Composition of meiobenthonic Platyhelminthes from brackish environments of the Galician and Cantabrian coasts of Spain with the description of a new species of *Djeziraia* (Polycystididae, Kalyptorhynchia)

CAROLINA NOREÑA[1], CRISTINA DAMBORENEA[2], ANNO FAUBEL[3] & FRANCISCO BRUSA[2]

[1]*Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain,* [2]*CONICET, División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, UNLP, La Plata, Argentina, and* [3]*Institute of Hydrobiology and Fishery Science, Hamburg, Germany*

Abstract
From 1997 to 1999, the fauna of free-living Platyhelminthes of the *rias* ecosystem was studied along the Galician and Cantabrian coast in northern Spain. In total, 72 platyhelminth species are listed in this study. Forty-two species represent new records for the Iberian Peninsula, three of which represent new genera records. A new species belonging to the genus *Djeziraia* (Polycystididae, Kalyptorhynchia), *Djeziraia longistyla* sp. nov., is described in this paper. In this broad-scale study, a large data set (27 localities) of the estuaries of northern Spain allowed an analysis of the turbellarian species assemblages and the relation of species distributions to salinity, conductivity, oxygen, temperature, and sediment characteristics. Species assemblages (species diversity) of each habitat of the brackish water ecotone are shown. The present study contributes to knowledge on the ability of adaptation of free-living Platyhelminthes to regimes of brackish water ecotones.

Keywords: *Northern Spain, brackish habitats, Platyhelminthes, turbellaria, new species, funeral records,* Djeziraia

Introduction

Research on the taxonomy and ecology of meiobenthonic organisms is well established for estuaries of northern and western Europe (Gourbault 1981; Warwick and Gee 1984; Ax 1991; Warwick et al. 1991; Mettam et al. 1994; Smol et al. 1994; Soetaert et al. 1994; Attrill and Thomas 1996; Blome 1996; Blome and Faubel 1996). In contrast, the meiobenthonic fauna of estuaries of the Iberian Peninsula is relatively unknown (Soetaert et al. 1995). Most studies in this region have been carried out in the delta of the Ebro River and deal with ecological aspects (Muñoz 1990; Ibañez et al. 1995, 1996, 1997; Guillen and

Correspondence: Francisco Brusa, División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, Paseo del Bosque s/n, 1900, La Plata, Buenos Aires, Argentina. Email: fbrusa@fcnym.unlp.edu.ar

Palanques 1997; Mikhailova 2003), whereas studies on the Galician and Cantabrian coasts of Spain have been focused on the distribution and composition of meiofaunal and macrofaunal communities (Palacio et al. 1992; Curras et al. 1993; García-Álvarez et al. 1993; Lastra et al. 1993; Sanchez-Mata et al. 1993; Garmendia et al. 1996; Arroyo et al. 2004; Borja et al. 2004; Garcia-Arberas and Rallo 2004). In Europe, taxonomic investigations on free-living Platyhelminthes have been mostly conducted in aquatic environments of Fennoscandia and estuarine areas of the North Sea (Nasonov 1926; Luther 1943, 1960, 1962, 1963; Karling 1963, 1974; Den Hartog 1977; Armonies 1987; Ax 1956a; 1995; Faubel and Warwick 2005). Comparable investigations are relatively scarce in Spain. Until now, only a few species were known and described from the Iberian Mediterranean coast (Gieysztor 1931; Steinböck 1954).

Hence, it was the aim of the present investigation to contribute to the knowledge of species composition and distribution of estuarine brackish water turbellarians along the Galician and Cantabrian coast. A brief account is given of the relationships between species distribution and community structure, and intrinsic abiotic factors during the study are shown. A new species, *Djeziraia longistyla* sp. nov. is described.

Material and methods

The northern region of the Iberian Peninsula, specifically the Galician and Cantabrian coast (Figure 1), is characterized by more than 26 *rias* of different sizes and depths. *Rias* are estuaries with important variations in salinity in the superficial waters due to evaporation or to the contribution of freshwater from rivers. These areas are usually very fertile, which, apart from the stratification, results in high oxygen consumption and therefore oxygen depletion, especially in the depressions on the estuary bottom (e.g. Ria de Vigo, Galicia) where anaerobic sediments have been deposited. In addition, the *rias* are subjected twice a day to tides, one of which is generally of greater amplitude. During high tide, the meiobenthonic organisms descend 5–10 mm into the sediment (Margalef 1989).

During the years 1997–1999, samples were taken (Project Fauna Iberica: SEUI-DGES PB95-0235) in the *rias* of the coastal region of northern Spain. Sixty-one sample sites were selected within the Comunidad de Galicia (March 1998 and March 1999), Comunidad de Asturias (October 1998), Comunidad de Cantabria (September 1997), and Comunidad de el País Vasco (July 1998). In addition to the sampling in the *rias*, the investigations were extended to the most important rivers contributing a great amount of freshwater to the system (e.g. Arnoia, Ribadil, Miño, Carvallo, Miñor, Oubia, Ferreras, Uncín, Ason, Ebro, Mieras, and Lea) (Table I; Figure 1).

The samples were obtained from sandy or muddy sediments in the eulittoral zone along the coastline and from the mouths of rivers during low tide using a small shovel to take surface sediments of about 320 cm^3 from an area of about 20×8 cm and a depth of 1–2 cm (samples 4, 5, 7–10, 13, 14, 18, 22–27; Table I; Figure 1). Three replicates were taken for each sediment sample. In general, below a sediment depth of 2 cm, the chemocline showed a strong contrast from aerobic to anaerobic sediment layers in the eulittoral and shallow sublittoral areas. Therefore, free-living Platyhelminthes were found only in the first 2 cm of sediment. Samples from stagnant waters (limnetic, brackish or marine), such as rock-pools covered with aquatic vegetation or detritus, were extracted with a plankton-net (mesh size 125 μm) (samples 12, 15, 16, 19–21; Table I). The Karaman–Chappuis method (Chappuis 1942) was applied to obtain samples from the gravel of river banks (samples 1–3, 6, 11, 17;

Figure 1. Location of the sample sites, with turbellarians (black points) and without turbellarians (white circles). The locality numbers correspond to those given in Tables I–III.

Table I). The water obtained with this method was filtered (approximately 30 litres) with a sieve of mesh size 125 μm. The individual number of each turbellarian species was recorded for all samples taken.

The samples were deposited in storage jars and transported to the laboratory. Individuals were separated under a dissecting microscope. Sexually mature specimens were identified alive and in squash preparations, i.e. flattened under the increasing pressure of the cover slip as the preparation dries. For anatomical study of the hard-structures such as stylets, whole mounts (polyvinyl–lactophenol) were prepared. For histological observation, specimens were fixed in Bouin's fixative. Sagittally prepared serial sections (4 μm thick) of sexually mature specimens were stained with Azan. Most of the individuals were also photographed (Video Graphic Printer UP-86 OCE) and filmed (Canovision EX-HI 8)

Table I. Sample sites with type of substrate.

Localities	Bottom
1. Arnoia River near O Viso; 42°12′N, 08°01′W	Stony, abundant marginal vegetation
2. Ribadil River in Riba de Mouro; 42°02′N, 08°19′W	Sandy-gravel
3. Carvallo (Tamuxo) River near Cumeira; 41°56′N, 08°50′W	Sandy-gravel
4. Beach in front of Monastery of Santa Maria de Oia; 41°59′N, 08°53′W	Sandy, rock-pools with brown algae
5. Ria de Bayona, ria-mouth; 42°7′N, 08°50′W	Muddy, green and brown algae
6. Ria de Bayona, America Beach; 42°8′N, 08°49′W	Sandy, brook
7. Ria de Arosa, in front of La Lanzada Beach; 42°27′N, 08°49′W	Sandy-muddy, with algae
8. Ria de Navia; 43°33′N, 06°43′W	Sandy-muddy, with periphyton
9. Ria de Villaviciosa near San Martin; 43°31′N, 05°23′W	Muddy, with green algae and periphyton
10. Ria de Villaviciosa near Badriñana; 43°30′N, 05°26′W	Muddy, with green algae
11. San Juan River in San Juan de Amandi; 43°28′N, 05°27′W	Stony with periphyton
12. Ria de Ajo; 43°28′N, 03°35′W	Muddy, abundant aquatic vegetation
13. Berria Beach near Santoña; 43°26′N, 03°28′W	Stony-sandy, green and red algae
14. Ria de Santoña near Escalante; 43°27′N, 03°30′W	Muddy, green algae
15. Ria de Rada near Rada; 43°23′N, 03°28′W	Muddy, detritus, algae and marginal vegetation
16. Ason River near Santa Ulalla Sanctuary; 43°20′N, 03°26′W	Stony, filamentous algae
17. Ason River near Barcena; 43°18′N, 03°27′W	Stony-sandy, periphyton and algae
18. Artificial spring in Ramales de la Victoria; 43°16′N, 03°27′W	Muddy, aquatic vegetation
19. Natural spring in Las Alisas-mountain-pass; 43°18′N, 03°39′W	Muddy, filamentous algae
20. Mieras River near Lierganes; 43°21′N, 03°43′W	Stony, aquatic vegetation
21. Pond near the Besaya River; 43°6′N, 04°04′W	Muddy, with detritus
22. Ria de Plentzia;43°24′N, 02°56′W	Sandy-muddy, filamentous algae
23. Ria de Guernica, San Antonio Beach; 43°23′N, 02°41′W	Muddy-sandy, with algae
24. Ria de Guernica near Kanala; 43°22′N, 02°40′W	Sandy-muddy, green and brown algae
25. Ria de Ondarroa; 43°19′N, 02°25′W	Muddy, with algae and aquatic vegetation
26. Zumaia Beach near Zumaia; 43°18′N, 02°15′W	Sandy
27. Ria Zumaia; 43°18′N, 02°14′W	Muddy, with green algae

under a microscope. The type material has been deposited in the Museo Nacional de Ciencias Naturales (MNCN), Madrid.

For characterization of the habitats, the abiotic factors of conductivity, temperature, oxygen concentration, and salinity (refractometric determination) were measured mainly in the water column (conductivity, T°: CRISON CDTM-523; oxygen, T°: YSI 33-SCT). Salinity regimes and classification were based on the Venice System (Karling 1974): limnetic (<0.5‰), oligohaline (0.5–5‰), mesohaline (5–18‰), polyhaline (18–30‰), euhaline (30–40‰).

Results

Meiobenthonic composition

Table II shows the distribution of the principal species found in localities along the Galician and Cantabrian coastal regions. Microturbellarians were found in 27 of the 61 sampling sites. Therefore, only these sites are considered in the faunistic approach presented. The total number of Turbellaria amounted to 72 species. From the total species number, 48 could be identified to species level and 13 could not be clearly identified (sexually immature juveniles) and are designated as "cf". Eleven taxa could be taxonomically determined to

Table II. Free-living Platyhelminthes collected in this study.

Species	Localities[a]	Environments
ACOELA		
Convoluta sp.	14	Brackish (meso)
Haploposthia sp.	22	Brackish (meso)
Mecynostomum auritum (Schultze, 1851)	27, 15, 9, 10	Brackish (olig)
Mecynostomum sp.	24	Brackish (meso)
Paramecynostomum diversicolor (Örsted, 1822)	9	Brackish (olig)
Philocelis karlingi (Westblad, 1946)	8, 9	Brackish (olig)
Philactinoposthia sp.	9	Brackish (olig)
Pelophila cf. *pachymorpha* Dörjes, 1968	14	Brackish (meso)
CATENULIDA		
Catenula lemnae Duges, 1812	1, 2	Limnetic
Rhynchoscolex simplex Leidy, 1851	1, 2, 11	Limnetic, brackish (olig)
Stenostomum cf. *karlingi* Luther, 1927	1	Limnetic
Stenostomum grabbskogense Luther, 1927	2	Limnetic
Stenostomum leucops (Duges, 1811)	17	Limnetic
Stenostomum sp.	3	Limnetic
MACROSTOMIDA		
Macrostomum balticum Luther, 1947	14	Brackish (meso)
Macrostomum curvituba Luther, 1947	27	Brackish (olig)
Macrostomum hamatum Luther, 1947	10	Brackish (olig)
Macrostomum hystricinum Beklemischev, 1951	9, 14	Brackish (olig)
Macrostomum obtusum (Vejdovsky, 1895)	14	Brackish (meso)
Macrostomum rostratum (Papi, 1951)	12, 15	Brackish (olig)
Macrostomum sp.	2, 3, 14, 21, 24, 27	Limnetic, brackish (olig)
Microstomum lineare Örsted, 1820	17	Limnetic
PROLECITHOPHORA		
Archimonotresis limophila Meixner, 1938	7	Brackish (poly)
Pseudostomum klostermani (Graff, 1874)	13	Euhaline
LECITHOEPITHELIATA		
Geocentrophora baltica (Kennel, 1883)	1	Limnetic
Geocentrophora sphyrocephala De Man, 1876	1	Limnetic
Prorhynchus stagnalis Schultze, 1851	1	Limnetic
PROSERIATA		
Archilina papillosa (Ax and Ax, 1977)	26	Brackish (meso)
Archilopsis unipunctata (Fabricius, 1826)	14, 15, 23	Brackish (olig)
Bothrioplana semperi Braun, 1881	1, 2, 17, 21	Limnetic
Monocelis lineata (Müller, 1774)	4, 22, 23, 27	Brackish (poly), brackish (olig)
Nematoplana cf. *nigrocapitula* Ax, 1966	6	Limnetic
Nematoplana coelogynoporoides Meixner, 1916	26	Brackish (meso)
Promonotus arcassonensis Ax, 1959	25	Brackish (meso)
Promonotus schultzei Meixner, 1920	8, 9, 10, 23	Brackish (olig)
Pseudomonocelis cf. *agilis* (Schultze, 1851)	24	Brackish (meso)
RHABDOCOELA		
Acrorhynchides cf. *robustus* (Karling, 1931)	10	Brackish (olig)
Baltoplana magna Karling, 1949	23	Brackish (meso)
Bresslauilla relicta Reisinger, 1929	25, 27	Brackish (olig)
Brunetia camarguensis (Brunet, 1965)	8	Brackish (olig)
Canetellia cf. *beauchampi* Ax, 1925	9	Brackish (olig)
Castrella truncata (Abildgaard, 1789)	21	Limnetic
Cheliplana cf. *setosa* Evdonin, 1971	9	Brackish (olig)
Djeziraia longistyla n. sp.	7	Brackish (poly)
Gyratrix hermaphroditus Ehrengberg, 1831	2, 3	Limnetic
Halammovortex nigrifrons (Karling, 1935)	24	Brackish (meso)

Table II. Continued.

Species	Localities[a]	Environments
Karchinorhynchus sp.	24	Brackish (meso)
Maehrenthalia cf. *intermedia* (Graff, 1882)	10	Brackish (olig)
Microdalyellia fusca (Fuhrmann, 1894)	3, 16, 19	Limnetic
Microdalyellia tennesseensis (Ruebush and Hayes, 1917)	18, 20	Limnetic
Phaenocora unipunctata (Örsted, 1820)	16, 18	Limnetic
Phonorhynchus cf. *helgolandicus* (Mecznikow, 1865)	10	Brackish (olig)
Placorhynchus octaculeatus Karling, 1931	5	Brackish (olig)
Pratoplana cf. *salsa* Ax, 1927	24, 27	Brackish (olig)
Promesostoma cf. *nynaesiensis* Karling, 1957	15	Brackish (olig)
Promesostoma marmoratum Schultze, 1851	5, 7, 8, 10, 23, 24	Brackish (olig)
Proschizorhynchella bivaginatus (Schilke, 1970)	8	Brackish (olig)
Proschizorhynchus triductibus Schilke, 1970	6	Limnetic
Provortex balticus (Schultze, 1851)	8, 23	Brackish (olig)
Provortex karlingi Ax, 1951	15	Brackish (olig)
Provortex pallidus Luther, 1948	8, 23	Brackish (olig)
Provortex sp. 1	4	Brackish (poly)
Provortex sp. 2	2	Limnetic
Provortex tubiferus Luther, 1948	10, 27	Brackish (olig)
Proxenetes flabellifer Jensen, 1879	15, 24	Brackish (olig)
Proxenetes simplex Luther, 1948	25	Brackish (meso)
Proxenetes unidentatus Den Hartog, 1966	10, 24	Brackish (olig)
Pseudograffilla cf. *hymanae* Mack-Fira, 1974	8, 9	Brackish (olig)
Ptychopera westbladi (Luther, 1920)	8, 9, 10, 24	Brackish (olig)
Utelga sp. 1	15	Brackish (olig)
Vejdovskya sp.	27	Brackish (olig)
Westbladiella obliquepharynx Luther, 1920	7	Brackish (poly)

olig, oligohaline; meso, mesohaline; poly, polyhaline. [a]Locality numbers correspond to those given in Table I.

the genus level. One species found in the polyhaline area of Ria de Arosa is new to science (*Djeziraia longistyla* sp. nov., description below). All species found are new records for the Galician and Cantabrian coast and 43 species represent new records for the Iberian Peninsula. The two sampling visits indicate high species richness for the area, with most species occurring in the freshwater/oligohaline and polyhaline/marine zones.

There are no species common to all Spanish districts. *Promesostoma marmoratum* is the species with the broadest distribution, always found in habitats with salinities between 0.5–5‰. Another species with a wide distribution found in oligohaline habitats is *Mecynostomum auritum*. Forty-seven species were found only in one of the sample localities (Table II) and 18 species live exclusively in limnetic areas (up to 0.5‰).

In the study area, one species, *Rhynchoscolex simplex*, appears to be ubiquitous without any definite relationship to a specific substrate. However, *Rhynchoscolex simplex* is a characteristic representative of freshwater sands, although the species is also abundant in stony areas of running waters (ubiquist—Table III). The species settles in the sandy interspaces between the stones and pebbles.

Some species were captured in only one type of habitat, and are therefore characteristic for the limnetic, brackish, or marine areas. Between them the most abundant species for the marine area was *Archilina papillosa*, in the limnetic environments *Stenostomum leucops* and *Microdalyellia tennesseensis*, and in the brackish environments *Macrostomum rostratum*.

On the other hand, the brackish habitats are characteristically overlapping zones for the marine and limnetic species. Therefore, 17 species captured in this study belong to these

Table III. Class of abundance: 1=1–5 specimens, 2=6–10 specimens, 3=11–20 specimens.

	Limnetic											Brackish												marine			
	Sandy			Stony				Muddy				Sandy					Muddy							Sandy		Stony	
Character Species / *Sites*	2	3	6	17	11	1	16	20	18	19	21	4	5	8	22	24	9	10	12	14	15	25	27	7	23	13	26
Ubiquitous																											
Rhynchoscolex simplex	3				3	2																					
Characteristic Species																											
Archilina papillosa																											3
Bresslauilla relicta																						1	1				
Gyratrix hermaphroditus	2	3																									
Macrostomum hystricinum																	1			1							
Macrostomum rostratum																			3		1						
Microdalyellia tennesseensis								3	3																		
Microstomum lineare				2																							
Nematoplana coelogynoporoides																											2
Promonotus arcassonensis																						3					
Proschizorhynchus triductibus			3																								
Provortex balticus														1											2		
Provortex pallidus														1											2		
Proxenetes simplex																						2					
Pseudostomum klostermani																										2	
Stenostomum leucops				3																							
Stenostomum sp.		3																									
Overlapping Species																											
Archilopsis unipunctata																				3	1				1		
Bothrioplana semperi	1			1		1					1																
Catenula lemnae	2					3																					
Mecynostomum auritum																	3	3			2	1	2				
Microdalyellia fusca		2					3			3																	
Monocelis lineata												3			3								3		3		
Phaenocora unipunctata							1		2																		
Phonorhynchus cf. *helgolandicus*														1				1									
Pratoplana cf. *salsa*																1							1				
Promesostoma marmoratum													3	2		2		2						3	2		

Table III. Continued.

	Limnetic											Brackish												marine			
	Sandy			Stony				Muddy				Sandy					Muddy							Sandy		Stony	
Character Species / *Sites*	2	3	6	17	11	1	16	20	18	19	21	4	5	8	22	24	9	10	12	14	15	25	27	7	23	13	26
Promonotus schultzei														3			2	2							1		
Provortex. karlingi																					2						
Provortex. tubiferus																		1					1				
Proxenetes flabellifer																1					3						
Proxenetes unidentatus																1		1									
Pseudograffilla cf. *hymanae*														1			1										
Ptychopera westbladi														2		3	2	1									
Habitat Aliens																											
Convoluta sp.																				1							
Haploposthia sp.															1												
Mecynostomum sp.																1j											
Nematoplana cf. *nigrocapitula*			1j																								
Paramecynostomum diversicolor																	2										
Pelophila cf. *pachymorpha*																				1							
Philactinoposthia sp.																	1										
Philocelis karlingi																	1										
Casual Species																											
Acrorhynchides cf. *robustus*																		1									
Archimonotresis limophila																								1			
Baltoplana magna																									1		
Brunetia camarguensis														1													
Canetellia cf. *beauchampi*																	1										
Castrella truncata											1																
Cheliplana cf. *setosa*																	1										
Geocentrophora baltica						1																					
Geocentrophora sphyrocephala						1																					
Halammovortex nigrifrons																1											
Karchinorhynchus sp.																1											
Macrostomum balticum																				1							
Macrostomum curvituba																							1				

Table III. Continued.

	Limnetic											Brackish												marine			
	Sandy			Stony				Muddy				Sandy					Muddy							Sandy		Stony	
Character Species / *Sites*	2	3	6	17	11	1	16	20	18	19	21	4	5	8	22	24	9	10	12	14	15	25	27	7	23	13	26
Macrostomum hamatum																		1									
Macrostomum obtusum																				1							
Maehrenthalia cf. *intermedia*																		1									
Placorhynchus octaculeatus													1														
Promesostoma cf. *nynaesiensis*																					1						
Prorhynchus stagnalis						1																					
Proschizorhynchella bivaginatus														1													
Pseudomonocelis cf. *agilis*																1											
Stenostomum cf. *karlingi*						1																					
Stenostomum grabbskogense	1																										
Utelga sp.																					1						
Vejdovskya sp.																							1				
Westbladiella obliquepharynx																								1			

overlapping areas. Within this group we find three species originally described for marine and brackish environments: *Archilopsis unipunctata*, *Monocelis lineata*, and *Promesostoma marmoratum*.

Another group is formed by species that can be found in different types of substratum, such as *Bothrioplana semperi*, *Microdalyellia fusca*, *Phaenocora unipunctata* in limnetic habitats and, for example, *Proxenetes flabellifer* or *Ptychopera westbladi* (see Table III) within brackish water habitats.

Alien species includes species that were found only once or in very small numbers, such as *Nematoplana* cf. *nigrocapitula*, a marine species, captured in a limnetic habitat (brook in America Beach, Ria de Bayona), and *Paramecynostmum diversicolor* and *Pelophila pachymorpha*, marine species but captured in brackish habitats.

The casual species represent a group of species with low individual number (sometimes only one individual). Their presence in the studied habitats is casual and their association with the locality cannot be asserted. Within this group we find species such as *Archimonotresis limophila*, *Brunetia camarguensis*, and *Castrella truncata* (Table III).

New species description

Suborder KALYPTORHYNCHIA
Family POLYCYSTIDIDAE
Subfamily DUPLACRORHYNCHINAE
Djeziraia longistyla sp. nov.
(Figure 2)

Type locality. Ria de Arosa (42°27′N, 08°49′W), Galicia, Spain, 9–11 March 1999.

Material. Holotype: one sagittally sectioned specimen (catalogue no. MNCN 4.01/38). Other material: photographs, video film.

Etymology. The species name refers to the long stylet of the male copulatory organ.

Description. Sexually mature individuals 0.9–1.2 mm long (Figure 2A). Body conical, rounded posteriorly. Colourless or light grey due to subepidermal pigment layer; whitish at posterior end due to well-developed caudal glands (in incident light). Well-developed proboscis glands and brain between the proboscis and the pharynx (Figure 2A). Two dark eyes clearly separated, posterior to the proboscis and surrounded by the brain.

The ciliary cover forms narrow furrows that run parallel to the longitudinal axis, only visible in slightly squashed individuals (Figure 2A, posterior end of the body).

The general organization of the epidermis and the basal membrane corresponds to the organization described for *Djeziraia pardii* Schockaert, 1971 (Schockaert 1971).

The proboscis is very small (0.045 mm long in living animals) and at the anterior end of the body. The pharynx (0.112 mm diameter) is directed forward, and lies in the anterior half of the body.

Male reproductive system with two elongated testes dorso-laterally at the posterior end of the pharynx. The male copulatory organ is composed of an unpaired vesicula seminalis, a vesicula granulorum or prostatic vesicle, and a long slender stylet (Figure 2B, C). The spherical vesicula seminalis is surrounded by a thin epithelium and weakly developed diagonal muscle layers while the prostatic vesicle is smaller and surrounded with a strong

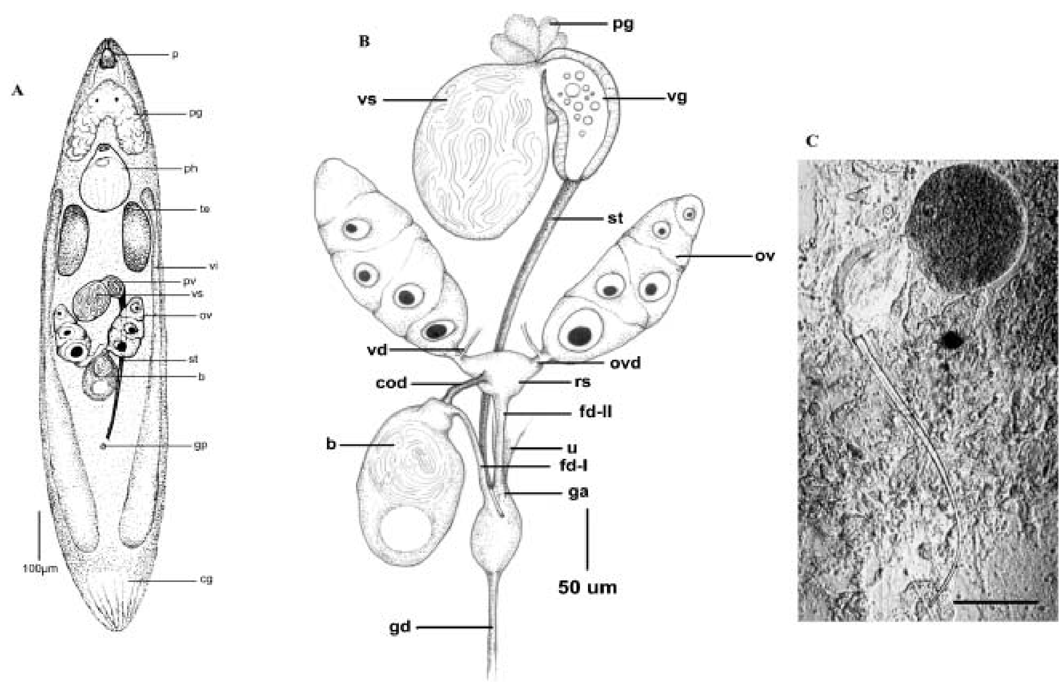

Figure 2. *Djeziraia longistyla* sp. nov. (A) General organization in dorsal view (from the living animal); (B) atrial organs; (C) stylet (photograph from the living animal). b, bursa; cg, caudal glands; cod, common oviduct; fd-I, female duct type I; fd-II, female duct type II; ga, common genital atrium; gd, genital duct; gp, gonopore; ov, ovary; ovd, oviduct; p, proboscis; pg, prostatic glands; ph, pharynx; pv, prostatic vesicle; rs, receptaculum seminis; st, stylet; te, testis; u, uterus; vi, vitellaria; vs, vesicula seminalis; vd, vitelloduct.

muscular layer consisting of spiral fibres. Both vesicles are clearly separated by a sphincter-like constriction. Extra-vesicular prostatic glands enter the prostatic vesicle proximally at the constriction. The stylet is a straight delicate tube, 250–300 µm long, slightly curved and with a terminal opening (Figure 2B, C).

In the female reproductive system, the bilateral vitellaria represent two large tubes running from the posterior end of the pharynx to the posterior end of the body (Figure 2A). The ovaries lie at the middle of the body, postero-laterally to the male copulatory organ. Ovaries connected with the single receptaculum seminis via short oviducts (Figure 2B). The receptaculum seminis passes communicates with the atrium genitale communis via the female duct (type II, after Artois and Schockaert 2005). The ovoid bursa is dorso-caudally located, and provided with a well-developed, muscular stalk or female duct type I and a distal common oviduct (Figure 2B). The female duct type I connects the bursa with the atrium genitale while the common oviduct connects the bursa with the receptaculum seminis. It is not discernible whether the distal part of the common oviduct and the proximal part of the female duct type I join the distal bursa together or separately (Figure 2B). In one animal the bursa contains a "ball" of compact sperm. The uterus lies ventrally and its opening is just ventral to the opening of the female duct type II. Eggs were not observed in the studied animals.

Female and male atrial organs (male copulatory organ, female ducts I and II, and uterus) open independently into the latero-frontal section of the small atrium genitale communis (Figure 2B). A long narrow genital duct leads from the distal part of the genital atrium to the common genital pore. This pore is situated at the beginning of the last third of the body.

Comparative discussion. Currently, the genus *Djeziraia* includes three species: *Djeziraia pardii* Schockaert, 1971 from Somalia (Indian Ocean), *D. incana* Artois and Schockaert, 2001 from the Galapagos Islands (Pacific Ocean), and *D. euxinica* (Mack-Fira, 1971) Schockaert, 1982 from the Black Sea and Mediterranean Sea.

The most conspicuous difference between *Djeziraia longistyla* sp. nov., *D. pardii*, and *D. euxinica* is the length of the stylet. The two last mentioned species have short stylets: 85 μm in *D. pardii* and 105–120 μm in *D. euxinica*. In *D. longistyla* sp. nov. the stylet is 250–300 μm long. The presence of a long, slender stylet (>150 μm) is shared with *D. incana*. *Djeziraia incana* has a stylet of 162–198 μm (up to 295 μm in one specimen) in length. Based on the length of the stylet, *D. longistyla* sp. nov. and *D. incana* are the most similar species within the genus *Djeziraia*. The differences between *D. longistyla* sp. nov. and *D. incana* concern the shape of the prostatic vesicle and the vesicula seminalis. *Djeziraia longistyla* sp. nov. shows a prostatic vesicle with a coat of strong spiral muscle layers, as in *D. pardii*, whereas *D. incana* has a thin-walled prostatic vesicle. As previously mentioned, both prostatic and seminalis vesicles are clearly separated in *D. longistyla* sp. nov. through a sphincter-like constriction. This prominent division between the vesicles is present in *D. pardii*, but not in *D. incana*.

On the other hand, *Djeziraia longistyla* sp. nov. and *D. incana* share a common oviduct that is surrounded by a thick circular muscle layer, as is the case in *D. pardii* ("diverticulum" after Schockaert 1971, or "insemination duct" after Artois and Schockaert 2001). However, the peculiar horns at the connection of the common oviduct to the recepaculum seminis (Schockaert 1971; Artois and Schockaert 2001) are absent in both *D. longistyla* sp. nov. and *D. incana*.

Environmental remarks

Relationships between environmental factors and turbellarian presence in the sample sites during the study are presented in Figures 3–5. The number of specimens is plotted against temperature, conductivity, and oxygen.

High levels of conductivity did generally indicate high levels of salinity. The sites with lower salinity values are areas that correspond to the brackish water ecotone. The substrates consisted of muddy, stony, or sandy sediments. In these areas Turbellaria abundance shows the highest values (cf. Figure 3). The maximum value at site 12 was essentially caused by the high abundance of *Macrostomum rostratum* (>30 individuals).

The temperature recordings (Figure 4) reflect the actual values at the time of sampling, which varied between 11.6 and 27.8°C at different times of day.

Dissolved oxygen ranged between 1 and 10 mg l^{-1} (Figure 4). The upper sediment layers (0–2 cm sediment depth) were well oxygenated and contrasted well against the lower greyish chemocline in which the oxygen values rapidly declined to very low values. Low values of oxygen correspond with sites from which turbellarian species were generally absent or which were very poor in species richness (Figure 4). That holds for the limnetic and marine areas, but in the brackish water ecotone there were some sites (10, 12, 25, and 27) with low oxygen values and high abundance values. This may be a result of a favourable combination of abiotic and biological factors.

In Figure 5, the percentage of species in each of the different types of substrate is shown. Habitats consisting of sand or mud each accounted for 29% of the species. The dominant

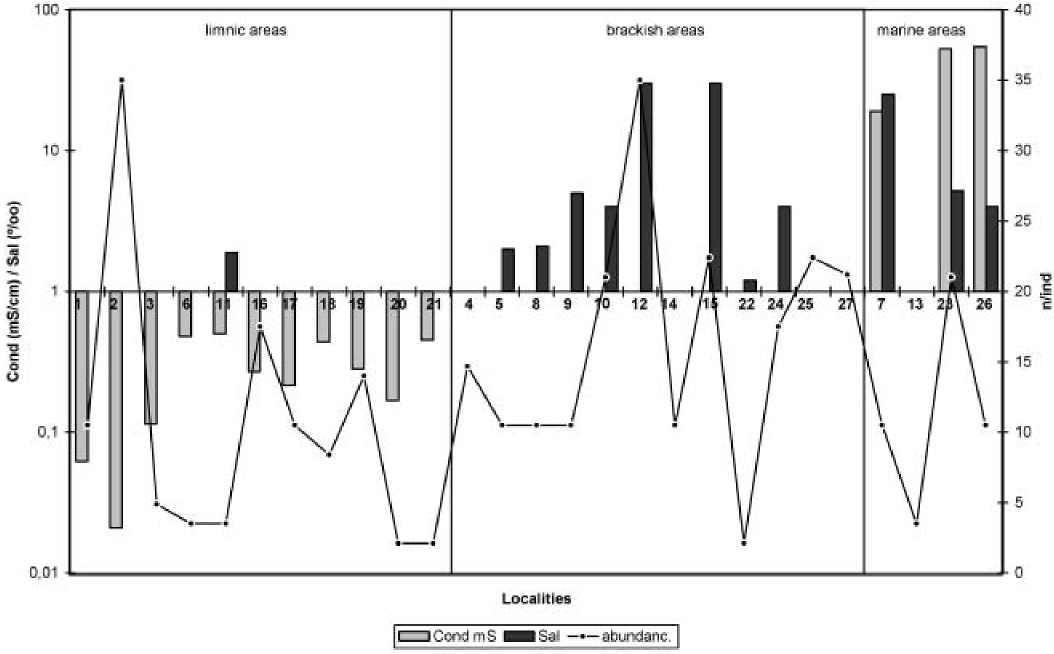

Figure 3. Number of turbellarians found in relation to the conductivity and to the salinity (conductivity data of the brackish localities were not considered, because they were punctual measurements).

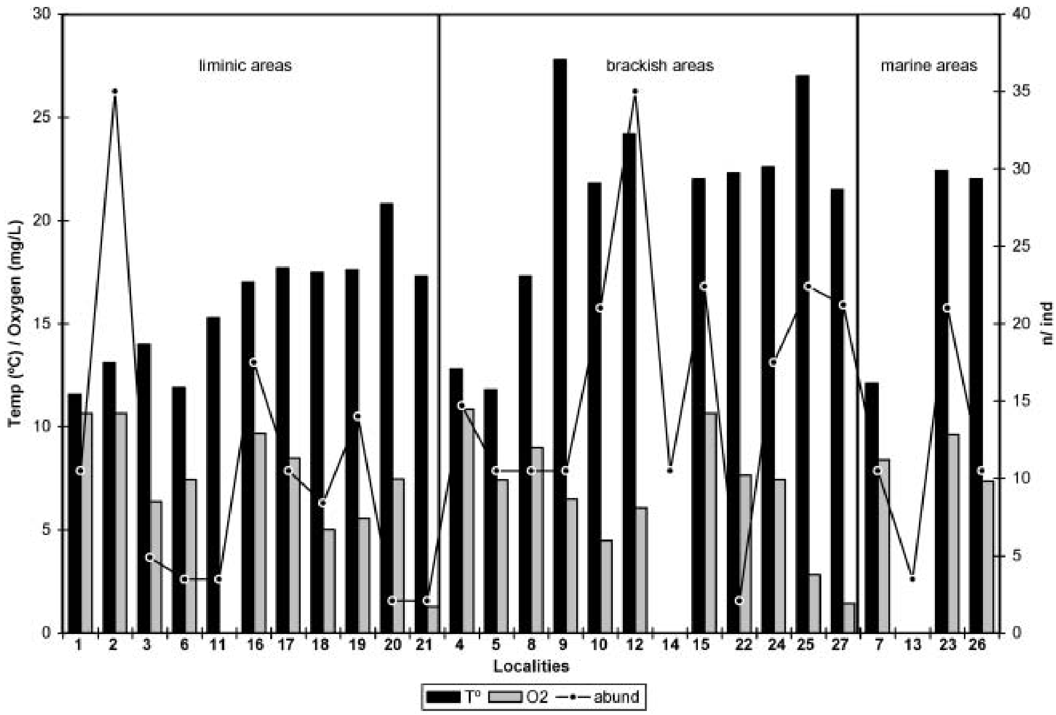

Figure 4. Number of turbellarians found in relation to the water temperature (black bars) and to the concentration of dissolved oxygen in the samples (grey bars) (the samples without dissolved oxygen and temperature data were not considered).

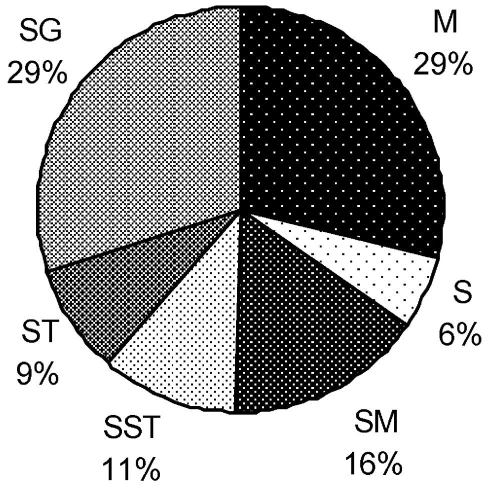

Figure 5. Percentage of turbellarians in relation to the substrate type. M, muddy; S, sandy; SG, sandy-gravel; SM, sandy-muddy; SST, sandy-stony; ST, stony.

substrate covering the bottoms of the sites sampled was mud, sometimes blended with sand. In relation to the occurrence of sandy-gravel sites (5% of the total of sample sites), the abundance of species appears to be relatively higher than in muddy habitats (Figure 4).

With respect to species richness (Figure 4), a clear gradient occurs from habitats arranged according to sand, stony, sandy stony, sandy muddy, and muddy. The lowest numbers of species were found in sands and in stony habitats. However, it is generally the case that muddy sediments are most densely settled. These results suggest that the presence of "Turbellaria", at the species level only, is closely related to characteristic types of substrates.

Discussion

The sampling sites were located on the coast and riverbanks of the *rias* along the Galician and Cantabrian coasts where the habitats range from limnetic (salinity between 0 and 0.5‰) to euhaline areas (e.g. Ria de Ajo, Ria de Rada, salinity of up to 30‰; Figure 3). The habitats considered as brackish (with variable salinity, between 0.5 and 30‰) are characterized by short-term salinity variations (shock-biotopes after Den Hartog 1964). These variations are normal occurrences in the *rias*. Therefore, only species with a high degree of adaptation to salinity variations would be expected to occur (Meixner 1943; Reuter 1961; Ax and Ax 1970).

For the distribution of the turbellarians and colonization of brackish habitats, two environmental factors appear to be fundamental: the substratum and the salinity. Most of the species were found in sandy, sandy-gravel, or sandy-stony substratum (Figure 5). Consequently, the interstitium as a tidal shelter and colonization medium appears to have been verified (Karaman 1955; Ax 1956a; Riemann 1968; Karling 1974). On the other hand, muddy bottom is also an adequate habitat for Platyhelminthes, but only in the first few centimetres of sediment and with the simultaneous presence of green algae or periphyton (Figure 5; Table I).

In relation to the salinity and the studied turbellarian fauna, some species were exclusive to limnetic habitats, a fact that coincides with previous records (e.g.

Microdalyellia tennesseensis, *Castrella truncata*, *Prorhynchus stagnalis*, *Geocentrophora baltica*, *G. sphyrocephala*, *Bothrioplana semperi*, *Phaenocora unipunctata*, and most species of Catenulida). However, most of the species were captured in brackish environments. Hence, some species described previously for marine and brackish-marine environments (i.e. *Nematoplana nigrocapitula* and *Proschizorhynchus triductibus*, respectively) were found in limnetic habitats. These species must be considered in the studied areas as alien species or casual species due to the scarce individual number (Table III), although the presence of *Nematoplana* sp. in oligohaline lagoons of the western Mediterranean is known (Ax 1956b). Likewise, some species that are undoubtedly limnetic, e.g. *Rhynchoscolex simplex* and *Bresslauilla relicta*, during this study were occasionally found in oligohaline water.

Other species known as marine were frequently found during this study in brackish or oligohaline/limnetic areas. This group of species includes: *Mecynostomum auritum*, *Paramecynostomum diversicolor*, *Pelophila* cf. *pachymorha*, *Archilopsis unipunctata*, *Monocelis lineata*, *Promonotus schultzei*, *Nematoplana coelogynoporoides*, *Pseudograffilla* cf. *hymanae*, *Canetellia* cf. *beauchampi*, *Provortex pallidus*, *P. balticus*, *P. tubiferus*, *Maehrenthalia* cf. *intermedia*, *Proxenetes simplex*, *P. unidentatus*, *P. flabellifer*, *Ptychopera westbladi*, *Acrorhynchides* cf. *robustus*, *Phonorhynchus* cf. *helgolandicus*, *Placorhynchus octaculeatus*, *Baltoplana magna*, *Karchinorhynchus* sp., *Proschizorhynchus triductibus*, and *Proschizorhynchella bivaginatus*.

Most of the species captured in brackish environments during this study are overlapping, casual or alien species (Table III), and are closely related to freshwater or marine habitats. This indicates that these species are adapted to changes in salinity. Thanks to this ability, the species of the brackish environments are in the best position to colonize marine or limnetic habitats. Finally, most of the species identified in this study are known from the coasts of Europe.

On the other hand, some of the species found were not known to dwell in this region, such as *Nematoplana* cf. *nigrocapitula*, *Cheliplana* cf. *setosa*, and *Archilina papillosa* (Pacific Ocean), and *Microdalyellia tennesseensis* (USA). Other freshwater species have a wider distribution, such as *Microstomum lineare*, *Geocentrophora baltica*, *Geocentrophora spyrocephala*, and *Gyratrix hermaphroditus*, common in Europe, North America, and other regions.

Acknowledgements

The authors thank Dr Dietrich Blome for stimulating and critical comments, Dr Tom Artois for his invaluable help in the identification of the genus *Djeziraia*, and Sarah Young for proofreading the English text. Last but not least, the authors thank the anonymous referees for their helpful comments and observations. Financial support was given by the Consejo Superior de Investigaciones Cientificas (CSIC) and the project Fauna Ibérica (SEUI-DGES PB95-0235).

References

Armonies W. 1987. Freilebende Plathelminthes in supralitoralen Salzwiesen der Nordsee: Oekologie einer borealen Brackwasser-lebensgemeinchaft. Microfauna Marina 3:81–156.

Arroyo NL, Maldonado M, Pérez-Portela R, Benito J. 2004. Distribution patterns of meiofauna associated with a sublittoral *Laminaria* bed in the Cantabrian Sea (north-eastern Atlantic). Marine Biology 144(2):231–242.

Artois TJ, Schockaert ER. 2001. Interstitial fauna of the Galapagos: Duplacrorhynchinae, Macrothynchinae, Polycystidinae, Gyratricinae (Platyhelminthes Polycystididae). Tropical Zoology 14:63–85.

Artois T, Schockaert ER. 2005. Primary homology assessment of structures in the female atrial system among species of the Polycystididae (Rhabditophora, Eukalytorhynchia). Invertebrate Biology 124:109–118.

Attrill MJ, Thomas RM. 1996. Long-term distribution patterns of mobile estuarine invertebrates (Ctenophora, Cnidaria, Crustacea: Decapoda) in relation to hydrological parameters. Marine Ecology Progress Series 143:25–36.

Ax P. 1956a. Die Einwanderung mariner Elemente der Mikrofauna in das limnische Mesopsammal der Elbe. Verhandlungen der Deutschen Zoologischen Gesellschaft 1956:428–435.

Ax P. 1956b. Les Turbellariés des Etangs Cotiers du Litoral Mediterranéen de la France Meridionale (Pyrénées-Orientales). Vie et Milieu (Actualités Scientifiques et Industrielles, Supplement 5) 1246:1–214.

Ax P. 1991. Northern circumpolar distribution of brackish-water plathelminthes. Hydrobiologia 227:365–368.

Ax P. 1995. Brackish-water Plathelminthes from the Faroe Islands. Hydrobiologia 305:45–47.

Ax P, Ax R. 1970. Das Verteilungprinzip des subterranen Psammon am uebergang Meer-Susswasser. Mikrofauna Meeresbodens 1:1–51.

Blome D. 1996. Inventory of the free-living marine nematodes of the east Frisian Wadden Sea including the estuaries of the rivers Ems, Jade, and Weser. Senckenbergiana Maritima 26:107–115.

Blome D, Faubel A. 1996. Eulittoral nematodes from the Elbe estuary: species composition, distribution and popultion dynamics. Archiv für Hydrobiologie, Supplement 110:107–157.

Borja A, Aguirrezabalaga F, Martinez J, Sola C, Garcia-Arberas L, Gorostiaga M. 2004. Benthic communities, biogeography and resources management. In: Borja A, Collins M, editors. Oceanography and marine environment of the Basque Country, Series 70. Elsevier Oceanography. Amsterdam: Elsevier. p 455–492.

Chappuis PA. 1942. Eine neue Methode zur Untersuchung der Grundwasserfauna. Universitas Francisco-Josephina "Kolozsvar". Acta Scientarum Mathematicarum Naturalium 6:1–7.

Curras A, Sanche-Mata A, Mora A. 1993. Estudio comparativo de la macrofauna bentónica de un fondo de zostera marina y un fondo arenoso libre de cubierta vegetal. Cahiers de Biologie Marine 35:91–112.

Den Hartog C. 1964. Typologie des Brackwassers. Helgolander Wissenschaftliche Meeresuntersuchungen 10:377–390.

Den Hartog C. 1977. Turbellaria from intertidal flats and saltmarshes in the estuaries of the southwestern part of the Netherlands. Hydrobiologia 52:29–32.

Faubel A, Warwick RM. 2005. The marine flora and fauna of the Isles of Scilly: free-living Plathelminthes ("Turbellaria"). Journal of Natural History 39:1–4.

García-Álvarez O, Miguez J, Fernandez S, Ortiz S, Veloso M. 1993. Poblamientos faunísticos intermareales de sustrato duro en la ría de La Coruña. Publicación Especial del Instituto Español de Oceanografía 11:267–274.

Garcia-Arberas L, Rallo A. 2004. Population dynamics and production of *Streblospio benedicti* (Polychaeta) in non polluted estuary on the Basque Coast (Gulf of Biscay). Scientia Marina 68:193–203.

Garmendia JM, Sanchez-Mata A, Mora J. 1996. Ecological seasonal study of molluscs of the ría de Ares-Betanzos (Galicia NW Spain). Iberus 14:115–123.

Gieysztor M. 1931. Contribution à la connaisance des Tuerbellariés rhabdocéles (Turbellaria, Rhabdocoela) d'Espagne. Bulletin de l'Academie Polonaise des Sciences et des Lettres, Classe des Sciences Mathematiques et Naturelles, Serie B, Sciences Naturelles 2:125–153.

Gourbault N. 1981. Les peuplements de nematodes du chenal de la baie de Morlaix (premiers donnees). Cahiers de Biologie Marine 22:65–82.

Guillen J, Palanques A. 1997. A historical perspective of the morphological evolutions in the lower Ebro River. Environmental Geology 30:174–180.

Ibañez C, Pont P, Prat N. 1997. Characterization of the Ebro and Rhone estuaries: a basis for defining and classifiying salt-wedge estuaries. Limnology and Oceanography 42:89–101.

Ibañez C, Prat N, Canicio A. 1996. Changes in the hydrology and sediment transport produced by large dams on the lower Ebro River and its estuary. Regulated Rivers: Research and Management 12:51–62.

Ibañez C, Rodrigues-Capitulo A, Prat N. 1995. The combined impacts of water quantity and quality on saline incursion: the dynamics of the salt wedge and the ecology of the lower Ebro River. In: Harper DM, Ferguson A, editors. The ecological basis for river manangements. New York: John Wiley & Sons. p 105–114.

Karaman T. 1955. Ueber einige Amphipoden des Grundwasses der jugoslawischen meeresküste. Acta Musei Macedonici Scientiarum Naturalium 2:223–241.

Karling TG. 1963. Die Turbellarien Ostfennoskandiens. V. Neorhabdocoela 3. Kalyptorhynchia. Fauna Fennica 17:1–101.

Karling TG. 1974. Turbellarian fauna of the Baltic proper. Fauna Fennica 27:3–101.

Lastra M, Sanchez-Mata A, Palacio J, Mora J. 1993. Dinámica temporal y producción secundaria de *Melinna palmata* Grube, 1870 en la bahía de Santander (N. de España). Cahiers de Biologie Marine 34:43–53.

Luther A. 1943. Untersuchungen an rhabdocoelen Turbellarien. IV. Ueber einige representanten der Familie Proxenetidae. Acta Zoologica Fennica 38:1–95.
Luther A. 1960. Die Turbellarien Ostfennoskandiens. I. Acoela, Catenulida, Macrostomida, Lecithoepitheliata, Prolecithophora and Proseriata. Fauna Fennica 7:1–155.
Luther A. 1962. Die Turbellarien Ostfennoskandiens. III: Neorhabdocoela 1. Dalyellioida, Typhloplanoida: Byrsophlebidae and Trigonostomidae. Fauna Fennica 12:1–71.
Luther A. 1963. Die Turbellarien Ostfennoskandiens IV. Neorhabdocoela 2. Typhloplanoida: Typhloplanidae, Solenopharyngidae und Carcharodopharyngidae. Fauna Fennica 16:1–163.
Margalef R. 1989. Ecología. Barcelona: Ediciones Omega.
Meixner J. 1943. Uber die Umbildung einer Turbellarienart nach Einwanderung aus dem Meere ins Süsswasser. Internationale Revue der Gesamten Hydrobiologie und Hydrographie 43:458–468.
Mettam C, Conneely ME, White SJ. 1994. Benthic macrofauna and sediments in Severn Estuary. Biological Journal of the Linnean Society 51:71–81.
Mikhailova MV. 2003. Transformation of the Ebro River delta under the impact of intense human-induced reduction of sediment runoff. Water Resources 30(4):370–378.
Muñoz I. 1990. Effects of river regulation on the lower Ebro River (NE Spain). Regulated Rivers: Research and Management 3:345–354.
Nasonov NV. 1926. Die Turbellarien Fauna des Leningrader Gouvernements. Bulletin de l'Academie des Sciences de l'URSS 1926:817–884.
Palacio J, Lastra M, Mora J. 1992. Distribución vertical de la macrofauna intermareal en la ensenada de Lourizan (ria de Pontevedra). Thalassas 9:49–62.
Reuter M. 1961. Untersuchungen über Rassenbildung bei *Gyratrix hermaphroditus* (Turbellaria, Neorhabdocoela). Acta Zoologica Fennica 100:5–32.
Riemann F. 1968. Die mikrofauna subterranear Lebensräume im Grenzbereich Meer-Süsswasser. Naturwissenschaftliche Rundschau 21:198–203.
Sanchez-Mata A, Lastra M, Mora J. 1993. Macrobenthic crustacean characterization of an estuarine area. Crustaceana 64:337–355.
Schockaert E. 1971. Turbellaria from Somalia. I. Kalyptorhynchia (Part 1). Monitore Zoologico Italiano 5:101–112.
Smol N, Willens KA, Govarre JCR, Sandee AJJ. 1994. Composition, distribution and biomass of meiobenthos in the Oosterschelde estuary (SW Netherlands). Hydrobiologia 282/283:197–217.
Soetaert K, Vincx M, Wittoeck J, Tulkens M. 1995. Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia 311:185–206.
Soetaert K, Vincx M, Wittoeck J, Tulkens M, Van Gansbecke D. 1994. Spatial patterns of Westerschelde meiobenthos. Estuarine, Coastal and Shelf Science 39:367–388.
Steinböck O. 1954. Sobre la misión del "plasmodio digestivo" en la regeneración de *Amphiscolops* (Turbellaria, Acoela). Publicación del Instituto de Biología Aplicada 17:101–117.
Warwick RM, Gee JM. 1984. Community structure of estuarine meiobenthos. Marine Ecology Progress Series 18:91–111.
Warwick RM, Goss-Custard JD, Kirby R, George CL, Pope ND, Rowden AA. 1991. Static and dynamic environmental factors determining the community structure of estuarine macrobenthos in SW Britain: why is the Severn estuary different? Journal of Applied Ecology 28:329–245.