1	3		1
				D. goat Gr (B)2	1	2		
			D. goat Gr (B)3	1	2		1	
	D. goat Gr (B)4	1				2		
European mouflon (<i>Ovis aries</i>)	Extremadura (Central-West Spain)	2	E. mouflon Ex 1	1	11	1		
			E. mouflon Ex 2	1	7	2		
	Sierra de Cazorla N.P. (Jaén, Southern Spain)	2	E. mouflonJa 1	1	9			
Iberian ibex (<i>Capra pyrenaica</i>)	Sierra Nevada N.P. (Granada, Southern Spain)	13	E. mouflonJa 2	1	4			
			I. ibex Gr 1	1	2			
			I. ibex Gr 2	5	3	10		
			I. ibex Gr 3	2	1	2	2	
			I. ibex Gr 4	1	5			
			I. ibex Gr 5	1	1	2	2	
			I. ibex Gr 6	3	4	2		
			I. ibex Gr 7	1	1	2		
			I. ibex Gr 8	1	2	2	1	
			I. ibex Gr 9	1	1	1		
			I. ibex Gr 10	1	5	2	2	
			I. ibex Gr 11	1	2	1		
			I. ibex Gr 12	1	1	1		
I. ibex Gr 13	1	2						

European mouflon were nearly identical. Thus, the identities were in the range 98.9–99.7% for 28S–1 kb, and 98.6–99.6% for 28S–1.5 kb. However, the sequences of larvae from Iberian ibex were less similar than those from the other hosts, with observed identities of 96.5–97.5% and 95.2–96.3% for 28S–1 kb and 28S–1.5 kb, respectively. Furthermore, a comparison with the 28S (rDNA) sequence from *O. ovis* available at GenBank (AJ551428) suggests that larvae from domestic sheep, domestic goats and European mouflon probably belong to this species. Thus, the 28S–1 kb and 28S–1.5 kb sequences were 98.5–98.9% and 96.6–97.5% identical, respectively. However, the sequences from *O. ovis* showed lower values of identity when compared to larvae from Iberian ibex (97.0–97.5% for 28S–1 kb, and 95.9–96.6% for 28S–1.5 kb).

3.2. Phylogenetic analysis

To perform phylogenetic reconstructions, our COI data (excluding 16 non-functional sequences) were combined with sequences of the same marker from several other Oestridae species available in GenBank.

The resulting phylogenetic trees all presented practically the same topology, regardless of the reconstruction method used and so support for most of the branches was very high in all cases. Fig. 1 shows the Bayesian analysis consensus tree. In this tree, species are grouped by genus and clustered into four subfamilies of the family Oestridae (Hypodermatinae, Oestrinae, Gasterophilinae and Cuterebrinae). The branches of the subfamilies Gasterophilinae, Hypodermatinae and Cuterebrinae are grouped and separated from the subfamily Oestrinae (Fig. 1a). Interestingly, all the sequences obtained in this work are grouped on one branch separated from the rest of the species of the subfamily Oestrinae (Figs. 1a and b). This branch is further split into two branches, one including only the sequences from Iberian ibex larvae and the other sequences from

larvae from domestic sheep, domestic goats and European mouflon, and the *O. ovis* sequence from GenBank (Fig. 1b). Hence, the phylogenetic reconstruction indicates clearly that the larvae analysed in this work belong to two different *Oestrus* species, namely, *O. ovis* that parasitizes domestic sheep, domestic goats and European mouflon, and another *Oestrus* sp. that parasitizes Iberian ibex.

4. Discussion

Identification of *Oestrus* species using the morphological features of larvae and adults is complicated, and so a molecular characterization of these parasites is required for species identification. However, molecular data from oestrid species are very scarce, and before this study there were only three sequences from *O. ovis* available in GenBank: COI, 28S (rDNA) and EF1 alpha gene.

Pérez et al. (1996) reported the parasitizing of Iberian ibex from Sierra Nevada National Park by *O. caucasicus* on the basis, above all, of the vein colour of the wings of imagoes. However, subsequent studies (Guiton et al., 2001; Pérez et al., 2006) have remarked on the great similarity in both morphology and biology between *O. ovis* and the species parasitizing Iberian ibex. Thus, the seemingly contradictory data regarding the identity of the species parasitizing Iberian ibex need to be more fully addressed.

Our analyses clearly indicate that all larvae from domestic sheep, domestic goats and European mouflon correspond to *O. ovis*. However, all larvae from Iberian ibex clearly belong to a different species, which should be included in the genus *Oestrus* due to its similarity to the *O. ovis* COI sequence. The identity values obtained in our study agree with the interspecific identity described previously for species belonging to the same genus in the family Oestridae. For example, the COI sequences taken from two species of *Gasterophilus* (*G. nasalis* and *G. intestinalis*) showed comparable interspecific identity (85.1–86.44%) (Pawlas-Opiela et al., 2010). In

species of the genus *Przhevalskiana*, the interspecific identity was wide-ranging, varying from 71.0% to 99.8% (Otranto and Traversa, 2004). Overall, the mean value for interspecific similitude of COI in the Oestridae family is 81.9%, while at the subfamily level the mean reported values are 86.8% in Hypodermatinae, 86.7% in Oestrinae, 90% in Gasterophilinae and 94.7% in Cuterebrinae (Otranto et al., 2005a).

As observed in the COI sequences, comparisons using the 28S (rDNA) also indicated that *O. ovis* was parasitizing all analysed host species except the Iberian ibex, which is probably the focus of a different *Oestrus* species. For this marker, identity percentages between the *O. ovis* sequences and those from the Iberian ibex larvae are lower than for the interspecific comparison between *Rhinoestrus purpureus* and *R. usbekistanicus*, which varied between 99.7% and 100% (Otranto et al., 2005a), and also between two closely related species of the genus *Gasterophilus* (*G. intestinalis* and *G. haemorrhoidalis*), which varied between 99.5% and 99.9% (Otranto et al., 2005b).

Previous molecular studies have demonstrated a strong divergence between the four subfamilies of Oestridae (Pape, 2001; Otranto et al., 2003a). All the sequences of larvae that were analyzed in this work are included in one branch that is clearly separated from the species of the genera *Rhinoestrus* and *Cephenemyia*. Within this branch, the sequences of the larvae collected from domestic sheep, domestic goats and European mouflon are separated with strong support from the sequences of the larvae from the Iberian ibex. This result reinforces the idea of the existence of two different species within the same genus: *O. ovis* and *Oestrus* sp. Interestingly, our analyses also show evidence of a differential host preference, as domestic goats from the eastern part of the Sierra Nevada Natural Park were parasitized by *O. ovis*.

Our data clearly reveal that the species parasitizing Iberian ibex is not *O. ovis*. However, in order to name this species, we still need to conduct further analyses at both morphological and molecular levels of specimens of *O. caucasicus*. On a merely speculative level, it cannot be ruled out that biogeographic isolation in the Iberian Peninsula might have given rise to the differentiation of a new parasite species.

Gravid *Oestrus* females are not thought to be strictly specific to their hosts and domestic animals are thought to become reservoirs and the main infestation source for wild fauna (Colwell, 2001). About 49% of these flies parasitize exclusively one host species (Price, 1980). Our results were somewhat unexpected. In the Iberian Peninsula *O. ovis* parasitizes different host species (both domestic and wild) belonging to the genera *Ovis* and *Capra* and, probably, also the aoudad (*Ammotragus lervia*) in southeastern Spain. However, *Oestrus* sp. seems to be strictly specific to the Iberian ibex from Sierra Nevada, since the sampled domestic goats from the Sierra Nevada mountain range harboured only *O. ovis* larvae. This suggests: (i) a possible long co-adaptation and/or co-evolution process between ibex and their oestrids, which might have resulted in *Oestrus*-host associations shaped by the biogeographical origin of wild ungulates and domestic breeds (Naderi et al., 2008); (ii) no hybridization between different *Oestrus* sympatric species; and that (iii): defensive host behaviour against attacks by gravid females for larviposition could also influence these host-parasite associations. More studies focused on the genetics of oestrid-host associations and on the behaviour of adult flies in the field are needed in order to test these hypotheses.

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