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The microhabitat preferences of water beetles in four rivers in Ourense province, Northwest Spain

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ABSTRACT

The microhabitat preferences of water beetles in four rivers in Ourense province, Northwest Spain

We analysed the microhabitat preferences of water beetle species in four rivers in Northwest Spain. In each river, we sampled 5 sites with different types of substrate. These sites were characterised in situ according to the predominant material type (macrophytes, moss, pebbles and sand). The occurrence of a substrate preference was verified from a comparative study of species richness and abundance among different microhabitats. The differences in abundance and richness between substrates and in the abundance of each species were tested with an ANOVA. The similarity between microhabitats was tested with non-metric multidimensional scaling (NMDS), and the correlation between fauna and substrates was verified with a correspondence analysis (CA). We observed different species distribution patterns, and these patterns reflected the microhabitat preference of each species. Both the ecological parameters and the correspondence analysis indicated that the preferred substrate for most of the species was moss, followed by pebbles.

Key words: Water beetles, substrates, preferences, rivers, Galicia.

RESUMEN

Preferencias de microhábitats en coleópteros acuáticos de cuatro ríos en la provincia de Ourense, Noroeste de España

Se analiza la preferencia de microhabitat de especies de coleópteros acuáticos en cuatro ríos del noroeste de España. En cada río se muestrearon 5 puntos en diferentes tipos de sustrato caracterizados in situ en función del tipo de material predominante (macrófitas, musgo, cantos-gravas y arena). La preferencia de sustrato fue verificada mediante un estudio comparado de riqueza y abundancia de especies entre los diferentes microhábitats. Las diferencias entre los diferentes sustratos para la abundancia y riqueza, así como para la abundancia de cada especie fueron testadas mediante un análisis ANOVA. La similitud entre microhábitats fue testada mediante un NMDS, mientras que la correlación entre la fauna y los sustratos, se verificó a partir de un análisis de correspondencias (CA). Se observaron diferentes patrones de distribución de las especies según su preferencia por determinados microhábitats. Tanto los parametros ecológicos de riqueza y abundancia como el análisis de correspondencias indican que el sustrato preferido por la mayoría de las especies fue el musgo, seguido de los cantos-gravas.

Palabras clave: *Coleópteros acuáticos, sustratos, preferencias, ríos, Galicia.*

INTRODUCTION

The study of river microhabitats is the key to understanding the structure of the assemblages inhabiting them and the correlation between species and the environment. In addition, the structure of communities and the ecological interactions that occur depend on the environmental variables (Illies & Botosaneanu, 1963) and the longitudinal gradient (Vannote *et al*., 1980; Minshall *et al*., 1985).

In studies of microhabitat association, it is important to consider the biotic and abiotic factors that determine the structure of aquatic communities. In this sense, inorganic and organic substrates are very important because they define microdistributions (Lloyd & Sites, 2000).

Aquatic beetles are considered to be good water quality indicators within these faunistic communities (García-Criado et al., 1999). These insects are widely distributed in running water (Smith *et al*., 2007), where they play an important role in trophic chains (Merritt & Cummins, 1996). Trophic diversity is a factor that makes beetles abundant and dominant in most freshwater environments, occupying different microhabitats formed by different substrates of the riverbed. In this sense, it is important to understand the interactions between these organisms and the environment where they live.

Several authors have used different groups of invertebrates as a model for analysing substrate preferences in rivers (Sheldon & Haick, 1981; Baptista *et al*., 2001; Crosa & Buffagni, 2002; Urbanic *et al*., 2005), but few previous studies have used water beetles as a model. Among these studies, the work of Lloyd & Sites (2000), who studied the association of three species of microhabitat Dryopoidea in the Missouri River (USA), can be highlighted.

The lack of such research in rivers in the Iberian Peninsula has motivated us to conduct the current study, whose purpose is to verify a possible correlation between the presence of water beetle species and the substrate by evaluating the degree of microhabitat preference shown by these species.

MATERIALS AND METHODS

Study area

This study was conducted in four rivers (Deva, Cadós, Tuño and Fragoso) located in southern Galicia, NW Spain (Fig. 1). The Deva and Tuño

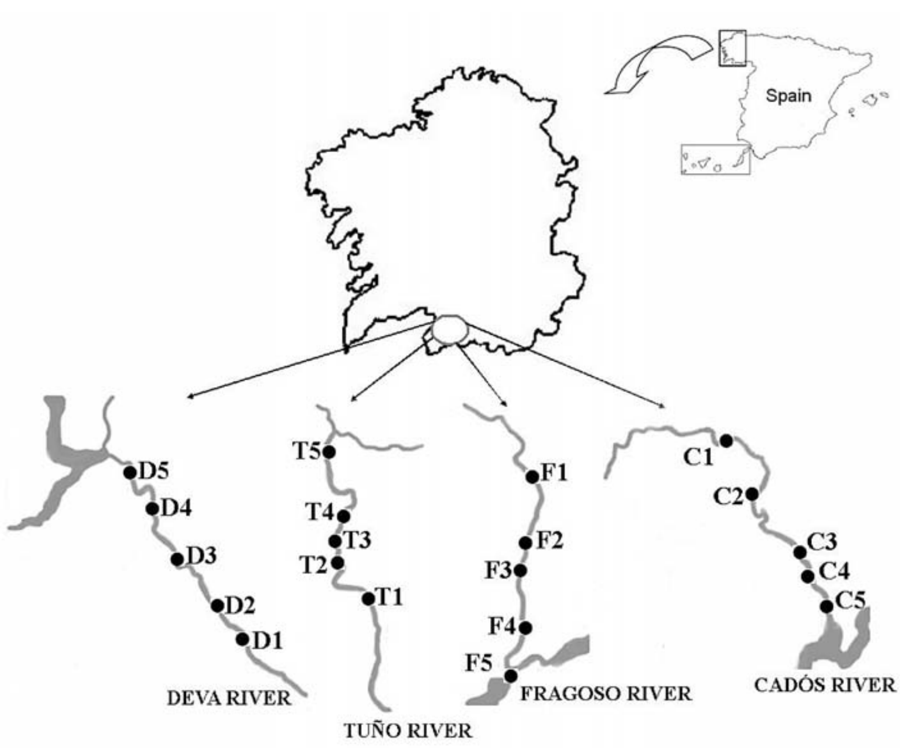

Figure 1. Location of the study area and sampling sites in the four rivers. *Localización del área de estudio y los puntos de muestreo en los cuatro ríos.*

Table 1. List of sampling sites with geographic data and coordinates. *Listado de los puntos de muestreo con los datos geográficos y coordenadas.*

River	Sampling site	Code	Altitude	UTM X	UTM Y
	Corbelle	C1	931	5813723869	46567385538
	Seoane	C2	768	5828905421	46550904208
Cadós	Pontenova	C3	697	5848731952	46529329982
	Xordos	C4	637	5848873810	46517486312
	Aguas abajo Central Cadós	C5	555	5857333375	46504693937
	Parada do Monte	F1	747	5788134605	46520147192
	Ponte do Groicio	F2	563	5791387083	46492544893
Fragoso	Ponte Abeleda	F3	545	5787222948	46482072459
	Requeixo	F4	419	5788295007	46453921633
	Grou	F5	410	5783543400	46438692400
	Santa Eufemia	T1	630	5808542671	46652495525
	Trasmiras	T2	486	5804987064	46670315590
Tuño	Pena Avegosa	T3	484	5805068379	46673247037
	O Outeiro	T4	384	5811502776	46690564631
	Ponte Madeiros	T5	298	5796436444	46718032278
	Retortoirio	D1	552	5757845968	46628952012
	Lavandeira	D2	329	5741678949	46652779154
Deva	Pena do Bugallo	D3	192	5729910636	46665118534
	Pontedeva	D4	93	5716285314	46686694927
	Ponte do Cantiño	D5	97	5712389185	46702078806

belong to the Miño and Arnoia river basins, respectively, whereas the Cadós and Fragoso belong to the Limia river basin. Table 1 shows the list of sampling sites with their names, codes (used below to identify the sampling sites), the river, altitude and UTM coordinates. The Tuño and Deva Rivers flow on schist materials, in which the predominant minerals belong to the group of silicates with high levels of silica, whereas the Fragoso and Cadós Rivers flow on materials that consist fundamentally of granite (Río Barja *et al.*, 1996). The dominant vegetation in the study area includes *Corylus avellana*, *Ulmus minor* Mill, *Fraxinus angustifolia* Vahl, *Alnus glutinosa* Gaertn and *Cornus sanguinea* L. These species form a riparian forest that is usually well structured.

Sampling methods

To perform this study, we selected 20 sampling sites, five in each river, distributed along the river course. We sampled all substrates present in the selected section of the river that were characterised by the predominant material. We identified a total of four substrate types: moss, macrophytes, sand and pebbles. Data were collected in eight sampling fields during one year, between July 2001 and June 2002, with two collections in each season, for a total of 160 samples.

The fauna was collected with a Surber net, 25 cm squared and 60 cm deep with 0.5 mm mesh, one sample for each substrate. The samples were fixed in the field with 4 % formaldehyde and taken to the laboratory. The specimens were identified according to standard procedures in entomology, using a stereomicroscope, a binocular microscope and different reference works, including Franciscolo (1979), Valladares (1988), Angus (1992), Prost *et al.* (1992), Gayoso *et al*. (1997) and Tachet *et al.* (2002). After the specimens were identified, they were preserved in 70° alcohol and deposited in the scientific collection of the Laboratory of Aquatic Entomology at Vigo University.

In addition, we measured several physical and chemical parameters in situ: water temperature, dissolved oxygen, pH, conductivity and total dis-

Table 2a. Abundance of species in the four substrate types and species codes in figure 3 (selected species in CA). *Abundancia de las especies en los cuatro tipos de sustrato y código de las especies en la figura 3 (especies seleccionadas en el CA).*

FAMILY	SPECIES	CODE	MA	MO	PE	SA
Haliplidae	*Haliplus lineatocollis*		0	0	1	0
Gyrinidae	*Orectochilus villosus*	24	12	5	35	7
Dytiscidae	*Oreodytes sanmarkii alienus*	21	0	1	5	2
	Hydroporus nigrita		0	0	0	1
	Stictotarsus bertrandi	22	1	0	6	1
	Deronectes ferruginens	23	2	4	6	5
	Graptodytes fractus		0	0	1	0
	Hydroglyphys geminus		0	1	2	0
	Scarodytes halensis		0	0	1	0
	Yola bicarinata bicarinata		0	1	1	1
	Nebrioporus carinatus		0	0	0	1
Hydrophilidae	*Anacaena lutescens*	26	2	8	1	0
Hydrochidae	*Hydrochus angustatus*	27	8	13	1	1
Helophoridae	*Helophorus flavipes*	30	1	2	0	1
Hydraenidae	*Hydraena iberica*	14	29	324	163	15
	Hydraena corinna	15	80	872	201	18
	Hydraena brachymera	16	65	542	122	4
	Hydraena testacea	17	28	338	42	4
	Hydraena hispanica	18	0	137	48	2
	Hydraena sharpi	19	12	393	21	1
	Hydraena barrosi		0	8	0	0
	Hydraena stussineri		0	2	0	0
	Hydraena unca		0	0	3	0
	Hydraena minutissima		0	1	0	0
	Ochthebius legionensis		0	1	0	0
	Ochthebius heydeni	20	1	14	0	0
	Limnebius lusitanus		0	1	1	0
	Limnebius evanescens		0	1	0	0
Elmidae	*Elmis aenea*	1	823	7059	375	116
	Elmis maugetii	2	148	1076	95	29
	Elmis rioloides	3	167	4067	210	46
	Elmis perezi	4	82	1326	50	21
	Limnius volckmari	5	31	11	180	131
	Limnius perrisi carinatus	6	62	52	485	231
	Limnius opacus	7	40	28	48	9
	Oulimnius bertrandi	8	75	475	211	70
	Oulimnius rivularis	9	35	133	41	162
	Oulimnius troglodites	10	6	91	198	90
	Oulimnius perezi		0	3	0	0
	Esolus angustatus	11	2	10	31	34
	Esolus parallelepipedus	12	10	16	170	37
	Dupophilus brevis	13	289	135	1508	334
	Stenelmis canaliculatus		0	0	1	0
Dryopidae	*Dryops luridus*	25	17	62	0	0
Scirtidae	*Cyphon* sp.	28	5	12	7	0
	Elodes sp.	29	5	23	6	1
Curculionidae	*Bagous* sp.		2	0	0	1

Table 2b. Physical, chemical and habitat variables of the rivers studied. *Variables físicas, químicas y del hábitat de los ríos estudiados.*

Variable	Mean ± standard deviation	Minimum	Maximum
Width m	5.94 ± 1.92	2.12	10.95
Depth m	0.87 ± 3.21	0.19	25.50
Altitude m	491.36 ± 215.94	93	931
Distance of Source m	11 012.56 ± 3748.09	4636	18 400
Stream Velocity m^2	3.18 ± 2.06	0.10	9.17
Temperature °C	10.44 ± 3.08	4.22	15.62
pH	6.69 ± 0.32	4.59	7.26
Conductivity $\mu S\ cm^{-1}$	0.42 ± 0.61	0.14	5.66
Dissolved oxygen %	103.98 ± 17.51	6.49	139.90
Dissolved oxygen mg/l	11.99 ± 8.11	5.43	82.07
TSS mg/l	22.11 ± 12.99	5.54	66.50

solved solids. We also measured several habitat parameters: width, depth, stream velocity, altitude and distance from the source (Table 2a and b).

Data analysis

We performed a series of statistical analyses to assess the microhabitat preferences of the water beetles found in the samples. For this purpose, we analysed the assemblage as a whole as well as each species.

The structure of the assemblage was evaluated for species richness (S) and abundance (N) for each substrate. These indices were selected because they potentially portray important characteristics of assemblages. An analysis of variance (one-way ANOVA) was used to test for significant differences between the four substrates in both richness measures and each species. The homogeneity of variance was tested with Levene's test. The ANOVA was conducted with SPSS version 19.

To determine the degree of correlation between the different water beetle species and the microhabitats colonised, we performed a correspondence analysis (CA). Prior to the CA, we refined the data, eliminating species present in 5 % or less of the samples. The selected species appear with a code in Table 2. The CA was conducted with CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

Finally, the similarity between sites was evaluated with non-metric multidimensional scaling (NMDS). This analysis generated a similarity matrix between substrates for different sampling sites. For this purpose, we used the Bray-Curtis similarity index for the standardised data (log *n*).

Table 3. Mean, SD and ranges of richness and abundance for the four different substrates. *Media, desviación estandar y rango de la riqueza y abundancia en los cuatro diferentes sustratos.*

Measures	Mean ± SD	Minimum	Maximum
Richness S			
Macrophytes	7.9 ± 6.3	0	19
Moss	16 ± 3.8	9	22
Pebbles	14.3 ± 3.8	8	22
Sand	6.7 ± 4.8	0	13
Abundance N			
Macrophytes	102 ± 160.3	0	505
Moss	862.4 ± 717.7	28	2986
Pebbles	213.9 ± 207.4	20	824
Sand	68.8 ± 92.0	0	319

RESULTS

Richness and abundance

We studied a total of 25 406 specimens belonging to 47 species of water beetles assigned to three families of Adephaga (Gyrinidae, Haliplidae, Dytiscidae) and eight Polyphaga (Helophoridae, Hydrochidae, Hydrophilidae, Hydraenidae, Elmidae, Dryopidae, Scirtidae and Curculionidae). The Hydraenidae and Elmidae were the families that were best represented in the study area, with 14 and 15 species, respectively (Table 2a).

Table 3 shows the mean, standard deviation (SD), maximum and minimum values of richness

Table 4. Significant ANOVA values ($p \leq 0.001$) for richness and abundance measures and species abundance with substrate types as factors. *Valores significativos (p ≤ 0.001) de ANOVA para las medidas de riqueza y abundancia y la abundancia de especies con tipo de sustrato como factor.*

Measures	F	p
Richness S	18.68	0.000
Abundance N	18.72	0.000
Elmis aenea	26.42	0.000
Elmis rioloides	9.24	0.000
Limnius perrisi carinatus	9.56	0.000
Hydraena corinna	15.55	0.000
Hydraena brachymera	6.77	0.000
Hydraena testacea	9.30	0.000
Dryops luridus	10.05	0.000

(S) and abundance (N) in each substrate measured in the 20 sampling sites during the annual cycle.

Both the species richness and abundance of aquatic beetles in each type of substrate and in the 20 sampling sites indicated that moss is the preferred substrate. This preference is especially evident from the abundance values, which are substantially higher in moss than in the other substrates: 3441 in the Cadós, 3401 in the Fragoso, 2429 in the Tuño and 7977 in the Deva. Moss was also the substrate with the highest species richness. Although we did not observe clear evidence of dominance, we observed higher species richness on moss in all rivers: 26 species in the Cadós, 27 in the Fragoso, 21 in the Tuño and 29 in the Deva.

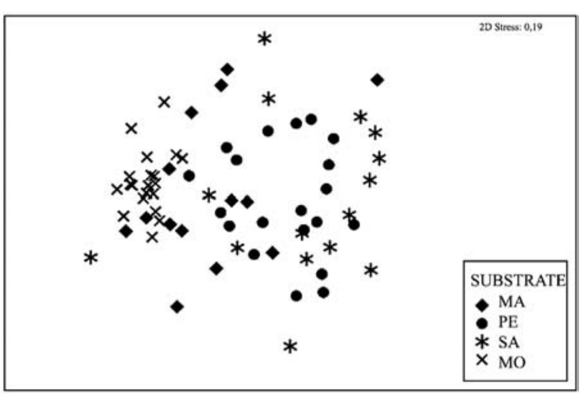

Figure 2. Non-metric multidimensional scaling (NMDS) based on species composition in the different substrates: MA (macrophytes) MO (moss), PE (pebbles) and SA (sand) in the 20 sampling sites. *Escalamiento multidimensional amétrico (NMDS) basado en la composición de especies en los diferentes sustratos MA (macrófitas) MO (musgo), PE (cantos-gravas) y SA (arena) en los 20 puntos de muestreo.*

Richness and abundance showed considerable variability among substrates as demonstrated by ANOVA. We also found significant variability ($p \leq 0.001$) among substrates in the abundance of seven species (Table 4).

The variation in species composition was high (Fig. 2), and the ordering of the sites remained constant during the year. A NMDS among substrates showed that moss (MO) represented a grouping that was relatively separate from the others, especially from sand (SA) and gravel (PE), which appeared to be correlated.

Figure 3 shows the results of the CA. The eigenvalues for axes I-IV were 0.566, 0.295, 0.249 and 0.195, respectively. The correlations for axes III and IV were low ($r < 0.5$), and only axes I and II were used for data interpretation. The cumulative percentage of variance explained for the species-habitats relation was 58.6 % for the first two axes. The first two canonical axes were significant, as shown by a Monte Carlo permutation test ($p = 0.002$). An overall Monte Carlo test also gave a significance of $p = 0.002$.

A large number of species appeared to be correlated with moss, including most species in the genera *Hydraena* and *Elmis*, *Ochthebius heydeni* and *Dryops luridus*, whereas *L. volckmari*, *L. perrisi carinatus* and *Oreodytes sanmarkii alienus* appeared to show an affinity for pebbles.

DISCUSSION

According to the results obtained, based on both the ecological parameters of richness and abundance and the correlation analysis, moss is the preferred substrate for most of the studied species of beetles. Note that several authors have documented the preference of species of aquatic beetles for microhabitats formed primarily by moss (Fernández-Díaz, 2003). According to Passos *et al.* (2003), substrates formed primarily by moss harbour an abundance of water beetles because they offer an abundant food source to herbivorous species.

Moss is associated with different species of Elmidae and Hydraenidae. The ANOVA demonstrated that most of these species also showed

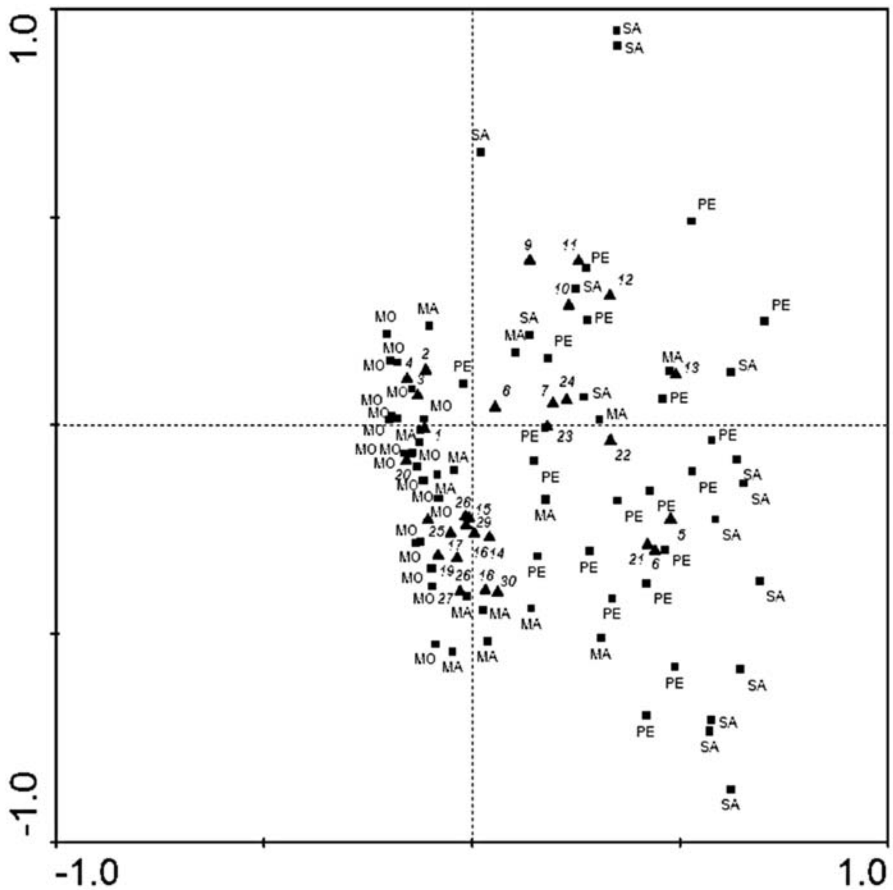

Figure 3. Correspondence analysis (CA) of the species in different microhabitats in the four rivers. *Análisis de Correspondencias (CA) de las especies en los distintos biotopos en los cuatro ríos.*

significant variability between substrates. This result confirms that the differing abundance of these species on different substrates may be indicative of microhabitat preferences.

The second most important substrate was found to be pebbles, which appear to be associated with a number of species. These species include *Limnius volckmari*, *Limnius perrisi carinatus* and *Oreodytes sanmarkii alienus*. These results are consistent with the findings of Garrido (1990), Sáinz-Cantero & Alba-Tercedor (1991), Gayoso *et al*. (1997) and Fernández-Díaz (2003).

The NMDS also found significant differences between the distributions of species on different substrates, especially moss, pebbles and sand. Pebbles and sand appear to be correlated on the side of the scaling diagram opposite that of the moss samples. The moss samples are close to each other in the diagram and form a separate group. This result may imply that the species that prefer this substrate are different from those that prefer the other substrates.

An analysis of the results by families shows that the family Hydraenidae exhibits a clear association of *H. brachymera*, *H. corinna*, *H. sharpi* and *H. testacea* with moss substrate. These results are consistent with the results of other studies conducted in different regions of the Iberian Peninsula. Specifically, Sainz-Cantero *et al*. (1987) have shown that *H. testacea* is primarily associated with moss, and Aguilera & Gerendas (1995) have found that moss is preferred by other species of the genus *Hydraena*, e.g., *H. sharpi*. In a study of the Órbigo River (Leon) by García-Criado *et al.* (1999), *H. hispanica* was captured primarily in moss. However, according to Fernández-Díaz (2003), this species was primarily associated with particular substrates (gravel, stones and sand) and to a lesser extent with macrophytes. In this study, *H. hispanica* appeared to show a preference for moss, as was observed in the Cadós and Fragoso Rivers. However, *H. iberica* appears to be associated with pebbles and moss, as previously shown by Valladares (1989), Fernández-Díaz (2003) and Aguilera & Gerend (1995).

In the Elmidae, the four species in the genus *Elmis* appeared to prefer a moss substrate. According to Sainz-Cantero & Tercedor Alba (1991), several *Elmis* species are very common on mosses and filamentous algae. These data are also consistent with the results of Fernández-Díaz (2003), who has shown that 3 species of *Elmis* (*E. aenea*, *E. rioloides* and *E. maugetii maugetii*) prefer moss. In this context, Berthelemy (1966) and Gayoso *et al.* (1997) consider that *E. aenea* is the *Elmis* species that shows the greatest affinity for a moss substrate.

The species of *Oulimnius* investigated in this study appeared to show no particular preference for any of the substrates tested. The exception was *O. bertrandi*, which was related to moss. Fernández-Díaz (2003) has linked *O. bertrandi* to moss, macrophytes, stone and sand but with a greater abundance on moss, as also shown by Gayoso *et al*. (1997).

Two species of *Limnius*, *L. volckmari* and *L. perrisi carinatus* appeared to be associated with pebbles, as previously observed by Sáinz-Cantero & Alba-Tercedor (1991), Gayoso *et al.* (1997) and Fernández-Díaz (2003).

D. brevis showed a preference for pebbles and macrophytes, as previously indicated by Gayoso *et al.* (1997) and Fernández-Díaz (2003). *E. parallepepidus* and *E. angustatus* were associated with pebbles and sand. According to Olmi (1969) and Fernández-Díaz (2003), these species are related to pebble substrates and submerged vegetation.

This study has enabled us to determine certain patterns of distribution of water beetle species in the rivers studied as a function of microhabitat. Note that moss was the preferred substrate for most species, particularly for the families Elmidae and Hydraenidae. A possible explanation for this preference is that the substrate provides stability, protection and food for these species, which appear to have adapted to fast-current areas covered primarily by this type of substrate (Nilsson, 1996). In this sense, this study highlights the importance of knowing the structure of rivers, which are composed of different microhabitats, each with its own characteristics and ecological functions. In this way, we can adequately conserve aquatic habitats and thus facilitate the maintenance of their biodiversity.

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