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Source: Florida Entomologist, 94(3) : 472-479

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.094.0311>

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## DIVERSITY AND COMMUNITY STRUCTURE OF OPIINAE (HYMENOPTERA: BRACONIDAE) IN THE FOREST ESTATE OF ARTIKUTZA (SPAIN)

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### ABSTRACT

The present work analyses the diversity of Opiinae (Hymenoptera: Braconidae) in the forest of the Artikutza Estate, within the Natural Park, Peñas de Aya, in the western Pyrenees. Specimens belonging to this subfamily were collected throughout two cycles and in two different habitats: mixed forest and beech forest. A total of 105 specimens, including 20 different species, were collected. Diversity and structure analysis indicated higher diversity for the beech forest habitat, although the community is composed of a few abundant species and many rare species. A relationship between the presence of Opiinae and an increase in temperature was also ascertained.

Key Words: diversity; parasitoids; protected area; Pyrenees; forest system

### RESUMEN

En el presente trabajo se analiza la diversidad de Opiinae (Hymenoptera: Braconidae) en la finca forestal de Artikutza ubicada en el Parque Natural Peñas de Aya y enclavada en el extremo occidental de los Pirineos. Durante dos ciclos se realizó la recolección de esta subfamilia en dos hábitats diferentes: bosque mixto y bosque de hayedo. En total fueron capturados 105 ejemplares pertenecientes a 20 especies. Posteriormente, mediante los análisis de diversidad y estructura se comprobó que el bosque de hayedo tiene mayor diversidad que el bosque mixto y que esta comunidad está compuesta por pocas especies abundantes y por bastantes raras. También se observó que la aparición de Opiinae está relacionada principalmente con el aumento de temperatura.

Translation provided by the authors.

The accelerated pace of environmental deterioration with the associated loss of whole taxonomic groups (Gaston 1991; Mayr 1992; Hawksworth & Ritchie 1993; Wilson 1994) underlines the critical importance of biodiversity studies to gain a deeper knowledge of the ecosystems and to implement measures that ensure the conservation of biodiversity and the maintenance of forested reserves (Pyle et al. 1981; Pearson & Casola 1992; Kremen et al. 1993).

The Braconidae are major regulators of phytophagous insect populations (LaSalle & Gauld 1992) and can be used to infer the presence or absence of their hosts (Matthews 1974; LaSalle & Gauld 1992). Some braconid species are economically important because of their potential for pest control (González & Ruiz 2000). The Braconidae are the second largest family within the Hymenoptera and the majority of species are primary parasitoids of the immature stages of Lepidoptera, Coleoptera and Diptera (Sharkey 1993).

Within the braconids, the subfamily Opiinae contains approximately 1,500 catalogued species

around the world (Wharton 1998). These are strictly koinobiont parasitoids of Diptera-Cyclorhapha (Wharton 1999), mainly of leaf miners and other larvae living in fruit. The hosts are known for only around 300 species, mostly within Agromyzidae, Anthomyiidae and Tephritidae (Fischer 1971, 1972, 1977, 1987; Shaw & Huddleston 1991).

There are currently numerous biodiversity studies in progress, but very few studies on braconid communities have focused on the Iberian Peninsula (Andorra, Spain and Portugal) (Nieves et al. 1991; Pujade 1996; Segade et al. 1997; Ros-Farré & Pujade-Villar 1998; González et al. 2000; Martínez de Murguía et al. 2001; Tomé et al. 2001; Falcó-Garí et al. 2006). Those that have done so have concentrated on the annual distribution of adult activity and relative abundance and diversity of biological strategies, always working at the subfamily or genus level.

Within this context, the present work aimed to analyze alpha, beta and gamma diversity and community structure of Opiinae in the forested

estate of Artikutza (Navarra), an area alternating between mixed and beech forests in the western Pyrenees and a site of enormous ecological value. Data on the subfamily's phenology and its relationship with environmental and climatic conditions were also studied.

MATERIAL AND METHODS

Area of Study

The present faunal study of Braconidae: Opiinae was carried out in the forest of the Artikutza Estate (30TWN972868 U.T.M.), which extends over 5 ha at an altitude between 575 and 652 m (Martínez de Murguía 2002, 2003). The estate is located within the Natural Park, Peñas de Aya (Guipuzcoa, Spain), in the westernmost spurs of the Pyrenees. Since its creation, the estate has gone through frequent processes of deforestation and regrowth, but is currently populated by two adjacent plant series: mixed forest and beech forest. The mixed forest contains secondary stands of *Pinus sylvestris* L., *Quercus petraea* L. and *Fagus sylvatica* L. By contrast, the beech forest has been only partially regrown and is surrounded by coniferae. Besides beech, there are isolated specimens of *Castanea sativa* L., *Taxus baccata* L., *Salix atrocinerea* Brot., *Fraxinus excelsior* L., *Betula celtiberica* Rothm. & Vasc., *Ulmus glabra* Hud. and *Sorbus aucuparia* (L.) Crantz. The oligotrophic nature of the soil produces a depauperate shrub and herbaceous stratum, in which the following species commonly occur: *Ilex aquifolium* L., *Blechnum spicant* (L.) Roth., *Deschampsia flexuosa* (L.) Trin., *Vaccinium myrtillus* L., *Euphorbia amygdaloides* L., *Daphne laureola* L., *Oxalis acetosella* L. and, in the brighter areas and clearing edges, *Crataegus monogyna* Jacq., *Corylus avellana* L., *Pyrus cordata* Desv., *Malus sylvestris* Miller, *Pteridium aquilinum* (L.) Kuhn., *Erica vagans* L. and *Asphodelus albus* Miller, among others.

The prominent climatic feature of the area is high precipitation, between 1,500 and 2,800 mm per annum. Rain is most abundant in winter and

scarcer during summer. The temperature is moderate, with an average between 8 and 17°C.

Opiinae were identified using van Achterberg's (1993) criteria. Subsequently, genera were determined with the keys of Tobias et al. (1986a, b) and Wharton et al. (1997), while species identifications were made with those of Fischer (1973).

Sampling Design and Data Collection

Sampling was carried out using Malaise traps, particularly apt at capturing flying insects because they operate by intercepting flight trajectories. On colliding with the trap, the insect tends to crawl or fly upwards, ending up in a jar full of preserving liquid that includes 75% alcohol and 5% acetic acid. The Malaise trap used in this study followed the bidirectional Townes model (203 cm maximum and 112 cm minimum height, 122 cm wide and 183 cm long). The traps were black in color, with a white ceiling and made of fine mesh (0.3 mm).

Sampling included the installation of three traps at each habitat: mixed forest (M-1, M-2 and M-3) and beech forest (H-1, H-2 and H-3). The heterogeneous distribution of trees and the differences in slope gave each trap specific conditions, the main features of which were as follows.

- M-1: On the edge of a clearing.
- M-2: In open forest.
- M-3: Under a mature beech tree in an area mostly populated with pine trees.
- H-1: Under a large beech tree.
- H-2: Near a small clearing and surrounded by a copse of beech shrubs.
- H-3: Under the shade of a mature beech tree.

The hill's orientation, orientation with respect to the North Pole, capture orientation, altitude, trap gradient and distance for each trap are shown in Table 1.

Sampling was carried out without interruption throughout two annual cycles from May 1995 to April 1997. These involved the collection of 46 samples per trap over 733 days (25 and 21 samples over 362 and 371 days respectively). During this period, jars were collected at intervals of 14

TABLE 1. CHARACTERISTICS OF THE LOCATION OF TRAPS IN TWO ADJACENT PLANT SERIES: MIXED AND BEECH FOREST (ARTIKUTZA, NAVARRA).

Trap	Hill orientation	North Pole orientation	Axis trap orientation	Altitude	Hill gradient
M-1	NE-SW	N230E	N-S	611 m	12°
M-2	NE-SW	N210E	N-S	631 m	15°
M-3	NE-SW	N216E	N-S	652 m	20°
H-1	NW-SW	N235E	N-S	576 m	19°
H-2	NW-SW	N210E	N-S	595 m	18°
H-3	NW-SW	N242E	N-S	620 m	9°

days, with the exception of seven occasions when they were collected after 28 days, adding to a grand total of 270 samples.

Meteorological data was collected by the meteorological station located in the village of Artikutza. There was abundant precipitation during the sampling period, 1,778 mm and 2,190 mm per annum, respectively. The rainiest months were Feb and Nov 1996 (372 and 636 mm, respectively), while Oct, Jun and Mar 1995 (23, 29 and 55 mm) were the driest. Temperature remained in the moderate range, with a minimum average of 5°C in Feb 1996 and a maximum of 20°C in Jul 1995. The second sampling period was colder and rainier than the first, particularly during the summer and autumn months.

#### Data Analysis

Once the Opiinae specimens had been identified, the alpha, beta and gamma biodiversity indexes for each trap and habitat were calculated 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 was used for valuing the 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, and establishing a functional relationship between number of species and total number of specimens (Moreno 2001).

Abundance is a concept used for valuing faunal composition of a given area (Magurran 1991). 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 (Moreno 2001; Magurran 1991; Villareal et al. 2004).

Dominance pertains to the occurrence of various taxa, and the dominance value was calculated with the Simpson index, often used to measure species dominance values in a given community; its negative thus representing equity or evenness. The Simpson index 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 1991).

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, the Jaccard and Complementarity indexes were used and cluster analyses were also performed. The Jaccard index relates the total number of species shared between two sites to the total number that are found in only one of the two sites, i.e., exclusive species. It is a qualitative coefficient, the interval of which varies from 0 when no species are shared between both sites to 1 when both sites have an identical species composition (Moreno 2001, Villareal et al. 2004). The 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 was 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 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).

The phenology of local braconids was also investigated to ascertain relationships between the presence of Opiinae and climatic conditions in the sampling area.

In order to complement the diversity analyses and inquire into community structure, log-series, log-normal and broken-stick models were also applied (Magurran 1991). 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).

Each of these models was applied to data obtained from the park to calculate the expected number of species, and the log<sub>2</sub> grouping of species according to abundance (Magurran 1991; Tokeshi 1993; Krebs 1999). To test the significance of the model outputs, the expected species values were compared with those of the observed species through chi-square analysis (Zar 1999).

#### RESULTS AND DISCUSSION

Sample collection resulted in the capture of 3,534 specimens of the Braconidae family, 105 (2.97%) of which belonged to the Opiinae subfamily.

Such a low percentage in the capture of Opiinae (2.97%) has also been observed in other studies worldwide, for example in Andorra (Falcó-Garí et al. 2006), Brazil (Scatolini & Penteado-Dias 2003; Cirelli & Penteado-Dias 2003) and Venezuela (Briceño et al. 2009), where the percentage of captures is also low (3.9%, 3.3%, 4.06% and 7.5%, respectively).

A total of 20 species were represented, including 6 genera: *Apodesmia irregularis* (Wesmael 1835), *Biosteres* sp.1, *Opius basirufus* Fischer 1958, *Opius flavitestaceus* Fischer 1958, *Opius fuscipennis* Wesmael 1835, *Opius graecus* Papp 1982, *Opius imitabilis* Telenga 1950, *Opius inflamatus* Fischer 1963, *Opius levis* Wesmael 1835, *Opius mendus* Papp 1982, *Opius nigricolor* Fischer 1960, *Opius pygmaeus* Fischer 1962, *Opius* sp.1, *Opius staryi* Fischer 1958, *Phaedrotoma daghoides* (Zaykov & Fischer 1983), *Utetes aemulus* (Haliday 1837), *Utetes curtipectus* (Fischer 1958), *Utetes* sp.1, *Xynobius aciculatus* (Thomson 1895), *Xynobius comatus* (Wesmael 1835) and *Xynobius holconotus* (Fischer 1958).

However, the species were not evenly distributed when different traps and habitats were considered separately. Thus, 10 species were identified in the mixed forest habitat (M) (9 in M-1, 4 in M-2 and 4 in M-3), whereas 17 were identified in the beech forest habitat (H) (3 in H-1, 12 in H-2 and 9 in H-3) (Table 2). In both types of forests, the areas with a larger number of species corresponded to clearings (M-1 and H-2), where plant, and consequently host species, diversity was higher.

The genus *Opius* was the most abundant with 54 examples, followed by the genera *Xynobius* (40) and *Apodesmia* (7). At the species level, *Xynobius comatus* was the most common, with 31 collected specimens (29.52%). 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 63 individuals were collected in the mixed forest (33 M-1, 19 M-2 and 11 M-3) and 42 in the beech forest (6 H-1, 17 H-2 and 19 H-3). In the mixed forest habitat, the trap with the most specimens was located in a clearing (M-1). In the beech forest habitat, however, while the trap placed in a clearing (H-2) showed a high number of captures, the trap with the most captures (H-3) was located on a slope under a large beech tree.

Alpha, beta and gamma diversity analyses were carried out with individual consideration of each habitat and trap.

Alpha Diversity. The beech forest habitat hosted a higher species richness with  $D_{Mg} = 4.281$ ,

TABLE 2. DISTRIBUTION OF THE VARIOUS OPIINAE SPECIES IN VARIOUS HABITAT.

Species	Trap Number			Type of Habitat			Total Specimens		
	M-1	M-2	M-3	H-1	H-2	H-3	ΣM	ΣH	Total
<i>Apodesmia irregularis</i>	2	1	0	0	1	3	3	4	7
<i>Biosteres</i> sp.1	0	0	0	0	1	0	0	1	1
<i>Opius basirufus</i>	1	0	0	0	0	0	1	0	1
<i>Opius flavitestaceus</i>	1	0	0	0	1	0	1	1	2
<i>Opius fuscipennis</i>	0	0	0	1	1	0	0	2	2
<i>Opius graecus</i>	0	0	0	0	3	7	0	10	10
<i>Opius imitabilis</i>	0	0	0	0	3	0	0	3	3
<i>Opius inflamatus</i>	0	0	0	0	2	1	0	3	3
<i>Opius levis</i>	15	5	2	0	1	2	22	3	25
<i>Opius mendus</i>	1	0	0	0	0	2	1	2	3
<i>Opius nigricolor</i>	0	0	0	0	0	1	0	1	1
<i>Opius pygmaeus</i>	0	0	0	1	0	0	0	1	1
<i>Opius</i> sp.1	0	0	0	0	1	0	0	1	1
<i>Opius staryi</i>	0	0	0	0	0	1	0	1	1
<i>Phaedrotoma daghoides</i>	0	0	0	0	1	0	0	1	1
<i>Utetes aemulus</i>	0	0	0	0	1	0	0	1	1
<i>Utetes curtipectus</i>	1	0	0	0	0	0	1	0	1
<i>Utetes</i> sp.1	1	0	0	0	0	0	1	0	1
<i>Xynobius aciculatus</i>	0	0	1	0	0	0	1	0	1
<i>Xynobius comatus</i>	10	10	5	4	1	1	25	6	31
<i>Xynobius holconotus</i>	1	3	3	0	0	1	7	1	8
Total specimens	33	19	11	6	17	19	63	42	105

Placement of malaise traps was as follows: M-1 on the edge of a clearing, M-2 in open forest, and M-3 under a mature beech tree in an area mostly populated with pine trees. The habitats were as follows: H-1 under a large beech tree, H-2 near a small clearing and surrounded by a copse of beech shrubs, and H-3 under the shade of a mature beech tree. M is the total number of specimens of a given species captured in the 3 traps. H is the total number of specimens of a given species captured in the 3 habitats.



while the mixed forest reached a value of 2.172. These values might be so discordant as a consequence of the identified species differing widely from one habitat to the other. At the trap level, H-2 had the highest richness of genera with  $D_{Mg} = 3.883$ , followed by H-3 and M-1 (2.717 and 2.288, respectively) (Table 3).

For analysis of proportional abundance, the Shannon-Wiener index and the Simpson dominance index were used (Table 3). The results obtained in the Shannon index for mixed forest (1.518) and beech forest (2.5) suggested a similar trend in the distribution of dominant genera; discrepancies were merely due to different numbers of rare genera (those represented by few specimens). This was also reflected by the Simpson index (0.7045 mixed forest and 0.8889 beech forest). At the trap level, H-2, H-3 and M-1 showed the highest equity values (2.364, 1.908 and 1.526, respectively) and in the Simpson index values, the ranking remained H-2 (0.8927), H-3 (0.8033) and M-1 (0.6924).

After conducting the analysis for the measurement of alpha diversity, it was observed that the traps located in clearings, where higher plant and

host species diversity were to be found, showed higher Opiinae diversity.

**Beta Diversity.** In order to obtain beta diversity (similarity/dissimilarity) values between the different areas under consideration, the Jaccard index was calculated (Table 4). The resulting value (0.318) indicated a certain degree of dissimilarity (as it was below 0.5) between the two habitats. At the trap level, the highest similarity in the represented genera was obtained by the combinations M-2/M-3 and H-1/H-3 (0.600), whereas M-1/H-1 showed the least similarity (0.083). These results were also observed in the Jaccard cluster obtained through cluster analysis with the correlation coefficient of  $r = 0.9293$  (Fig. 1).

On the other hand, the Complementarity Index (C) suggested that the mixed and beech forest habitats showed a fair degree of complementarity (0.681), but also indicated the presence of different genera at each habitat. At the trap level, the combinations M-1/H-1 and H-1/H-3 showed the highest complementarity with a value of 0.916, suggesting a very different array of collected genera, while M-2/M-3 showed the least complemen-

TABLE 3. DIVERSITY AND ABUNDANCE VALUES OF THE COLLECTED OPIINAE SPECIES.

	Trap Number			Type of Habitat			Total Caught	
	M-1	M-2	M-3	H-1	H-2	H-3	ΣM	ΣH
Species	9	4	4	3	12	9	10	17
Specimens	33	19	11	6	17	19	63	42
Shannon I.	1.526	1.136	1.241	0.8676	2.364	1.908	1.518	2.5
Simpson I.	0.6924	0.626	0.6777	0.5	0.8927	0.8033	0.7045	0.8889
Margalef I.	2.288	1.019	1.251	1.116	3.883	2.717	2.172	4.281

Placement of malaise traps was as follows: M-1 on the edge of a clearing, M-2 in open forest, and M-3 under a mature beech tree in an area mostly populated with pine trees. The habitats were as follows: H-1 under a large beech tree, H-2 near a small clearing and surrounded by a copse of beech shrubs, and H-3 under the shade of a mature beech tree. M is the total number of specimens of a given species captured in the 3 traps. H is the total number of specimens of a given species captured in the 3 habitats.

TABLE 4. COMPARISON OF COMPLEMENTARITY (C) AND JACCARD INDEX VALUES FOR OPIINAE SPECIES IN EACH TRAP.

	M-1	M-2	M-3	H-1	H-2	H-3		
M-1		0.4	0.272	0.083	0.222	0.428	Jaccard	
M-2	0.6		0.6	0.166	0.23	0.4		
M-3	0.727	0.4		0.166	0.142	0.272		
H-1	0.916	0.833	0.833		0.153	0.083		
H-2	0.777	0.769	0.857	0.846		0.294		
H-3	0.571	0.6	0.727	0.916	0.705			
			Complementarity					

Placement of malaise traps was as follows: M-1 on the edge of a clearing, M-2 in open forest, and M-3 under a mature beech tree in an area mostly populated with pine trees. The habitats were as follows: H-1 under a large beech tree, H-2 near a small clearing and surrounded by a copse of beech shrubs, and H-3 under the shade of a mature beech tree. M is the total number of specimens of a given species captured in the 3 traps. H is the total number of specimens of a given species captured in the 3 habitats.

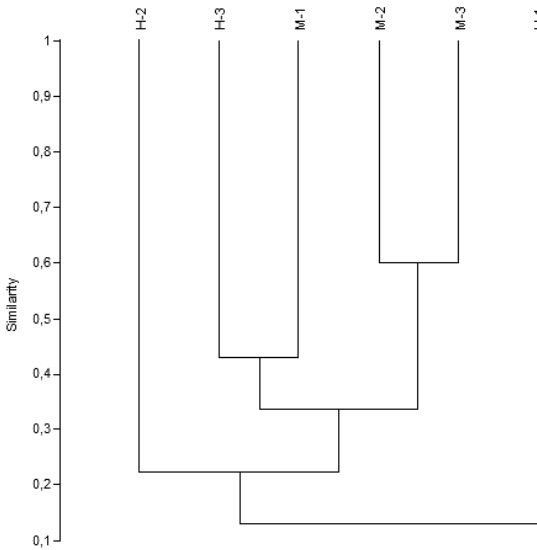


Fig. 1. Cluster analysis reflecting the number of Opiinae species captured in each trap. Placement of malaise traps was as follows: M-1 on the edge of a clearing, M-2 in open forest, and M-3 under a mature beech tree in an area mostly populated with pine trees. The habitats were as follows: H-1 under a large beech tree, H-2 near a small clearing and surrounded by a copse of beech shrubs, and H-3 under the shade of a mature beech tree.

tarity (0.400) as a result of stronger similarity between their genera.

**Gamma Diversity.** Gamma diversity at the habitat level reached a value of 21, largely contributed by the beech forest habitat, which exhib-

ited the most diversity with 17 registered species. At the trap level, gamma diversity showed a value of 20.499, as expressed in the total number of species represented in each community. According to these values, the trap showing the most diversity was H-2 with 12 species, whereas that showing the least diversity was H-1 with 3 species.

Seasonal patterns of abundance and diversity could be discerned (Fig. 2). In the Forest Estate of Artikutza, Opiinae were most numerous between late Apr and late Oct. Results also showed that temperature increase (spring and summer) was reflected in a corresponding increase in specimen abundance. On the other hand, increased rainfall during the winter months caused a significant decrease in temperature and consequently in the number of specimens.

The analysis of the Opiinae community structure showed compliance with log-series and log-normal models, since the p-value obtained for each (0.825 and 0.262) was greater than 0.05. This indicates an unstable community composed of a few abundant species and a large number of rare species (Table 5). These results showed that habitat did not determine community structure because the sampling area presented very specific botanical and faunal composition and climatic conditions.

To conclude, the beech forest had a higher diversity of Opiinae even though the number of captured specimens was clearly lower. The individual analysis of each trap, on the other hand, showed that clearings, where plant host species diversity was greater, had higher specific diversity. Finally, we can say that this community was composed of a few abundant and a large number of rare spe-

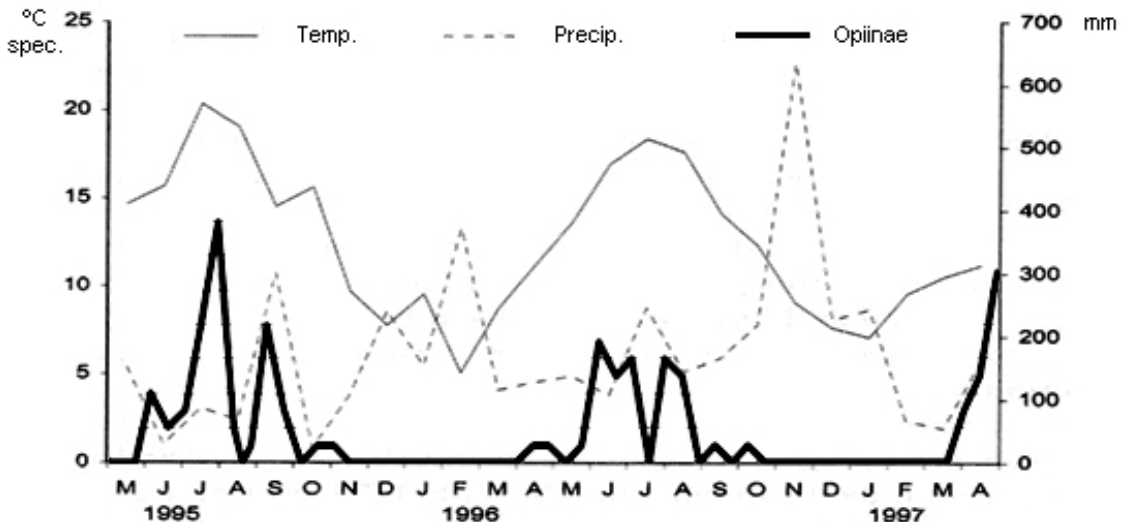


Fig. 2. Relationship between climatic conditions and seasonal abundance of the Opiinae.

TABLE 5. OBSERVED AND EXPECTED FREQUENCIES OF SPECIES (EXP F) ACCORDING TO ABUNDANCE MODELS (LOG-SERIES, LOG-NORMAL AND BROKEN-STICK) FOR THE OPIINAE COMMUNITY IN THE FOREST OF THE ARTIKUTZA ESTATE.

Class	Log-series		Log-normal		Broken-stick		
	Exp f	Obs f	Exp f	Obs f	Exp f	Obs f	
0	—	—	2.66	0	—	—	
1	10.76	13	7.97	13	6.11	13	
2	3.59	3	2.97	3	4.21	3	
3	3.18	2	2.28	2	4.85	2	
4	2.21	1	1.27	1	3.18	1	
5	1.01	2	0.52	2	0.67	2	
6	0.22	0	0.16	0	0.01	0	
7	0.01	0	0.04	0	0.00	0	
		$\chi^2 = 2.864$ $p = 0.825$			$\chi^2 = 7.678$ $p = 0.262$		
					$\chi^2 = 13.937$ $p = 0.016$		

cies and that there was a clear relationship between the abundance of Opiinae and climatic conditions.

#### ACKNOWLEDGMENTS

We would like to thank Leticia Martínez de Murguía for the donation of the specimens of Braconidae stored at the Laboratorio de Entomología y Control de Plagas of Valencia University for their analysis and identification.

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