# Diversity and Community Structure of Opiinae (Hymenoptera, Braconidae) in Mediterranean Landscapes of Spain

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# ABSTRACT

The aim of the present work was to analyse the diversity of Opiinae (Hymenoptera) in three Spanish protected Mediterranean natural parks affected by bioclimatic conditions: La Font Roja, Las Lagunas de la Mata-Torrevieja and La Tinença de Benifassà. Sampling was conducted between 2004 and 2005. During this period, 226 specimens, belonging to 32 different species, were captured. Eleven species captured are new records for Spain: *Ademon urinator*, *Biophthora rosica, Biosteres analis, Biosteres spinaciae, Eurytenes (Xynobiotenes) scutellatus, Opius (Agnopius) tirolensis, Opius (Misophthora) bulgaricus, Opius (Nosopoea) cingulatoides, Opius (Nosopoea) speciosus, Opius (Nosopoea) viennensis and <i>Xynobius caelatus*. Alpha, beta and gamma diversities, as well as the structure of the communities were subsequently analysed. Our results indicate that Tinença de Benifassà and Torrevieja have higher diversity than Font Roja. Based on analysis of structural models, the latter communities were observed to be unstable and are composed of only a few abundant species and a large number of rare species.

Key words: Braconidae, Opiinae, diversity, Mediterranean landscapes, Spain.

# INTRODUCTION

Mediterranean forests are rich in evergreen species, frequently intersected by areas of brushwood, pasture, farming and ranching. In close proximity to these areas, however, it is often possible to identify zones that have regained their highly diverse natural communities after the cessation of human intervention. This favours the proliferation of hotspots in Mediterranean ecosystems (Myers *et al.*, 2000). Despite the huge resistance posed by Mediterranean biotopes to human pressure, isolation and fragmentation are unavoidable (Pungetti, 2003), resulting in the emergence of isolated patches within the landscape.

This wide variety of landscapes, there causes a large diversity of phytophagous insects causing damage, and therefore, a wide variety of regulators. Among them, Braconidae are major regulators of phytophagous insect populations (LaSalle and Gauld, 1992; Falcó-Garí *et al.*, 2014; Khajeh *et al.*, 2014) and they are the second largest family within the Hymenoptera. The majority of braconid species are primary parasitoids of the immature stages of Lepidoptera, Coleoptera and Diptera (Shaw and Huddleston, 1991). Within the braconids, Opiinae is one of the largest subfamilies

containing 1,968 described species known in the world fauna (Yu et al., 2012; Khajeh et al., 2014). The species of Opiinae are an assemblage of small parasitic wasps strictly koinobiont parasitoids of cyclorrhaphous Diptera (Wharton, 1997), mainly of leaf miners and other larvae living in fruits. The hosts are known for only around 300 species, mostly within Agromyzidae, Tephritidae, Anthomyiidae, Ephydridae, Scatophagidae Drosophilidae and Psilidae (Fischer, 1971a,b, 1972, 1977, 1987; Shaw and Huddleston, 1991; Beyarslan and Fischer, 2011; Peris-Felipo et al., 2014a; Khajeh et al., 2014). The genera Opius Wesmael 1835 and Phaedrotoma Forster 1862 are the largest genera, and more than half of the recorded hosts belong to the Agromyzidae (Shaw and Huddleston, 1991; Beyarslan and Fischer, 2011; Khajeh et al., 2014), which are considered as pests of economic importance (Capinera, 2001). Several species of the subfamily Opiinae are considered as important biocontrol agents of leaf mining Agromyzidae and fruit-infesting Tephritidae (Fischer, 1971b; Greathead, 1975; Wharton, 1984, 1989, 1997; Neuenschwander et al., 1987; Schuster and Wharton, 1993; Salvo and Valladares, 1995). However, host-parasitoid relationships and worldwide distribution could be greater due to the lack of studies realized. On the other hand, Opiinae is closely related to Alysiinae, based on the morphological data and the type of parasitism on cyclorrhaphous Diptera (Wharton, 1988; Quicke and van Achterberg, 1990) and also molecular analyses (Dowton et al., 1998; Khajeh et al., 2014).

Despite many different community studies around Braconidae have been carried out worldwide, for example in Brazil (Cirelli and Penteado-Dias, 2003; Scatolini and Penteado-Dias, 2003), Venezuela (Briceño *et al.*, 2009) or in the Iberian Peninsula (Falcó-Garí *et al.*, 2006; Peris-Felipo and Jiménez-Peydró, 2011; Jiménez-Peydró and Peris-Felipo, 2011; Peris-Felipo *et al.*, 2013; Pérez-Rodríguez *et al.*, 2013; Peris-Felipo *et al.*, 2014b; Falcó-Garí *et al.*, 2014), these communities have been insufficiently analysed.

Within this context, this work analyses the alpha, beta and gamma diversity and the community structure of the Opiinae in three Mediterranean Natural Parks of Valencia (Spain).

# MATERIAL AND METHODS

### Study Area

The Natural Parks selected were Natural Park of La Font Roja (Font Roja), Natural Park of Las Lagunas de la Mata-Torrevieja (Torrevieja) and Natural Park of La Tinença de Benifassà (Tinença); all located within the Comunidad Valenciana and each with peculiar microclimate conditions (Fig. 1). The climatic and orographic descriptions were given by Peris-Felipo and Jiménez-Peydró (2012).

Climate conditions of La Font Roja Natural Park (north of Alicante province), are classified as upper sub-humid, with annual rainfall between 600-1,000 mm in the north face; while the south face is dry, with anual rainfall between 350-600 mm. Due to high average temperatures throughout the year (15- 20°C), and the low average rainfall, the park is classified as dry and thermo-Mediterranean.



Fig. 1. Distribution of Natural Parks in sampled.

Las Lagunas de la Mata-Torrevieja Natural Park is located to the south of Alicante province. The climate is arid with an annual rainfall below 300 mm and high temperatures.

La Tinença de Benifassà Natural Park is located to the north of Castellon province. Climate conditions are continental humid, with annual average temperaturas below 12°C: freezing conditions are posible throughout most of the year. Rainfall varies in different zones according to topographical features and the annual precipitation ranges from 600 to 1,000 l/m2. The park is contained within the supramediterranean bioclimate.

### Sampling design and data collection

Sampling stage covers the period from April 2004 to December 2005. During this period, in each natural park, a Malaise trap to collected specimens was placed. Weekly, each area was visited to sampling with entomological net and replace the trap drop. Specimens captured were preserved in 70% ethanol until final preparation.

Once separated, the specimens were determined by subfamily keys of van Achterberg (1993) to work only with Opiinae specimens. Subsequently, genera were determined with the keys of Tobias *et al.* (1986) and Wharton *et al.* (1997), while species identifications were made with those of Fischer (1972). The studied specimens are deposited with a bar code labels in the Entomological Collection at the University of Valencia (Valencia, Spain; ENV).

### Data analysis

Once all specimens of Opiinae were identified, alpha, beta and gamma biodiversity indexes were calculated for each trap and habitat to gain insight into the richness, abundance, dominance and complementarity values of each area.

Alpha diversity reflects the richness in species of a homogeneous community. This sort of diversity was measured by taxa richness, abundance and dominance.

Taxa richness: used for valuing richness of sampling areas. It was measured using the Margalef index, a measure of specific richness that transforms the number of species per sample into the proportion to which the species are added by expansion of the sample, establishing a functional relationship between number of species and total number of specimens (Moreno, 2001).

Species richness estimators: It was measured using Chao 2 to know what percentage of the total known species of possible species (Moreno, 2001).

Abundance: used for valuing faunal composition of a given area (Magurran, 1988). This was undertaken using the Shannon-Weaver index because it measures equity, indicating the degree of uniformity in species representation (in order of abundance) while considering all samples. This index measures the average degree of uncertainty that predicts which species an individual randomly picked from a sample belongs to (Magurran, 1988; Moreno, 2001; Villareal *et al.*, 2004).

Dominance: occurrence of genera or dominance value was calculated with the Simpson index, often used to measure species dominance values in a given community, its negative thus representing equity. It measures the representativity of the most important species without considering the other species present. It expresses the probability that two individuals randomly picked from a sample will belong to the same species (Magurran, 1988).

Community structure: In order to complement the diversity analyses and enquire into community structure, *log-series, log-normal* and *broken-stick* models were also applied (Magurran, 1988). The *log-series* model represents a community composed of a few abundant species and a high number of rare species. The *broken-stick* model refers to maximum occupation of an environment with equitable sharing of resources between species. Finally, the *log-normal* reflects an intermediate situation between the two (Soares *et al.*, 2010). Using the data obtained from the parks, each of these models was applied to calculate the expected number of species, log<sub>2</sub> grouping species according to abundance (Magurran, 1988; Tokeshi, 1993; Krebs, 1999). To test the significance of the models, the expected species values were compared with those of the observed species through chi-square analysis (Zar, 1999).

Beta diversity is the degree of change or substitution in species composition between different communities within the same landscape. In order to measure beta diversity, Jaccard and Complementarity indexes were used and cluster analyses were also performed.

Jaccard index: relates the total amount of shared species to the total amount of exclusive species. It is a qualitative coefficient, the interval of which will go from 0 when no species are shared between both sites to 1 when both sites have an identical composition (Moreno, 2001; Villareal *et al.*, 2004).

Complementarity index: indicates the degree of similarity in species composition and abundance between two or more communities (Moreno, 2001; Villareal *et al.*, 2004).

Cluster analysis: employed to calculate the degree of correlation based on similarity/ dissimilarity. For the calculation of these values, statistics-processing software PAST was used (Hammer *et al.*, 2001).

Finally, gamma diversity measurement indicates the diversity value of all environments under study, as expressed in the richness indexes for each area (alpha diversity) and the difference between them (beta diversity) (Schluter, Ricklefs, 1993; Villareal *et al.*, 2004).

### RESULTS

A total of 32 species were represented, including 8 genera: *Ademon* Haliday, 1833, *Biophthora* Forster, 1862, *Biosteres* Forster, 1862, *Eurytenes* Forster, 1862, *Opius* Wesmael, 1835, *Phaedrotoma* Forster, 1862, *Utetes* Forster, 1862 and *Xynobius* Forster, 1862. Also, seven unknown species where collected: one for *Biosteres* and six for *Opius*. Species captured and its distribution of each Natural Parks are provide in Table 1.

However, the species were not evenly distributed when different parks were considered separately. Thus, 19 species were identified in Tinença, 18 species in Torrevieja, and 6 were identified in Font Roja (Table 2).

The genus *Opius* was the most abundant with 134 examples, followed by the genus *Phaedrotoma* (55) and *Eurytenes* (19). At the species level, *Opius* (*Opiostomus*) *clausus* was the most common, with 75 collected specimens (33.18%). The *Opius* genus includes around 800 catalogued species, whose host specificity is unknown (Wharton 1988). On the other hand, when analyzing the number of captures, it was observed that 138 specimens were collected in Torrevieja, followed by Tinença and Font Roja with 73 and 15 specimens, respectively.

The obtained results together with Simpson, Berger-Parker, Shannon-Wiener and Margalef indexes (Table 2) indicated that one or more species dominated the community structure in the case of high population levels and that Tinença was the area with the highest species richness.

Nevertheless, the analysis of parametric models exposed that Font Roja presented compliance with a log-series, log-normal and broken stick models, presenting p-value of 0.486, 0.510 and 0.173, respectively. These results are due to the reduce capture obtained during sampling period, only 15 specimens. However, Tinenca present compliance with log-series and log-normal models, presenting approximately the same p-value (0.539 and 0.229 respectively). This fact could be indicating two types of behaviour. On the one hand this community could be unstable and composed by a few abundant species and a large number of rare species. On the other hand the number of specimens could be conditioned by a large number of factors associated with the high temperatures and low rainfalls characteristic of this area, forcing the species to adapt to these strict conditions. Finally, results obtained for Torrevieja exposed that presented compliance with a log-series model, presenting p-value of 0.073, indicating that these communities are unstable and composed by few abundant species and a large number of rare species. These results show that it is not the habitat what determines the community structure, as the sampling area presents very specific climatic conditions and botanical and faunal composition.

Table 1. Distribution of species captured by Natural Park.

Species	Font Roja	Tinença	Torrevieja	Total
Ademon urinator (de Stefani, 1902)	0	0	1	1
Biophthora rossica (Szepligeti, 1901)	0	0	3	3
Biosteres analis (Wesmael, 1835)	0	0	1	1
Biosteres sp. 1	0	0	1	1
Biosteres spinaciae (Thomson, 1895)	0	1	0	1
Eurytenes (Xynobiotenes) scutellatus (Fischer, 1962)	0	0	19	19
Opius (Agnopius) tirolensis Fischer, 1958	1	0	5	6
Opius (Allophlebus) staryi Fischer, 1958	0	1	0	1
Opius (Cryptonastes) gracilis Fischer, 1957	2	0	0	2
Opius (Misophthora) bulgaricus Fischer, 1959	0	1	0	1
Opius (Nosopoea) cingulatoides Fischer, 1959	0	3	0	3
Opius (Nosopoea) maculipes Wesmael, 1835	0	0	1	1
Opius (Nosopoea) speciosus Fischer, 1959	0	1	0	1
Opius (Nosopoea) viennensis Fischer, 1959	7	0	0	7
Opius (Opiognathus) propodealis Fischer, 1958	2	0	0	2
Opius (Opiostomus) clausus Fischer, 1958	0	0	75	75
Opius (Opiothorax) vindex Haliday, 1837	1	0	2	3
Opius orbiculator (Nees, 1811)	0	0	5	5
<i>Opius</i> sp. 1	0	1	1	2
Opius sp. 2	0	3	1	4
Opius sp. 3	0	2	1	3
<i>Opius</i> sp. 4	0	1	1	2
<i>Opius</i> sp. 5	0	1	8	9
<i>Opius</i> sp. 6	0	6	1	7
Phaedrotoma biroi (Fischer, 1960)	0	7	0	7
Phaedrotoma depeculator Forster, 1862	0	2	0	2
Phaedrotoma diversiformis (Fischer, 1960)	0	8	0	8
Phaedrotoma exigua (Wesmael, 1835)	0	30	8	38
Utetes (Frekius) imitabilis (Telenga, 1950)	0	1	0	1
Xynobius aciculatus (Thomson, 1895)	2	1	0	3
Xynobius caelatus (Haliday, 1837)	0	2	4	6
Xynobius rudis (Wesmael, 1835)	0	1	0	1
TOTAL	15	73	138	226

In order to obtain the beta diversity, the Jaccard index  $(I_J)$  (Table 3) was calculated. The values obtained by the Jaccard index indicated a certain degree of dissimilarity between the Natural Parks; Torrevieja and Font Roja being the most similar ( $I_J = 0.275$ ). However, applying the Principal Component Analysis (PCA) shows that there are species of each Natural Park while there are others that are usually present shared (Fig. 2). Table 2. Diversity and abundance values for collected Opiinae.

	Font Roja	Tinença	Torrevieja
Species	6	19	18
Specimens	15	73	138
Simpson I.	0.720	0.795	0.674
Berger-Parker	0.466	0.411	0.543
Shannon I.	1.523	2.184	1.744
Margalef	1.846	4.195	3.450

Table 3. Jaccard index and Complementarity index values for Opiinae between Natural Parks.

	Tinença	Font Roja	Torrevieja	
Tinença		95.8%	72.4%	tarity
Font Roja	0.041		90.9%	nplemen
Torrevieja	0.090	0.275		Con
	Jaccard			



Fig. 2. Principal Component Analysis (PCA) reflecting relationship between Natural. Parks.

Additionally, the indices of species replacement were determined using the Complementarity index (Table 2), showing Font Roja and Torrevieja as the areas with the highest replacement. The reason of this is the distance, as these natural parks are closer to each other compared with the one at Tinença.

Finally, gamma diversity reached a value of 31.92, practically identical to the total species richness observed in the three Natural Parks (species number = 32).

# DISCUSSION

Regarding the faunistic study, eleven species captured are new records for Spain: Ademon urinator, Biophthora rosica, Biosteres analis, Biosteres spinaciae, Eurytenes (Xynobiotenes) scutellatus, Opius (Agnopius) tirolensis, Opius (Misophthora) bulgaricus, Opius (Nosopoea) cingulatoides, Opius (Nosopoea) speciosus, Opius (Nosopoea) viennensis and Xynobius caelatus.

While, regarding the biodiversity study, it is possible to see that the Natural Park of La Tinenca de Benifassà and Torrevieja present greater species diversity, followed by Font Roja. Also, Torrevieja presents the major number of specimens captured, followed by Tinenca and Font Roja. On the other hand, the analysis of the structure of the network has showed that the Font Roja shows a model of community that matches with log-series, log-normal and broken stick models. These results are due to the reduce capture obtained during sampling period. Tinenca presents compliance with log-series and log-normal models meanings that the community has two types of behaviour. On the one hand this community could be unstable and composed by a few abundant species and a large number of rare species. On the other hand the number of specimens could be conditioned by a large number of factors associated with the high temperatures and low rainfalls characteristic of this area, forcing the species to adapt to these strict conditions. Finally, Torrevieja shows a log-series model. This demonstrates that the community structure is not the habitat what determines the community structure, as the sampling area presents very specific climatic conditions and botanical and faunal composition. Furthermore, when comparing parks, it can be seen that Font Roja and Tinença show the most similarities between each other, whilst Font Roja and Tinenca show a larger group of species that complement each other.

On the other hand, checking with the studies realized in other areas of Spain as Artikutza about the Opiinae show that this group was the most abundant captured with approximately 2.97% (Jiménez-Peydró and Peris-Felipo, 2011). The information about the abundance is very interesting due to the relationships that these parasitic wasps have with their hosts. This information could be used to estimate the biodiversity appearing in each area.

Finally, we conclude that, these studies are of extreme relevance because the braconids can be used as biodiversity indicators thanks to their biology and the tri-trophic relationships they maintain with their hosts and plants. Consequently, studies of Braconidae in different areas and DNA-barcode analyses are highly recommended in order to increase our knowledge of this large and still unknown group.

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