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Diatom communities in thermo-mineral springs of Galicia (NW Spain)

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Abstract

The species composition of diatom assemblages was studied in five unpolluted thermo-mineral springs in Galicia (NW Spain). Three are considered hot and two cold, and some contain hydrogen sulphide. A total of 68 taxa (24 genera) have been recorded. The Shannon–Wiener diversity index (H') ranged between 0.63 and 2.01. The nMDS ordination showed that diatom assemblage composition was influenced mostly by conductivity, temperature and hydrogen sulphide concentration. Diatom assemblages found in the Galician springs differed from those in springs of other geographical locations with similar physical and chemical characteristics. The most species-rich genera were *Nitzschia* and *Achnanthidium* with 10 and 8 species, respectively. *Achnanthidium exiguum* and *Achnanthidium saprophilum* had the widest distribution. *Denticula thermalis* and *Achnanthidium caledonicum* were found in environments with relatively low mineralization and low temperature, whereas *Rhopalodia gibberula*, *Rhopalodia operculata* and *Fragilaria crotonensis* were found in mineral-rich springs with high temperatures. *Achnanthidium exiguum*, *A. saprophilum*, *Achnanthes coarctata*, *Achnanthes exig*ua var. *elliptica*, *Mayamaea atomus*, *Eunotia implicata*, *Gomphonema minusculum*, *Gomphonema minutum* and *Cosmioneis pusilla* were present in the spring with high H_2S content.

Keywords:
Biodiversity, Diatoms, Environmental parameters, Galicia, Thermo-mineral springs, Thermal-sulphur waters

Introduction

The outstanding importance of springs for the conservation of freshwater biodiversity is increasingly being recognized (Cantonati et al. 2012). Since they originate underground, most permanent freshwater springs have fairly stable physical and chemical characteristics. Due to this, and despite their small size, springs have great ecological value. They are very interesting ecotones for studies of biological communities because their biota is often enriched by rare taxa and endemics, which from a biogeographic point of view can be very important (Kilroy et al. 2007). For these reasons, springs are unique aquatic habitats for the study of microalgal floras, especially diatoms, which are often dominant. In addition, the particular conditions that occur in some springs make them ideal places to study specific algal assemblages in extreme natural conditions, such as high temperature, low pH and the presence of hydrogen sulphide (Hambrook et al. 1999, Denicola 2000, Quintela et al. 2013).

Galicia (NW Spain) is an area with extensive thermo-mineral groundwater resources, many of them with therapeutic value, constituting an important resource for human utilization. More than 300 springs are registered in Galicia, of which 22 have been used for therapeutic purposes since ancient times (Roman thermal baths) (Xunta de Galicia 1995). The regional geologic diversity ensures high variability in the hydrochemical facies of its mineral waters, and for this reason many different spring types are found, including the commoner, cold and moderately mineralized, thermal or saline springs. In addition, one characteristic of this area is the presence of many sulphur springs that are formed where deep waters rise through igneous and metamorphic rocks of high sulphide content, where conditions can be anoxic. Many of these springs are also unpolluted by nutrients.

However, despite the importance and usefulness of these environments, the biota of the thermo-mineral springs in Galicia has been little studied (Noguerol 1984), especially in relation to diatoms. For this reason, the main objective of this work was to describe the current taxonomic composition and structure of the epilithic diatom assemblages in certain springs in Galicia, determining the relationships between their environmental variables and their species composition.

Methods

Location and description of the sampled springs

Five springs in Galicia (NW Spain) were selected for this study. Galicia is located in the so-called wet Iberia with an oceanic climate. Its main features are the regularity of rainfall during the year, from 1000 to 1500 mm per year and mild temperatures with low annual oscillation. The selected springs are representative of the broader range of springs that can be found within the Hesperian massif. In addition, they are considered unchanged since ancient times and are currently used for balneotherapy. Three thermal springs, As Burgas (42°20′04″N, 7°51′55″W), Outariz (42°20″56″N, 7°54′58″W) and Cuntis (42°38′10″N, 8°33′45″W), and two cold-water springs, Guitiriz (43°10′41″N, 7°53′32″W) and Augas Santas of Pantón (42°30′58″N, 7°36′08″W) were studied (Fig. 1). Three of these springs (Cuntis, Pantón and Guitiriz) are considered sulphur springs.

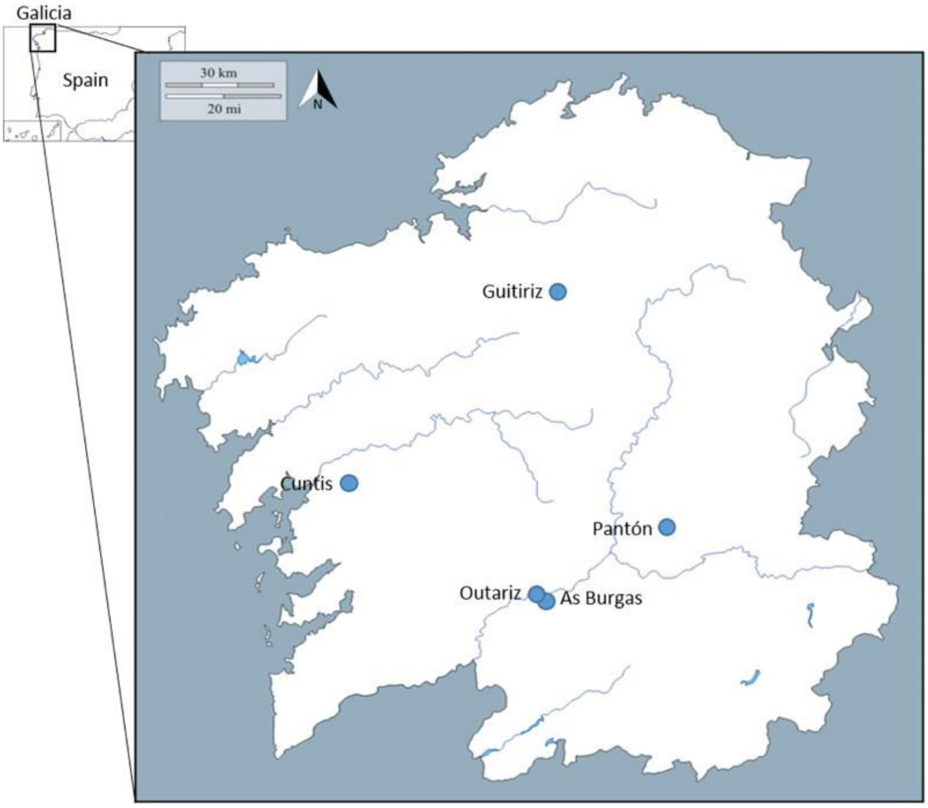

Fig. 1. Maps showing the geographical locations of the five springs selected for the present work.

The As Burgas and Outariz springs are characterized by high temperature (60–65 and 56–62°C, respectively) and are located in the city of Ourense, at the bottom of a valley excavated by the Miño River. Both springs arise through a dense network of fissures (Delgado-Outeiriño et al. 2009). Lithologically, they are situated on granite crystalline rocks covered by alluvial soils and their waters emerge at the highest temperature of all the thermal springs in Galicia. With a flow rate of 50 L s^{-1}, As Burgas flows through a nineteenth century granite building, comprising a niche with a central pillar that sits in a 7 cm deep basin. This basin receives the water that overflows from the upper 1 cm deep basin. Some of the water that overflows from the lower basin runs through a narrow channel where the water accumulates. The samples from this spring were taken by scraping the surface of the granite stones (Fig. 2). The Outariz spring emerges directly among rocks at the margin of the Miño River with a flow rate of 10 L s^{-1}, running through the rocks down to the river. The samples were taken from the rock surfaces (Fig. 3).


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Figs 2–6. Photographs illustrating the morphology of the springs and the sampling sites. Fig. 2. As Burgas. Fig. 3. Outariz. Fig. 4. Guitiriz. Fig. 5. Aguas Santas of Pantón. Fig. 6. Cuntis.

Guitiriz and Augas Santas of Pantón springs are considered cold (14–17 and 19–20°C, respectively) and are located in the Ollo de Sapo domain where there is great rock diversity, in addition to the dominant granite massifs (Corral & López 2010). Guitiriz comprises an artificial stone fountain with a semi-circular basin fed by water from two pipes. The basin is about 90 cm long, 50 wide and 20 deep, and excess water drains into an underground sink. The flow rate is 0.5 L s^{-1}. Samples were taken from the walls of the fountain (Fig. 4). The Aguas Santas of Pantón spring emerges directly from the rocks with a flow rate of 1.5 L s^{-1}. Its waters are collected in a rectangular basin through two pipes. The water drains continuously through a hole in the bottom of the basin. Samples were taken from the walls of this basin (Fig. 5).

The Cuntis spring experiences deep hydrothermal flows and intermediate chemical evolution passing through granites and biotitic granodiorites, and its water is considered warm to hot (48–54°C). Samples from this spring were collected from a granite channel, 20 cm wide, 15 cm deep and 8 m long, through which water flows continuously at 0.6 L s^{-1}. The thermal water of this spring emerges inside a nearby building, passing through a hole in the wall (Fig. 6).

Physical and chemical characteristics

Water temperature, pH and conductivity were measured *in situ* at four different points in each spring, as close as possible to the diatom sampling sites, with portable instruments calibrated in the field. Temperature and pH were measured with an HI 8014 Crison-Hanna meter and conductivity was measured using an HI 8633 probe EC meter. Water samples were collected in 1 L polyethylene bottles to allow major ions, nutrients and trace elements to be analysed in the laboratory following standard procedures and methods (Apha 2005).

Sampling and identification of diatoms

The algae were scraped off the stones, edges and bottom of the springs with a sterile knife, according to European recommendations (Kelly et al. 1998). Four samples were collected from each spring. The samples were immediately preserved with formalin (4% final concentration) and stored in plastic tubes. The samples were cleaned of organic matter following standard methods (Comité European de Normalisation 2003). Permanent diatom slides were made with Naphrax®. Diatoms were examined using a light microscope (LM) (Olympus BX51) with a ×100 oil immersion DIC objective. Approximately, 400 valves were counted in each sample. Light photographs of the most abundant taxa were taken with a DP70 Olympus camera. Diatoms were identified using standard floras and related literature (Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b, 2000, Lange-Bertalot 1993, 1999, Potapova & Hamilton 2007, Hofmann et al. 2013, Trobajo et al. 2013).

Data and statistical analysis

For the numerical analyses, diatom count data were expressed as relative abundances. Species diversity was calculated using the Shannon–Wiener diversity index (H') (Shannon & Weaver 1963). A non-metric multidimensional scaling technique (nMDS) with Bray–Curtis distance measure, 100 maximum iterations and square-root transformation of diatom data was used to explore variation of diatom assemblages in the studied springs. Environmental variables were correlated to the nMDS axes using environmental vector fitting (envfit procedure). Environmental variables were first transformed using square-root transformation and standardized. Redundant environmental variables were eliminated taking into account those that obtained a value greater than 0.78 in a Pearson correlation matrix. With the variables retained in the analysis, the fit (R^2) of each variable to the ordination using the envfit function (see below) was assessed with a Monte-Carlo analysis of 999 permutations. nMDS and correlation with environmental variables (envfit function) were performed using the BiodiversityR library (Kindt & Coe 2005) in the R programming environment v. 3.3.1 (R Core Team 2016).

Results

Environmental parameters

There was great variability in temperature and conductivity (Table 1), but pH did not vary much and was always above 7.0 in all the springs (weak alkaline or alkaline springs). Carbonate and bicarbonate were the prevalent anions in most of the springs. The Pantón, Cuntis and, to a lesser extent, Guitiriz samples had higher proportions of sulphate. The percentage of Na and K ions was high in all samples, unlike Ca and Mg ions which were very low in all springs. SiO_2 was also high, especially in Cuntis and As Burgas.

Table 1. Range of the physical and chemical characteristics obtained at the sampling points ($n = 4$) in the five springs.

Parameter/Spring	Cuntis	Pantón	Guitiriz	As Burgas	Outariz
Temperature (°C)	40–43	19–20	13–17	37–42	40–44
pH	8.7–8.9	7.9–9.0	8.4–9.5	7.5–7.8	7.2–7.8
Conductivity (µS cm^{-1})	487–526	674–689	340–354	835–864	556–580
F^- (mg L^{-1})	18–22	20–29	14–17	12–14	11–17
Cl^- (mg L^{-1})	55–59	39–58	24–29	18–24	19–26
HCO_3^- (mg L^{-1})	45–76	180–232	49–87	512–646	316–327
CO_3^{2-} (mg L^{-1})	22–94	24–30	<d.l.-6	< d.l.	<d.l.-2
SO_4^{2-} (mg L^{-1})	45–48	64–85	15–37	3–11	9–20
S^{-2} (mg L^{-1})	21–37	18–20	11–66	< d.l.	<d.l.–1
Li^+ (mg L^{-1})	0.1–1	3–3.1	0.5–0.8	4–5	0.8–1.2
Na^+ (mg L^{-1})	104–109	129–133	68–83	202–260	136–142
K^+ (mg L^{-1})	3–4	4.9–5.1	0.8–1	9–11	5–5.3
Mg^{2+} (mg L^{-1})	0.04–1	2–3	0.1–0.9	1–3	0.9–1
Ca^{2+} (mg L^{-1})	1.8–3	4–7	1.2–4	3–7	2–7
Mn^{2+} (mg L^{-1})	< d.l.	<d.l.–0.012	< d.l.	0.04	0.02
NH_4^+ (mg L^{-1})	03–05	4.4–5.4	0.92	0.7–1.01	0.39–055
SiO_2 (mg L^{-1})	89–95	36–42	18–26	84–88	61–92
Fe^{2+} (µg L^{-1})	<d.l.–0.04	72–182	<d.l.–0.33	35–53	<d.l.–3
Ba^{2+} (µg L^{-1})	0.6–1	6–6.2	< d.l.	44–94	0.1–2.3
Al^{3+} (µg L^{-1})	7–19	30–33	< d.l.	19–29	<d.l.-12
H_2S (mg L^{-1})	7–8	2.4–2.7	1.3–1.5	< d.l.	< d.l.

Note: d.l. = detection limit.

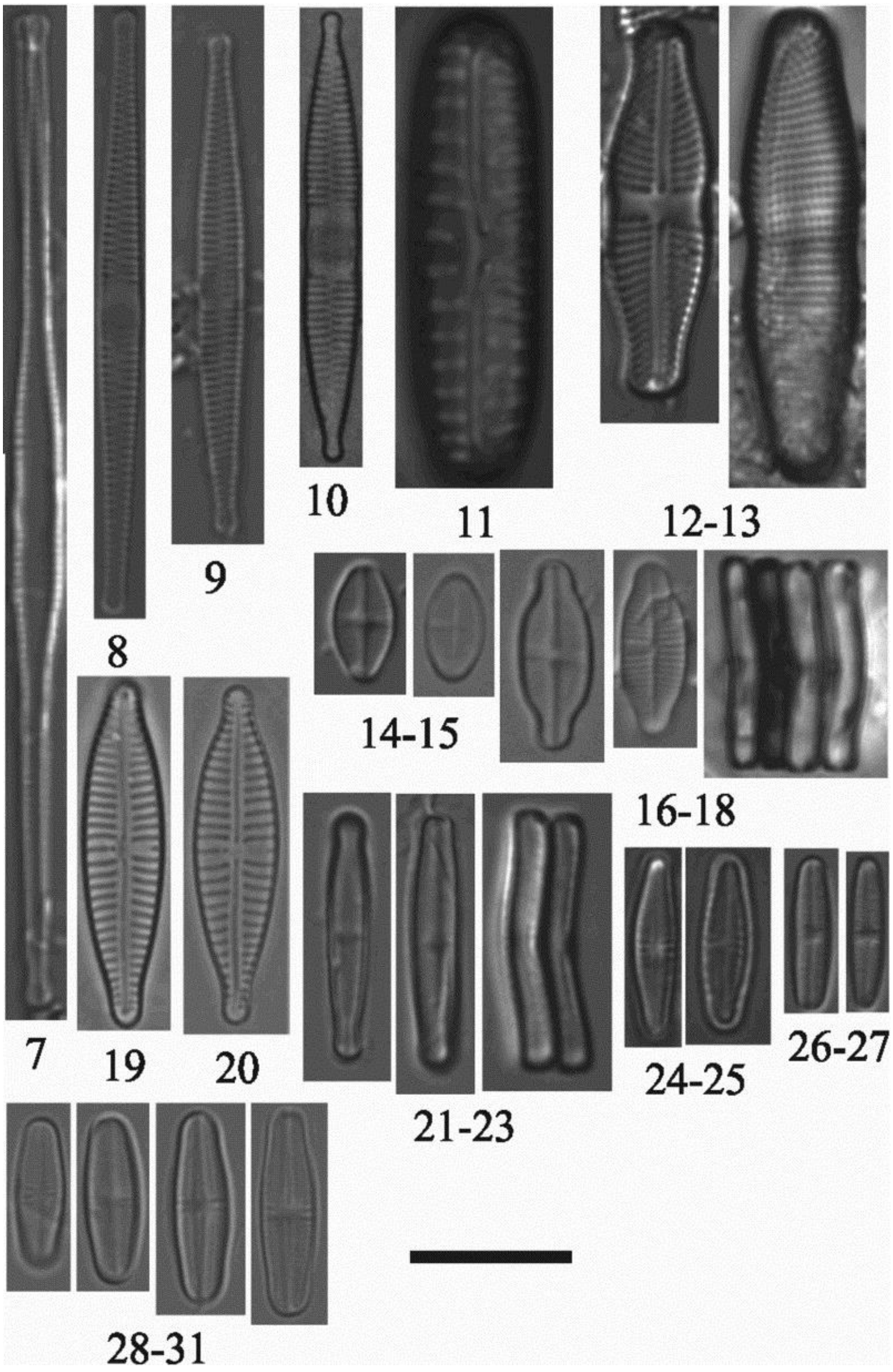
Table 2. Species composition and relative abundance of diatoms in the studied springs.

Taxon name	Abbreviations	As Burgas	Cuntis	Guitiriz	Pantón	Outariz
Achnanthes coarctata (Brébisson) Grunow	AchCo	0	0–1	0	0	0
Achnanthes exigua var. *elliptica* Hustedt	AchEx	0–1	0–1	0	0	0
Achnanthidium caledonicum (Lange-Bertalot) Lange-Bertalot	AchCal	0	0	1–3	0	0
Achnanthidium exiguum (Grunow) Czarnecki	AchExi	2–4	3–5	0–1	0	0–1
Achnanthidium exile (Kützing) Heiberg	AchExil	0	0	0	0	1
Achnanthidium lanceolatum Brébisson ex Kützing	AchLan	0	0–1	0–1	0	0–1
Achnanthidium lineare W. Smith	AchLi	0	0	0–1	0–1	0–1
Achnanthidium minutissimum (Kützing) Czarnecki	AchMi	1	0	0	1	1–2
Achnanthidium saprophilum (H. Kobayasi & S. Mayama) Round & L. Bukhtiyarova	AchSa	0–1	1	0	1–3	0–1
Achnanthidium subhudsonis (Hustedt) H. Kobayasi	AchSub	0	0	0	0	0–1
Amphora pediculus (Kützing) Grunow ex A. Schmidt	AmPe	0	0	0	0	0–1
Caloneis vasileyevae Lange-Bertalot, Genkal & Vekhov	CaVasi	0	0–1	0	0	0
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	CocPla	0–1	0	0	0	0–1
Cocconeis pseudolineata (Geitler) Lange-Bertalot	CocPsel	0	0	0	0	0–1
Cocconeis pseudothumensis Reichardt	CocPseth	0–1	0	0	0	0
Cosmioneis pusilla (W. Smith) D.G. Mann & A.J. Stickle	CosPu	0	0–1	0	0	0
Ctenophora pulchella (Ralfs ex Kützing) D.M.Williams & Round	CtePul	0	0	0	0	1
Cyclotella meneghiniana Kützing	CyMen	0	0	0	0	0–1
Denticula thermalis Kützing	DenTher	0	0	3–5	0	0
Diadesmis contenta (Grunow) D.G. Mann	DiadCont	0–1	0–1	0	0	0–1
Diatoma mesodon (Ehrenberg) Kützing	DiatMes	0	0	0	0	0–1
Encyonema minutum (Hilse) D.G. Mann	EnMi	0	0	0	0	0–1
Encyonema silesiacum (Bleisch) D.G. Mann	EnSi	0	0	0	0	1
Eolimna minima (Grunow) Lange-Bertalot & W. Schiller	EoMi	0	0–1	0	0	0–1
Eunotia implicata Nörpel, Lange-Bertalot & Alles	EuImp	0	0–1	0	0	0
Fallacia vitrea (Østrup) D.G. Mann	FaVi	0–1	0	0	0	0–1
Fragilaria acus (Kützing) Lange-Bertalot	FraAc	0	0	0	0–2	0–1
Fragilaria crotonensis Kitton	FraCro	0	0	0	0	2–4
Fragilaria pararumpens Lange-Bertalot, G. Hofmann & Werum	FraPar	0	0	0	0	0–1
Fragilaria perminuta (Grunow) Lange-Bertalot	FraPer	0	0	0	0	0–1
Fragilaria rumpens (Kützing) G.W.F. Carlson	FraRu	0	0	0	0	0–1
Gomphonema capitatum Ehrenberg	GomCa	0	0	0	0	0–1
Gomphonema clavatum Ehrenberg	GomCla	0	0	0	0	0–1
Gomphonema exilissimum Lange-Bertalot & E. Reichardt	GomExi	0	0	0	0	0–1
Gomphonema minusculum Krasske	GomMis	0	0–1	0	0	0
Gomphonema minutum (C. Agardh) C. Agardh	GomMin	0	0–1	0	0	0
Gomphonema parvulum (Kützing) Kützing	GomPar	0	0	0	0	0–1
Halamphora veneta (Kützing) Levkov	HaVe	0–1	0	0	0	0
Hannaea arcus (Ehrenberg) R.M. Patrick	HanAr	0	0	0	0	0–1
Luticola goeppertiana (Bleisch) D.G. Mann	LuGoe	0	0	0	0–1	0
Mayamaea atomus (Kützing) Lange-Bertalot	MaAto	0	0–1	0	0	0
Navicula cincta (Ehrenberg) Ralfs	NaCin	0	0	0	0	0–1
Navicula cryptotenella Lange-Bertalot	NaCry	0	0	0	0	0–1
Navicula gregaria Donkin	NaGre	0–1	0	0	0	0–1
Navicula salinicola Hustedt	NaSal	0–1	0	0	0	0

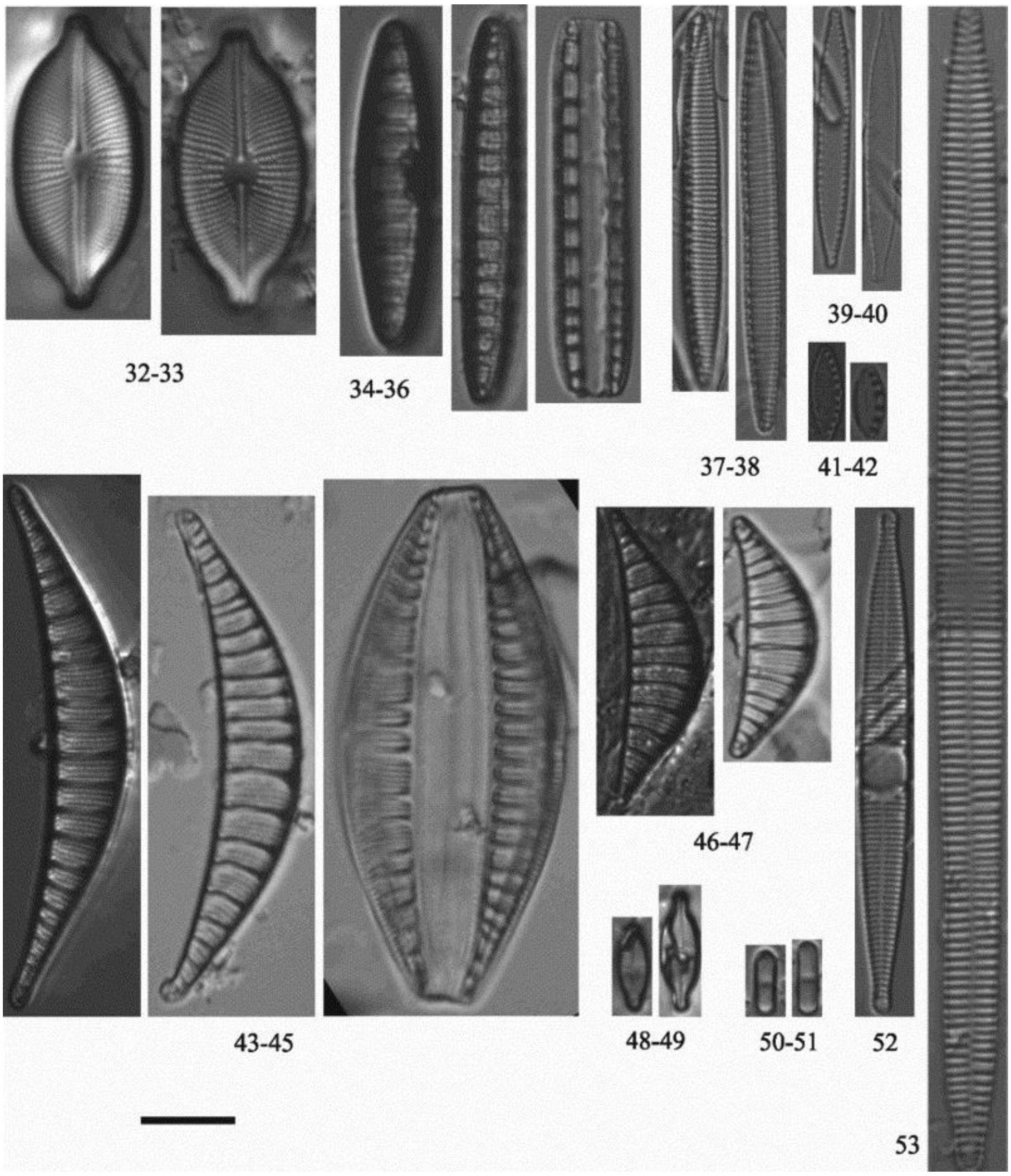
Table 2. Species composition and relative abundance of diatoms in the studied springs.

Taxon name	Abbreviations	As Burgas	Cuntis	Guitiriz	Pantón	Outariz
Navicula tripunctata (O.F. Müller) Bory de Saint-Vincent	NaTri	0–1	0	0	0	0
Navicula veneta Kützing	NaVen	0	0	0	0	0–1
Nitzschia amphibia Grunow	NitAmp	0–1	0	0	1–2	0–1
Nitzschia bulnheimiana (Rabenhorst) H.L. Smith	NitBul	0	0	0	0	0–1
Nitzschia clausii Hantzsch	NitCla	0	0	0	0–1	0
Nitzschia fonticola (Grunow) Grunow	NitFon	0	0	0	0–1	0–1
Nitzschia hantschiana Rabenhorst	NitHan	0	0	0–1	0	0
Nitzschia inconspicua Grunow	NitInc	1	0–1	0	0	0
Nitzschia palea (Kützing) W. Smith	NitPa	0–1	0	0	0	0–1
Nitzschia palea var. *debilis* (Kützing) Grunow	NitPaD	0	0–1	0	1–2	0
Nitzschia thermalis (Ehrenberg) Auerswald	NitTh	0	0–1	0	0	0
Nitzschia valdestriata Aleem & Hustedt	NitVal	0	0–1	0	0	0
Pinnularia borealis Ehrenberg	PinBo	0	0–1	0	0	0
Pinnularia schoenfelderi Krammer	PinSch	0	0–1	0	0	0
Punctastriata sp.	Pun	0	0	0	0	0–1
Rhopalodia gibberula (Ehrenberg) Otto Müller	RhoGib	1–3	0	0	0	0
Rhopalodia operculata (C. Agardh) Håkanasson	RhoOp	1	0	0	0	0
Sellaphora parapupula Lange-Bertalot	SePar	0	0	0	0	0–1
Sellaphora seminulum (Grunow) D.G.Mann	SeSe	0–1	0–1	0	0–1	0
Staurosira dubia Grunow	StDu	0	0	0	0	0–1
Staurosira venter (Ehrenberg) Cleve & Moeller	StVen	0–1	0	0	0	0
Tabellaria flocculosa (Roth) Kützing	TaFlo	0	0	0	0	0–1
Ulnaria biceps (Kützing) Compère	UlBi	0	0	0–1	0–1	0
Number of species in each spring	19	20	7	11	40	
Number of genera	11	12	4	6	18	

Note: Relative abundance of diatoms: 0 – absence, 1 – (0–20)% relative abundance, 2 – (21–40), 3 – (41–60); 4 – (61–80), 5 – (81–100).



Figs 7–31. LM micrographs of the sampled material. Fig. 7. *Fragilaria crotonensis*. Fig. 8. *Fragilaria pararumpens*. Fig. 9. *Fragilaria perminuta*. Fig. 10. *Fragilaria rumpens*. Fig. 11. *Pinnularia borealis*. Figs 12, 13. *Achnanthes coarctata* (Fig. 12 – raphe valve, Fig. 13 – rapheless valve). Figs 14–18. *Achnanthidium exiguum* (Figs 14, 15 – raphe valve, Figs 16, 17 – rapheless valve, Fig. 18 – girdle view). Fig. 19. *Gomphonema exilissimum*. Fig. 20. *Gomphonema parvulum*. Figs 21–23. *Achnanthidium caledonicum* (Fig. 21 – raphe valve, Fig. 22 – rapheless valve, Fig. 23 – girdle view). Figs 24, 25. *Achnanthidium minutissimum*. Figs 26, 27. *Achanthidium lineare*. Figs 28–31. *Achnanthidium saprophilum* (Figs 28, 29 – rapheless valve, Figs 30, 31 – raphe valve). Scale bar = 10 µm.



Figs 32–53. LM micrographs. Figs 32, 33. *Cosmioneis pusilla*. Figs 34–36. *Denticula thermalis*. Figs 37, 38. *Nitzschia amphibia*. Figs 39, 40. *Nitzschia palea* var. *debilis*. Figs 41, 42. *Nitzschia inconspicua*. Figs 43–45. *Rhopalodia gibberula*. (Fig. 45 – girdle view). Figs 46, 47. *Rhopalodia operculata*. Figs 48, 49. *Achnanthes exigua* var. *elliptica*. Figs 50, 51. *Diadesmis contenta*. Fig. 52. *Ctenophora pulchella*. Fig. 53. *Fragilaria acus*. Scale bar = 10 µm.

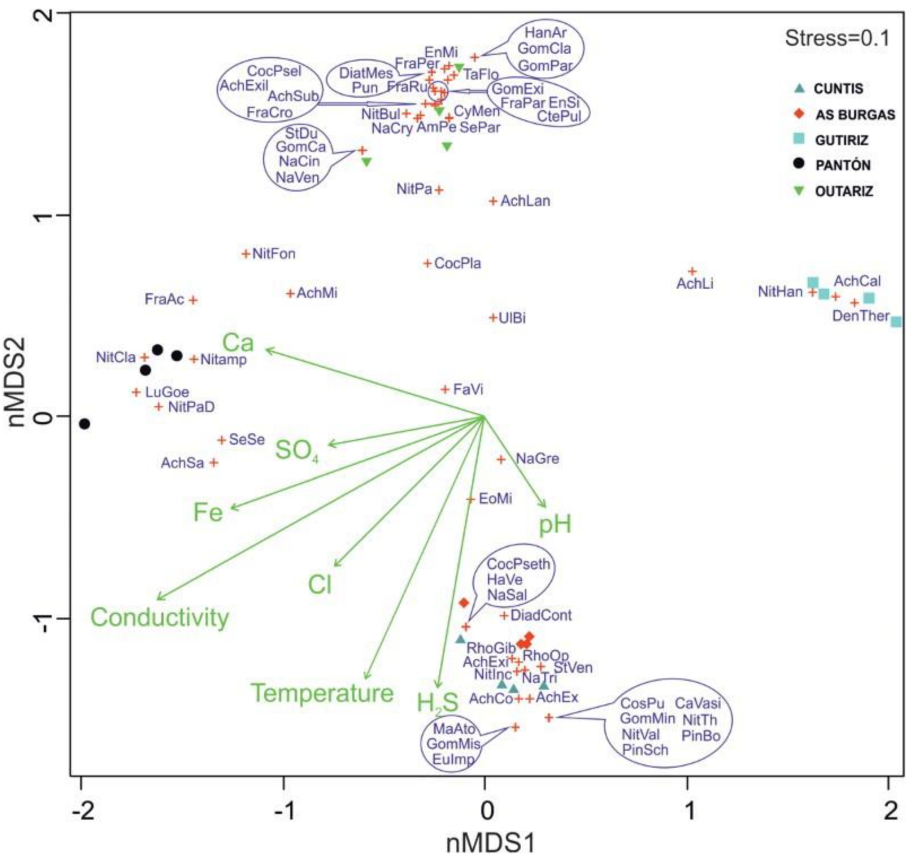


Fig. 54. The non-metric multidimensional scaling (nMDS) ordination diagram of the springs based on species composition. The eight environmental variables tested are shown as green arrows, with orientation and length indicating direction of greatest change and strength of correlation, respectively. Correlation data are shown in Table 3.

Nutrient levels (nitrate, nitrite, phosphate) were very low in the five springs, below detection limits (~0.01 mg L^{-1}). Trace element concentrations were compared with the United States Environmental Protection Agency guidelines (Usepa 1985 USEPA) for water quality criteria. Under these guidelines, critical concentrations of Al, Fe and Mn ions for aquatic life are 87, 1000 and 1090 µg L^{-1}, respectively, which, when exceeded, could harm aquatic organisms. None of the studied water samples had concentrations that exceeded these limits (Table 1).

After eliminating the redundant environmental variables, eight remained for further analysis by nMDS. Thus, conductivity, which measures total ionic concentration, summarized the information from the concentrations of Li, Na, K, HCO_3, Ba and Al ions. In addition, it was found that SiO_2 concentration correlated with temperature.

Diatom assemblages

In total, 68 diatom taxa from 24 genera were found. In the hot springs, diatoms only occurred in the samples from shallow water or from damp ground located at or close to the springs, and at temperatures below 45°C (Table 1).

A taxonomic list of the recorded diatom species found with their relative abundances is presented in Table 2. Generic richness ranged from 4 to 18, whereas species richness ranged from 7 to 40. The maximum number of identified taxa (40) was found in Outariz and the minimum number (7) in Guitiriz. The most species-rich genera were *Nitzschia* Hassall and *Achnanthidium* Kützing with 10 and 8 species, respectively. The most common species were *Achnanthidium exiguum* (Grunow) Czarnecki (Figs 14–18) and *Achnanthidium saprophilum* (Kobayasi & Mayama) Round & L. Bukhtiyarova (Figs 28–31), which were found in four of the five springs. Seventy-two percent of the taxa were only found in one of the springs. Fourteen diatom species reached at least 10% relative abundance in at least one sample: *Fragilaria crotonensis* Kitton (Fig. 7), *A. exiguum* (Figs 14–18), *Achnanthidium caledonicum* (Lange-Bertalot) Lange-Bertalot (Figs 21–23), *Achnanthidium minutissimum* (Kützing) Czarnecki (Figs 24, 25), *A. saprophilum* (Figs 28–31), *Cosmioneis pusilla* (W. Smith) Mann & Stickle (Figs 32, 33), *Denticula thermalis* Kützing (Figs 36–38), *Nitzschia amphibia* Grunow (Figs 37, 38), *Nitzschia palea* var. *debilis* (Kützing) Grunow (Figs 39, 40), *Rhopalodia gibberula* (Ehrenberg) O. Müller (Figs 43–45), *Achnanthes exigua* var. *elliptica* Hustedt (Figs 48, 49), *Diadesmis contenta* (Grunow) D.G. Mann (Figs 50, 51), *Ctenophora pulchella* (Ralfs ex Kützing) Williams & Round (Fig. 52) and *Fragilaria acus* (Kützing) Lange-Bertalot (Fig. 53).

Outariz, Cuntis and As Burgas had the highest species diversity, whereas Pantón and Guitiriz had low-diversity assemblages. Outariz stands out, having 40 different taxa and an *H′* index of 2.01 ± 0.56. This contrasts with Guitiriz, a cold-water spring with moderate mineralization, in which only seven taxa were identified and the H′ index was lowest, 0.63 ± 0.29. The other springs had intermediate *H′*values: Pantón 1.55 ± 0.35, As Burgas 1.33 ± 0.13 and Cuntis 0.72 ± 0.58.

The nMDS ordination (Fig. 54) shows the differences in species composition among the five different springs forming four clusters, with Cuntis and As Burgas in the same cluster, indicating a greater degree of similarity between them. Following the removal of redundant environmental variables, eight variables were included in the analysis and fitted to the ordination space. Based on R^2-values (Table 3), conductivity, temperature and hydrogen sulphide had the strongest relationship with species composition.

Table 3. Relationships between the species ordination scores (nMDS) and the fitted environmental variables.

	nMDS1	nMDS2	R^2	*p*
Temperature	−0.97133	−0.23773	0.331	0.049*
pH	0.56649	−0.82407	0.0467	0.666
Conductivity	−0.85003	−0.52673	0.5339	0.003**
Cl	−0.99603	−0.08905	0.2206	0.114
SO_4	−0.53535	−0.84463	0.0895	0.45
Ca	−0.11424	−0.99345	0.2041	0.134
Fe	−0.94765	0.31932	0.2911	0.050*
H_2S	−0.69844	−0.71567	0.3251	0.044*

Note: (*): $p < 0.05$; (**): $p < 0.01$.

The position of the diatom taxa in the nMDS ordination diagram showed that the species associated with lower conductivity and temperature were *D. thermalis*, *A. caledonicum* and *Nitzschia hantzschiana* Rabenhorst. These conditions occurred in Guitiriz samples. These species also appear to tolerate the presence of H_2S, but at low levels. By contrast, species such as *R. gibberula, Rhopalodia operculata* (C.A. Agardh) Håkanasson, *Pinnularia borealis* Ehrenberg, *Nitzschia thermalis* (Ehrenberg) Auerswald, *F. crotonensis*, *Navicula salinicola* Hustedt and *Achnanthes coarctata* (Brébisson) Grunow were associated with more mineral-rich environments and higher temperatures.

Discussion

The Galician springs showed diatom populations that varied in terms of composition and abundance. The number of taxa found was higher in hot springs (63 in 3 sites) than in the cold springs (15 in 2 sites). This suggests that warm environments maintain a more diverse diatom population. However, 11 taxa were common to both hot and cold springs, demonstrating their great thermal amplitude. In the springs whose waters emerge at high temperatures, diatoms only occurred in samples collected where temperatures were below 45°C. This is in accordance with the results of Smith et al. (2013) who studied the algal component of Hot Springs National Park (Arkansas, USA.), only observing diatoms in two springs at lower temperatures (<40°C). Patrick et al. (1969) found that an average temperature of 34–38°C resulted in a shift of dominance in the algal flora from diatoms to cyanobacteria. However, there are examples of diatoms that occur at higher temperatures, as in hot springs from Kuril and Sakhalin Islands (Russia). At these locations, the largest number of diatom taxa was recorded in springs and reservoirs with water temperatures from 37°C to 60°C (Nikulina & Kociolek 2011).

In the Galician springs, *A. exiguum* was the most common and abundant species. It was particularly abundant in the hot springs of Cuntis and As Burgas, with a relative abundance of a 90% in some samples. This species is considered typical of hot spring environments (Fairchild & Sheridan 1974). It has a wide ecological amplitude and has been found in many different types of water, including industrial and other wastewater. Its occurrence has been recorded several times in high-temperature habitats (Quintela et al. 2013). In addition, this species is found in environments with moderate to elevated electrolyte content (Krammer & Lange-Bertalot 1991b). Our results are in agreement with these observations; however, this species was scarce in the samples collected from Outariz whose temperature and conductivity were also high, but instead, it was also found in Guitiriz samples (cold spring). This is not surprising since Fairchild & Sheridan (1974) found that the maximum and minimum temperatures at which this species grew were 44°C and 10°C, respectively.

As in studies carried out on different springs in Europe (Delgado et al. 2013, Lai et al. 2016), *Nitzschia* was also the most represented genus in the Galician springs, by 10 species. *Nitzschia* is a widely distributed genus with a large number of species, several of which are ecologically important. They are very common (often the most abundant taxa) in different types of inland, coastal and marine waters (Trobajo et al. 2013). The predominant species in the Galician springs was *N. amphibia*, which was not only abundant (around 35%) in the samples from the cold Pantón spring, but also was found (with lower abundance, <4%) in the hot springs of As Burgas and Outariz. Although this species predominates in cold environments, for example in cold-water, low conductivity springs in eastern Spain (Aboal et al. 1998), it can occur in high-temperature environments (Mannino 2007). Owen et al. (2008) found that the optimum temperature for this species was 24.75°C.

Another interesting genus found in three of our springs was *Gomphonema* Ehrenberg, whose species are relatively common in freshwater diatom communities, although most occurrences are in rivers and lakes, rarely in springs. As in other aquatic systems in Europe (Wojtal 2003), *Gomphonema* was represented in the Galician springs, although it was not very abundant. More

Gomphonema species were found in the Outariz spring, but none were detected in the Guitiriz and Pantón samples. *Gomphonema parvulum* (Kützing) Kützing is widespread and very common in waters with a wide range of trophic characteristics (Abarca et al. 2014), but whether or not this species is ubiquitous and cosmopolitan remains controversial. In this study it was only found in the Outariz spring.

Achnanthidium minutissimum sensu lato was found in the outflow channels of Outariz and Pantón and was an important component of the diatom populations from these springs, although with a relative abundance below 20%. It was also found in As Burgas, but with even lower (<2%). *Achnanthidium minutissimum* was seventh in order of abundance in these springs, indicating that it is a typical species for these environments. Our data indicate that the temperature does not seem to be an important factor in its distribution and it is also widely distributed in springs in the Alps (Cantonati & Lange-Bertalot 2010, Gesierich & Kofler 2010, Mogna et al. 2015), on Majorca (Delgado et al. 2013), and in many alkaline springs elsewhere in Europe (Wojtal & Sobczyk 2012, Kollár et al. 2015, Luc & Oosterlynck 2015). Its widespread occurrence throughout Europe contributes to the generally accepted idea that it is a ubiquitous taxon (Ector 2011). However, a complexity of forms has been attributed to this species and new taxa described from its varieties (Potapova & Hamilton 2007, Van de Vijver & Kopalová 2014). Nevertheless, moderate abundance of *A. minutissimum* in a river (0–25%) has been considered as an indicator of no disturbance (Stevenson & Bahls 1999). In fact, it was used with good results to characterize the quality of the waters of the Guadalquivir River (Martín et al. 2010) and for the assessment of Mediterranean rivers (Almeida et al. 2014). Similarly, *A. minutissimum* was also found in karst springs of Wyżyna Krakowsko-Czętochowska Upland in southern Poland (Wojtal & Sobczyk 2012) where it was dominant in epilithic samples from springs located away from villages with lower specific conductivity. Mackay et al. (2012) consider this species to be sensitive to organic pollution and nutrient enrichment, which agrees with its presence in the unpolluted Galician springs. Therefore, all these data seem to support the idea that this species is a good indicator of good water quality and reinforces interest in this species as a bioindicator.

Although the diatom communities found in Galicia showed some similarities (especially the species of wide distribution) with other, more or less similar springs, diatom populations in the Galician springs differed in composition and abundance (Table 2). Thus, alpine springs with some similar characteristics to Guitiriz (cold water, low conductivity and slightly alkaline pH) were dominated by *A. minutissimum*, *Meridion circulare* (Greville) C.A. Agardh var. *circulare*, *Diatoma mesodon* (Ehrenberg) Kiitzing, *Denticula tenuis* Kiitzing, *Encyonema sublangebertalotii* Lange-Bertalot & M.Cantonati, *Planothidium lanceolatum* (Brébisson ex Kutzing) Lange-Bertalot, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi or *Achnanthidium dolomiticum* M.Cantonati & Lange-Bertalot (Cantonati & Lange-Bertalot 2010). None of these species was found in Guitiriz, in which *D. thermalis*, *A. caledonicum* and *Achnanthidium lineare* W. Smith were the most abundant species. However, it is noteworthy that the alpine springs emerge through carbonate rocks and therefore have higher calcium and magnesium values of than those of Guitiriz (Galician springs mainly emerge on igneous and metamorphic rocks). Similarly, in cold springs on Majorca, also on calcareous substrata the diatom assemblages were characterised by *A. minutissimum*, *A. pyrenaicum*, *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, *Cymbella vulgata* Krammer, *Diploneis separanda* Lange-Bertalot, *Encyonopsis minuta* Krammer & Reichardt, *Gomphonema lateripunctatum* Reichardt & Lange-Bertalot and *Navicula cryptotenella* Lange-Bertalot (Delgado et al. 2013). Only the cold Pantón spring, which had the highest calcium and magnesium values (Table 1), showed a certain similarity with those calcareous springs, owing to the presence of *A. minutissimum*, although the other species were different. Similarly, high-temperature Galician springs did not coincide with other sites within a similar temperature range. In the same way that the geothermal floras found elsewhere (Iceland, New Zealand and Kenya) vary in terms of composition, diversity and abundance (Owen et al. 2008), the Galician springs also vary. For example, of the most common diatoms found in Sicilian thermal-sulphur waters (*A. pediculus*, *Cocconeis placentula* Ehrenberg, *Gomphonema minutum* (C.A. Agardh) C.A. Agardh, *Navicula cryptotenella* Lange-Bertalot, *Navicula tripunctata* (O.F. Müller) Bory, *N. amphibia*, *Rhoicosphenia abbreviata* (C.A. Agardh) Lange-Bertalot, *Gomphonema gracile* Ehrenberg and *Nitzschia commutata* Grunow (Mannino 2007), only *N. amphibia* occurred in As Burgas and Outariz. Even *D. contenta*, abundant in the cold springs of Majorca, was also found in the samples from the three high-temperature Galician springs.

These results seem to indicate that it is difficult to establish a characteristic diatom flora for spring waters. In fact, none of the taxa were common to the five springs and only two (*A. exiguum* and *A. saprophilum*) were found in four springs. In addition, 73.5% of the taxa were only found in a single spring. It should be added that some of the taxa in these Galician springs were common, widely distributed species (probably habitat generalists) with broad ranges of tolerance to the major environmental variables.

However, there are other species for which the physical and chemical characteristics of the individual spring environment may be more decisive, controlling their distribution. Thus, different combinations of temperature and conductivity could explain the differences in the composition of diatom assemblages in the Galician springs. However, these factors are insufficient to explain the differences when the assemblages are compared with those from similar environments elsewhere. The Cuntis spring can be considered similar to other hot spring systems, in Iceland, New Zealand or Kenya (Owen et al. 2008), however its assemblages were different. One of the physical and chemical properties that characterize the Cuntis spring is its high H_2S content. Hydrogen sulphide content showed good correlation with the nMDS ordination and could be partially responsible for the differences observed. Hambrook et al. (1999) noted that important differences in the flora and fauna of regionally nearby springs in Ohio were due to the presence of H_2S in one of them. This toxic compound is considered an important factor in the distribution of the diatom populations because of their varying tolerance to it (Admiraal & Peletier 1979). In fact, of the 20 species in Cuntis, 11 were found exclusively there (especially *C. pusilla*, *A. coarctata* and *Mayamaea atomus* (Kützing) Lange-Bertalot, Table 2). Of the remaining nine, *N. palea* var. *debilis* was also abundant in Pantón, another sulphur spring. It is also noteworthy that *N. thermalis* (found in Cuntis) was tolerant to a concentration of H_2S of 0.9 mM in unialgal cultures in the laboratory (Admiraal & Peletier 1979). Some of the species found in this spring coincide with those found in other springs of similar characteristics (temperature and presence of H_2S), as are the thermal-sulphur waters of Fiume Caldo (Northwestern Sicily) (Mannino 2007), for example, *G. minutum* and *Nitzschia inconspicua* Grunow. However, as indicated above, the similarities are few, perhaps determined by the difference in conductivity; about five times greater in Fiume Caldo than in Cuntis. On the other hand, there are also differences in the diatom populations between Cuntis and a sulphidic spring in Slovenia (Eleršek & Mulec 2014), although in this case the conductivities are very similar. However, these springs differ in temperature and pH. Therefore, it is difficult to attribute a unique role to H_2S in determining species composition in Cuntis compared to other springs containing this compound. This is mainly due to the absence of data on H_2S content in springs, but the species found in Cuntis could be a useful for studying this type of environment. The lower diversity of diatoms in Cuntis could also be related to H_2S content compared with Outariz. Both springs had very similar temperatures and conductivities (Table 1), but species diversity was greater in Outariz. The species whose relative abundances differentiated the springs were *A. exiguum*, *F. crotonensis*, *A. minutissimum* and *C. pulchella*. *Fragilaria crotonensis* was abundant in the Outariz samples and was found in lakes that have a high sulphur content due to mining (Hamilton et al. 2015); however, it was not detected in the Cuntis samples. On the other hand, the presence (with relative abundance) of *A. exiguum* in the sulphidic Cuntis spring is an indicator of its wide distribution and resistance to stressors.

Conclusions

Diatom assemblages found in the Galician springs showed great diversity. As in other springs, diatoms occurred at temperatures below 45°C. A significant number of species were common to cold and hot springs. The environmental variables that had more influence on the distribution of diatoms were conductivity, temperature and H_2S content. This last variable could be important in determining the diatom composition in the sulphur spring of Cuntis. With respect to these variables it can be concluded: (1) *A. exiguum* and *A. saprophilum* are widely distributed; (2) *D. thermalis, A. caledonicum* and *N. amphibia* are common in low mineral content environments; (3) *R. gibberula* and *R. operculata* are abundant in mineral-rich environments with higher temperatures; and (4) *A. exiguum*, *A. saprophilum*, *A. coarctata*, *A. exigua* var. *elliptica*, *M. atomus*, *E. implicata*, *G. minusculum*, *G. minutum* and *C. pusilla* are tolerant to H_2S.

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