Biodiversity within the subfamily Alyssinae (Hymenoptera, Braconidae) in the Natural Park Peñas de Aya (Spain)

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ABSTRACT. Biodiversity within the subfamily Alyssinae (Hymenoptera, Braconidae) in the Natural Park Peñas de Aya (Spain). The study of parasitoid Hymenoptera is of significance for the assessment of diversity in a given area because of their role in the regulation of insects populations. The present work analyses diversity within Alysiinae (Hymenoptera, Braconidae) in the Forested Estate of Artikutza, located in the Natural Park Peñas de Aya, western Pyrenees, Spain. Collection of specimens was spread over two years and was carried out in two different habitats: mixed forest and beech forest. A total of 2,270 specimens, belonging to 22 separate genera, were captured. Subsequently, alpha, beta and gamma diversities were analysed, and the beech forest was proven to host greater diversity than the mixed forest. A sampling strategy was adopted for the analysis of Alysiinae phenology and its relationship with environmental climatic conditions; as a result, a direct relationship between phenology and temperature was attested.

KEYWORDS. Diversity; forest system, parasitoids; protected area; Pyrenees.

RESUMO. Biodiversidade na Alyssinae subfamília (Hymenoptera, Braconidae) no Parque Natural Peñas de Aya (Espanha). O estudo dos himenópteros parasitóides é significativo para uma avaliação da diversidade em determinada área por causa do seu papel no controle das populações de insetos. O presente trabalho analisa a diversidade no taxon Alysiinae (Himenóptero, Braconidae) na área de floresta de Artikutza, localizada no Parque Natural de Peñas de Aya, Pirinéus ocidentais, Espanha. A coleta de espécimes dividiu-se em dois anos e foi realizada em dois habitats diferentes: floresta mista e floresta de faias. No total, foram capturados 2.270 exemplares pertencentes a 22 gêneros distintos. Subsequentemente, as variedades alfa, beta e gama foram analisadas, comprovando-se que a floresta de faias apresenta uma diversidade maior que a floresta mista. Adotou-se a estratégia de amostra para análise da fenologia da Alysiinae e da sua relação com as condições climáticas ambientais, de onde se concluiu que existe uma relação direta entre a fenologia e a temperatura.

PALAVRAS-CHAVE. Área protegida; diversidade; parasitóides; Pirineus; sistema florestal.

In land environments, the information provided by arthropods can be very valuable for the adoption of measures aimed at guaranteeing the diversity and welfare of protected forests (Pyle *et al.* 1981; Pearson & Cassola 1992; Kremen *et al.* 1993), especially insects with a high sensitivity to alterations in environmental resources and conditions. Parasitoid hymenoptera of the Braconidae family, with around 14,890 catalogued species, are especially pertinent in this respect due to their particular biology (Wharton *et al.* 1997).

Braconidae are the second largest family within Hymenoptera, the majority of species are primary parasitoids of immature stages of Lepidoptera, Coleoptera and Diptera (Sharkey 1993). These wasps are of enormous ecological interest because of their role in controlling the population of phytofagous insects, causing direct effects in the host species' population size and indirect ones in the diversity and survival of host plants (La Salle & Gauld 1992; Torezan-Silingardi 2011). Additionally, they can indicate presence or absence of those populations (Matthews 1974; LaSalle & Gauld 1992). Finally, some species can also be relevant from an economic point of view due to their potential for pest control (González *et al.* 2000; Elpino-Campos *et al.* 2007).

Because of the type of relationship established between Braconidae populations and host species, and the effect that climatic factors and human activity possess upon this, we can consider that Braconidae (especially those adopting koinobiont strategies in which not paralyze its guest or only momentarily) are a valid parameter for the determination of human effect on these communities and the assessment of specific diversity within a region (Delfín & Burgos 2000).

The subfamily Alysiinae, with 1,500 catalogued species worldwide, and including two tribes, Alysiini and Dacnusini, has a prominent position within the Braconidae family (Dolphin & Quicke 2001). The Alysiini interact with a wide variety of cyclorrhapha hosts, often in humid habitats and ephemeral substrata, laying their eggs in the host's larvae or eggs. Dacnusini, by contrast, are almost exclusively specialised in leaf and stem miners, such as Agromyzidae, Ephydridae and Chloropidae. Specimens of the generas *Aphaereta* Förster, 1862 and *Alysia* Latreille, 1804, belonging to the Alysiini tribe, have been bred and freed in biological control programs involving Calliphoridae and Muscidae, while some Dacnusini have been successfully employed to control pests of leaf miners and Tephritidae (Wharton *et al.* 1998).

Although many faunal and diversity studies around Braconidae have been carried out worldwide, for example in Brazil (Cirelli & Penteado-Dias 2003; Scatolini & Penteado-Dias 2003) or Venezuela (Briceño *et al.* 2009), in the Iberian Peninsula (Andorra, Spain and Portugal) these communities have been insufficiently analysed (Nieves & del Castillo 1991; Pujade-Villar 1996; Segade *et al.* 1997; Ros-Farré & Pujade-Villar 1998; González *et al.* 2000; Martínez de Murguía *et al.* 2001; Tomé *et al.* 2001), with the exception of a study about this family in the Pyrenees (Falcó-Garí *et al.* 2006).

Within this context, the present work analyses alpha, beta and gamma diversity of Alysiinae in the Forested Estate of Artikutza (Navarra), selected for its location, south-west Pyrenees, and its habitat which comprises alternating pine and beech forest and thus possesses enormous ecological value. Data on the subfamily's phenology and its relationship with environmental and climatic conditions are also offered.

MATERIAL AND METHODS

Area of study. The present faunal study on Alysiinae Braconidae population was carried out in the Natural Park Peñas de Aya (Guipuzcoa, Spain) located at the western end of the Pyrenees. This natural park has an extension of 6,913 ha, and a maximum altitude of 834 m. Due to its large dimensions a study area of 5 ha was selected from within the Forested Estate of Artikutza (30TWN972868 U.T.M.). The estate lies at an altitude between 575 and 652 m (Martínez de Murguía *et al.* 2001) and has gone through frequent processes of deforestation and repopulation in the past, but is currently populated by two adjacent plant series: mixed forest and beech forest. The mixed forest is a repopulated secondary forest including *Pinus sylvestris* L., *Quercus petraea* L. and *Fagus sylvatica* L. On the other hand, the beech forest has been partially repopulated and is surrounded by other repopulated conifer forest. Other species are represented by isolated specimens, as *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 soil oligotrophic nature produces a poor shrubbery and herbaceous stratum, in which the following species should be highlighted: *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 main climatic feature for the area is the high precipitation regime, between 1,500 and 2,800 mm. Rain is more abundant in winter, and scarcer during summer. The temperature is moderate, with an average between 8 and 17°C (Fig. 1).

For the identificacion of Alysiinae, the criteria indicated in Achterberg (1993), Tobias *et al.* (1986 a, b) and Wharton *et al.* (1997) were followed.

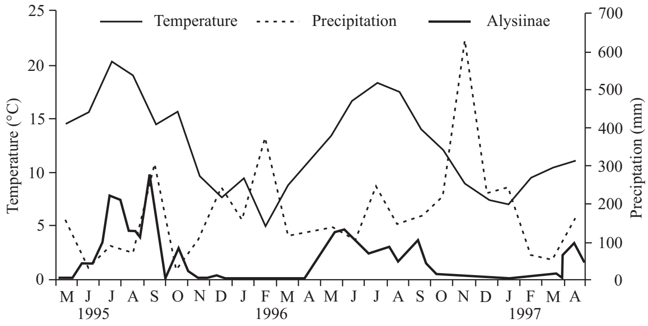

Fig. 1. Relationship between climatic conditions and Alysiinae phenology.

Sampling design and data collection. The sampling of parasitoid wasps was carried out with Malaise traps, Townes design, characterised for being bi-directional (203 cm maximum height, 112 cm minimum, 122 cm wide and 183 cm long), and black in colour, with a white ceiling and fine mesh (0.3 mm). This trap works on the principle that many species of flying insects tend to adopt an ascending trajectory when confronted with an obstacle. By doing so, the insects end up in a jar full of preserving liquid that includes 75% of alcohol, 20% of distilled water and 5% acetic acid.

The sampling included the installation of three traps in 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 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 layer of beech shrubs; H-3: Under the shade of a mature beech tree on a landing.

Sampling was carried out without interruption during a two-years period, from May 1995 to April 1997. During this period, jars were collected at intervals of 14 days, with the exception of seven occasions, in which they were collected after 28 days, with a total of 270 samples.

Data analysis. Once the Alysiinae specimens had been identified, alpha, beta and gamma biodiversity indexes for each trap and habitat were calculated, in order to gain insight into the richness, abundance, dominance and complementarity values for each area. Additionally, the phenological study of all captured specimens was undertaken, by comparing the climatic conditions on the sampling area in order to determine the possible relationship between temperature and the presence of Alysiinae.

Alpha diversity was measured by taxa richness, abundance and dominance.

Taxa richness: used for valuing richness on sampling areas. It was measured using the Margalef index (Moreno 2001).

Abundance: used for valuing faunal composition of a given area (Magurran 1991). This was measured using the Shannon index, because it measures equity, indicating the degree of uniformity in species representation (in order of abundance) while considering all samples (Moreno 2001; Magurran 1991; Villarreal *et al.* 2004).

Dominance: genera occurrence or dominance value was calculated using the Simpson index, often used to measure species dominance values in a given community, its negative thus representing equity (Magurran 1991).

In order to measure beta diversity, the Jaccard and Complementarity indexes were used.

Jaccard index: relates the total amount of shared species with the total amount of exclusive species (Moreno 2001; Villarreal *et al.* 2004).

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

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

RESULTS AND DISCUSSION

During the sampling period, 78,229 specimens of hymenoptera were collected, including 3,534 specimens in the Braconidae family and 2,270 (64.23%) in the Alysiinae subfamily.

The captured specimens fall into a total of 22 genera of Alysiinae: *Alysia* Latreille 1804, *Aphaereta* Förster 1862, *Aristelix* Nixon 1943, *Asobara* Förster 1862, *Aspilota* Förster 1862, *Chorebus* Haliday 1833, *Coelinidea* Viereck 1913, *Coelinius* Nees 1818, *Coloneura* Förster 1862, *Cratospila* Förster 1862, *Dacnusa* Haliday 1833, *Dapsilarthra* Förster 1862, *Dinotrema* Förster 1862, *Exotela* Förster 1862, *Laotris* Nixon 1943, *Orthostigma* Ratzeburg 1844, *Pentapleura* Förster 1862, *Phaenocarpa* Förster 1862, *Synaldis* Förster 1862, *Synelix* Förster 1862, *Tates* Nixon 1943 and *Trachyusa* Ruthe 1854 (Table I).

The number of genera indentified varies from habitat to habitat and from trap to trap. For example, 17 genera were identified in the mixed forest habitat (15 in M-1, 14 in M-2 and 11 in M-3), while 20 were identified in the beech forest habitat (12 in H-1, 15 in H-2 and 14 in H-3).

The genus Dinotrema was the most commonly captured, with 1,278 specimens, followed by Chorebus (338), Orthostigma (239) and Aspilota (139). The genus Dinotrema is noted for its cosmopolitism and its abundance within the Alysiinae (Wharton *et al.* 1997), as well as for being a primary parasitoid of Phoridae and Platypezidae (Diptera).

The abundance differed depending on the kind of forest observed, 1,593 specimens were captured in mixed forest (528 M-1, 550 M-2 and 485 M-3) while 707 were captured in beech forest (243 H-1, 236 H-2 and 228 H-3).

When calculating alpha, beta and gamma diversity values, each habitat and each trap must be considered separately.

Alpha diversity. According to our observations, beech forest is richer in number of genera represented, with D_{Mg} = 2.896; against 2.176 in mixed forest. These values are so close because the number of genera identified in both habitats is

Table I. Distribution of Alysiinae specimens per habitat.

Genera	M-1	M-2	M-3	H-1	H-2	H-3	Total
Alysia	11	0	1	0	0	1	13
Aphaereta	0	0	0	0	0	1	1
Aristelix	1	0	0	1	0	0	2
Asobara	6	2	0	0	1	4	13
Aspilota	41	19	25	16	11	27	139
Chorebus	51	120	55	40	49	23	338
Coelinidea	0	0	0	1	2	0	3
Coelinius	0	2	1	0	1	1	5
Coloneura	0	0	0	1	1	2	4
Cratospila	8	5	4	4	4	2	27
Dacnusa	49	5	8	4	10	16	92
Dapsilarthra	8	2	2	2	1	2	17
Dinotrema	302	320	311	119	113	113	1278
Exotela	0	0	0	0	1	0	1
Laotris	1	3	0	0	1	0	5
Orthostigma	24	45	60	47	33	30	239
Pentapleura	1	0	0	0	0	1	2
Phaenocarpa	2	1	1	0	0	0	4
Synaldis	15	23	17	7	7	5	74
Synelix	0	1	0	0	0	0	1
Tates	0	0	0	1	0	0	1
Trachyusa	8	2	0	0	1	0	11
Total	528	550	485	243	236	228	2270

also similar. On the other hand, at trap level, H-2 is the trap showing the greatest richness in number of genera, with D_{Mg} = 2.562, followed by H-3 and M-1 (2.212 and 2.081 respectively) (Table II).

Table II. Diversity and abundance of Alysiinae captured.

	M-1	M-2	M-3	H-1	H-2	H-3	M	H
Species	109	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

For the analysis of proportional abundance, the Shannon-Wiener and the Simpson dominance indexes were calculated (Table II). According to the Shannon index (1.438 for mixed forest and 1.633 for beech forest), both habitats show a similar trend regarding distribution of dominant genera; significant differences are limited to rare genera, that is, those represented by a few specimens. The Simpson index reflects a similar picture (0.6099 for mixed forest and 0.7038 for beech forest). When analysing these values at trap level, H-3, H-2, M-1 and H-1 were observed to be the traps showing more equity (1.639, 1.607, 1.515 and 1.511 respectively), while in the Simpson index the ranking is H-3 (0.7043), H-2 (0.7027), H-1 (0.6898) and M-1 (0.6299).

Beta diversity. Beta diversity (similarity/dissimilarity) was obtained using values between the different areas under consideration, with the Jaccard index (Table III). The value obtained comparing both habitats (0.681) shows again a certain degree of similarity between environments. At the trap level M-1/M-2 and M-2/H-2 (0.705) show the strongest similarity, while M-2/H-1 are the least similar (0.444).

Table III. Values of the Complementarity (C) and Jaccard index for Alysiinae in each trap.

	M-1	M-2	M-3	H-1	H-2	H-3	
M-1		0.400	0.272	0.083	0.222	0.428	Jaccard
M-2	0.600		0.600	0.166	0.230	0.400	
M-3	0.727	0.400		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	0.600	0.727	0.916	0.705		
	Complementarity						

On the other hand, the complementarity index (C) shows a low level of complementarity between mixed and beech forest (0.375) indicating that the genera present in both habitats are similar. On the other hand, at the trap level M-2/H-1 show a higher degree of complementarity, with a value of 0.555, indicating differences in the genera collected, while M-2/H-2 show lower complementarity (0.294) as a consequence of a similar representation of genera in both cases.

Gamma diversity. Gamma diversity at the habitat level reaches a value of 24.527, largely contributed to by the beech forest habitat, which shows the bigger diversity with 22 collected genera. At the trap level, gamma diversity attains a value of 21.7323, as expressed in total number of genera identified in each community. According to these values, M-1 and H-2 are the traps showing higher diversity with 15 genera, and M-3 was the fewer diverse, with 11.

The data collected were also used to analyse the seasonal evolution of Alysiinae. The results show that Alysiinae are present between late April and late October each year. Subsequently, the data was also used to assess the possible relationship between their presence and climatic conditions, no relationship could be attested between precipitation levels and number of captured Alysiinae, but a coincidence between rise in temperatures and increased numbers of captures could be certified.

Finally, after the analysis of all data collected and the estimation of alpha, beta and gamma diversity values in both habitats, mixed forest and beech forest, we conclude that the latter shows a higher diversity of Alysiinae than mixed forest, even though the number of captured specimens is clearly lower. We also conclude that the presence of this subfamily of Braconidae is connected with climatic conditions.

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