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Characterizing the complete mitochondrial genome and rDNA of *Oestrus* sp. (Diptera: Oestridae): the bot fly parasitizing the Iberian ibex, *Capra pyrenaica*

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Species of the genus *Oestrus* Linnaeus, 1758 (Diptera: Oestridae) are obligate parasites that affect the nasal and pharyngeal cavities of mammals, causing significant harm to both livestock and wildlife. In this study, the complete mitogenome of *Oestrus* sp., a bot fly parasitizing the Iberian ibex (*Capra pyrenaica* Schinz, 1838), has been sequenced and assembled. A comparison with the mitogenome of *Oestrus ovis* Linnaeus, 1758 revealed a high degree of similarity in terms of gene organization and nucleotide composition, and phylogenetic analyses confirmed the close evolutionary relationship between them. Additionally, the entire rDNA cistron, including the internal transcribed spacers (ITS1 and ITS2), was characterized for both species, revealing considerable variation in the length of ITS1, while the 18S, 5.8S, and 28S rRNA genes showed high sequence conservation. These results provide information about molecular markers of 2 *Oestrus* species. Furthermore, the findings offer insights that could facilitate the diagnosis and management of parasitic infestations of Oestridae species in both domestic and wild hosts.

Keywords: Capra pyrenaica, mitogenome, rDNA, *Oestrus ovis*, *Oestrus* sp

Introduction

Oestrids (Oestridae), also known as nasal/pharyngeal bots, are obligate parasites of mammals in the larval stage. Females of the subfamily Oestrinae are larviparous, depositing or ejecting packets of larvae onto the nostrils of the host. The larvae then reach the nasal, pharyngeal, and frontal cavities of the host, where they feed on cellular debris and mucosal secretions until maturity (Wood 1987, Colwell 2001). Apart from the mechanical damage to the host's mucous membranes caused by Oestrinae larvae, they may cause dilatation in the retropharyngeal pouches, degenerative changes, and squamous metaplasia in the nasal and olfactory mucosae. Submucosal edema with leukocyte infiltration (mainly eosinophils) may also occur (Jaganath et al. 1989). *Cephenemyia jellisoni* Townsend, 1941 larvae have been reported to reach unusual locations, such as the ventral nasal meatus and eustachian tube in *Cervus elaphus* Linnaeus, 1758 (Artiodactyla: Cervidae), potentially causing neurologic disease (Foreyt et al. 1994). In bighorn sheep, *Ovis canadensis* Shaw, 1804 (Artiodactyla: Bovidae), cases of

chronic sinusitis, presumably caused by secondary infections associated with oestrosis, have been frequently reported, and can affect the host's skull, sometimes leading to death (Allen and Bunch 1982).

Within the subfamily Oestrinae, the genus *Oestrus* Linnaeus, 1758, is of particular concern due to its impact on both livestock and wild hosts (Colwell 2001, Otranto and Stevens 2006), as well as its zoonotic potential (Hall and Wall 1995, Panadero-Fontán and Otranto 2015). Currently, 4 *Oestrus* species are formally recognized: *Oestrus ovis* Linnaeus, 1758, *Oestrus variolosus* Loew, 1863, *Oestrus aureoargentatus* Rodhain, and Bequaert, 1912, and *Oestrus caucasicus* Grunin, 1948. The identities of *Oestrus bassoni* Zumpt, 1961, and *Oestrus macdonaldi* Geodelst, 1912, remain under discussion (Colwell et al. 2006). *Oestrus ovis*, which parasitizes sheep and domestic goats worldwide, has also been reported to parasitize Caprinae, such as Alpine ibex (*Capra ibex* Linnaeus, 1758), aoudad [*Ammotragus lervia* (Pallas, 1777)], argali (*Ovis ammon* Linnaeus, 1758), Asiatic ibex [*Capra sibirica* (Pallas, 1776)], bighorn sheep (*O. canadensis* Shaw, 1804) European mouflon (*Ovis gmelini* Blyth, 1841) and Nubian ibex (*Capra nubiana* Cuvier, 1825) (Grunin 1957, Capelle 1966, Wetzel and Bauristhene 1970, Howard 1980, Moreno et al. 1999, Colwell et al. 2006, Barroso et al. 2017, Sánchez et al. 2017). Larvae of *O. caucasicus* have been reported from Caucasian tur [*Capra cylindricornis* (Blyth, 1841)], Asiatic ibex and Iberian ibex (*Capra pyrenaica* Schinz, 1838) (Grunin 1957, Pérez et al. 1996).

Morphologically, *O. ovis* and *O. caucasicus* are very similar to each other, except for the distinctive black wing veins in the imagoes of *O. caucasicus* and the dorsal spinulation of third-instar larvae, with variable number of small denticles in the II segment in *O. ovis* and 2 to 3 fairly regular rows of spines in segments III to V and lateral groups of spines in segments VI to VIII in *O. caucasicus* (Grunin 1957, Zumpt 1965, Colwell 2001, Guitton et al. 2001). Moreover, their life cycles are quite similar (Pérez et al. 2006, 2016). In this context, molecular characterization has become a powerful and versatile tool for both diagnosis and larval identification (Otranto et al. 2000).

In eukaryotes, nuclear ribosomal DNA (rDNA) is usually organized in long tandem repeats. These repeats are arranged head-to-tail in large rDNA clusters that form nucleolar organizing regions in chromosomes (Gokhman and Kuznetsova 2024). Until now, only partial sequences of the rDNA unit have been described for species in the Oestridae family (Moreno et al. 2015, de la Fuente et al. 2021). In the same way, only a few mitogenomes have been described for this family. For the genus *Oestrus*, only 2 fragments of the 28S rRNA gene have been described in *O. ovis* and *Oestrus* sp. (Stevens 2003, Moreno et al. 2015), and the complete mitochondrial genome of *O. ovis* was recently characterized (Aleix-Mata et al. 2021b).

The limited molecular data available for Oestridae has led researchers to question the exact taxonomic status of species affecting certain hosts. Moreno et al. (2015), for instance, did not rule out the possibility that the species parasitizing the Iberian ibex represents a distinct, previously unclassified species, and identified it provisionally as *Oestrus* sp. Therefore, this study aimed (i) to characterize the complete mitochondrial genome of *Oestrus* sp. and compare it with that of *O. ovis*, and (ii) to describe the complete rDNA unit of both *Oestrus* species, providing a basis for further phylogenetic analysis and species identification.

Material and Methods

Larvae Collection and Storage

Larvae of *Oestrus* sp. were collected from the head of Iberian ibex (*C. pyrenaica*) from Sierra Nevada National Park (Granada, Southern Spain) (37°14′N to 36°54′N; 2°37′3°39′W), and larvae of *O. ovis* were obtained during the necropsy of a domestic goat (*Capra hircus*), which shares habitat with the Iberian ibex during the summer in this protected area. Larvae were fixed in 70% to 90% ethanol and stored at 4 °C until use. They were morphologically identified based on the shape of the posterior peritremes and dorsal and ventral spinulation (Zumpt 1965, Colwell et al. 2006).

Extraction of Genomic DNA and Illumina Sequencing

Genomic DNAs, from 3 larvae of *Oestrus* sp. and *O. ovis*, were extracted using the ZR Tissue & Insect DNA MiniPrep kit (Zymo Research, Irvine, California, USA). The total genomic DNA of 1 larva of *Oestrus* sp. was sequenced using the Illumina Hiseq 2000 platform by Novogene (Cambridge, UK). Approximately 3 µg of DNA were used to construct a library of 750-bp-length fragments. A total of 2 Gb of paired-end reads (2 × 250 bp) were obtained. Data from the Illumina sequencing of *O. ovis* were obtained from a previous study describing the mitogenome (Aleix-Mata et al. 2021b).

Although, the entire larvae, excluding the digestive tract, were macerated to extract the DNA, other specimens are deposited in the Department of Experimental Biology at the Jaén University (Jáen, Spain) (https://www.ujaen.es/departamentos/bioexp/; Contact email: abaca@ujaen.es).

Mitochondrial Genome Assembly and Annotation

Raw reads from *Oestrus* sp. were quality-trimmed, and sequencing adapters were removed using Trimmomatic v.0.36 (Bolger et al. 2014). A set of 1.8 million clean reads was employed for mitogenome assembly via NOVOplasty v.3.7 (Dierckxsens et al. 2017), using the mitogenome of *O. ovis* (Aleix-Mata et al. 2021b) as reference. The *COI* gene from the mitogenome of *O. ovis* (Aleix-Mata et al. 2021b) served as the seed to initiate the assembly. The resulting *Oestrus* sp. mitogenome was annotated with MITOS (http://mitos.bioinf.unileipzig.de) (Bernt et al. 2013) and tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE) (Lowe and Eddy 1997), with manual refinement by comparison with other mitogenomes of Oestridae species. The circular representation of the assembled mitogenome was created using the OrganellarGenomeDRAW tool (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Greiner et al. 2019).

rDNA Assembly and Internal Transcribed Spacers Cloning and Sequencing

The complete rDNA cistrons, including the 18S rRNA, internal transcribed spacers (ITS1), 5.8S rRNA, ITS2, and 28S rRNA genes (excluding the IGS), were assembled using the previously sequenced fragments of the 28S rDNA from *Oestrus* sp. and *O. ovis* (GenBank accession numbers: KP974971, KP974986, KP974937, KP974975) as seeds, with NOVOPlasty v.3.7 (Dierckxsens et al. 2017).

In addition, we amplified by PCR, cloned and sequenced the ITS1 region of *O. ovis* and the ITS2 regions of both *O. ovis* and *Oestrus* sp. The ITS regions were amplified by PCR using specific primer pairs as previously described (de la Fuente et al. 2021): CAS18sF1 (5′-TACACACCGCCCGTCGCTACTA-3′) and CAS5p8sB1d (5′-ATATGCGTTCAAAATGTCGATGTTCA-3′) for ITS1, and CAS5p8sFt (5′-TGAACATCGACATTTTGAACGCATAT-3′) and CAS28sB1d (5′-TTCTTTTCCTCCCCTAATTAATATGCTTAA-3′) for ITS2 (Ji et al. 2003). Amplicons were resolved in 1% agarose gels stained with ethidium bromide, isolated from the gel using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), and cloned in Z-Competent *Escherichia coli* JM109 (Zymo Research) using the pGEM-T Easy Vector (Promega, Madison, Wisconsin, USA). Positive

clones were Sanger sequenced with T7 and SP6 universal primers. The sequences obtained with NOVOPlasty and from positive clones were aligned using BioEdit v.7.7.1 (Hall 1999) and Clustal Omega (Madeira et al. 2024) to confirm the sequence composition of both ITS1 and ITS2 regions. Repetitive elements were identified with RepeatMasker (http://www.repeatmasker.org/), and repeat finder (https://www.novoprolabs.com/tools/repeats-sequences-finder).

Phylogenetic Analysis

For the phylogenetic analysis, we used the complete mitogenome of *Oestrus* sp., along with the mitogenomes of 17 species from the family Oestridae available in GenBank (Accession numbers: *Cephenemyia trompe* (Modéer, 1786) (MN814272), *Cephenemyia stimulator* (Clark, 1815) (NC_059850), *O. ovis* Linnaeus, 1758 (NC_059851), *Rhinoestrus usbekistanicus* Gan, 1947 (MN833259), *Hypoderma lineatum* (De Villiers, 1789) (GU584123), *Hypoderma sinense* Pleske, 1926 (NC_071819), *Hypoderma bovis* Linnaeus, 1758 (NC080982), *Hypoderma pantholopsum* Ahmed, Simsek, Saki, Kesik and Kilinc, 2017 (PP078819) *Cephalopina titillator* (Clark, 1816) (MN833258), *Gyrostigma rhinocerontis* (Owen, 1830) (MK045312), *Gasterophilus pecorum* (Fabricius, 1794) (KU578262), *Gasterophilus nigricornis* (Loew, 1863) (MG920506), *Gasterophilus nasalis* (Linnaeus, 1758) (MG920505), *Gasterophilus intestinalis* (De Geer, 1776) (KU236026), *Gasterophilus inermis* (Brauer, 1859) (MG920503), *Gasterophilus haemorrhoidalis* (Linnaeus, 1758) (MG920502), *Dermatobia hominis* (Linnaeus, 1781) (AY463155)). As an outgroup, we used *Sarcophaga tuberosa* Pandellé, 1896 from the family Sarcophagidae (Accession number MK820723). Sequences were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Madeira et al. 2024), and poorly aligned positions and divergent regions were removed using Gblocks program v.0.91.1 (https://ngphylogeny.fr/tools/tool/276/form) (Talavera and Castresana 2007). Phylogenetic relationships were inferred using the Maximum-Likelihood (ML) method in MEGA 11 (Tamura et al. 2021), with node support assessed by 1,000 bootstrap replicates. The best-fit nucleotide substitution model (GTR + G + I) was selected as it yielded the lowest Bayesian Information Criterion value using MEGA 11.

Results and Discussion

Mitogenome of *Oestrus* sp.

The mitochondrial genome (GenBank: PQ516713) of *Oestrus* sp. has a total length of 16,769 bp, with a base composition of A (39.4%), C (16.4%), T (35.7%), and G (8.5%) (A + T content 75.1%). It exhibits a positive AT skew of 0,049 and a negative GC skew of –0,317. These characteristics are very similar to those of the *O. ovis* mitogenome, which has a length of 16,584 bp and a base composition of A (39.6%), C (15.9%), T (36.2%), and G (8.3%) (A + T content 75.8%), with a positive AT skew of 0,045 and a negative GC skew of –0,314 (Aleix-Mata et al. 2021b). The A + T content, as well as the positive AT skew and the negative GC skew, are similar to the mitogenomes of other dipteran species (Junqueira et al. 2004). The gene organization is identical to that of mitogenomes of *O. ovis* and other Oestridae species (Aleix-Mata et al. 2021a, b, Gao et al. 2016, Li et al. 2020), containing the typical 13 protein-coding genes (PCGs) (*COI-III*, *ND1-6*, *ND4L*, *CYTB*, *ATP6*, and *ATP8*), 2 rRNA genes (12S, and 16S), 22 tRNA genes, and a control region (CR or D-loop region) (Fig. 1 and Table 1).

The lengths of the PCGs were in the following decreasing order: *ND5* > *COI* > *ND4* > *CYTB* > *ND2* > *ND1* > *COIII* > *COII* > *ATP 6* > *ND6* > *ND3* > *ND4L* > *ATP8*. Most of the genes are encoded on the heavy strand; of the PCGs, 9 (*ATP6*, *ATP8*, *CYTB*, *COI*, *COII*, *COII*, *ND2*, *ND3*, and *ND6*) are transcribed on the heavy strand, while only 4 (*ND1*, *ND4*, *ND4L*, and *ND5*) are transcribed on the light strand. The 2 rRNA genes are transcribed on the light strand, and of the tRNAs, 14 are transcribed in the heavy strand and eight in the light strand (Table 1 and Fig. 1).

The PCGs of *Oestrus* sp. mitogenome were identical in length to those of *O. ovis*; however, there are some differences in the start codons among them. In fact, in *Oestrus* sp. 7 genes start with ATG, 5 with ATT, and 1 with TCG, whereas in *O. ovis*, 7 genes start with ATG, 3 with ATT, 1 with TCG, 1 with ATA, and 1 with GTG (Table 1; Supplementary Table S1) (Aleix-Mata et al. 2021b). The start codon TCG, which differs from the standard invertebrate mitochondrial code, is a common feature in the *COI* gene in both *Oestrus* species. This feature has also been described in the mitogenomes of species from the families Calliphoridae, Tephritidae, and Culicidae (Junqueira et al. 2004, Gao et al. 2016).

Regarding stop codons, there is no variation between the PCGs of the mitogenomes of *Oestrus* sp. and *O. ovis*. In both species, 7 genes use TAA, 3 use the incomplete T–, and 3 use TAG (Table 1; Supplementary Table S1) (Aleix-Mata et al. 2021b).

The variation in lengths of the tRNA genes ranged from 62 to 72 bp in both *Oestrus* sp. and *O. ovis*; however, 7 tRNAs differ in length between the 2 species (Table 1; Supplementary Table S1) (Aleix-Mata et al. 2021b).

The rRNA 12S gene has the same length in both species (789 bp), while 16S gene has lengths of 1,328 bp in *Oestrus* sp. and 1,326 bp in *O. ovis* (Table 1; Supplementary Table S1) (Aleix-Mata et al. 2021b). Both rRNA genes exhibit high A + T content, with 79.5% for 16S rRNA and 77.4% for 12S rRNA. The rRNAs of *O. ovis* also have similar A + T contents (80.0% and 77.3%, respectively), and high A + T content is common in these sequences across other species (Junqueira et al. 2004).

In the *Oestrus* sp. mitogenome, overlapping regions between genes occurred in some genes, varying from 1 to 7 nucleotides, while intergenic spaces also varied between 2 and 126 nucleotides (Table 1). The *O. ovis* mitogenome also exhibits overlapping and intergenic spaces in the same positions, including 1 additional overlapping position; however, some intergenic spaces differ in length from those in *Oestrus* sp. (Supplementary Table S1).

The D-loop region has a length of 1,659 bp in *Oestrus* sp., which is slightly longer than the 1,598 bp of *O. ovis*. The A + T content is 87.0% and 87.3%, respectively. The identity percentage between *Oestrus* sp. and *O. ovis* is 79.9%. The variation in the length of this region is the main factor explaining the differences in mitogenomes size among Oestridae species (Junqueira et al. 2004).

The phylogenetic tree (Fig. 2), using the *Oestrus* sp. mitogenome along with the 17 complete and available mitogenomes of species from the family Oestridae in GenBank, and *S. tuberosa* (Diptera: Sarcophagidae) (Kai et al. 2019) as an outgroup, shows the 4 subfamilies of Oestridae with their genera and species grouped into clearly and well-differentiated branches with high statistical support. The mitogenome of the specimen of *Oestrus* sp. appears grouped in a node with the mitogenome of *O. ovis*. The species *R. usbekistanicus* is also grouped in a node with them. This described node is associated with the node of the species from the genus *Cephenemyia*. This association among the genera *Oestrus*, *Rhinoestrus*, and *Cephenemyia* has been documented in other phylogenetic analyses (Pape 2001, Moreno et al. 2015, Pape et al. 2017). In general, all data align with the previous analyses of the phylogenetic relationships among genera in the family Oestridae (Moreno et al. 2015, de la Fuente et

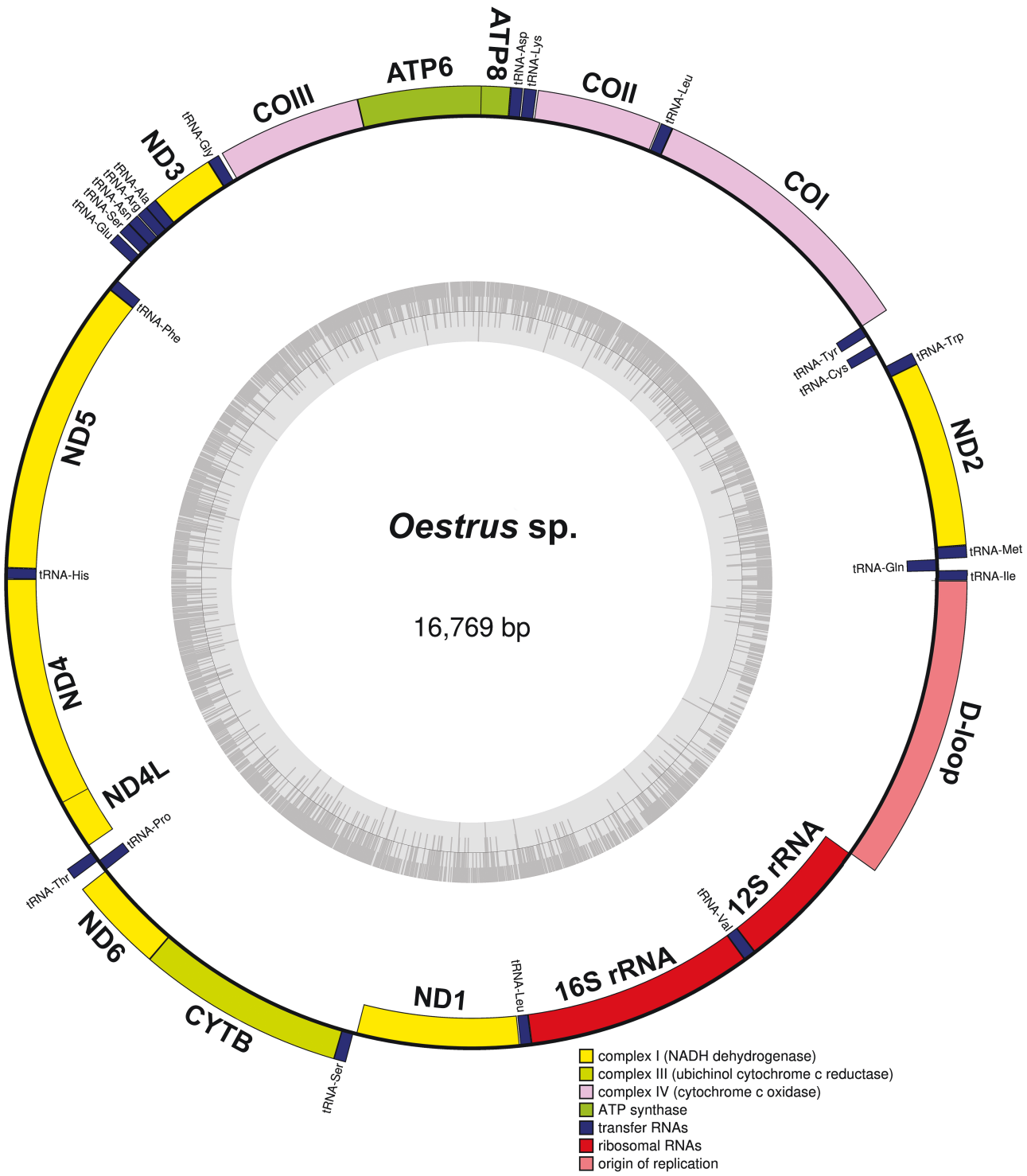

Fig. 1. Graphical map of the mitochondrial genome of *Oestrus* sp. Genes encoded by the heavy strand (H) are shown outside the circle and those encoded by the light strand (L) are shown inside the circle. The inner ring shows the GC content of this genome.

al. 2021). However, in the cladogram the subfamily Oestrinae has been spliced in 2 separate groups, because *C. titillator* (Clark, 1816) clusters with the stomach bot flies (Gasterophilinae). This association has been described in phylogenetic analyses of *C. titillator* using mitogenome genes (Shamsi et al. 2023, Yao et al. 2025). However, the association between *C. titillator* and species of Oestrinae is also described using mitogenome genes (Li et al. 2020, Chen et al. 2023).

These findings indicate that mitogenome sequences could be an important molecular marker for phylogenetic studies of this group of species and other species in general.

rDNAs Sequences

The complete cistron sequences of rDNA, measuring 10,838 and 9,697 bp in length, were assembled from the *Oestrus* sp. and *O. ovis*

Table 1. Gene organization of the *Oestrus* sp. mitogenome

Gene	Position from	Position to	Length (bp/aa)	Intergenic nucleotides	Anticodon	Start codon	Stop codon	Strand
$tRNA^{Ile}$	1	65	65	−3	GAT			H
$tRNA^{Gln}$	63	131	69	−1	TTG			L
$tRNA^{Met}$	131	198	68		CAT			H
ND2	199	1,215	1,017 (338aa)	−2		ATT	TAA	H
$tRNA^{Trp}$	1,214	1,281	68	82	TGA			H
$tRNA^{Cys}$	1,364	1,426	63	56	GCA			L
$tRNA^{Tyr}$	1,483	1,547	65	−2	GTA			L
COI	1,546	3,079	1,534 (511aa)			TCG	T--	H
$tRNA^{Leu}$	3,080	3,146	67	4	TAA			H
COII	3,151	3,834	684 (227aa)	6		ATG	TAA	H
$tRNA^{Lys}$	3,841	3,911	71	4	CTT			H
$tRNA^{Asp}$	3,916	3,981	66		GTC			H
ATP8	3,982	4,143	162 (53aa)	−7		ATT	TAA	H
ATP6	4,137	4,814	678 (225aa)	−1		ATG	TAA	H
COIII	4,814	5,602	789 (262aa)	19		ATG	TAA	H
$tRNA^{Gly}$	5,622	5,687	66		TCC			H
ND3	5,688	6,041	354 (117aa)	−2		ATT	TAG	H
$tRNA^{Ala}$	6,040	6,103	64	−1	TGC			H
$tRNA^{Arg}$	6,103	6,164	62	3	TCG			H
$tRNA^{Asn}$	6,168	6,231	64		GTT			H
$tRNA^{Ser}$	6,232	6,299	68	13	GCT			H
$tRNA^{Glu}$	6,313	6,376	64	126	TTC			H
$tRNA^{Phe}$	6,503	6,570	68		GAA			L
ND5	6,571	8,302	1,732 (577aa)			ATG	T--	L
$tRNA^{His}$	8,303	8,366	64		GTG			L
ND4	8,367	9,705	1,339 (446aa)	−7		ATG	T--	L
ND4L	9,699	9,992	294 (97aa)	37		ATG	TAA	L
$tRNA^{Thr}$	10,030	10,094	65		TGT			H
$tRNA^{Pro}$	10,095	10,162	68	2	TGG			L
ND6	10,165	10,689	525 (174aa)	−1		ATT	TAA	H
CYTB	10,689	11,825	1,137 (378aa)	−2		ATG	TAG	H
$tRNA^{Ser}$	11,824	11,891	68	16	TGA			H
ND1	11,908	12,852	945 (314aa)	4		ATT	TAG	L
$tRNA^{Leu}$	12,857	12,921	65		TAG			L
16S rRNA	12,922	14,249	1,328					L
$tRNA^{Val}$	14,250	14,321	72		TAC			L
12S rRNA	14,322	15,110	789					L
D-loop	15,111	16,769	1,659					

genomes (GenBank accession numbers: PQ562418 to PQ562419), which included the 18S, 5.8S, and 28S rRNA genes, as well as the ITS1 and ITS2. The 5′ and 3′ ends of the rRNA genes and ITSs were determined using the sequence of *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) rDNAs as a reference (Accession number: M21017) (Tautz et al. 1988). Hence, the rDNA unit sequences described here [excluding the external transcribed spacers and intergenic spacer (IGS)] represent the first such sequences reported in species of the Oestridae family.

An analysis of the *Oestrus* sp. and *O. ovis* sequences revealed low levels of genetic variation in the 18S, 5.8S, and 28S rRNA genes, while the ITSs of both species exhibited greater variability. The rRNA genes of both *Oestrus* species are very similar in length, sequence, and A + T content. The identity percentages were high, ranging from 97.80% for 28S rRNA gene to 99.90% for 18S rRNA gene (Table 2).

The ITS1 sequences exhibited the highest variation in size; in fact, the ITS1 of *Oestrus* sp. is over 1,000 bp longer than that of *O. ovis*, with an identity percentage of the aligned fragment between the ITS2s of both species at 78.21%. However, the ITS2 fragments are very similar in size for both species, with an identity percentage of 79.30%, which is lower compared to the rRNA genes (Table 2).

The 2 PCR-amplified clones of ITS1 from *O. ovis* (GenBank accession numbers: PQ521220 to PQ521221) confirmed the assembled ITS1 sequence and displayed different lengths (1,167 and 1,148 bp) due to variations in microsatellite sequences, achieving 95% identity. Similarly, the 2 cloned sequences for ITS2 from *O. ovis* and *Oestrus* sp. (GenBank accession numbers: PQ521222 to PQ521225) confirmed the assembled ITS2 sequences, showing low variation in length (1,444 and 1,441 bp for *Oestrus* sp. and 1,453 and 1,448 pb for *O. ovis*). The identity percentages between the ITS2 clones of both species range from 76.16% to 76.84%. In all the ITS2 sequences, we identified the conserved 2S rRNA gene with 30 bp (TGCTTGGACTACATATGGTTGAGGGTTGTA) (de la Fuente et al. 2021).

Regarding the size variation of the ITSs in Oestridae, the sequences available in GenBank ranged between 487 and 1,631 bp for ITS1 and between 361 and 1,043 bp for ITS2 (de la Fuente et al. 2021). Therefore, the ITS1 of *Oestrus* sp. at 2,294 bp and the ITS2 of both species are the largest described for species within this group. In addition, in 4 species of the Calliphoridae genera, the ITS2 ranged from 310 to 337 bp (GilArriortua et al. 2014, 2015). Generally, in most eukaryotes, the length of both ITSs is less than

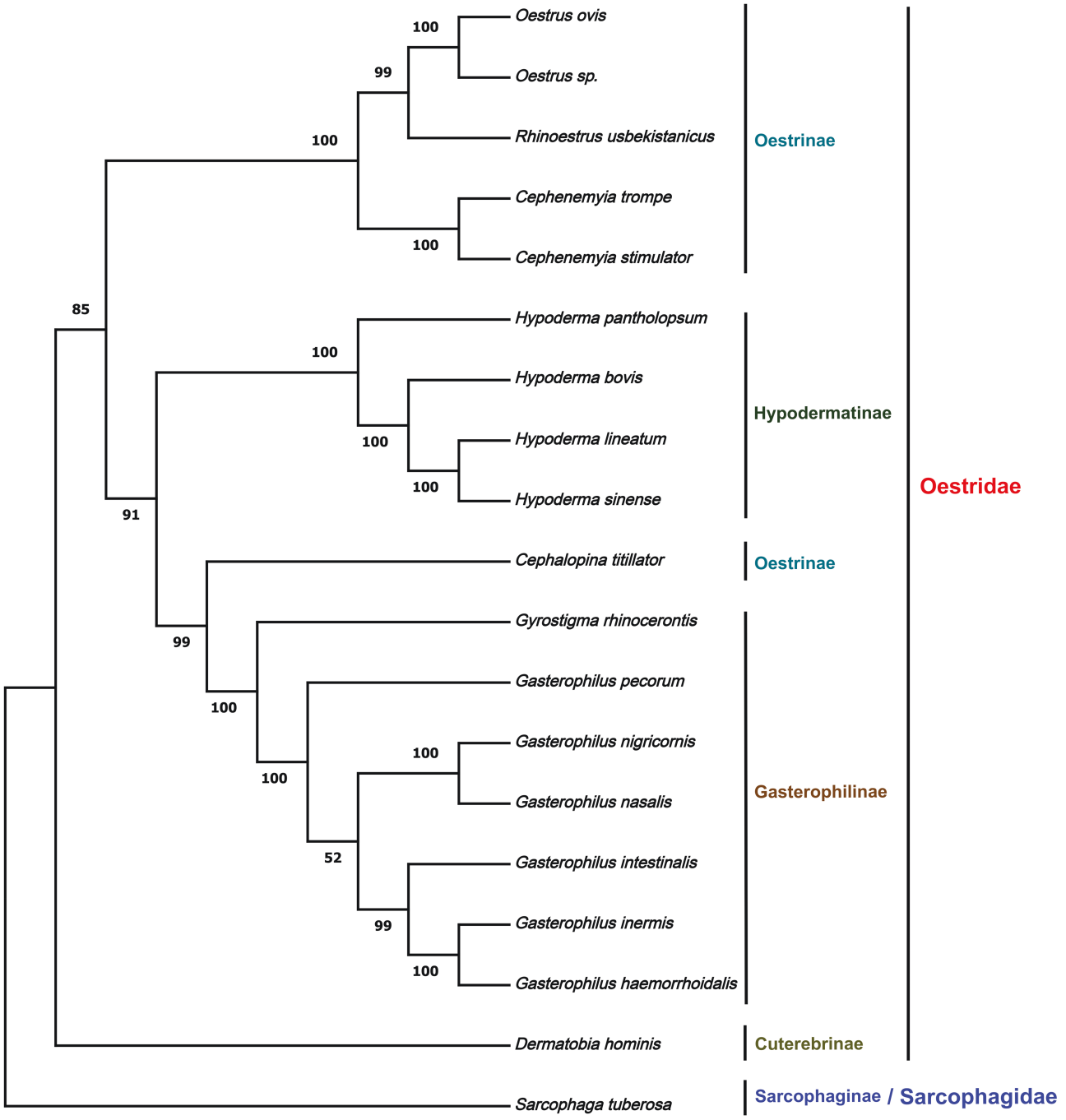

Fig. 2. ML phylogenetic consensus tree using the complete mitochondrial genomes. The data at the nodes correspond to Bootstrap values and represent the statistical support for them. The tree has been performed using the nucleotide substitution model GTR + G + I.

500 bp (Coleman 2015), although there are exceptional shorter and longer examples of ITSs in some eukaryotes. Specifically, the size of ITS1 varies between 41 bp in the protozoan parasite *Giardia lamblia* (Lambl 1859) (Diplomonadida: Hexamitidae) and 2,572 bp in the coleopteran *Exochumus quadripustulatus* (Linnaeus, 1758) (Coleoptera: Coccinellidae), while ITS2 varies between 55 bp in *G. lamblia* and 1,547 bp in the mite *Aponomma concolor* (Neumann, 1899) (Parasitiformes: Ixodidae) (Boothroyd et al. 1987, von der Schulenburg et al. 2001, Hlinka et al. 2002).

The RepeatMasker program identified several simple repeats in the ITSs of both species. In the ITS1 of *Oestrus* sp. 8 simple repeats were found, representing 16.96% of the total, while the ITS1 of *O. ovis* contained 5 simple repeats, accounting for 19.45% of the total. In the ITS2, 6 simple repeats were identified, representing 19.13% in *Oestrus* sp. and 22.62% in *O. ovis*. The presence of simple repeat in the ITS sequences of Oestridae species has been previously described, for example, in *C. stimulator* (Clark, 1815), *Cephenemyia auribarbis* (Meigen, 1824), and *Pharyngomyia picta* (Wiedemann, 1824) (de la Fuente et al. 2021).

The findings presented here show that *Oestrus* sp. is very closely related to *O. ovis*, as previously demonstrated (Moreno et al. 2015). However, the proposed correspondence between *Oestrus* sp. and

Table 2. Characteristic of the assembled rDNA of *Oestrus* sp. and *Oestrus ovis*

Region	*Oestrus* sp.		Oestrus ovis				Variable sites
	Length	A + T content (%)	Length	A + T content (%)	Pairwise identity (%)	Pairwise distance	
Assembled rDNA	10,838	65.21	9,697	64.97	90.69	0.0400	1,728
18S rRNA	2,012	57.26	2,012	57.16	99.90	0.0010	2
ITS1	2,294	71.71	1,167	73.86	78.21[a]	0.3247	1,453
5.8S rRNA	123	49.59	123	50.41	99.19	0.0082	1
ITS2	1,443	71.59	1,441	73.91	79.30	0.1116	257
28S rRNA	4,334	62.97	4,333	62.94	97.80	0.0115	74

[a]Value corresponding to the sequence fragment that aligns between the ITS2s of both species.

O. caucasicus, as suggested by Pérez et al. (1996), requires further confirmation through an analysis of molecular data from *O. caucasicus*. In fact, future work to determine whether *Oestrus* sp. and *O. caucasicus* are the same species or 2 different species depends on obtaining adult specimens or larvae of *O. caucasicus*. These specimens would allow accurate comparison of morphological traits with specimens of *Oestrus* sp. Likewise, these specimens could bring authoritative sequence data for molecular markers of *O. caucasicus*, which could then be compared with those of *Oestrus* sp. The results of these morphological and molecular analyses would allow definitive determination of whether *Oestrus* sp. is *O. caucasicus* or a new, yet undescribed species.

Finally, the molecular data presented here could be useful for the identification of larvae, phylogenetic analyses, and comparative analyses with mitogenomes and rRNA genes in other Oestridae species.

Supplementary material

Supplementary material is available at *Journal of Medical Entomology* online.

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