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Distribution and taxonomic notes of *Eunotia* Ehrenberg 1837 (*Bacillariophyceae*) in rivers and streams of Northern Spain

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ABSTRACT

Distribution and taxonomic notes on *Eunotia* Ehrenberg 1837 (*Bacillariophyceae*) in rivers and streams of Northern of Spain

Between August and October of 2003, a total of 397 samples of epilithic diatoms were collected from different stations belonging to the control network of the North Hydrographical Confederation of Spain (CHN). One hundred and seventeen sites with *Eunotia* diatom species were found. Galicia, with 78 localities, was the region with higher representation. Eighteen taxa of the *Eunotia* genus were identified, 17 of them to the species level. *Eunotia minor* (Kützing), Grunow in van Heurck and *Eunotia implicata* Nörpel, Lange-Bertalot & Alles were the most common species, present in 59 % and 50.4 % of the samples, respectively. Conductivity and water acidity were the most important factors in determining *Eunotia* distribution in the studied area. The main objective of this work was to contribute to the knowledge, from the taxonomic and distribution viewpoints, of the diatoms of the genus *Eunotia* Ehrenberg that were found in the Iberian Peninsula. It is the first comprehensive study on the *Eunotia* genus of northern Spain.

Key words: Diatoms, *Eunotia*, distribution, ecology, Northern Spain Basins.

RESUMEN

Distribución y notas taxonómicas de* Eunotia *Ehrenberg 1837* (Bacillariophyceae) *en ríos y arroyos del norte de España

Entre agosto y octubre del 2003, se recogieron un total de 397 muestras de diatomeas epilíticas en distintas estaciones pertenecientes a la red de control de la Confederación Hidrográfica del Norte (CHN). En un total de 117 localidades se encontraron diatomeas del género Eunotia*. Galicia, con 78 estaciones, es la región con más representación. 18 taxones del género* Eunotia *han sido determinados, 17 de ellos a nivel específico.* Eunotia minor *(Kützing) Grunow in van Heurck y* Eunotia implicata *Nörpel, Lange-Bertalot & Alles han sido las especies con más representación encontrándose en el 59 % y 50.4 % respectivamente de las muestras. La conductividad y la acidez del agua han resultado ser factores importantes que determinan la distribución de especies del género* Eunotia *en la zona de estudio. El objetivo principal de este trabajo es contribuir al conocimiento desde el punto de vista taxonómico y de distribución de las especies del género* Eunotia *Ehrenberg en la península Ibérica. Corresponde al primer trabajo exhaustivo del género* Eunotia *en el norte de España.*

Palabras clave: *Diatomeas,* Eunotia, *distribución, ecología, cuencas del Norte de España.*

INTRODUCTION

Eunotia is the best represented genus of the *Eunotiaceae* family (*Bacillariophyceae*) in the Iberian inland waters. This is essentially a freshwater diatom genus frequently associated with acidic waters (Slàdeček, 1986; Patrick, 1948; Descy, 1979; Alles *et al*., 1991; Cameron, 1995; Carter & Flower, 1988; Pierre, 1996) and oligotrophic or dystrophic situations (Patrick & Reimer, 1966; Descy, 1979; Koyabaysi *et al*., 1981; Lange-Bertalot & Metzeltin, 1996).

The diatom genus *Eunotia* is unusual among raphid diatoms in that the raphe slits are short, often rudimentary, and not integrated into the primary pattern centre; instead, they lie to one side of it (Mann, 1984; Round *et al*., 1990). This and other characteristics, particularly the presence of rimoportulae (labiate processes), are consistent with the hypothesis that *Eunotia* is a basal lineage within the raphid group (Mann *et al*., 2003). In girdle view they have a characteristic rectangular shape. In valve view they are asymmetrical along the apical axis. Usually the dorsal margin is more or less convex while the ventral margin is straight or somewhat concave.

Eunotia is a large genus that comprises around 200 species (Van Landingham, 1969). The genus has a world-wide distribution, although numerous species are restricted to tropical areas, due to their environmental water preferences: low pH and conductivity being the most abundant and diverse genus in these areas (Coste & Ricard, 1982; Torgan & Delani, 1988; De Oliveira & Steinitz-Kannan, 1992; Metzeltin & Lange-Bertalot, 1998; Sala *et al*., 2002; Díaz-Castro *et al*., 2003).

Despite its abundance in freshwater habitats, literature concerning *Eunotia* species is scant. There are few studies about the specie's valve structure; we can find some references on fine structure for 52 *Eunotia* species in the bibliographic compilation of Gaul *et al*. (1993). Some studies have pointed out that the difficulties on the identification of the species of *Eunotia* are due to its great variability in the size and shape of the valve throughout the life cycle (Mayama, 1992; Mayama & Kobayasi, 1991). This morphological variation is associated with size reduction during the vegetative cell division (Round, 1972), or appears to be more a function of the clone genotype than the environmental conditions (light intensity, temperature, and agitation) (Steinman & Sheath, 1984). On the other hand, there are unsolved taxonomical problems because of resemblances between different taxa (Mayama, 1997), and those difficulties increase with very small species (Petersen, 1950). However, Krammer & Lange-Bertalot (1991) have done a modern and exhaustive revision of the group on materials collected in European temperate environments, which has made the identification of some *Eunotia* species easier.

From an historical point of view, papers on diatoms in the studied area are scarce, some

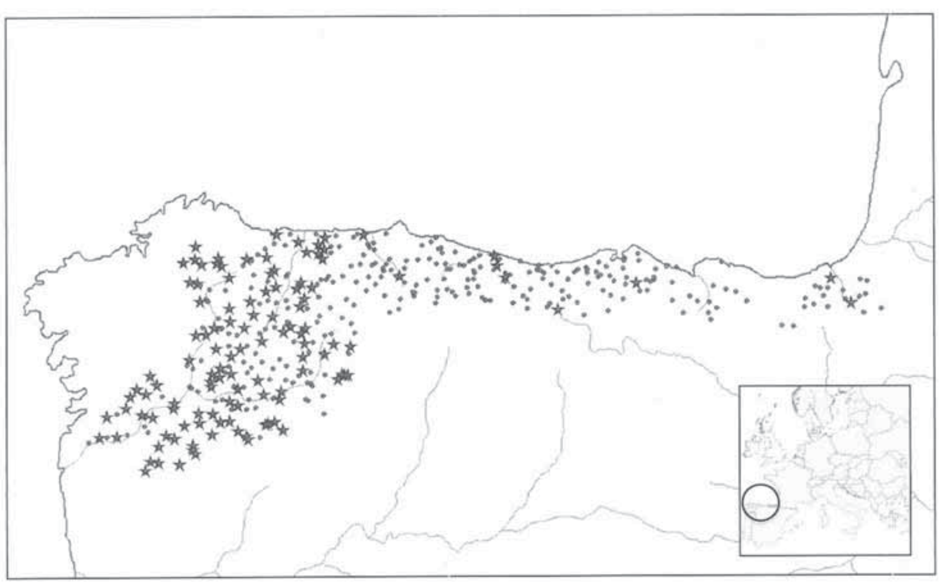

Figure 1. Geographical distribution of the 397 sites where epilithic diatoms were sampled (filled circles) and the 117 localities where *Eunotia* (*Bacillariophyceae*) species were found (filled stars). *Localización geográfica de las 397 localidades donde se han muestreado diatomeas epilíticas (círculos llenos) y las 117 localidades donde se han encontrado especies del género* Eunotia (Bacillariophyceae) *(estrellas llenas).*

disperse studies have been published so far by Trúan (1885), West (1911), and Gonzalez-Guerrero (1942). The first important contribution to the diatom flora of this area was performed by Margalef (1946, 1950, 1956a, 1956b, 1958). In recent years, doctoral theses (Alonso-Fernández, 1989) and reports to assess running water quality by using diatoms (Antelo, 1991; Ector *et al*., 1992; Aboal *et al*., 2003b) were carried out in this zone, increasing the knowledge of benthic diatom flora. Nevertheless, the genus *Eunotia* has not been studied in detail in this region.

The aim of this study is to present a taxonomic description of the *Eunotia* species found and to give an account of their ecological preferences and distribution in the studied area; it represents the first comprehensive *Eunotia* study of northern Spain.

STUDY AREA AND METHODS

The studied area is located inside the Water Quality Surveillance Network of the North Hydrographical Confederation of Spain (CHN). It comprises a total length of 9000 km with an area of 38,384 km^2. It includes all the rivers and streams which run into the Cantabrian Sea and the Atlantic Ocean in the North of Portugal, excluding those rivers which flow entirely in Galicia and the Basque Country. Diatom samples were collected in different ecotypes due the habitats heterogeneity in the studied basins. The sites were determined taking into account the main rivers in order to have a great representation of geographic areas and eco-regions. Miño, Nalón, Navia, Nervión and Limia were the best represented basins.

A single sample per site was collected between August and October 2003 in 397 stations (Fig.1). Epilithic diatom samples were taken and treated and permanent diatom slides were prepared following standard methods (European Committee for Standardization 2003, 2004). At least 5 small submerged boulders from the main flowing water and well-lighted part of the river were brushed to collect diatoms. Samples were preserved in 4 % formaldehyde. Organic matter was eliminated by oxidation with hydrogen peroxide to obtain clean frustule suspension. Diluted hydrochloric acid was added to remove the calcium carbonate in order to avoid late precipitation, which could make frustule observation and counting difficult. After distilled water cleanings, permanent slides were then mounted on cover slips with *Naphrax*® for examination by light microscopy (LM) using an oil-immersion lens at 1000x magnification. A complete taxa list was made and a minimum of 400 valves from each slide were counted to calculate species relative abundance (Prygiel & Coste, 1993). These data were processed with the software *OMNIDIA,* version 4.1 (Lecointe *et al.* 1993, 1999), which provided the resulting values for 13 diatoms water quality indices per each inventory. In order to estimate the pollution level of the sampling sites we chose the Specific Pollution Sensitivity Index (SPI, Coste in Cemagref, 1982). The SPI is considered a general pollution evaluator. This index is based on the Zelinka & Marvan formula (Zelinka & Marvan, 1961), which is a weighted average of species indicator values. This index has been chosen because SPI has usually been considered as the reference index for long time (Descy & Coste, 1991). It has already been tested in the Iberian Peninsula rivers (e.g. Sabater, 2000; Almeida, 2001; Gomà *et al.,* 2004; 2005) with successful results and it takes into account all known taxa. The index range is from 1 to 20, 1 being the worst quality and 20 the best.

Samples with a total percentage of *Eunotia* species higher than 15 % were examined by scanning electron microscopy (SEM). SEM was carried out with a Hitachi S-2300 operated at 10 Kv. Photographs were taken of taxa with LM or SEM when possible. Conductivity and pH were measured *in situ* using multi-parametric sensors.

To describe the pH preferences of each *Eunotia* species identified, we used the pH classification of van Dam *et al*. (1994) which is derived from Hustedt (1938-1939), who used the term acidobiontic for diatoms having their pH optimum at pH < 5.5; acidophilus when mainly occurring at pH < 7; circumneutral for diatoms having their pH optimum around 7; alkaliphilous when mainly occurring at pH > 7 and alkalibiontic when exclusively occurring at

Table 1. List of the collecting sites in the studied area from which *Eunotia* species had been found with abundances over 5 %, indicating the site code, river, region, basin, and village. The sites are ordered from higher to lower abundance in *Eunotia* species. *Lista de las localidades estudiadas donde las especies de* Eunotia *presentaron una abundancia superior al 5 %, indicando el código de estación, río, región, cuenca y localidad. Las localidades están ordenadas de mayor a menor abundancia en especies de* Eunotia.

SITE CODE	RIVER	REGION	BASIN	VILLAGE	% *EUNOTIA*
MI-078	CAYOSO	GALICIA	MIÑO	CARBALLEDA	69.70
MI-004	TAMOGA	GALICIA	MIÑO	ABADIN	56.13
MI-500	LABRADA	GALICIA	MIÑO	GUITIRIZ	51.34
EO-001	CUBILLEDO	GALICIA	EO	BALEIRA	42.43
MI-117	RUBIN	GALICIA	MIÑO	PUEBLA DEL BROLLON	38.06
MI-002	SAN MARTIN	GALICIA	MIÑO	VILLALBA	30.02
MI-005	TAMOGA	GALICIA	MIÑO	VILLALBA	28.88
MI-138	ARNOYA	GALICIA	MIÑO	BAÑOS DE MOLGAS	27.91
NA-027	AVIOUGA	ASTURIAS	NAVIA	IBIAS	25.62
NAL-029	PUMAR	ASTURIAS	NALON	CANGAS DE NARCEA	24.38
NE-001	NEGRO	ASTURIAS	NEGRO	LUARCA	23.88
MI-049	LA FRAGUA	CASTILLA-LEÓN	MIÑO	VILLAGATON	18.95
MI-060	LA VEGA	CASTILLA-LEÓN	MIÑO	CANDIN	15.78
MI-001	SAN MARTIN	GALICIA	MIÑO	XERMADE	15.57
LI-004	VIDUEIRO	GALICIA	LIMIA	RAIRIZ DE VEIGA	15.16
NE-002	NEGRO	ASTURIAS	NEGRO	LUARCA	15.07
MI-151	TEA	GALICIA	MIÑO	COVELO (O)	13.60
MI-061	ANCARES	CASTILLA-LEÓN	MIÑO	CANDIN	13.52
LI-002	NOCELO	GALICIA	LIMIA	XINZO DE LIMIA	13.17
NA-025	MEIRO	ASTURIAS	NAVIA	COAÑA	12.27
MI-088	CONSO	GALICIA	MIÑO	VILARIÑO DE CONSO	9.83
MI-003	LADRA	GALICIA	MIÑO	VILLALBA	9.56
MI-057	CUA	CASTILLA-LEÓN	MIÑO	PERANZANES	8.37
ES-003	ESVA	ASTURIAS	ESVA	TINEO	7.98
MI-087	CAMBA	GALICIA	MIÑO	VIANA DO BOLO	7.66
MI-132	ARENTEIRO	GALICIA	MIÑO	CARBALLIÑO (O)	7.42
MI-090	REQUEJO	GALICIA	MIÑO	VEIGA (A)	7.42
MI-050	RIAL	CASTILLA-LEÓN	MIÑO	TORRE DEL BIERZO	7.31
LI-007	SALAS	GALICIA	LIMIA	MUIÑOS	7.16
MI-119	CINSA	GALICIA	MIÑO	MONFORTE DE LEMOS	6.55
MI-147	CIERVES	GALICIA	MIÑO	RIBADAVIA	5.95
MI-089	CONSO	GALICIA	MIÑO	VILARIÑO DE CONSO	5.72
MI-133	VIÑAO	GALICIA	MIÑO	IRIXO (O)	5.50
NA-020	VILLANUEVA	ASTURIAS	NAVIA	VILLANUEVA DE OSCOS	5.15

Table 2. Alphabetical list of the studied sites where the most relevant *Eunotia* species were identified (abundances higher than 5 % in at least one sample), indicating the site code, the values of the environmental variables, the SPI value, and the relative abundance of each *Eunotia* species. For the *Eunotia* code see Table 3. *Lista en orden alfabético de las localidades estudiadas donde las especies mas relevantes de Eunotia fueron identificadas (abundancias superior al 5 % en al menos una muestra). Indicando el valor de las variables estudiadas, valor del IPS y la abundancia relativa de cada especie de Eunotia. Para saber el código Eunotia ver Tabla 3.*

CODE	ALTITU. (m)	SLOPE (%)	TEMP. (°C)	DIS. OXI. (mg./l)	COND. (μS/cm)	pH	SPI	EBIL	EEXI	EFOR	EIMP	EINC	EMIN	EPEC	ESUB
EO-001	624	2.38	15.03	9.11	36.00	6.79	19.50	—	—	—	19.11	—	20.35	—	—
ES-003	298	2.94	18.15	8.22	35.00	4.70	18.90	—	0.44	—	1.11	—	—	—	6.43
LI-002	631	1.25	14.07	6.69	111.60	6.50	14.00	—	—	3.17	5.85	—	3.90	—	—
LI-004	638	0.87	12.94	7.70	58.70	6.77	17.50	1.47	—	1.22	8.80	0.49	1.71	—	—
LI-007	703	0.79	15.80	7.35	34.60	6.57	18.60	—	1.19	—	2.63	1.43	0.24	—	1.43
MI-001	457	0.62	14.47	9.88	42.00	6.37	18.50	—	2.43	—	8.76	4.38	—	—	—
MI-002	484	0.04	14.45	9.31	51.00	5.30	19.30	—	—	—	15.58	7.00	0.45	—	—
MI-003	424	0.23	14.21	8.62	61.00	5.56	17.90	0.25	0.49	—	4.66	—	0.49	3.68	—
MI-004	440	1.03	13.97	9.41	34.00	3.75	18.90	—	—	—	—	—	25.48	—	29.68
MI-005	419	0.33	14.84	8.06	46.00	6.73	18.70	6.68	3.10	5.73	1.91	4.77	6.68	—	—
MI-049	851	4.75	11.23	10.39	93.00	5.94	19.20	—	15.96	—	0.25	2.24	0.50	—	—
MI-050	783	2.74	13.60	9.23	40.00	6.47	15.70	—	—	—	—	—	7.31	—	—
MI-057	1075	2.32	12.00	9.98	11.20	5.57	19.40	1.40	—	—	—	—	5.58	—	1.40
MI-060	1086	3.71	11.60	10.04	17.60	6.21	19.50	—	—	—	—	—	12.38	—	—
MI-061	824	2.20	13.00	10.06	23.00	5.59	19.30	—	2.33	—	—	—	11.19	—	—
MI-078	409	1.83	15.00	9.24	518.00	4.36	19.30	—	69.70	—	—	—	—	—	—
MI-087	734	0.96	18.85	8.79	21.80	6.75	19.40	—	0.48	0.24	3.59	2.87	0.48	—	—
MI-088	1080	3.33	14.20	6.35	17.70	7.05	16.90	—	—	—	2.21	—	6.39	—	—
MI-089	708	3.33	15.25	7.90	22.50	6.46	17.30	—	0.23	—	1.37	—	4.12	—	—
MI-090	968	2.74	13.46	7.98	23.00	6.42	18.30	—	0.24	—	2.39	—	4.55	—	—
MI-117	481	2.71	14.42	9.46	39.00	6.68	19.50	—	1.89	—	34.99	1.18	—	—	—
MI-119	275	0.64	17.10	6.55	105.20	4.66	15.90	0.49	0.49	0.24	1.21	0.97	2.18	—	—
MI-132	396	0.15	14.24	8.49	30.20	6.22	15.90	—	—	—	6.55	—	—	—	0.87
MI-133	522	1.79	12.49	8.59	38.60	7.31	18.50	—	—	—	3.67	—	—	—	0.46
MI-138	531	0.51	15.48	8.07	28.70	5.95	17.50	7.21	1.86	—	16.51	—	2.33	—	—
MI-147	105	6.53	17.83	8.49	25.90	6.36	19.80	—	—	—	2.38	—	—	—	—
MI-151	475	9.67	16.39	8.79	19.30	6.26	19.80	—	—	—	—	—	10.02	—	0.95
MI-500	472	0.43	14.44	9.47	38.00	6.00	19.70	7.54	10.71	0.49	16.06	9.98	—	—	6.33
NA-020	534	2.52	14.20	9.04	50.60	5.78	18.40	—	—	—	—	—	5.15	—	—
NA-025	205	1.93	16.70	8.74	57.60	5.39	18.20	—	2.45	—	—	—	—	5.32	—
NA-027	665	3.89	13.11	9.83	16.00	5.82	19.20	—	—	—	—	—	24.38	—	—
NAL-029	572	3.06	13.13	9.35	27.00	6.75	16.70	—	—	—	—	—	24.38	—	—
NE-001	211	2.94	15.39	9.81	30.00	3.67	16.80	—	—	—	—	—	23.88	—	—
NE-002	25	1.00	17.14	8.05	44.00	5.36	18.70	—	2.74	—	—	4.34	7.99	—	—

Table 3. Alphabetical list of the *Eunotia* taxa identified in the studied region, indicating the number of localities where they have been found and the number of the previous references for each one in the Iberian Peninsula and in the studied area. *Lista en orden alfabético de los taxones de* Eunotia *identificados en la zona de estudio indicando el número de localidades donde se han encontrado y el número de citas previas para cada una en la península Ibérica y en el área de estudio.*

Taxon Code	TAXON	N. Sites	N. References	
			Iberian peninsula	Studied area
EBIL	*Eunotia bilunaris* (Ehrenberg) Mills	15	42	13
EBLI	*Eunotia bilunaris* var. *linearis* (Okuno) Lange-Bertalot & Nörpel	2	1	1
EBMU	*Eunotia bilunaris* var. *mucophila* Lange-Bertalot & Nörpel	3	4	0
EEXI	*Eunotia exigua* (Brebisson ex Kützing) Rabenhorst	22	22	5
EFOR	*Eunotia formica* Ehrenberg	11	8	3
EIMP	*Eunotia implicata* Nörpel, Lange-Bertalot & Alles	59	2	2
EINC	*Eunotia incisa* Gregory	21	10	1
EUIN	*Eunotia intermedia* (Krasske ex Hustedt) Nörpel & Lange-Bertalot	4	1	1
EMIN	*Eunotia minor* (Kützing) Grunow in Van Heurk	69	23	4
EUPA	*Eunotia paludosa* Grunow	1	1	0
EPEC	*Eunotia pectinalis* (Dillwyn, O.F. Müller, Kützing) Rabenhorst	3	64	10
EPUN	*Eunotia pectinalis* var. *undulata* (Ralfs) Rabenhorst	3	11	5
EPRA	*Eunotia praerupta* Ehrenberg	1	17	5
ESOL	*Eunotia soleirolii* (Kützing) Rabenhorst	1	1	0
ESUB	*Eunotia subarcuatoides* Alles, Nörpel & Lange-Bertalot	13	2	2
ETEN	*Eunotia tenella* (Grunow) Hustedt	13	16	2
EVEN	*Eunotia veneris* (Kützing) De Toni	1	5	1

pH > 7. Diatoms with no apparent optimum in pH values are considered indifferent in this classification, nevertheless diatoms which are insensitive to pH are extremely rare, even diatoms which occur over a very wide pH-range are rarely indifferent.

A principal component analysis (PCA) was performed to classify the sample stations according to their environmental variables (conductivity, altitude, water temperature, dissolved oxygen, slope, pH, and pollution levels as assessed with the SPI diatom index). In order to relate the *Eunotia* species data to these environmental variables, a Canonical Correspondence Analysis (CCA) was carried out. Both the PCA and the CCA were computed with the MVSP version 3.13n software. Other physical and chemical characteristics of the sampled sites were not available for our sampling stations to improve the CCA and PCA analysis results. These analyses were carried out only in those samples where the most relevant *Eunotia* species were identified (abundances higher than 5 % in at least one sample) (Table 2).

The maps were drawn using the ArcMap software (ESRI®).

RESULTS

Up to 117 sites with *Eunotia* species were found (Fig. 1). However, only in 34 was their abundance higher than 5 % (Table 1). Galicia, with 78 localities, was the region with higher representation, followed by Asturias with 21 and Castilla-León with 14; Cantabria and Navarra with 2 sites each and the Basque Country with none, are less represented regions. The basin with most occurrences of *Eunotia* species was Miño, followed by Navia, Nervión, and Limia.

Up to 17 taxa were identified at a specific level in the studied area. The taxa identified have different occurrence and distribution; only 8 of the identified *Eunotia* species have abundances higher than 5 % in at least one sample and 6 were found with abundances never higher than 1 %. Table 2 shows the most relevant *Eunotia* species identified (abundances higher than 5 % in at least one sample) and figure 2 shows the distribution of the best represented *Eunotia* species in the studied area. All the species except *E. soleirolii* were previously reported in the floristic list of Aboal *et al.* (2003a). *E. soleirolii* was mentioned for the first time in Goma *et al.* (2004). The number of localities for each taxon and the number of its previous references in the Iberian Peninsula and in the studied area are showed in Table 3.

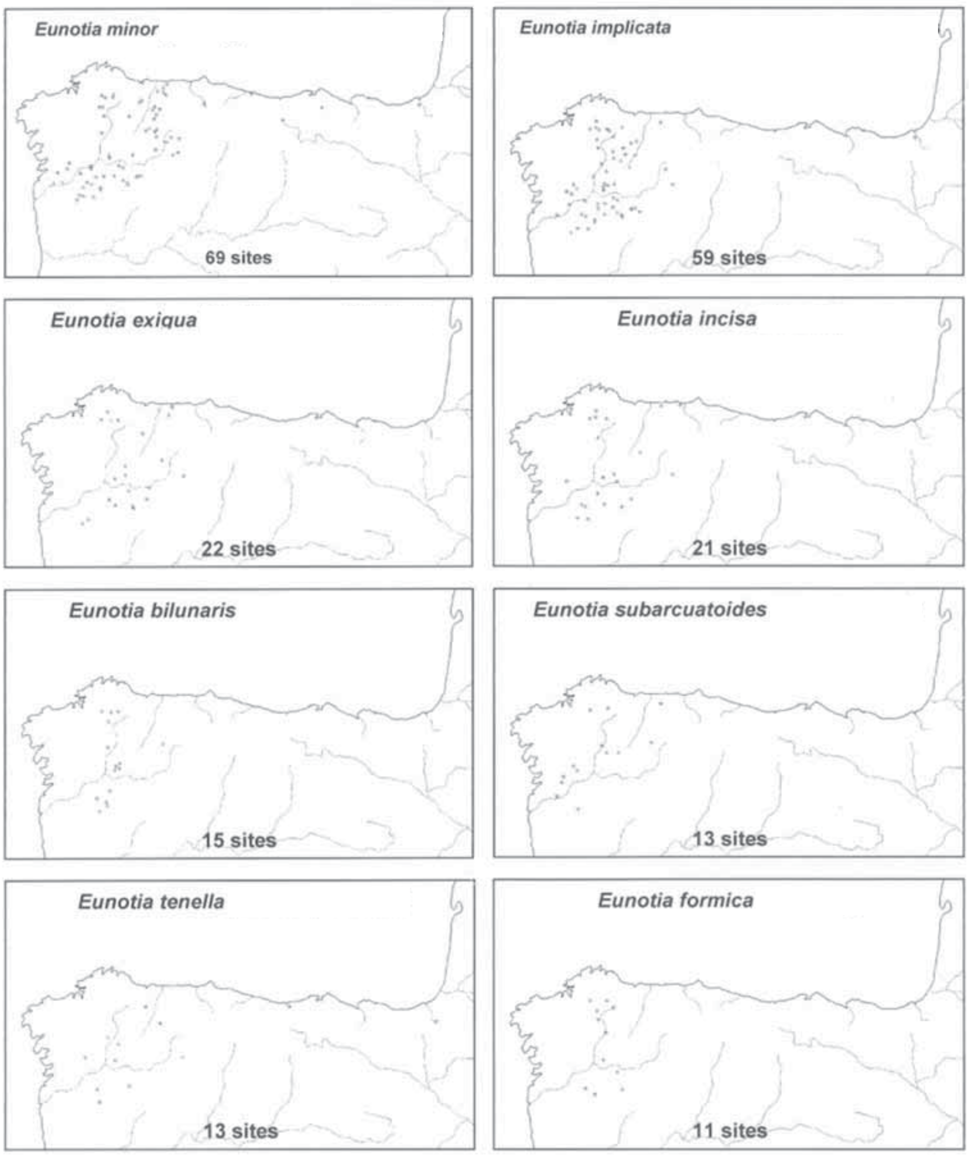

Figure 2. Distribution of the more frequent *Eunotia* (*Bacillariophyceae*) species in the studied basins. *Distribución de las especies del género* Eunotia (Bacillariophyceae) *más frecuentes en las cuencas estudiadas.*

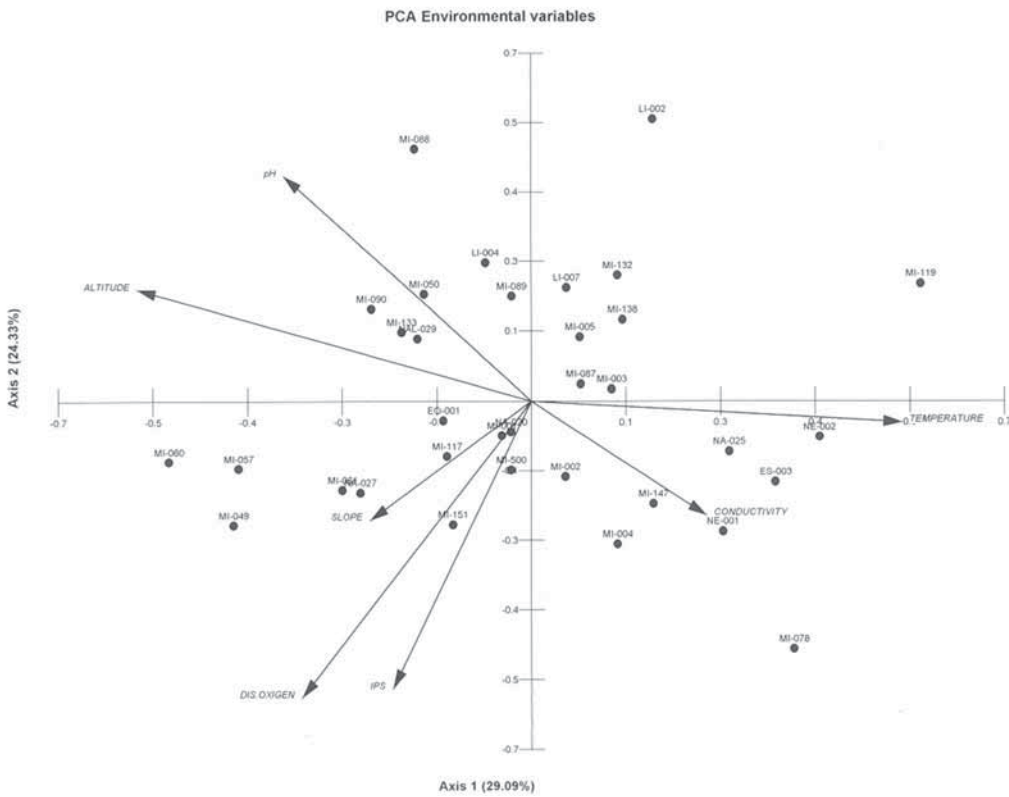

Figure 3. Results of the principal component analyses (PCA) of the environmental variables in the sites where the most relevant *Eunotia* species were identified (abundances higher than 5 % in at least one sample). The percentage of variance explained by the PCA axes is indicated in brackets. *Resultados del análisis de componente principales (ACP) de las variables ambientales en las localidades donde las especies de* Eunotia *más relevantes fueron identificadas (abundancias superiores al 5 % en al menos una muestra). El porcentaje de varianza explicada por cada eje del ACP se indica entre paréntesis.*

Observations and descriptions

Eunotia bilunaris (Ehrenberg) Mills 1934 (Fig. 3: A-B, Fig. 4: D)

Krammer & Lange-Bertalot 1991, 180, Fig. 137, 139 (1-4); Metzeltin & Witkowski 1996, 98, Fig. 33:14; Lange-Bertalot & Metzeltin 1996, 136, Fig.9: 1-4

This species is characterized by the lunate appearance of its valve, the small terminal nodules and its indistinct raphe. It often produces abnormal forms which have the margins indented in several ways. These have been described as varieties. Striae: 13-18/10 µm. Length: 20-150 µm. Breadth: 3-6 µm. This species is widely distributed in waters with a low mineral content; commonly found in acid waters, but may occur in slightly alkaline waters (Patrick & Reimer, 1966). Two optima in pH values which are almost equally developed, one around 4.0-4.3, the other one around or beyond pH 7 (Alles *et al.*, 1991). This taxon has no apparent optimum in pH (Van Damm *et al.*, 1994).

In the studied area, the species was collected in habitats with pH: 4.7-7.5, conductivity: 11.2-132 µS/cm, altitude: 275-1075 m asl, SPI: 12.3-19.7. Its maximum abundance was between 6.7 %-7.5 % in the Miño basin with pH: 5.9-6.7, conductivity 28.7-46 µS/cm, altitude: 419-531 m asl, SPI: 17.5-19.7

Eunotia bilunaris var. ***linearis*** (Okuno) Lange-Bertalot & Nörpel 1991

Krammer & Lange-Bertalot, 1991, 180: Fig. 137: 13-16.

Valves very slender, elongated with parallel margins, slightly bent to almost straight, often wavily-crooked, more or less capitately widened on the ends, half-circular rounded on the apical valve margin. Transapical striae dense and regular. Raphe in the valve surface with a stroke-shaped appendage, which begins on the raphe end and runs back proximally, parallel with the apical axis. Striae: 9-12/10 µm. Length: 44-205 µm. Breadth: 3.5-5.5 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

This taxon is not very well represented in this region, it was found only in two sites with an abundance < 0.5 %.

Eunotia bilunaris var. ***mucophila*** Lange-Bertalot & Nörpel 1991

Krammer & Lange-Bertalot, 1991, 180: Fig. 138: 20-24.

Cells in girdle view rectangular; laterally asymmetrical (lunate) in valve view along the apical axis. Valves bent, gradually narrowing towards rounded apices. Ventral margin concave and dorsal margin convex. Valves less arcuate in smallest individuals. Striae uniseriate, divergent

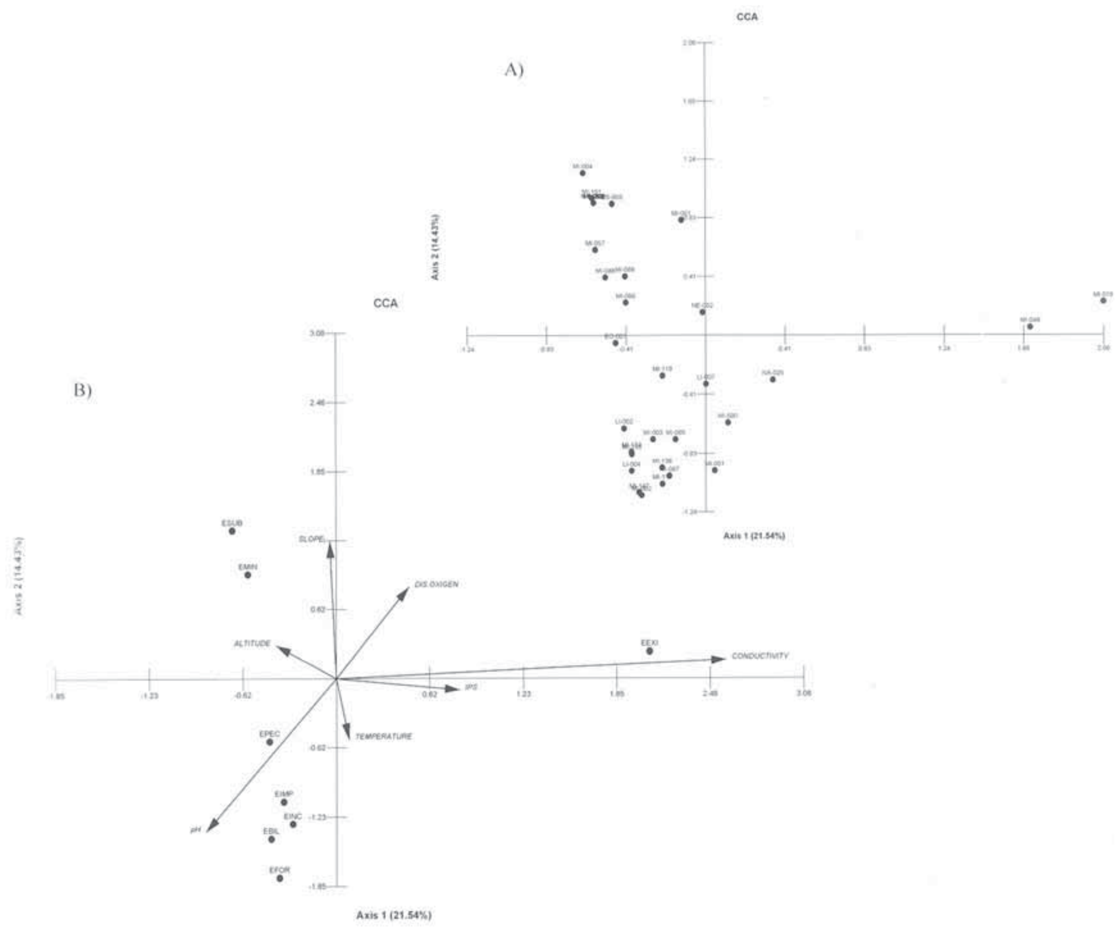

Figure 4. Results of a Canonical Correspondence Analysis (CCA) based on the most relevant *Eunotia* species (abundances higher than 5 % in at least one sample) with respect to environmental variables. Sampled sites (A) and *Eunotia* species (B) (abbreviation codes are given in Table 3) are displayed on the CCA axis in separated plots. The percentage of variance explained by axis of the CCA is indicated in brackets. *Resultados del análisis canónico de correspondencias (ACC) de las especies más relevantes de* Eunotia *(abundancias superiores al 5 % en al menos una muestra) respecto a las variables ambientales. Las localidades (A) y las especies de* Eunotia *(B) (la abreviatura de los códigos están en la Tabla 3) están representados en ejes de ACC en gráficos separados. El porcentaje de varianza explicada por cada eje del ACC se indica entre paréntesis.*

from the ventral margin to the dorsal one. Striae: 20-28/10 µm. Length: 10-70 µm. Breadth: 1.9-2.7 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

This taxon is not very well represented in this region, it was found only in three sites with abundance lower than 0.5 %.

Eunotia exigua (Brébisson ex Kützing) Rabenhorst 1864 (Fig. 3: G-I, Fig. 4: E)

Krammer & Lange-Bertalot 1991, 199, Fig. 153: 5-43; Hustedt 1957, 240; Hustedt 1959, 285, Fig. 751: a-r; Schoeman 1973, 75; Patrick *et al*. 1975, 215, Fig. 13: 17-18; Patrick & Reimer, 1966, 215, 13:17-18.

Ventral margin concave. Dorsal margin usually more strongly convex. Margins of the valve usually not parallel. Valve gradually broadened toward the centre. Dorsal margin strongly reflexed to form capitate, more or less truncate ends. Terminal nodules on ventral margin at the apices of the valve. Striae parallel, 20-25/10 µm. Length: 10-26 µm. Breadth: 2-4 µm. Often found associated with mosses in acid water of low mineral content (Patrick & Reimer, 1966). This species tolerates high and abrupt variations of pH values (Alles *et al*., 1991). Acidobiontic: optimal occurrence at pH < 5.5 (Van Damm *et al*., 1994).

In the studied area the species was collected in habitats with pH: 4.4-7.4, conductivity: 17.4-518 µS/cm, altitude: 25-968 m asl, SPI: 15.9-19.7. Its highest abundance is 69.7 % in the river Cayoso (Miño basin) in Carballeda (Galicia) with pH: 4.4, conductivity 518 µS/cm, altitude: 409 m asl, SPI: 19.3.

Eunotia formica Ehrenberg 1843 (Fig. 3:T)

Krammer & Lange-Bertalot 1991, 209, Fig. 152: 8-12A; Hustedt 1930, 186, Fig. 257; Hustedt 1957, 242; Hustedt 1959, 308, Fig. 775; Germain 1981, 90, Fig. 31: 29-31; Lange-Bertalot & Metzeltin 1996, 144, Fig. 13: 1-2.

This species is easily recognized by its wedge-capitated ends and the swelling at the centre of the valve. Valve usually linear, sometimes slightly bent. Striae parallel, often not equidistant; in many cases broken near the ventral margin. Striae: 6-12/10 µm. Length: (12)35-200(300) µm. Breadth: 7-14 µm. It is typically found in acid to circumneutral soft waters, standing or usually slow-flowing waters (Patrick & Reimer 1966). Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

In the studied area the species was collected in habitats with pH: 4.7-7.3, conductivity: 21.8-177 µS/cm, altitude: 275-734 m asl, SPI: 14-19.7. Its highest abundance is 5.7 % in river Tamoga (Miño basin) in Villalba (Galicia) with pH: 6.7, conductivity 46 µS/cm, altitude: 419 m asl, SPI: 18.7.

Eunotia implicata Nörpel, Lange-Bertalot & Alles 1991 (Fig. 3: C, Fig. 4: B, I)

Krammer & Lange-Bertalot 1991, 197, Fig. 143: 1-9A, Fig. 144: 6.

Ventral margin slightly concave. Dorsal margin distinctly convex, often with two shallow ondulations. Valve somewhat narrower at the ends than at the centre. Apices of the valve rounded; slightly narrower but not distinctly set off from the main body of the valve. Terminal nodules near, but not at the ends of the valve; distinct but not large. Striae straight; almost perpendicular to ventral margin at the centre of the valve, but sometimes curved at the ends. Striae, usually 14-16/10 µm at the centre, to about 22 in 10 µm at the ends. Length: 20-40 µm. Breadth: 3-6 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

In the studied area the species was collected in habitats with pH: 4.3-7.9, conductivity: 4.17-720 µS/cm, altitude: 76-1356 m asl, SPI: 12.3-20. Its highest abundance is between 15.6 %-35 % in conditions with pH: 5.3-6.8, conductivity 28.7-51 µS/cm, altitude: 472-624 m asl, SPI: 19.3-19.7.

Eunotia incisa Gregory 1854 (Fig. 3: O-R, Fig. 4: C,K)

Krammer & Lange-Bertalot 1991, 221, Fig. 161: 8-19, Fig. 162: 1-2, Fig. 163: 1-7; Metzeltin & Witkowski 1996, 100, Fig.34:1; Lange-Bertalot & Metzeltin 1996, 150, Fig. 8-15.

This species is characterized by the apices of the valve undifferentiated from the main body of the valve and by the very fine striae. Frustules in girdle view rectilinear. Frustules in valve view with straight ventral margin and convex dorsal margin. Apices of the valve usually acute, sometimes rounded. Striae more

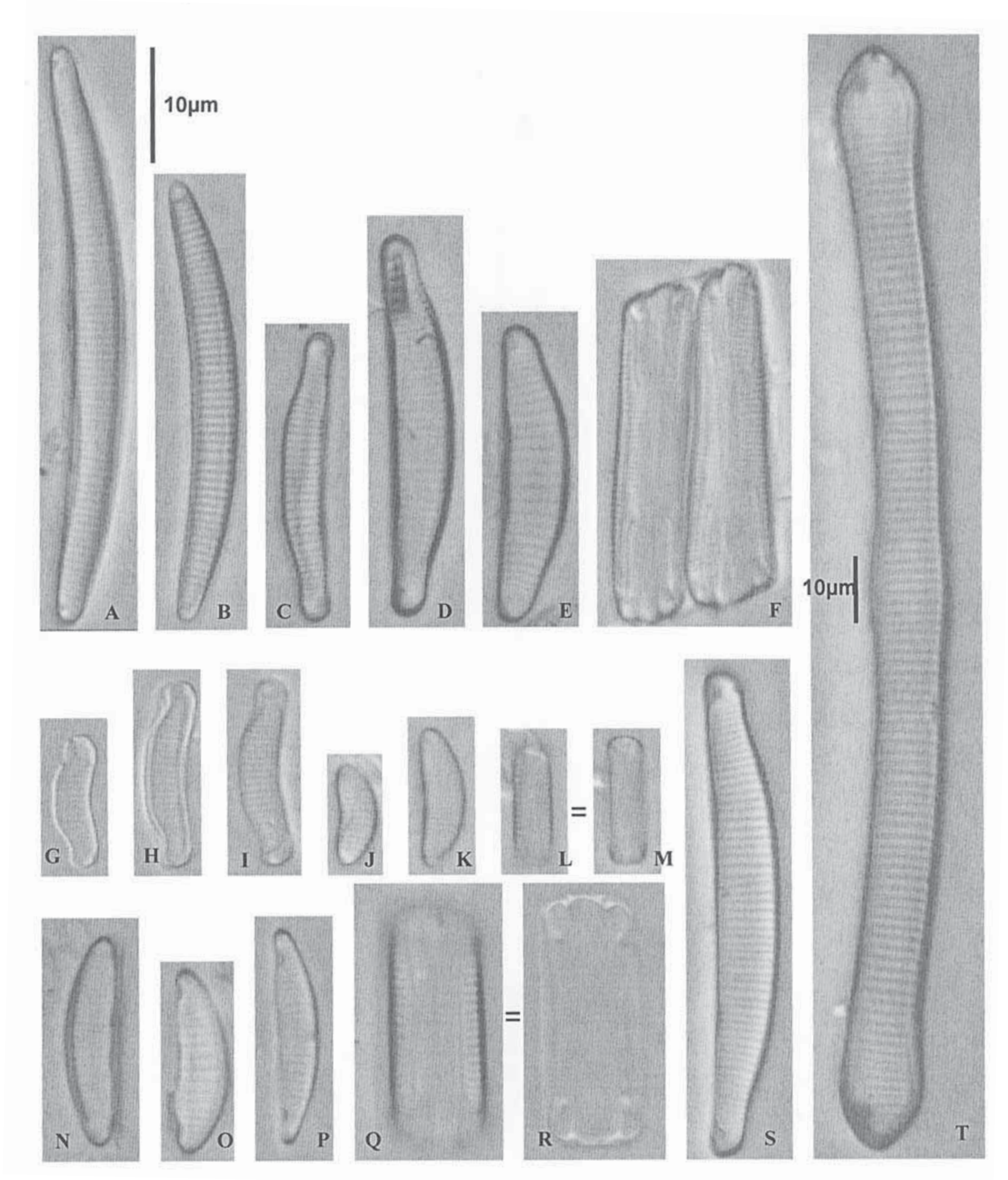

Figure 5. A-B: *Eunotia bilunaris*; C: *Eunotia implicata*; D-F: *Eunotia minor*; G-I: *Eunotia euxigua*; J-M: *Eunotia subarcuatoides*; N: *Eunotia intermedia*; O-R: *Eunotia incisa*; S: *Eunotia* cf. *veneris*; T: *Eunotia formica*. A-E, G-K, N-P; S-T: External valve view; F, L-M, Q-R: Frustule in girdle view, detail of girdle bands. *A-E, G-R, N-P; S-T: vista externa de la valva; F, L-M, Q-R: Vista lateral del frústulo, detalle de las bandas interpleurales.*

distant at the centre of the valve than at the ends, 13-17/10 µm. Length: 15-50 µm. Breadth: 4-7 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al.*, 1994).

In the studied area the species was collected in habitats with pH: 4.7-6.8, conductivity: 4.17-194 µS/cm, altitude: 25-975 m asl, SPI: 15.5-19.8. Its highest abundance is between 7 %-10 % in conditions with pH: 5.3-6, conductivity 38-51 µS/cm, altitude: 472-484 m asl, SPI: 19.3-19.7.

Eunotia intermedia (Krasske ex Hustedt) Nörpel & Lange-Bertalot 1991 (Fig. 5: N)

Krammer & Lange-Bertalot 1991, 215, Fig. 143: 10-15.

Ventral margin straight, wall thickened in areas halfway between the centre of the valve and the ends. Dorsal margin convex. Ends not distinctly formed, but confluent with the rest of the valve. Terminal nodules distinct, near the ends of the valve. Striae parallel, 14-16/10 µm at the centre of the valve, more numerous toward the ends. Length: 14-45 µm. Breadth: 3.5-5 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al.*, 1994).

In the studied area the species was collected in habitats with pH: 5.4-7.3, conductivity: 17.6-112 µS/cm, altitude: 205-1086 m asl, SPI: 18.2-19.8 with abundance never higher than 3.4 %.

Eunotia minor (Kützing) Grunow in van Heurck 1881 (Fig. 3: D-F, Fig. 4: A, J)

Krammer & Lange-Bertalot 1991, 196, Fig. 142: 7-15, Fig. 144: 5; Lange-Bertalot & Metzeltin 1996, 144, Fig. 13: 18-21, Fig. 103:11.

Cells in girdle view thick, rectangular; laterally asymmetrical (lunate) in valve view along the apical axis. Valves bent with subrostrate large round apices. Ventral margin slightly concave but slightly inflated at the centre; dorsal margin convex. Striae uniseriate, on the ventral mantle more numerous and alternating, 9-15/10 µm. Length: 20-60 µm. Breadth: 4.5-8 µm. Optimal abundance around pH 7 (Alles *et al.*, 1991). Acidophilus: mainly occurring at pH < 7 (Van Damm *et al.*, 1994).

In the studied area the species was collected in habitats with pH: 3.7-8.6, conductivity: 4.17-544 µS/cm, altitude: 14-1295 m asl, SPI: 12.3-19.9. Its maxim abundance is between 10-25.5 % in conditions with pH: 3.8-6.8, conductivity 19.3-36 µS/cm, altitude: 211-1086 m asl, SPI: 16.7-19.8.

Eunotia paludosa Grunow 1862 Krammer & Lange-Bertalot 1991, 203, Fig. 155: 1-20, 22-37; Hustedt 1930, 178, Fig. 228; Grunow 1862, 336, Fig. 6: 10; Lange-Bertalot & Metzeltin 1996, 152, Fig. 17: 48-19, Fig. 103:12.

Valve slightly arcuate, ventral and dorsal sides parallel, ends subcapitated. Striae parallel along the valve face, radial at one pole. Raphe oblique at the valve face, raphe endings slightly expanded. Girdle bands with several rows of poroids. Striae: 19-25/10 µm. Length: 6-60 µm. Breadth: 2-3(4) µm. Often associated to mosses in acid waters of low mineral content, also in bogs and small streams (Patrick & Reimer, 1966). Acidobiontic: optimal occurrence at pH < 5.5 (Van Damm *et al.*, 1994).

One single occurrence in the Meiro River (Navia basin) in Coaña (Asturias).

Eunotia pectinalis (Dillwyn?,O. F. Müller?, Kützing) Rabenhorst 1864 (Fig. 6 H)

Krammer & Lange-Bertalot 1991, 193, Fig. 141: 1-7, Fig. 143: 1; Metzeltin & Witkowski 1996, 98, Fig. 33:1; Lange-Bertalot & Metzeltin 1996, 146, Fig. 14: 5-7.

This is quite a variable taxon whose morphological variability is well documented (Geitler, 1932; Steinman & Sheath, 1984). The varieties which are recognized represent the extremes of series of variations from the typical form. Rabenhorst gives Dillwyn as the authority for this specific name. However, Dilwyn names Müller as the authority in his citation of this name. It is impossible to be sure from the original description and plates that either Müller or Dillwyn actually were working with this taxon. Frustules rectangular in girdle view. Valve elongate, slightly curved or bent. Ventral margin straight or slightly concave, sometimes slightly swollen at the centre of the valve. Valve narrowed toward broadly attenuated, truncate rounded ends. Striae parallel slightly radiate at the ends. Striae: 7-12/10 µm at the centre of the valve, to 14 in 10 µm at the ends of the valve. Length: 17-140 µm. Breadth: 5-10 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al.*, 1994).

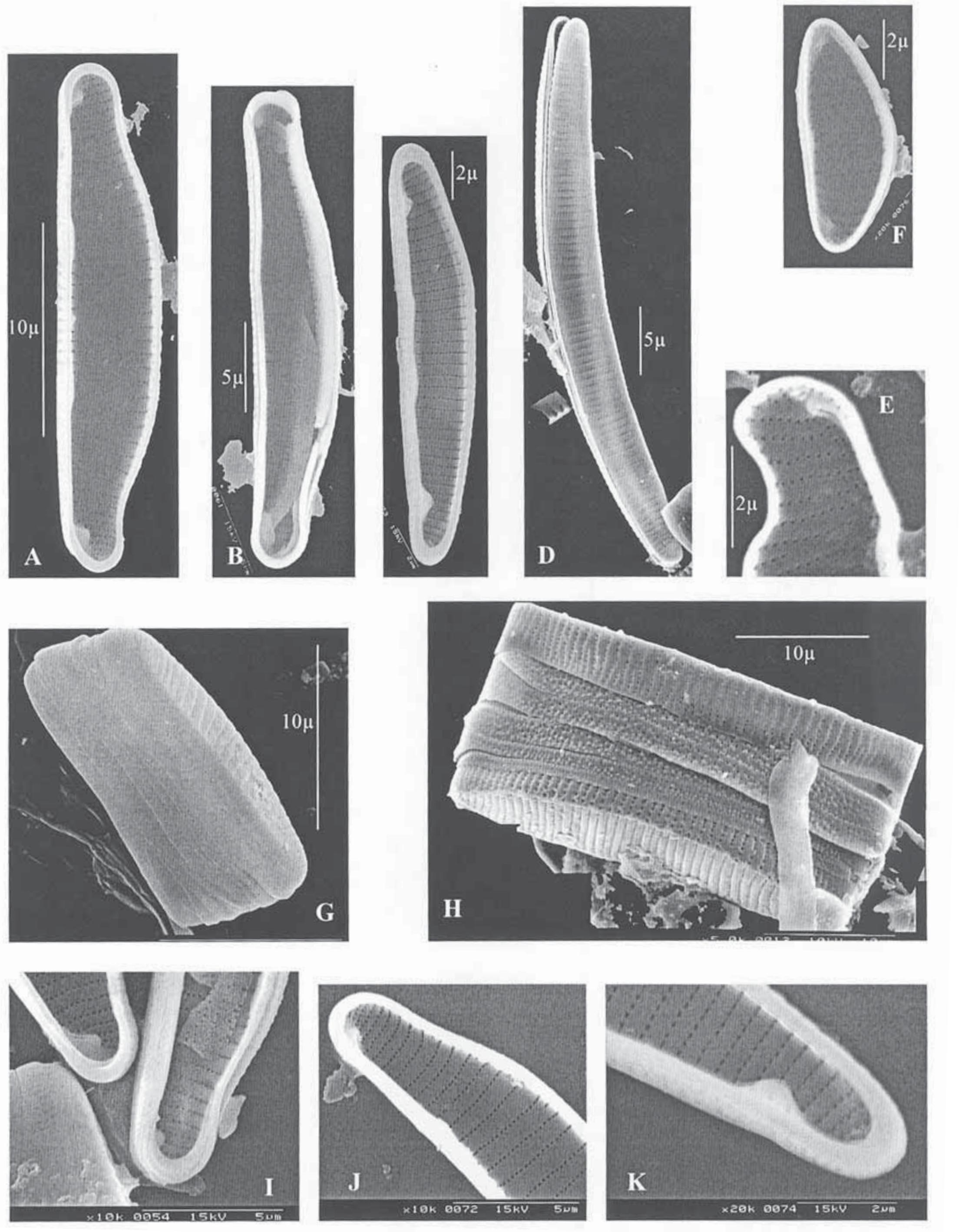

Figure 6. A, J: *Eunotia minor*; B, I: *Eunotia implicata*; C, K: *Eunotia incisa*; D: *Eunotia bilunaris*; E: *Eunotia exigua*; F-G: *Eunotia subarcuatoides*; H: *Eunotia pectinalis*. A-C, G-H: Internal view K-M: Internal view, detail of rimoportula and striae; D: External valve view; G-H: Frustule in girdle view, detail of girdle bands. *A-C, G-H: Vista interna K-M: Vista interna, detalle de la rimoportula y las estrias; D: Vista de la valva externa; G-H: Frústulo en vista lateral, detalle de las bandas interpleurales.*

In the studied area the species was collected in habitats with pH: 5.4-6.02, conductivity: 52-61 µS/cm, altitude: 205-444 m asl, SPI: 17.7-18.2 with abundance never higher than 5.3 %.

Eunotia pectinalis var. ***undulata*** (Ralfs) Rabenhorst 1864

Hustedt 1930, 182, Fig. 240; Hustedt 1959, 298, Fig. 763: i; Hustedt 1957, 241; Hustedt 1981, 98, Fig. 35: 3-11; Fig. 37: 1-4; Schoeman 1973, 78; Patrick & Reimer 1966, 206, Fig. 12: 11; Krammer & Lange-Bertalot 1991, 193, Fig. 141: 1-5.

This variety is easily distinguished by the many undulations, three or more, of the dorsal margin and the central swelling of the ventral margin. It is quite variable in outline. This variety prefers circumneutral waters of low mineral content (Patrick & Reimer 1966). Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

This taxon is not very well represented in this region, it was found only in three sites with abundance never higher than 0.5 %.

Eunotia praerupta Ehrenberg 1843 sensu lato

Krammer & Lange-Bertalot 1991, 186, Fig. 148, 149: 1-7; Van Heurck 1880-1885, 143, Fig. 34: 19; Okuno 1974, 11, Fig. 845-846; Hustedt 1930, 174, Fig. 211; Hustedt 1957, 239; Hustedt 1959, 280, Fig. 747 A: a-e; Germain 1981, 88, Fig. 30: 7-8; Schoeman 1973, 81; Ehrlich 1995, 49, Fig. 8: 12-13; Metzeltin & Witkowski 1996, 98, Fig. 33:6; Lange-Bertalot & Metzeltin 1996, 142, Fig. 12: 12-13; Patrick & Reimer 1966, 193, Fig. 10: 14.

This species can be easily recognized by the convexity of the dorsal margin and by the characteristic truncate-rostrate to slightly capitate ends. Frustules in girdle view, rectangular. Ventral margin usually slightly concave at the centre, straight at the ends of the valve; sometimes almost straight throughout the entire length. Striae parallel: 6-13/10 µm. Length: 20-100 µm. Breadth: 4-15 µm. Usually in northern or mountainous localities in acid to circumneutral waters (Patrick & Reimer, 1966). Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

One single occurrence in Tremor River (Miño basin) in Torre del Bierzo (Castilla-León).

Eunotia soleirolii (Kützing) Rabenhorst 1864

Krammer & Lange-Bertalot 1991, 194, Fig. 142:1, 2-6; Patrick & Reimer 1966, 208, Fig. 13: 1-2. The diagnostic character is the internal septation which seem to be a result of an abnormal division of the frustule. Valve linear-lunate in shape. The dorsal and ventral margins are parallel. Ends not differentiated clearly from the main part of the valve. It forms filaments in which the frustules contain internal septae. Striae: 12-14/10 µm. Length: 30-65 µm. Breadth: 3-4 µm. Occurs predominantly in periodic water-bodies as well as under alkaline or acidic conditions, high or low electrolyte content (Patrick & Reimer, 1966). Circumneutral: mainly occurring at pH values about 7 (Van Damm *et al*., 1994).

One single occurrence in Meiro River (Navia basin) in Coaña (Asturias).

Eunotia subarcuatoides E. Alles, M. Nörpel & Lange-Bertalot 1991 (Fig. 3 : J-M, Fig. 4 : F-G)

Krammer & Lange-Bertalot 1991, 214, Fig. 138: 1-9, Fig. 145:6. Alles *et al.* 1991, 188, Fig. 4: 1-36.

Narrow linear in girdle view. Valves slender, bow-shaped, as a rule strongly curved, with more or less parallel margins. The dorsal line somewhat strongly sloping toward the ends, valves therefore slightly narrowed towards the end; ends rounded. End nodules small, raphe slightly developed. Small forms, rarely over 30 µm long, strongly narrowed near the ends. Valves therefore strongly lanceolate, often only weakly bent. Striae: 18-23/10 µm. Length: 6-35(40) µm. Breadth: 2.7-4.5 µm. This species tolerate high and strong variations of pH-values (Alles *et al*., 1991). Acidobiontic: optimal occurrence at pH < 5.5 (Van Damm *et al*., 1994).

In the studied area the species was collected in habitats with pH: 3.8-7.7, conductivity: 11.2-720 µS/cm, altitude: 76-1075 m asl, SPI: 14-19.9. Its highest abundance was 29.7 % in Tamoga river (Miño basin) in Abadin (Galicia) with pH: 3.8, conductivity 34 µS/cm, altitude: 440 m asl, SPI: 18.9.

Eunotia tenella (Grunow) Hustedt in A. Schmidt *et al*. 1913

Krammer & Lange-Bertalot 1991, 202, Fig. 154: 23-30; Hustedt 1930, 175, Fig. 220; Hustedt 1957, 240; Hustedt 1959, 284, Fig. 749; Germain 1981, 90, Fig. 31: 1-7, 22-26.

This species is characterized by its distinct terminal nodules on the ventral margin close to the apices. Ventral margin slightly concave. Dorsal margin narrows toward rostrate, rounded or somewhat capitated ends and varies in its degree of convexity. Margins sometimes almost parallel or swollen in the middle portion. Striae: 14-16/10 µm at the centre to 20 in 10 µm at the ends. Length: 6-37 µm. Breadth: 2-4 µm. Acidophilous: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

In the studied area the species was collected in habitats with pH: 3.8-7.9, conductivity: 16-632 µS/cm, altitude: 250-1080 m asl, SPI: 14-19.9. Its maxim abundance is 3 % with pH: 6.8, conductivity: 36 µS/cm, altitude: 624 m asl, SPI: 19.5.

Eunotia veneris (Kützing) De Toni 1892 (Fig. 3: S)

Krammer & Lange-Bertalot 1991, 222, Fig. 163: 14-19.

Rectangular plate-shaped in girdle view, commonly shifted into rhombic shape. Valves half-lanceolate with straight ventral margin and symmetrically shallow, convex dorsal margin. Dorsal line bow-shaped; imperceptibly or not at all depressed near the ends. Ends quite sharply rounded. End nodules shifted proximally on the ventral line. Transapical striae 13-15/10 µm in the middle, closer near the apices. Length: 15-50 µm. Breadth: 4-6 µm. Acidophilus: mainly occurring at pH < 7 (Van Damm *et al*., 1994).

One single occurrence in the river Cares (Deva-Cares basin) in Cabrales (Asturias).

Environmental variables

The sites where we found *Eunotia* species are characterised by low to neutral pH. These optimal occurrences are according to van Damm *et al.* (1994) classification: species acidobiontic (optimal occurrence at ph < 5.5): *Eunotia exigua,* pH: 4.36, abundance: 69.70 %; *E. subarcuatoides,* pH: 3.75, abundance: 29.68 %, and species acidophilous (mainly occurring at pH < 7): *E. implicata* pH: 6, abundance: 16.06 %; *E. minor* pH: 6.75, abundance: 24.38 %.

A principal component analysis was carried out on the obtained environmental variables results to establish the environmental characteristics in those sites where the most relevant *Eunotia* species were identified (abundances higher than 5 % in at least one sample) (Table 2). Figure 5 shows the distribution of the variables in the space formed by the first two components, which explain a total of 53.41 % of variance. The first component (29.09 % of variance) is determined by altitude and also, with a negative correlation, by pH, dissolved oxygen, and slope. Other variables like temperature and conductivity are positively correlated to this component. The antagonism and extreme position of temperature and altitude along this axis was to be expected, the water temperature is lowered when altitude above sea level increases. The opposed position between conductivity and altitude along this axis could be interpreted in relation to the fact that in the upper stretches of the river, the rainfall is greater and the salt content lower. Dissolved oxygen and slope correlate with altitude. Taking into account these environmental variable correlations, the first component ordered the stations according to an altitudinal sequence and corresponds to an increase in water temperature and mineralization on the opposite direction.

The second axis (24.33 % of the variance) may be characterized by the parameters which determined the biological quality of water. Globally we observed that the highest notes of SPI (meaning very good quality) correspond to sites that were situated on the down site of the diagram. In those localities, we observed that a good oxygen dissolution rate combined with high slope provides a high self-purification power to the river and could explain the high SPI notes obtained. Rivers with moderate to null slopes are very sensitive to eutrophication and show much lower valves in the SPI diatom index (Tison *et al*., 2007). Therefore, this axis is related to the pollution levels of the studied area.

The Canonical Correspondence Analysis (CCA) allowed relating the most relevant *Eunotia* species identified (abundances higher than 5 % in at least one sample) to the environmental parameters (Fig. 6). The first two axes explained 35.96 % of the variance. The first axis (21.54 % of variance explained) was strongly correlated with conductivity; thereby the species were sorted along this axis following a water salinity gradient. The species which showed a highly positive correlation with this variable was *Eunotia exigua* with a clear tendency for waters with a certain mineral content. The other *Eunotia* species, with a poorly mineralized water preference, were grouped on the left side of the diagram. The second axis (14.43 % of variable explained) was positively correlated with dissolved oxygen and slope. Another important factor in the analysis was pH, and was important on both axes; the CCA analysis revealed a strong positive influence on some *Eunotia* species such as *Eunotia subarcuatoides* and *E. minor.* The altitude and the water temperature had no clear influence on the *Eunotia* species distribution.

SUMARY AND CONCLUSIONS

Although *Eunotia* species occurred in 30 % of the samples studied, only in 8.8 % of the sites was their abundance over 5 %. The analysis of the samples showed that the genus *Eunotia* is well represented in the Galicia region. The basin with more occurrences of *Eunotia* species was Miño, followed by Navia, Nervión, and Limia. *Eunotia minor* and *Eunotia implicata* were the most geographically frequent species, occurring in 59 % and 50.4 % of the samples respectively. All of the taxa were previously mentioned in the floristic list of Aboal *et al*. (2003a) except *E. soleirolii*, which was cited for the first time in Goma *et al.* (2004). The lack of *Eunotia* species identification in the Basque Country can be due to the few sampling stations in this zone (the 4.5 % in the whole studied area) and the neutral to alkaline pH values of these stations as well.

The localities where we found species of *Eunotia* are characterized by low to neutral pH and moderate conductivity, sometimes with extremely low values. The ecological characteristics given in the literature for the majority of the studied taxa agree with our observations except for *E. minor* which shows a large range of variability in pH values that is rather wider than those mentioned in van Damm *et al*. (1994) and Alles *et al.* (1991).

The distribution of *Eunotia* species in the studied area was related to some dominant environmental factors. As suggested by the results of the CCA, derived from *Eunotia* data analyses, these were conductivity and pH. However, other factors affect *Eunotia* species presence in some rivers and streams in the studied area such as slope, dissolved oxygen and pollution levels. However, the altitude and the water temperature have no clear influence. Among the sets of these environmental parameters, conductivity plays the most important role, showing a high influence on *Eunotia exigua*. The pH had effects on the distribution of *Eunotia* species, the species were ordinated mainly along the second axis following an acidity gradient where *Eunotia subarcuatoides* and *E. minor*, in the upper side of the diagram, showed a clear preference for acidic waters. Altitude, based on the CCA analysis, contributes poorly to the prediction of *Eunotia* species in the studied area. In reality, hydro-morphological characteristics such as slope or dissolved oxygen values discriminate more than altitude. On the other hand, altitude was related to temperature variation but had minor influence on *Eunotia* species presence compared to pollution (assessed by SPI diatom index) and mineral content (assessed by conductivity).

According to the values obtained with the SPI diatom index, all the sampling sites had good or very good biological quality, so the anthropogenic pressure in the studied area is low. In agreement with this, a great deal of these localities could be close to reference conditions and so, we can consider that their pH value is highly linked to geology (Meybeck, 1986).

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