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RESEARCH ARTICLE

Mitochondrial genome and morphological variability of *Bactrocera oleae* (Diptera: Tephritidae) from an organic olive orchard in Southern Spain

[version 1; peer review: awaiting peer review]

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Abstract

Background

The olive fruit fly, *Bactrocera oleae*, is the most damaging pest of olive production. This study aimed to characterize the intraspecific morphological variability and assemble the complete mitochondrial genome of a population from an organic olive orchard in Órgiva (Granada, southern Spain), a region showing high biodiversity and habitat heterogeneity.

Methods

A total of 140 adults emerging from naturally infested olives were examined for morphological variation (thoracic pigmentation, abdominal banding, abdomen shape, body size, and ovipositor morphology). Morphometric analyses were performed to identify distinct groups. Longevity and fecundity were assessed on two olive varieties (Picual and Picholine). The complete mitochondrial genome, reflecting the local population of this biotope, was assembled using Oxford Nanopore sequencing from a pooled subset of 40 individuals selected among the 140 adults.

Results

Marked morphological variation was observed. Morphometric analyses revealed six distinct groups, primarily separated by body size

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and ovipositor traits, with significant differences in longevity (25–32 days) and fecundity (36–52% on Picual; 32–49% on Picholine). A complete circular mitogenome of 15,810 bp was assembled (GenBank accession number: PZ179621). The Órgiva haplotype differed from the Italian reference by 21 nucleotide substitutions, including 11 non-synonymous changes in ND and COX genes, and clustered within the Mediterranean clade in phylogenetic analyses.

Conclusions

This study provides a high-quality consensus mitochondrial genome and a comprehensive baseline of morphological and fitness variation of *B.oleae* from a traditional organic olive orchard, offering valuable resources for comparative studies, haplotype tracking, population monitoring, and research on phenotypic plasticity in Mediterranean organic olive systems

Keywords
Bactrocera oleae; olive fruit fly; organic olive orchard, intraspecific morphological variation; mitochondrial genome; phenotypic plasticity



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1. Introduction

Olive cultivation is a cornerstone of Mediterranean agriculture, providing substantial economic, cultural and environmental benefits while supporting high levels of biodiversity. However, olive production remains severely constrained by the olive fruit fly, *B. oleae* (Rossi, 1790) (Diptera: Tephritidae), the most economically damaging pest of olives worldwide. Larval feeding inside the fruit causes premature drop, yield losses frequently exceeding 30–50 %, and significant degradation of olive oil quality through increased acidity and microbial contamination.[1,2] For decades, chemical control of *B. oleae* has relied heavily on synthetic insecticides. This intensive use has promoted the selection of pre-existing resistant alleles in natural populations, resulting in the widespread evolution of insecticide resistance[3,4] Moreover, such practices have caused significant negative impacts on non-target organisms, including natural enemies, while raising growing concerns for human health and environmental sustainability. In this context, organically managed and low-input olive groves have emerged as valuable model systems for developing more sustainable integrated pest management (IPM) strategies. A recent systematic map of control methods for the olive fruit fly highlights the urgent need for diversified approaches that go beyond conventional chemical reliance.[5] Production systems that rely less on synthetic insecticides and promote habitat heterogeneity and plant diversity generate complex ecological conditions, with diverse alternative host plants, microclimatic variation, and increased biodiversity.[6] These factors strongly shape *B. oleae* behaviour and population dynamics. In such environments, alternative food resources, together with cultural practices, pheromone-based mass trapping, and biological control, can influence adult survival and fecundity under organic olive production.[7] In heterogeneous environments, adult flies interact with a variety of landscape elements, and the availability of alternative resources can influence their movement and local abundance.[8] Some tephritid species also show that, during host-searching, adults can exploit a wide range of alternative food sources, including crop pollen.[9] In agro-diversified agroecological systems, Ceratitis capitata, like other tephritid species, is known to exhibit pronounced phenotypic plasticity.[10] Such ecological complexity can shape morphological traits and generate substantial intraspecific variation, highlighting the importance of detailed morphological studies in entomology.[11] Previous research has addressed the evolution of morphological traits,[12,13,14,15] whereas other studies have concentrated on the development and application of geometric morphometric approaches.[16,17] In B. oleae specifically, functional morphology in response to fruit composition has been evidenced through sensory analyses.[18] A recent study by Pajač Živković et al.[19] demonstrated the utility of geometric morphometrics in capturing cultivar-specific morphological adaptations of *B. oleae.* The authors showed that olive cultivars differ in their susceptibility to the insect, and may influence population variability. Moreover, as reported by Pajač Živković et al.,[19] this phenotypic plasticity of *B. oleae* such as wing shape and sexual dimorphism may enhance dispersal and oviposition efficiency in olive fruits. Accurate identification and monitoring of locally adapted populations therefore represent a significant challenge for organic olive production.

Although fruit characteristics clearly play a major role in determining olive susceptibility to *B. oleae* attack, the insect's own morphological responses remain poorly documented. Only a limited number of studies have described intraspecific morphological variation within olive agroecosystems, and very few have quantified how such variation may influence performance traits in *B. oleae.* This gap is particularly evident across the Mediterranean basin, where diverse production systems and environmental conditions could promote substantial phenotypic differentiation.

Within defined environments such as organically managed systems, investigating morphological variability in *B. oleae* is especially relevant. Beyond descriptive accounts, determining whether this variability has a detectable genetic basis remains an open question that has not yet been systematically addressed. Establishing such connections would provide a valuable framework for linking morphological diversity with genomic data and strengthening future comparative studies across cultivars, orchards, and geographic regions.

Population genetic studies have revealed substantial polymorphisms and complex colonization histories of *B. oleae* across the Mediterranean basin and olive groves areas and.[20,21] These patterns have been supported by the early development of polymorphic microsatellite markers.[22] More recently, genomic technologies have opened new avenues for pest management by providing insights into population structure, gene flow, and potential adaptive mechanisms.[23,24] Complete mitochondrial genomes, in particular, offer higher resolution for detecting fine-scale haplotype diversity compared to partial gene fragments.[25] Although conventional markers have revealed relatively high genetic diversity combined with limited geographic structure and high gene flow among Mediterranean populations,[26,27] mitogenomic data from organically managed olive groves remain scarce.

In the present study, *B. oleae* adults were collected from a traditional organic olive orchard in Órgiva (Granada, southern Spain). We documented morphological variability within this local population, focusing on traits associated with fecundity and survival potential on two local olive varieties (Picual and Picholine), and assembled a complete mitochondrial genome using Oxford Nanopore long-read sequencing (GenBank accession PZ179621). This work provides both a high-quality reference mitogenome and a detailed morphological baseline from an organic biotope,

offering a valuable foundation for future comparative analyses and the development of region-specific molecular tools to support sustainable integrated pest management strategies against *B. oleae.*

2. Materials and methods

2.1 Description of the sampling site

The study was conducted in a traditional, organically managed olive orchard located near Órgiva, Granada Province, southern Spain (36°53′47.56″N, 3°24′01.46″W; elevation 401 m a.s.l.) (Figure 1). The orchard is situated on mountain slopes and comprises approximately 450 olive trees of the varieties Picual and Picholine, each around 60 years old. It is embedded within a heterogeneous Mediterranean agroecosystem that includes mixed crops such as citrus, carob, and pomegranate, together with forest trees, hedgerows, and patches of natural vegetation. Management follows low-input organic practices, with minimal use of synthetic pesticides and fertilizers, relying mainly on pheromone-based mass trapping and other ecological methods.

2.2 Sample collection and rearing protocol

B. oleae adults were obtained from naturally infested olive fruits collected from about 180 trees of the traditional Lechín and Picual varieties in the study orchard. Sampling was conducted between September and December 2024, a period characterized by moderate infestation levels of approximately 30–40%. Infested fruits were collected and placed in transparent plastic containers covered with aerated fabric to ensure ventilation and prevent mould development. They were then transferred to larger containers fitted with a fine-mesh zippered lid and maintained in controlled-environment chambers at 25 °C and 70 % relative humidity (reduced to ~65 % during pupation) under a 16:8 h (L:D) photoperiod until adult emergence. Emerging adults were examined to document morphological carateristics and subsequently preserved at −20 °C in 70% ethanol prior to DNA extraction.

2.3 Imaging procedure and morphological characterization

A total of 140 *B. oleae* adults were examined using an Olympus SZX61 stereomicroscope at 50× magnification, equipped with an integrated digital camera. All images were captured under standardized lighting conditions and processed with GIMP (v2.10) to standardize background, brightness, and contrast while preserving natural morphological features. A calibrated 2 mm scale bar was included in each photograph to enable accurate morphometric measurements.



Figure 1. Area of sampling in southern Spain. The red polygon outlines the olive orchard (~450 olive trees of the varieties Picual and Picholine) in Órgiva (36°53′47.56″N, 3°24′01.46″W, Granada). The inset map (upper left) indicates the position within Spain. Satellite imagery from Google Earth (2026). Mapping and annotations performed with GIMP 3.0.8-2.

The following morphological traits were quantified for each individual: thoracic pigmentation, abdominal banding intensity, abdomen shape (length-to-width ratio), ovipositor morphology (females only), and body length measured from the anterior margin of the head to the tip of the abdomen. Thoracic pigmentation was scored on a scale of 1–5, where 1 represented light yellowish-brown uniform coloration and 5 indicated dark reddish-brown with strongly contrasting pigmented zones. Abdominal banding intensity was scored on a similar 1–5 scale, ranging from absent or very faint (1) to wide, strongly marked black transverse bands (5). Abdomen shape was quantified as the length-to-width ratio. Ovipositor morphology was assessed qualitatively on a scale of 1–4 (1 = slender and straight; 4 = short, robust, curved or pointed). Six morphological groups were initially defined based primarily on thoracic pigmentation and abdominal banding scores. All measurements and scorings were performed by the same observer to ensure consistency. Statistical analyses were subsequently conducted to verify and refine this preliminary classification, assessing whether the observed morphological groupings were supported by quantitative data.

2.4 Statistical and multivariate analyses

Data normality was evaluated using the Shapiro–Wilk test and non-parametric tests were applied. Differences in body length and abdomen L/W ratio among the six morphological groups were analyzed using the Kruskal–Wallis test, followed by Dunn's post-hoc test for multiple comparisons ($p < 0.05$). To explore overall morphological variation and validate the group structure, a Principal Component Analysis (PCA) was performed on standardized variables to identify the main axes of variation. Hierarchical clustering using Ward's method and Euclidean distance was then applied to the PCA scores to classify individuals into clusters. All analyses and visualizations were performed in R v4.5.2[28] using the packages FactoMineR, factoextra, and ggplot2. Significance was set at $p < 0.05$.

2.5 Adult longevity and fecundity assays

To assess potential differences in fitness-related traits among the six morphological groups, longevity and oviposition performance were evaluated under controlled laboratory conditions. For each morphological group, three replicates were prepared, each consisting of three females selected from the 140 emerged adults plus two males from the same rearing batch (to ensure mating). Thus, a total of 9 females and 6 males per morphological group were tested. Replicates were maintained in aerated containers constructed from fine muslin fabric, each fitted with an access door and provisioned with 30 healthy, fresh olives (15 Picual and 15 Picholine). Olives were replaced every 5 days. Adults were provided with a continuous source of food consisting of diluted honey on cotton wool and water. The experiment was conducted under under the laboratory's ambient temperature and humidity conditions.

Longevity was recorded daily until the death of all individuals. Oviposition was evaluated by counting the total number of punctures (stings) on the olives and distinguishing between fertile punctures (those containing an egg) and sterile punctures.

The percentage of fertile punctures was calculated as:

$$\%\text{fertile punctures} = (\text{number of fertile punctures}/\text{total number of punctures}) \times 100$$

This was calculated separately for Picual and Picholine cultivars. Statistical analyses were performed using one-way ANOVA to test for differences among morphological groups, followed by Tukey's HSD post-hoc tests to identify pairwise differences in longevity and fecundity.

2.6 DNA extraction, sequencing and basecalling

High-molecular-weight genomic DNA was extracted from a pool of 40 adult *B. oleae* individuals, selected from the 140 morphologically characterized specimens and preserved in 96% ethanol. This pooling approach ensured sufficient high-quality DNA for Oxford Nanopore long-read sequencing while providing a representative consensus mitogenome of the Órgiva population.

Specimens were first rinsed thoroughly three times with nuclease-free water to remove residual ethanol, gently air-dried on sterile filter paper at room temperature, snap-frozen in liquid nitrogen, and stored at −80°C until extraction. Approximately 25 mg of pooled tissue was mechanically pulverized in a pre-chilled mortar and pestle. DNA extraction was performed using the MagAttract HMW DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions for insect tissue, with minor modifications only in the initial tissue homogenization step to optimize yield from pooled samples.

DNA quality and quantity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) for purity ratios (A260/A280 ≈ 1.8–2.0 and A260/A230 > 1.8) and a Qubit 4.0 fluorometer (Invitrogen) for accurate double-stranded DNA concentration. Only extracts meeting high-quality thresholds were used for library preparation.

Sequencing libraries were prepared using the Native Barcoding Expansion 1–24 (EXP-NBD114) and the Ligation Sequencing Kit (SQK-LSK114) (Oxford Nanopore Technologies). Sequencing was performed on a PromethION P2 Solo instrument equipped with a FLO-PRO114M flow cell. Basecalling was conducted with Guppy v6.5.7 using the "super-accurate" (SUP) model. This generated 3.11 Gb of raw data from 2,391,348 reads (mean read length 1,301 bp). A total of 37,405 reads (53 Mb) mapped to the *B. oleae* reference mitochondrial genome, providing an average coverage depth of approximately 3,600×. Sequencing and library preparation were carried out by Secugen S.L. (Madrid, Spain).

2.7 Mitochondrial genome mapping, assembly, annotation

Nanopore long reads were mapped to the *B. oleae* reference mitochondrial genome (GenBank CM090314.1) using minimap2 v2.28 with the "map-ont" preset. Mapped reads were extracted and assembled de novo with Flye v2.9.5 in high-accuracy mode (--nano-hq). The consensus sequence was polished with Medaka using the r1041_e82_400bps_sup model. Functional annotation was performed with MITOS2 v2.1.9 using the NCBI invertebrate mitochondrial genetic code (translation table 5). Nucleotide composition and codon usage were calculated in MEGA v11.[29] Statistical analyses were performed in R v4.5.2,[28] including a one-way ANOVA to assess variability among genes, with standard deviations computed from raw gene-level values.

2.8 Comparative and phylogenetic analyses

2.8.1 COI Distance analysis

The cytochrome c oxidase subunit I (COI) gene was extracted from the assembled mitogenome and aligned with COI sequences from closely related *Bactrocera* and *Dacus* species retrieved from GenBank. Pairwise genetic distances were calculated under the Tamura–Nei 93 (TN93) substitution model[30] using MEGA v11[29] to evaluate species-level divergence and the diagnostic utility of COI for *B. oleae* identification. Pairwise mitochondrial genome distances from full alignments were visualized as a heatmap with hierarchical clustering. Distance matrices were generated in R v4.5.2[28] using the pheatmap package v1.0.12[31] with Euclidean distance metrics and complete linkage, and refined with ggplot2 v3.5.[32]

2.8.2 Phylogenetic reconstruction

To position the Órgiva *B. oleae* mitogenome within Tephritidae, complete mitochondrial genomes of 19 representative species were used. The dataset included 14 Bactrocera species (*B. dorsalis, B. cucurbitae, B. zonata, B. carambolae, B. tryoni, B. correcta, B. scutellata, B. limbifera, B. melastomatos, B. umbrosa, B. tuberculata, B. caudata, B. biguttula, B. ritsemae*), three Dacus species (*D. bivittatus, D. longicornis, D. trimacula*), *Ceratitis capitata* as a related outgroup, and *Drosophila melanogaster* as a distant outgroup. All accessions and references are listed in Table S1. Sequences were aligned using MAFFT v7 with the L-INS-i algorithm. The best-fit nucleotide substitution model was identified using ModelTest in MEGA X. A Maximum Likelihood phylogenetic tree was reconstructed in MEGA X with 1,000 bootstrap replicates and rooted with *D. melanogaster*. Tree visualization was performed using MEGA X. Finally, the newly assembled *B. oleae* mitogenome was aligned with the Italian reference sequence (NC_005333.1) using MAFFT v7. Pairwise nucleotide differences were identified and classified as synonymous or non-synonymous using MEGA v11 under the invertebrate mitochondrial genetic code.

3. Results and analyses

3.1 Morphological analyses and adult performances

3.1.1 Definition of morphological groups

From the rearing protocol, 140 adult *B. oleae* successfully emerged from naturally infested olives collected from the organic orchard in Órgiva. All specimens exhibited the diagnostic traits of the species, with body length ranging from 4.2 to 5.1 mm (mean ± SD: 4.68 ± 0.23 mm). Compared to the typical Spanish phenotype described by Ruiz Castro (1948)[33] (Figure 2), characterized by a yellowish-brown thorax, pale scutellum, and elongated abdomen with subtle lateral markings, the emerged adults displayed notable intraspecific variation in thoracic pigmentation, abdominal banding, abdomen shape, and ovipositor morphology. Quantitative assessment allowed the classification of all 140 individuals into six distinct morphological groups based primarily on thoracic pigmentation and abdominal banding intensity (Table 1). Representative specimens of each morphological group were photographed to illustrate this variation (Figure 3A–L). Group 1 (Typical light form) was the most abundant (27.9%), whereas Group 3 (Compact orange form) was the rarest (6.4%). Thoracic pigmentation scores ranged from 1 (light yellowish-brown, uniform) to 5 (dark reddish-brown with strongly contrasting zones; overall mean 3.1 ± 1.3). Abdominal banding intensity ranged from 1 (faint or absent) to 5 (pronounced transverse black bands; overall mean 2.9 ± 1.4). Abdomen length-to-width ratios varied between 1.50 (compact) and 2.00 (elongated). Among females, ovipositor morphology ranged from slender and straight to short and blunt. Body length differed significantly among groups (Kruskal–Wallis test, $H = 28.4$, $p < 0.001$), with

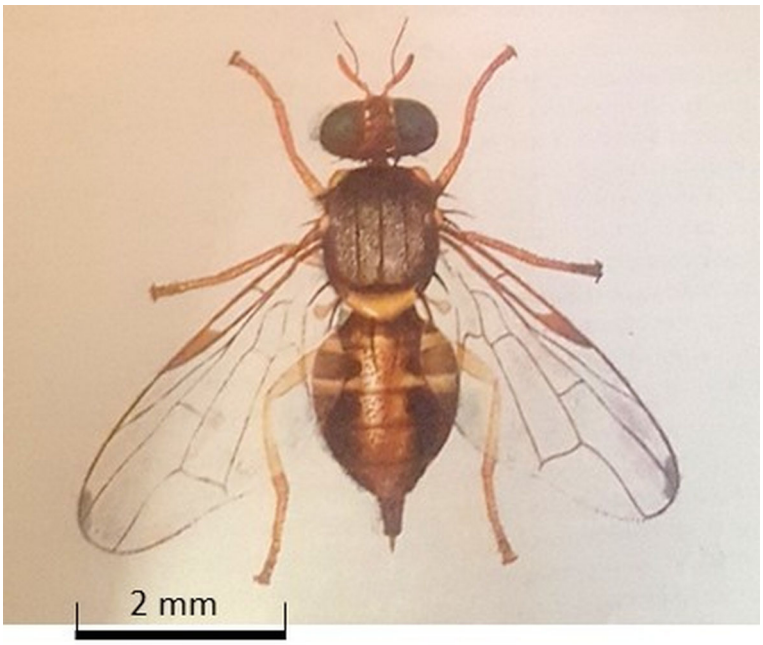


Figure 2. Reference specimen of the olive fruit fly *Dacus oleae*, as described by Ruiz Castro (1948) in Fauna entomológica del olivo en España.

individuals in Groups 3 and 6 being significantly smaller than those in Groups 1, 4, and 5 (Dunn's post-hoc test, $p < 0.05$). The abdomen L/W ratio also varied significantly among groups ($H = 45.7$, $p < 0.001$), with Group 6 showing the most compact shape.

Principal Component Analysis (PCA) revealed that the first two dimensions accounted for 95.4% of the total variance (Dim1 = 87.5%, Dim2 = 7.9%), with Dim1 driven by thorax length, thorax width, and ovipositor length, reflecting overall body size, while Dim2 was associated with abdomen proportions (Figure 4). Building on these results, hierarchical clustering using Ward's method identified six well-defined clusters (Figure 5), which corresponded closely to the morphological groups defined by pigmentation and banding scores. The clusters were primarily separated along Dim1, confirming body size as the dominant axis of differentiation. The different analyses reveal that Groups 3 and 6 were characterized by smaller body size and compact abdominal morphology, whereas Groups 1, 4, and 5 were distinguished by larger thoracic dimensions and elongated ovipositors."

3.1.2 Longevity and fecundity performance

Significant morphological variation was observed among the 140 emerged *B. oleae* adults, which were clustered into six distinct groups based on thoracic pigmentation, abdominal banding, body shape, and ovipositor morphology (see Table 1 and Figure 3 for representative specimens). Adult longevity and fecundity potential differed significantly among the six morphological groups (Table 2). Mean longevity ranged from 24.9 ± 2.1 days (Compact orange form) to 31.7 ± 1.8 days (Dark thoracic form). One-way ANOVA revealed significant differences in longevity among groups ($F = 4.87$, $df = 5$, $p = 0.003$).

Fecundity, expressed as the percentage of fertile punctures, also varied significantly among morphological groups on both olive cultivars. On cv. Picual, the percentage of fertile punctures ranged from $35.6 \pm 4.1\%$ (Compact orange form) to $51.7 \pm 2.9\%$ (Brown elongated thorax) ($F = 8.24$, $df = 5$, $p < 0.001$). A similar pattern was observed on cv. Picholine, with fertile puncture rates ranging from $32.4 \pm 3.7\%$ to $48.5 \pm 3.4\%$ ($F = 7.65$, $df = 5$, $p < 0.001$). Post-hoc Tukey's HSD tests showed that groups 4 (Brown elongated thorax) and 5 (Pronounced abdominal banding), which possessed characteristically long ovipositors, exhibited significantly higher percentages of fertile punctures compared to groups with shorter or blunter ovipositors (groups 3 and 6).

3.2 Mitochondrial genome assembly and gene length variation across tephritidae

Oxford Nanopore sequencing of the pooled sample of 40 adults generated 3.11 Gb of long-read data, yielding a high-quality circular mitochondrial genome of 15,810 bp with an average coverage depth of ~3,600×. (GenBank accession

Table 1. Morphological variation among 140 B. oleae adults collected from the organic olive orchard at Orgiva, southern Spain. Values are presented as mean ± SD or range. Groups were defined primarily by thoracic pigmentation and abdominal banding intensity scores.

Morphological Group*	Description	Thoracic pigmentation (1–5)	Abdominal banding (1–5)	Abdomen L/W ratio	Ovipositor morphology (♀)	Body length (mm)**	n (%)	Representative specimens (Figure 3)
1) Typical light form	Brown thorax with faint markings, slender body and pale abdomen	4–5	3–5	1.80–1.95	Slender, straight	4.75 ± 0.22[a]	39 (27,9)	B, I
2) Dark thoracic form	Dark reddish-brown thorax, contrasting pigmentation	4–5	2–3	1.65–1.80	Robust, slightly short	4.65 ± 0.18[ab]	23 (16.4)	E, K
3) Compact orange form	nearly square thorax, compact and rounded abdomen, characteristic bright orange	1–2	1–2	1.50–1.65	Short and blunt	4.50 ± 0.25[b]	9 (6.4)	G
4) Brown elongated thorax	Elongated brown thorax, dark transverse bands on the abdomen	2–4	2–4	1.70–1.85	characteristically l ong	4.80 ± 0.20[a]	26 (18.5)	H
5) Pronounced abdominal banding	Brown thorax, strong transverse black bands, compact abdomen	3–4	4–5	1.85–2.00	Long, straight	4.85 ± 0.15[a]	24 (17.1)	F, L
6) Dark compact form	Blackish robust thorax, white scutellum, rounded compact abdomen	1–3	1–2	1.55–1.70	Short to medium, robust	4.59 ± 0.27[b]	19 (13.6)	C, J
Overall	-	**3.1 ± 1.3**	**2.9 ± 1.4**	**1.74 ± 0.16**	-	**4.68 ± 0.23**	**140 (100)**	A–L

*Groups were defined primarily by thoracic pigmentation and abdominal banding intensity scores
**Different letters within the same column indicate significant differences among groups (Kruskal–Wallis test followed by Dunn's post-hoc test, $p < 0.05$)

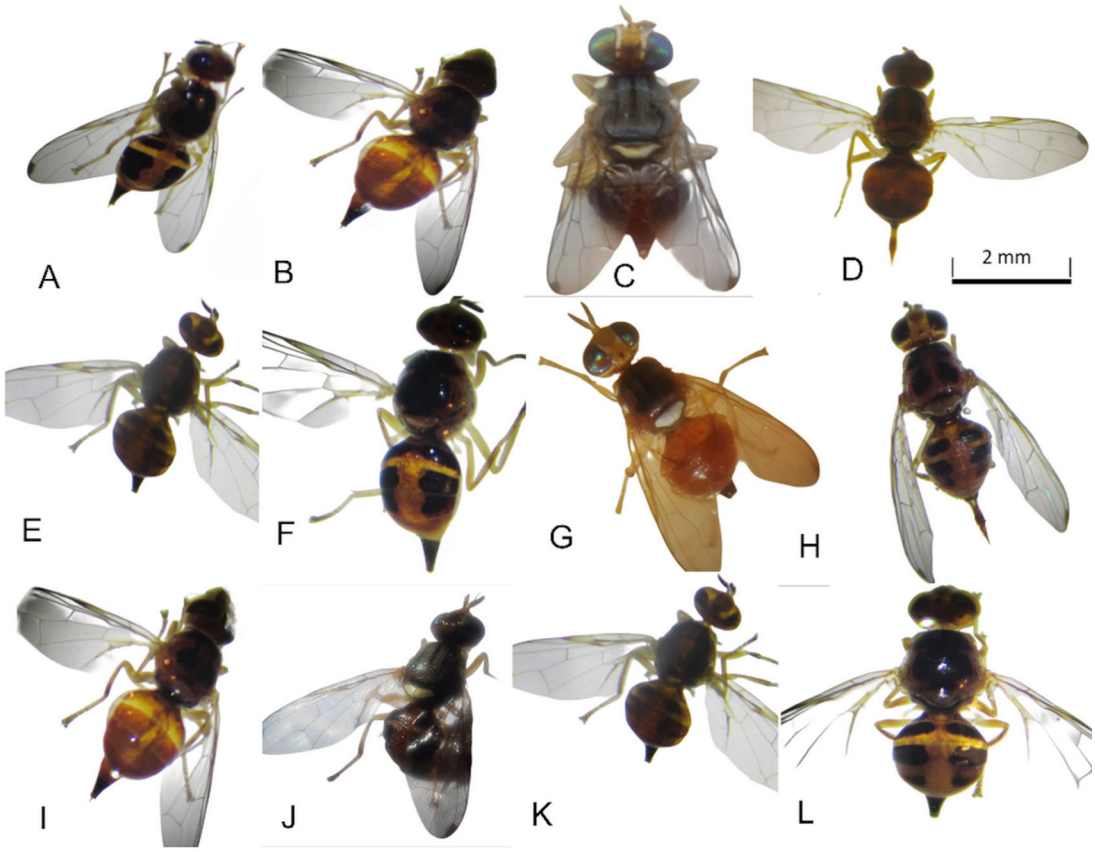
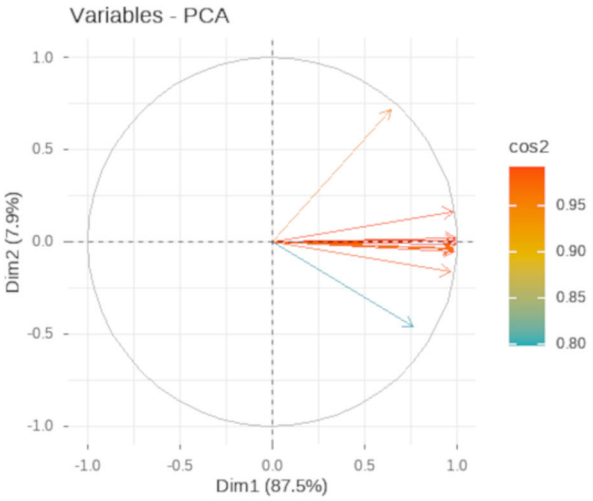


Figure 3. Representative specimens of the six morphological groups of *B. oleae* adults emerged from the organic olive orchard in Órgiva, southern Spain. Panels A–L show representative specimens of the six morphological groups defined in Table 1. Scale bar = 2 mm. Olympus SZX61 stereomicroscope and processed with GIMP (v2.10).



	Variable	Cos2
Thorax.L	Thorax.L	0.985
Thorax.W	Thorax.W	0.985
Pigmentation	Pigmentation	0.963
Abdomen.L	Abdomen.L	0.956
Abdomen.W	Abdomen.W	0.973
Banding..	Banding..	0.931
Ovip.L	Ovip.L	0.992
Ovip.Abd	Ovip.Abd	0.586
Corps.L	Corps.L	0.415
Poids	Poids	0.962

Figure 4. Principal Component Analysis (PCA) of morphological traits in B. oleae performed on 140 adults collected from the organic olive orchard at Orgiva. Generated in R v4.5.2 using ggplot2.

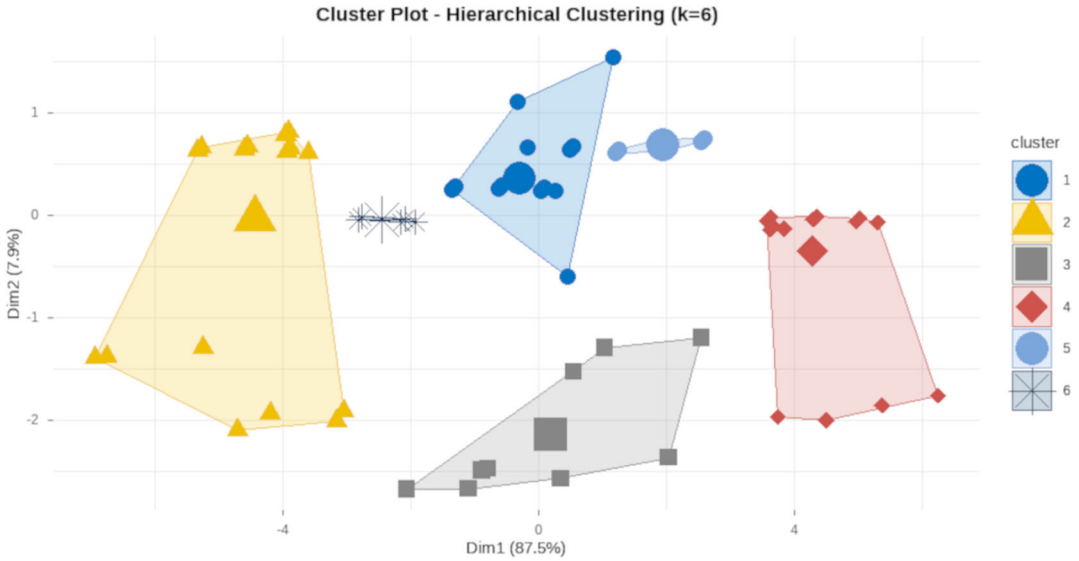


Figure 5. Hierarchical clustering of morphological traits in B. oleae performed on 140 adults collected from the organic olive orchard at Orgiva using Ward's method, generated in R v4.5.2 with ggplot2.

Table 2. Longevity and fecundity performance of six morphological groups of *B, oleae* females under laboratory conditions.

% Fertile punctures on Picholine Mean ± SE	% Fertile punctures on Picual Mean ± SE	Longevity (days) Mean ± SE	Morphological Group
38.7 ± 2.9^{b}	42.1 ± 3.2^{b}	28.3 ± 1.4^{ab}	1) Typical light form
41.2 ± 3.1^{ab}	45.3 ± 2.8^{ab}	31.7 ± 1.8^{a}	2) Dark thoracic form
32.4 ± 3.7^{c}	35.6 ± 4.1^{c}	24.9 ± 2.1^{b}	3) Compact orange form
48.5 ± 3.4^{a}	51.7 ± 2.9^{a}	29.8 ± 1.5^{ab}	4) Brown elongated thorax
46.9 ± 2.8^{a}	49.8 ± 3.0^{a}	30.4 ± 1.6^{a}	5) Pronounced abdominal banding
36.8 ± 3.3^{bc}	39.4 ± 3.5^{bc}	27.1 ± 1.9^{ab}	6) Dark compact form
40.8 ± 1.7	44.0 ± 1.8	28.7 ± 0.8	**Overall**

Note: Values are means ± standard error of the mean (SEM) from 3 replicates per group (9 females/group). Different superscript letters within the same column indicate statistically significant differences (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$).

PZ179621) (Figure 6). The assembly contained the complete set of 37 mitochondrial genes (13 protein-coding genes, 22 tRNAs, 2 rRNAs, and one A+T-rich control region) in the typical Tephritidae gene order. Nucleotide composition was strongly AT-biased (72.6%), consistent with other *Bactrocera* species.

Comparison of the 13 protein-coding genes (PCGs) across Tephritidae mitogenomes revealed strong structural conservation. Most genes showed identical or nearly identical lengths, with modest variation mainly restricted to ND1, ND3, ND5, COX1, and ND6. The Órgiva mitogenome (PZ179621) matched the length patterns of previously published B. oleae sequences. ND3, ND4L, and ND6 were the most conserved (median lengths ≈350 bp, ≈290 bp, and ≈520 bp), whereas ND1 and ND5 showed broader interspecific variation (≈940 bp and ≈1720 bp, with deviations up to ~100 bp). Mean values closely matched medians, indicating minimal skew in the distributions (Figure 7).

The Órgiva consensus mitogenome was aligned with three publicly available *B. oleae* mitogenomes (NC_005333.1, AY210702, AY210703). Pairwise nucleotide divergence ranged from 0.000% to 0.202% (Figure 8). Compared to the Italian reference NC_005333.1, the Órgiva haplotype differed by 21 nucleotide substitutions (0.133% divergence), including 11 non-synonymous changes located mainly in ND (ND1, ND5, ND6) and COX (COX1, COX2) genes. Comparative analysis with the additional mitogenomes confirmed low but detectable intraspecific variation, with no insertions, deletions, or genomic rearrangements observed.

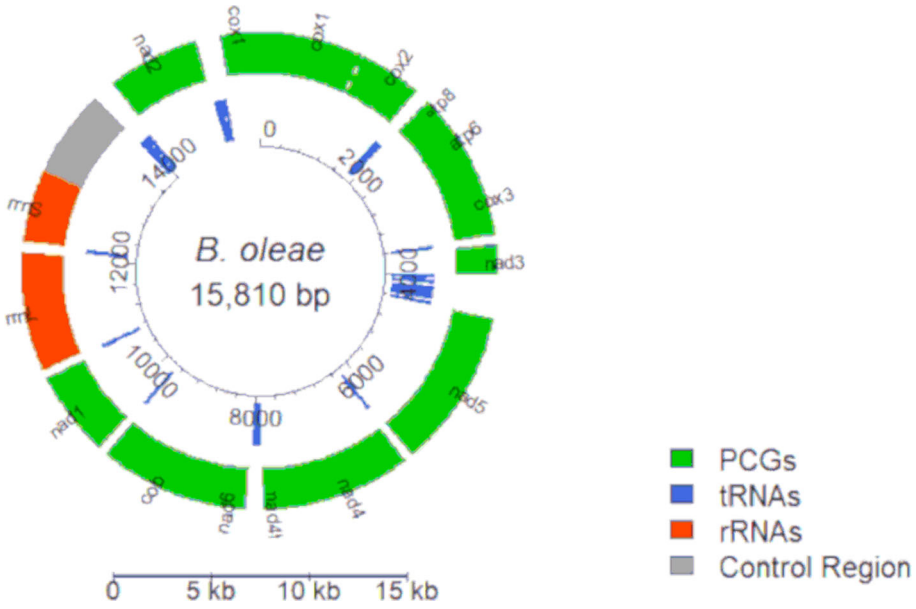


Figure 6. Circular map of the complete mitochondrial genome of Bactrocera oleae (isolate 25OT00063; 15,810 bp). Protein-coding genes (green), tRNAs (blue), rRNAs (orange), and the A+T-rich control region (grey) are shown. Genes on the majority (J) strand are transcribed clockwise, while those on the minority (N) strand are transcribed counterclockwise. Map generated in R v4.5.2 using the circlize package.

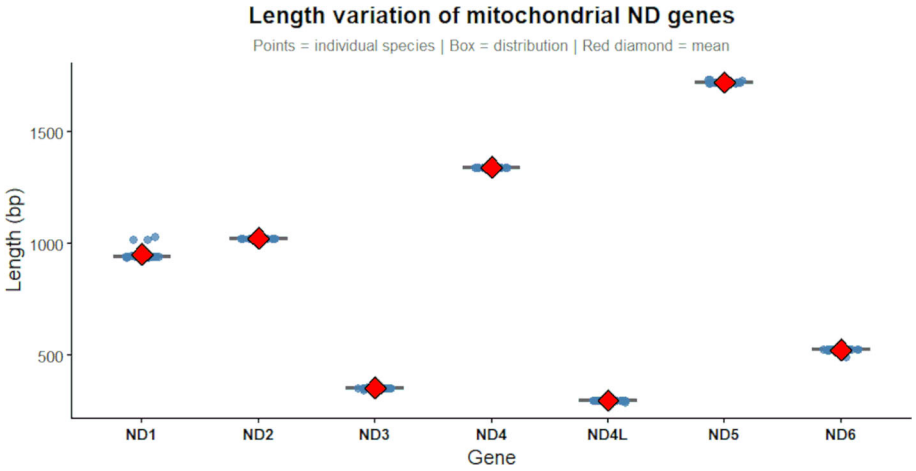


Figure 7. Length variation of mitochondrial NADH dehydrogenase (ND) genes across the Tephritidae species. Red diamonds indicate mean lengths. Generated in R v4.5.2 using ggplot2.

3.3 Phylogenetic placement and comparative analysis

The multiple sequence alignment of 21 Tephritidae mitogenomes, with Drosophila melanogaster as outgroup, revealed the expected combination of conserved and variable regions typical of insect mitochondrial DNA. The ~15.8 kb alignment contained ~65% conserved sites and ~20% parsimony-informative positions, providing strong phylogenetic signal. Clear genus-level patterns were detected: *Bactrocera* sequences were highly homogeneous across PCGs, *Dacus* showed lineage-specific motifs, and Ceratitis displayed distinct substitutions toward the 3′ ends of several genes. As expected, Drosophila was strongly divergent, confirming its suitability as an outgroup.

Maximum Likelihood analyses based on the complete mitogenome alignment produced a well-resolved topology (Figure 9). *Bactrocera* formed a strongly supported monophyletic clade, clearly separated from Dacus and from the outgroup taxa *Ceratitis capitata* and *Drosophila melanogaster*. The Órgiva mitogenome (PZ179621) clustered with full support (bootstrap = 100%) within the Mediterranean *B. oleae* lineage, showing closest affinity to the reference sequence

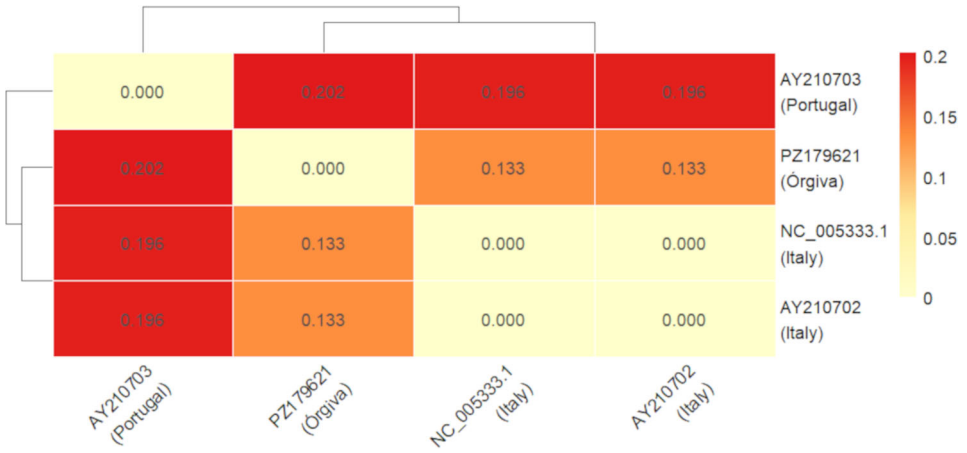


Figure 8. Pairwise nucleotide divergence heatmap of the Órgiva consensus mitogenome of B. oleae (PZ179621) compared with three reference mitogenomes (NC_005333.1, AY210702 and AY210703). Generated in R v4.5.2 using the ggplot2 package

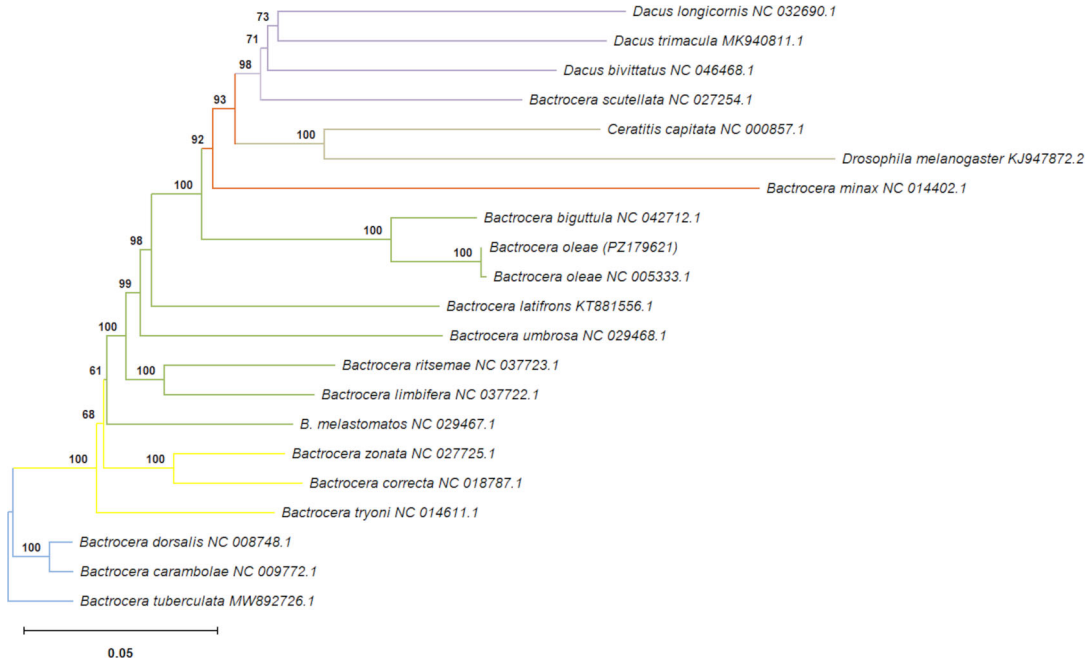


Figure 9. Maximum Likelihood phylogenetic tree of Tephritidae based on complete mitogenomes (21 taxa), with Drosophila melanogaster as outgroup. The Órgiva haplotype (PZ179621) is highlighted in green. Bootstrap values (≥ 70 %) are shown at nodes. The scale bar indicates substitutions per site.

NC_005333.1. This Mediterranean subclade was clearly distinct from the Asian/African *Bactrocera* group. Bootstrap values (1,000 replicates) confirmed the robustness of the topology, with ≥98–100% support for most major nodes. Lower support at a few deep nodes did not affect the placement of the Órgiva sequence or the separation between *Bactrocera* and *Dacus.*

4. Discussion

This study was carried out in a structurally complex, organically managed olive orchard in southern Spain, characterized by mixed crops, forest patches, and low chemical inputs, representing a typical Mediterranean agroecosystem. This traditional landscape supports high local biodiversity and provides an excellent setting for documenting morphological variation and generating genomic ressources, including the reference mitogenome. These datasets provide a valuable baseline for future comparative research on *B. oleae* species in Mediterranean olive systems. Field sampling of the

autumnal generation, conducted from September to December 2024, revealed moderate to high infestation levels (30–40% of fruits affected) in the Lechín and Picual cultivars. Such infestation rates are typical of organically managed olive orchards with limited pesticide inputs. The observed differences in cultivar susceptibility to *B. oleae* infestation are consistent with fruit traits such as morphology, maturation timing, and pericarp hardness, which are known to influence oviposition preference and larval performance.[34,35,36] This influence may also be combined with factors such as cuticle size and volatile compounds in the fruits.[37]

In this study, adults of *B. oleae* reared from naturally infested olives collected in a traditional organic orchard provided a representative sample for documenting intra-specific morphological diversity. All individuals exhibited the diagnostic traits of the species; however, clear intraspecific variation was observed in thoracic pigmentation, abdominal banding, abdomen proportions, and especially ovipositor morphology. The integration of qualitative and quantitative descriptors allowed the classification of adults into distinct morphological groups. Multivariate analyses strongly supported this structure: Principal Component Analysis (PCA) identified body size and ovipositor traits as the primary axes of variation and hierarchical clustering revealed six well-defined morphological groups confirmed the robustness of the classification, with thorax and ovipositor characters emerging as the strongest discriminating variables.

This descriptive framework is consistent with the broader use of morphometric methods in entomology. Multivariate approaches, particularly geometric morphometrics, have proven effective in characterizing functional morphology, sexual dimorphism, and host-associated phenotypic plasticity in various insect groups. In Tephritidae, such techniques have been successfully applied to reveal phenotypic variation and host-induced morphological changes in species such as *B. dorsalis* and *C. capitata.*[10,38,39]

The marked phenotypic diversity, observed among flies emerging from olive cultivars (primarily Lechín and Picual) during the same sampling period and within the same biotope, appears to reflect a certain degree of phenotypic plasticity in response to heterogeneous microhabitats and reduced chemical pressure—conditions typically associated with organic farming. Such variation contrasts with the more uniform morphology historically reported for Spanish populations,[33] who described a typical light form (yellowish-brown thorax and pale abdomen) that remains the most frequent phenotype in vast monoculture olive plains and conventional production systems. More recently, studies have demonstrated cultivar-specific morphological adaptations in B. oleae in olive orchards, with cultivar-specific susceptibility.[19] Importantly, the occurrence of morphological diversity within the same microhabitat may be attributed to additional factors, including the organic system and habitat heterogeneity. Organic compounds and environmental conditions have been shown to strongly influence oviposition preference and offspring performance in container-breeding insects, providing insights into host selection mechanisms that may also apply to *B. oleae.*[40]

The six morphological groups identified showed differences in body size and ovipositor traits, which were associated with variations in longevity and reproductive potential. Adults belonging to groups with elongated ovipositors exhibited both higher longevity and greater reproductive success across Picual and Picholine cultivars, whereas compact forms tended to live shorter and produced fewer fertile punctures. These results suggest a functional link between morphological variation and fitness-related traits, highlighting the adaptive significance of phenotypic plasticity in this organic biotope. Similar associations between ovipositor morphology and reproductive success have been reported in *B. oleae* populations under controlled laboratory conditions[41] and more broadly in tephritid flies where oviposition depth influences host exploitation and fecundity.[42]

Taken together, these findings emphasize that morphological diversity in *B. oleae* is not merely a descriptive trait but carries direct implications for fitness and population dynamics. By linking ovipositor morphology to reproductive success, this study highlights how phenotypic plasticity may enhance adaptability in heterogeneous organic olive systems, thereby shaping both pest pressure and management strategies.

The observed variability in thoracic pigmentation, abdominal banding, and ovipositor morphology, combined with the high-quality mitogenome assembly, underscores the potential of integrating morphological and genomic datasets. Such integration provides a basis for developing molecular markers and comparative tools, but this remains a hypothesis that requires verification through longer-term sampling and cultivar-specific analyses. Fruit traits such as cuticle thickness and volatile composition are known to influence olive fly tolerance[37] and documenting these factors alongside morphological descriptors will be essential for future work. Establishing robust baselines of morphological diversity and genomic data will ultimately facilitate monitoring of population structure, haplotype distribution, and gene flow between orchards managed under different production systems.

Oxford Nanopore long-read sequencing of a pooled sample of 40 adults, selected to represent the observed morphological diversity, yielded a high-quality circular mitochondrial genome of 15,810 bp (PZ179621), assembled de novo with an average coverage of ~3,600×. This consensus haplotype represents the predominant mitochondrial lineage present in the Órgiva organic population. The mitogenome displays the typical insect quadripartite architecture, including the full complement of 37 genes (13 protein-coding genes, 22 tRNAs, 2 rRNAs, and one A+T-rich control region) and a strongly AT-biased nucleotide composition (72.6 %), consistent with other *Bactrocera* species.

Comparative analysis with publicly available B. oleae mitogenomes indicates that the Órgiva haplotype exhibits only modest intraspecific variation. The Órgiva haplotype differs from the Italian reference (NC_005333.1) by only 21 nucleotide substitutions including 11 non-synonymous changes, mainly in ND and COX genes. This limited divergence is consistent with the generally low mitochondrial variability reported across Mediterranean populations. Similar patterns of high sequence similarity with other regional haplotypes further support the genetic homogeneity of *B. oleae* in this area. Phylogenetic reconstruction based on complete mitogenomes placed the Órgiva haplotype with maximum bootstrap support (100 %) within the Mediterranean clade of B. oleae, confirming its genetic coherence with the broader Mediterranean group.[25,26] Genomic studies of this pest can further strengthen this approach; for instance, physical mapping of the *B. oleae* genome has enabled the localization of key genes on polytene chromosomes, offering a valuable foundation for genomic and cytogenetic investigations.[43]

Based on the proposed grouping classification, the combination of morphological polymorphism (six groups among 140 individuals) with low mitochondrial genetic divergence strongly suggests that the observed variation is primarily driven by phenotypic plasticity rather than deep genetic differentiation. Although 11 non-synonymous substitutions were identified in ND and COX genes, these may hint at local adaptation to organic conditions—characterized by minimal use of pesticides—and microhabitat heterogeneity. Notably, *B. oleae* shows possible susceptibility to cultivar diversity,[19] is influenced by microclimatic and soil heterogeneity[44] and may interact with the endosymbiont *Candidatus Erwinia* dacicola.[45,46] However, the present dataset does not allow firm conclusions regarding the functional or adaptive significance of these substitutions. Nevertheless, the findings provide valuable support for the development of morphological and genetic study protocols.

This study was conducted in a traditional organic orchard during the autumnal generation and relied on pooled sequencing, yielding a consensus haplotype. While this approach provides a robust baseline, it also highlights the need for future individual-level analyses to capture intra-population mitochondrial diversity and haplotype frequencies. The present work delivers a morpho-genomic case study from a well-characterized agroecosystem, integrating field observations, quantitative morphological assessment, and mitogenomic data. The newly assembled mitogenome represents a high-quality dataset that expands the limited genomic resources currently available for Bactrocera oleae. Beyond its descriptive value, the reference sequence establishes a baseline for the development of molecular markers, haplotype tracking, and comparative analyses across Mediterranean olive systems.

While the results do not allow firm conclusions regarding local genetic adaptation, they underscore the value of integrating detailed morphological characterization with high-quality genomic data. The Órgiva population, although genetically consistent with the broader Mediterranean *B. oleae* lineage, exhibits clear local morphological variation. This integrated morpho-genomic approach provides a robust baseline for future comparative studies across cultivars, orchards, and management systems (organic versus conventional).

Ultimately, this work contributes a high-quality reference mitogenome and a reproducible framework for morphological analysis. These resources are particularly relevant for organic and low-input olive systems, where sustainable pest management is essential, and will facilitate the development of region-specific molecular tools to support effective, biodiversity-friendly strategies across the Mediterranean basin.

5. Conclusions

This study provides a high-quality reference mitogenome for *B. oleae* (15,810 bp, GenBank PZ179621) together with a detailed morphometric characterization of 140 adults collected from a traditional organic olive orchard in southern Spain. Multivariate analyses (PCA, hierarchical clustering, and LDA) supported the identification of six distinct morphological groups showing notable variation in thoracic pigmentation, abdominal banding, abdomen shape, and ovipositor morphology.

Adult longevity and fecundity assays revealed significant differences among these morphological groups. Flies with elongated ovipositors consistently exhibited higher longevity and greater reproductive success across Picual and Picholine cultivars, whereas compact forms tended to live shorter and produced fewer fertile punctures. These findings

establish a functional link between morphological variation and fitness-related traits, underscoring the adaptive significance of phenotypic plasticity in this organic biotope.

Although the pooled nature of the mitogenome limits direct genotype–phenotype linkages, the very low genetic divergence (0.133 %) from Mediterranean references suggests that the observed phenotypic diversity is primarily driven by plasticity rather than strong genetic differentiation. These resources offer a valuable foundation for future comparative analyses, haplotype tracking, population monitoring, and investigations into phenotypic plasticity versus genetic adaptation in Mediterranean organic olive systems

Ethics and consent

Ethical approval and consent were not required

Data and software availability

Underlying data

GenBank: Complete mitochondrial genome of *Bactrocera oleae* isolate from Órgiva, Granada, Spain. Accession number: PZ179621. Available at: https://www.ncbi.nlm.nih.gov/nuccore/PZ179621[47]

Zenodo: Morphometric and mitochondrial data of *Bactrocera oleae* from olive orchards in Granada (2024) Available at: https://doi.org/10.5281/zenodo.22332986[48]

The version 1 contains the following underlying data:

- bactrocerarecap_dataset.csv (Morphometric measurements: thorax, abdomen, pigmentation, oviposition rate, etc.)
- Calidad_lecturas_DNA mit_NanoPlot-report.html (Quality report of DNA reads generated by NanoPlot)
- Calidad_Secuenciación Dacus oleae_NanoPlot-report.html (Sequencing quality report)
- Figure_1.png (Photograph of the organically managed olive orchard in Órgiva, Granada, Spain)
- PZ179621.1 Bactrocera oleae isolate.txt (Complete mitochondrial genome sequence in FASTA format)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Extended data

Zenodo: Morphometric and mitochondrial data of *Bactrocera oleae* from olive orchards in Granada (2024). Available at: https://doi.org/10.5281/zenodo.22820610[49]

The version 2 contains the following extended data:

- Bactrocera_oleae_Orgiva_ML_tree.nwk (Phylogenetic tree in Newick format.nwk)
- Circular_map_Bactrocera_oleae_Orgiva.pdf.png (Circular map of the complete mitochondrial genome of *Bactrocera oleae* from Órgiva)
- circular_map_Bactrocera_oleae_Orgiva.R (R script used to generate the circular map)
- gene_lengths.csv (Lengths of mitochondrial protein-coding genes for 21 Tephritidae species)
- Length_variation_ND_genes.png (Length variation of mitochondrial ND genes across Tephritidae species)
- Length_variation_ND_genes.R (R script used to generate the ND genes length variation plot)
- Phylogenetic_tree_Bactrocera_oleae_Orgiva_PZ179621.tiff (Maximum Likelihood phylogenetic tree of Tephritidae mitochondrial genomes)

- tree_session_Bactrocera_oleae_ Orgiva.mtsx (MEGA session files (.mtsx) containing the parameters of the phylogenetic analyses)

Zenodo: Morphometric and mitochondrial data of *Bactrocera oleae* from olive orchards in Granada (2024).

Supplementary Table_S1 containing the list of complete mitochondrial genomes used in the phylogenetic analysis, including species, GenBank accession numbers, and corresponding references

Available at: https://doi.org/10.5281/zenodo.22899886[50]

The version 3 contains the following extended data:

- Table_S1_mitochondrial_genomes_phylogenetic_analysis.xlsx

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Software and Code availability

All software used for bioinformatics and statistical analyses in this study consists of established and publicly available tools and software packages.

Statistical analyses and data visualization were performed in R (version 4.5.2) using the ggplot2 package.

Sequence alignments and phylogenetic analyses were performed using MEGA X.

The circular map of the mitochondrial genome and the length variation plot of ND genes were generated in R (version 4.5.2) using the packages circlize, dplyr, tidyr and ggplot2.

Statistical analyses and data visualization were also performed in R (version 4.5.2) using the ggplot2 package.

All alignment files, MEGA session files, phylogenetic trees, R scripts and related files are available in the Zenodo repository: https://doi.org/10.5281/zenodo.22820610[49]

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References

1. Daane KM, Johnson MW: **Olive fruit fly: Managing an ancient pest in modern times.** *Annu. Rev. Entomol.* 2010; **55**: 151–169.
PubMed Abstract | Publisher Full Text

2. Amr A, Sadder MT, Alsakarneh NA: **Olive fruit fly Bactrocera oleae infestation of olives: Effect on quality and detection in olive oil.** *Jordan J. Agric. Sci.* 2023; **19**: 56–69.
Publisher Full Text

3. Vontas J, Hernández-Crespo P, Margaritopoulos JT, *et al.*: **Insecticide resistance in tephritid flies.** *Pestic. Biochem. Physiol.* 2011; **100**: 199–205.
Publisher Full Text

4. Majeed A, Rasheed MT, Akram J, *et al.*: **Insecticide resistance and eco-friendly management of Bactrocera oleae.** *J. Plant Dis. Prot.* 2025; **132**: 10.
Publisher Full Text

5. Balampekou EI, Koutsos TM, Menexes GC, *et al.*: **Pest management pathways: Control strategies for the olive fruit fly (Bactrocera oleae)—A systematic map.** *Agronomy.* 2024; **14**: 2929.
Publisher Full Text

6. Brooker RW, Hawes C, Iannetta PPM, *et al.*: **Plant diversity and ecological intensification in crop production systems.** *J. Plant Ecol.* 2023; **16**: rtad015.
Publisher Full Text

7. Stavrianakis G, Sentas E, Zafeirelli S, *et al.*: **Utilizing olive fly ecology towards sustainable pest management.** *Biology.* 2025; **14**: 125.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Ortega M, Moreno N, Fernández CE, *et al.*: **Olive landscape affects Bactrocera oleae abundance, movement and infestation.** *Agronomy.* 2022; **12**: 4.
Publisher Full Text

9. McQuate GT, Jones GD, Sylva CD: **Assessment of corn pollen as a food source for two tephritid fruit fly species.** *Environ. Entomol.* 2003; **32**: 141–150.
Publisher Full Text

10. Lemic D, Benítez HA, Bjeliš M, *et al.*: **Agroecological effect and sexual shape dimorphism in medfly Ceratitis capitata.** *Zool. Anz.* 2020; **288**: 118–124.
Publisher Full Text

11. Betz O: *Insect Ecomorphology: Linking Functional Insect Morphology to Ecology and Evolution.* London, UK: Academic Press; 2025. 978-0-443-18544-1.
Publisher Full Text

12. de Souza JMGA, Lima-Filho PAD, Molina WF, *et al.*: **Wing morphometry and acoustic signals in sterile and wild males: Implications for mating success in Ceratitis capitata.** *Sci. World J.* 2015; **2015**: 526969.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

13. Bonduriansky R: **Convergent evolution of sexual shape dimorphism in Diptera.** *J. Morphol.* 2006; **267**: 602–611.
PubMed Abstract | **Publisher Full Text**

14. Benítez HA, Bravi R, Parra LE, *et al.*: **Allometric and non-allometric pattern in sexual dimorphism discrimination of wing shape in the parasitoid, Ophion intricatum: Do two male morphotypes coexist?.** *J. Insect Sci.* 2013; **13**: 143.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

15. Le Roy C, Debat V, Llaurens V: **Adaptive evolution of butterfly wing shape: From morphology to behaviour.** *Biol. Rev.* 2019; **94**: 1261–1281.
PubMed Abstract | **Publisher Full Text**

16. Breuker CJ, Gibbs M, Van Dongen S, *et al.*: **The use of geometric morphometrics in studying butterfly wings in an evolutionary ecological context.** *Morphometrics for Nonmorphometricians.* Berlin/Heidelberg, Germany: Springer; 2010; pp. 271–287.
Publisher Full Text

17. Benítez HA, Lemic D, Bažok R, *et al.*: **Evolutionary directional asymmetry and shape variation in Diabrotica v. virgifera (Coleoptera: Chrysomelidae): An example using hind wings.** *Biol. J. Linn. Soc.* 2014; **111**: 110–118.
Publisher Full Text

18. Solinas M, Rebora M, De Cristofaro A, *et al.*: **Functional morphology of Bactrocera oleae Gmel. (Diptera: Tephritidae) tarsal chemosensilla involved in interactions with the host plant.** *Entomologica.* 2001; **35**: 103–123.

19. Pajač Živković I, Čirjak D, Hojsak L, *et al.*: **Assessing cultivar-specific susceptibility of Bactrocera oleae.** *J. Insect Sci.* 2025; **25**: 13.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

20. Di Rienzo V, Sion S, Taranto F, *et al.*: **Genetic flow among olive populations within the Mediterranean basin.** *PeerJ.* 2018; **6**: e5260.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

21. Dogaç E, Kandemir İ, Taşkın V: **The genetic polymorphisms and colonization process of olive fly populations in Turkey.** *PLoS ONE.* 2013; **8**: e56067.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

22. Augustinos AA, Stratikopoulos EE, Zacharopoulou A, *et al.*: **Polymorphic microsatellite markers in the olive fly, Bactrocera oleae.** *Mol. Ecol. Notes.* 2002; **2**: 278–280.
Publisher Full Text

23. Zhang Y, Li H, Feng S, *et al.*: **Mitochondrial phylogenomics reveals the evolutionary and biogeographical history of fruit flies (Diptera: Tephritidae).** *Entomol. Gen.* 2023; **43**: 359–368.
Publisher Full Text

24. Al Naggar Y, Fahmy NM, Alkhaibari A, *et al.*: **Mechanisms and genetic drivers of resistance of insect pests to insecticides and approaches to its control.** *Toxics.* 2025; **13**: 681.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

25. Nardi F, Carapelli A, Dallai R, *et al.*: **The mitochondrial genome of the olive fly Bactrocera oleae.** *Insect Mol. Biol.* 2003; **12**: 605–611.
PubMed Abstract | **Publisher Full Text**

26. Lantero E, Matallanas B, Callejas C: **Vast gene flow among Spanish populations of Bactrocera oleae.** *Insects.* 2022; **13**: 642.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

27. Lantero E, Matallanas B, Ochando MD, *et al.*: **Current status of the main olive pests: Integrated pest management strategies and genetic tools.** *Appl. Sci.* 2023; **13**: 12078.
Publisher Full Text

28. R Core Team: *R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing.* Vienna, Austria: 2025.
Reference Source

29. Tamura K, Stecher G, Kumar S: **MEGA11: Molecular Evolutionary Genetics Analysis version 11.** *Mol. Biol. Evol.* 2021; **38**: 3022–3027.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

30. Tamura K, Nei M: **Estimation of nucleotide substitutions in mitochondrial DNA.** *Mol. Biol. Evol.* 1993; **10**: 512–526.
Publisher Full Text

31. Kolde R: **pheatmap: Pretty Heatmaps; R package version 1.0.12.** 2019. (accessed on 5 September 2026).
Reference Source

32. Wickham H: *ggplot2: Elegant Graphics for Data Analysis.* New York, NY, USA: Springer-Verlag; 2016.

33. Ruiz Castro A: *Fauna Entomológica del Olivo en España. Vol. I: Generalidades, Coleoptera y Diptera.* Madrid, Spain: Instituto Español de Entomología, CSIC; 1948; 182.

34. Iannotta N, Noce ME, Scalercio S: **Olive fruit fly infestation and cultivar susceptibility in Calabria.** *J. Appl. Entomol.* 2007; **131**: 647–653.
Publisher Full Text

35. Gonçalves MF, Torres LM, Pereira JA: **Influence of fruit traits on oviposition preference of the olive fly Bactrocera oleae.** *Sci. Hortic.* 2012; **145**: 127–135.
Publisher Full Text

36. Karakoyun NS, Uçkun AA: **Susceptibility of olive cultivars to olive fly (Bactrocera oleae) and parameters influencing cultivar selection.** *J. Entomol. Zool. Stud.* 2023; **11**: 185–193.
Publisher Full Text

37. Taiti C, Lombardo L, Godino G, *et al.*: **Exploring morphological aspects, cuticle size and volatile compounds in the fruits of four olive cultivars as possibly interdependent components of Bactrocera oleae tolerance.** *Front. Plant Sci.* 2025; **16**: 1681993.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

38. Pieterse W, Benítez HA, Addison P: **The use of geometric morphometric analysis to illustrate the shape change induced by different fruit hosts on the wing shape of Bactrocera dorsalis and Ceratitis capitata (Diptera: Tephritidae).** *Zool. Anz.* 2017; **269**: 110–116.
Publisher Full Text

39. Reis M, Siomava N, Wimmer EA, *et al.*: **Conserved and divergent aspects of plasticity and sexual dimorphism in wing size and shape in three Diptera.** *Front. Ecol. Evol.* 2021; **9**: 660546.
Publisher Full Text

40. Allgood DW, Yee DA: **Oviposition preference and offspring performance in container breeding mosquitoes: Evaluating the effects of organic compounds and laboratory colonisation.** *Ecol. Entomol.* 2017; **42**: 506–516.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

41. Yokoyama VY: **Olive fruit fly (Diptera: Tephritidae) in California: Longevity, oviposition, and development in canning olives in the laboratory and greenhouse.** *J. Econ. Entomol.* 2012; **105**: 186–195.
PubMed Abstract | **Publisher Full Text**

42. Galvão-Silva FL, Oliveira JPM, Araújo AS, *et al.*: **Oviposition activity of an invasive fruit fly species at different densities in laboratory.** *Entomol. Exp. Appl.* 2026; **174**: 45–52.
Publisher Full Text

43. Drosopoulou E, Nakou I, Mavragani-Tsipidou P: **The Bactrocera oleae genome: Localization of nine genes on the polytene chromosomes of the olive fruit fly (Diptera: Tephritidae).** *Genome.* 2015; **57**: 573–576.
PubMed Abstract | **Publisher Full Text**

44. Catalano GA, Pirrello G, D'Urso PR, *et al.*: **Suitability maps of Bactrocera oleae presence by SDM based on pedo-climatic and topographic predictors data in Sicily.** *Agronomy.* 2026; **16**: 501.
Publisher Full Text

45. Ben-Yosef M, Pasternak Z, Jurkevitch E, *et al.*: **Symbiotic bacteria enable olive flies (Bactrocera oleae) to exploit intractable sources of nitrogen.** *J. Evol. Biol.* 2014; **27**: 2695–2705.
PubMed Abstract | **Publisher Full Text**

46. Capuzzo C, Firrao G, Mazzon L, *et al.*: **'Candidatus Erwinia dacicola', a coevolved symbiotic bacterium of the olive fly Bactrocera oleae (Gmelin).** *Int. J. Syst. Evol. Microbiol.* 2005; **55**: 1641–1647.
PubMed Abstract | **Publisher Full Text**

47. Blibech I: **Bactrocera oleae isolate Blibech_Granada_2024 mitochondrion, complete genome. GenBank accession PZ179621.** 2026.
Reference Source

48. Blibech I, Cabrero-Sañudo FJ, Ruiz de la Cuesta JL: Morphometric and mitochondrial data of Bactrocera oleae from olive orchards in Granada (2024). [Dataset]. *Zenodo.* 2026.
Publisher Full Text

49. Blibech I, Cabrero-Sañudo FJ, Ruiz de la Cuesta JL: Morphometric and mitochondrial data of Bactrocera oleae from olive orchards in Granada (2024). [Dataset]. *Zenodo.* 2026.
Publisher Full Text

50. Blibech I, Cabrero-Sañudo FJ, Ruiz de la Cuesta JL: **Morphometric and mitochondrial data of Bactrocera oleae from olive orchards in Granada (2024). Supplementary Table_S1.** *Zenodo.* 2026.
Publisher Full Text