Acta Bot. Croat. 68 (2), 263–283, 2009

CODEN: ABCRA 25
ISSN 0365–0588

Epilithic diatoms (Bacillariophyta) from cloud forest and alpine streams in Bolivia, South America 3: diatoms from Sehuencas, Carrasco National Park, Department of Cochabamba

EDUARDO A. MORALES[1,2,3*], ERIKA FERNÁNDEZ[4], PATRICK J. KOCIOLEK[5]

[1] Herbario Criptogámico Universidad Católica Boliviana, Carrera de Ingeniería Ambiental, Casilla de Correos 5381, Cochabamba, Bolivia

[2] Patrick Center for Environmental Research, The Academy of Natural Sciences of Philadelphia, PA 19103-1195, USA

[3] Unidad de Limnología y Recursos Acuáticos, Universidad Mayor de San Simón, Casilla de Correos 992, Cochabamba, Bolivia

[4] Centro de Biodiversidad y Genética, Universidad Mayor de San Simón, Casilla de Correos 538, Cochabamba, Bolivia

[5] Museum of Natural History, UCB 218, University of Colorado, Boulder, CO 80309, USA

Studies on Bolivian diatoms are scarce and they do not represent the great geographic variability of the country. One of the regions with the highest biological diversity in Bolivia is the Yungas (cloud forest), a 90.500 km^2 strip located between the Andean Puna and the Amazonian lowlands. The Carrasco National Park is the park with the largest extension of Yungas within its boundaries. This park is located east from Cochabamba, the third largest city in Bolivia, and has an area of ca 6.226 km^2, serving as a refuge to 5.000 recorded species of plants and more than 300 species of vertebrates. Very little is known about the aquatic biota in the zone and there are no studies on diatoms. One of the preferred tourist spots within the park is Sehuencas, located 17°31'42" S and 65°16'17" W and characterized by numerous lotic waterbodies. The present work was carried on 5 epilithic samples from which 118 species, varieties and forms were identified using light (LM) and scanning electron microscopy (SEM). Forty-two (36%) of these taxa were not found in the literature for South America or other regions of the world. This high percentage of unknown taxa suggests a high potential for the contribution of new organisms to science, many of which are possibly endemic to the region, thus justifying an additional effort to preserve the aquatic habitats in the park. Two new species are described herein (*Fragilaria cochabambina* Morales sp. nov. and *Achnanthidium sehuencoensis* Morales

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* Corresponding author, e-mail: edu.morales2006@gmail.com

sp. nov.) and comparisons with published morphologically similar taxa are presented. Unknowns remain undescribed until larger populations that allow detailed LM and SEM studies are found.

Key words: Diatom, *Fragilaria cochabambina*, *Achnanthidium sehuencoensis,* ultrastructure, cloud forest, Yungas, Carrasco National Park, Bolivia

Introduction

Bolivian diatoms have been poorly studied and a review of the literature (MORALES et al. 2008) shows that from the 54 references published for specific algal groups, only 12 (22%) deal with diatoms. Furthermore, this literature is largely restricted to the Altiplano (high mountain plateau) and the lowlands of the Amazon. The taxonomy presented in these publications is poor and names based on European floras have been force-fitted giving the impression that the flora is not too diverse and that it does not differ too much from other parts of the world. However, the recent works of Lange-Bertalot and collaborators (METZELTIN and LANGE-BERTALOT 1998, RUMRICH et al. 2000, METZELTIN and LANGE-BERTALOT 2007), among others, show that the diatom flora in South America, except for a few cosmopolitan elements, is indeed distinct.

The South American Cloud Forest (or Peruvian-Bolivian Yungas) is one of the most biologically diverse ecosystems in South America (IBISH and MERIDA 2003, NAVARRO and MALDONADO 2004). It runs along the Eastern Cordillera of the Andes, a branch of the mountainous chain that projects in a southeastern direction, between 13 °–17 °S and 69 ° –63 °W. The wide ranges in geographic and climatic characteristics favor the development of a diverse set of habitats populated by a highly diverse flora and fauna. The Yungas is also characterized by hundreds of small and medium-sized rivers and streams with diverse physical and chemical characteristics (NAVARRO and MALDONADO 2004), but the consequences of such a diversity on the aquatic biota are less known, and in the case of algae, completely neglected. Two of the most recent surveys on soft-bodied algae and diatoms (MCCLINTIC et al. 2003 and MORALES and VIS 2007, respectively) show that the potential of the Yungas for the contribution of new species to science, many of which could be endemic to the region, is very high.

The conservation efforts directed to the Yungas have been considerable, from both the Bolivian government and many international organizations (PARKS WATCH 2005). At least 4 national parks contain portions of the Yungas within its territories, Carrasco National Park in the department of Cochabamba being the park with the highest proportion of Yungas within its borders. However, all conservation practices have concentrated on terrestrial ecosystems and there is no clear intention (national or international) to preserve lotic and lentic systems in the area.

The current paper presents a taxonomic assessment, using LM and SEM, of the epilithic diatom flora in five courses of water located in Sehuencas, a region within Carrasco National Park, which has received considerable attention from tourists and sport fishermen. The increased inflow of people has had a visually striking impact on the local aquatic systems, but no scientific assessments have been produced to measure such an impact. We propose that diatoms offer an opportunity to seriously consider conservation practices of aquatic systems within the park.

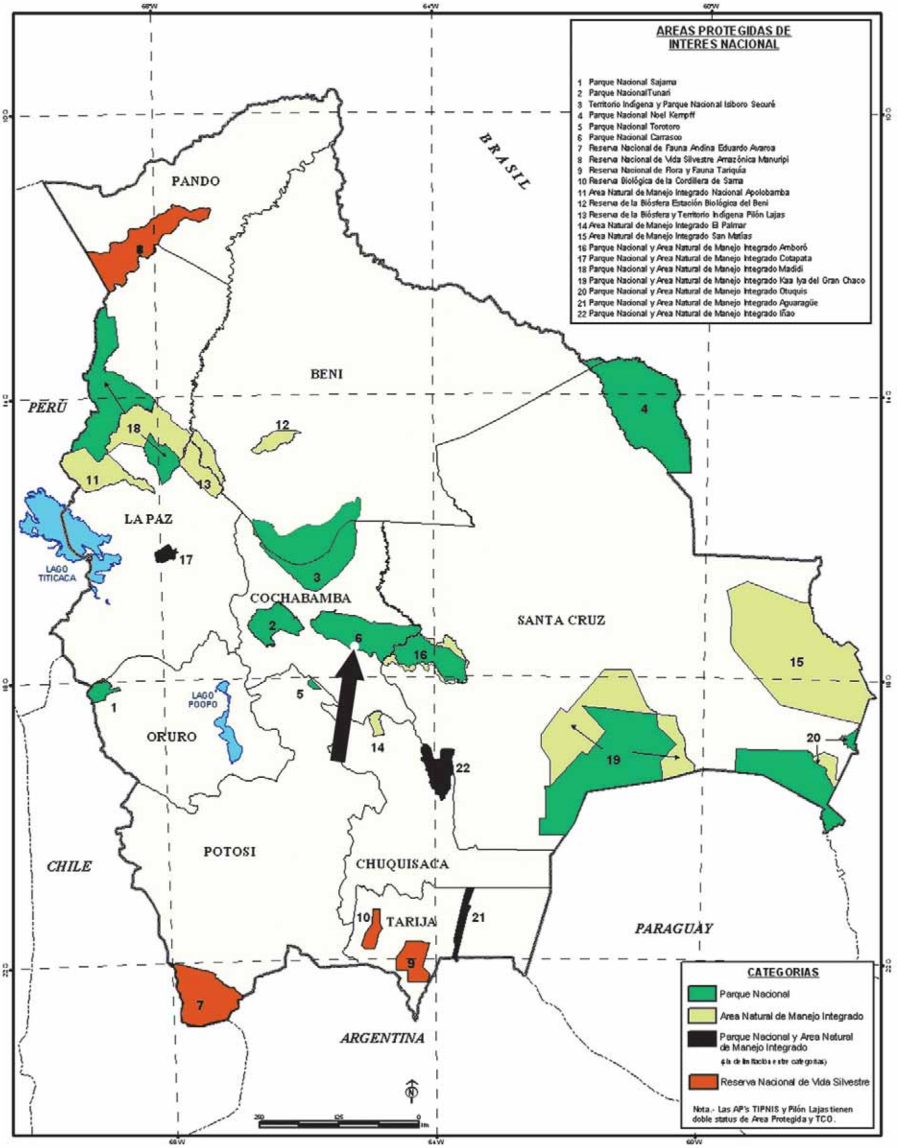


Fig. 1. Map of Bolivia showing the national parks system. Carrasco National Park is located in the center of the country in the department of Cochabamba (number 6). Sehuencas is depicted by a white spot pointed by the black arrow. Modified from www.sernap.gov.bo (visited October, 2008).

Materials and methods

Epilithic material from two medium-sized rivers and 3 streams in Sehuencas (about 150 km from the city of Cochabamba) (Tab. 1) were studied as part of a larger project directed to a preliminary assessment of the epilithic diatom diversity in the cloud forest of Bolivia.

Samples were collected in 2007, scraping with a stiff metal brush, five medium-sized rocks selected from each water course, rinsing scrapings with river water onto a plastic tray, and transferring the mixture into plastic bottles for transport to the laboratory. Samples were fixed with about four drops of formalin per 200 mL of sample, sufficient to kill zooplankton and arrest algal growth. The preservation of samples was not sought since our interest was concentrated on inorganic diatom remains. Location and physicochemical data were collected directly from the stream at the same time samples were collected (Tab. 1).

Tab. 1. Information data for the five lotic systems considered in this work. Descriptions of the sites were done directly in the field as samples were collected.

River / Stream	Geographic location and altitude	Water physico-chemistry	Description
Río Ibirizu	17° 29.8" S, 65° 16.4" W 2.200 m.a.s.l.	T 12 °C; pH 6.8; Cond. 31 µS/cm	Clear water. Width of channel variable (about 50 m in some places). Depth very variable, but no more than 50 cm. Fast flowing current. Bed formed by rocks of different sizes, even large boulders are present. Water contains debris, mainly leaves from nearby trees; logs are also present. Area affected by camping.
Stream 1	17° 31.6" S, 65° 16.2" W 2.400 m.a.s.l.	T 10 °C; pH 6.9; Cond. 31 µS/cm	Clear water. Width of channel variable (about 2 m at sampling site). Depth variable, but no more than 30 cm. Stream cuts off secondary road, water flows fast, but sample was collected from a pool a few steps upstream. Bed composed of fine pebbles, small stones and large boulders. Area affected by road traffic (about one car every hour during the day).
Stream 2	Not determined, 2.450 m.a.s.l.	T 10 °C; pH 6.1; Cond. 20 µS/cm	Clear water. Width of channel variable (about 50 cm at sampling site). Depth variable, but no more than 5 cm. Thread of water coming downhill on the side of the secondary road. Many wet rocks and pebbles, as well as silts characterize the area. Moss and algal growth evident on wet rocks. Coordinates were not determined due to heavy cloud cover, but site is about 400 m up the road from Río Sehuencas.
Río Sehuencas	17° 32.6" S, 65° 16.12" W 2.600 m.a.s.l.	T 10.2 °C; pH 6.7; Cond. 23 µS/cm	Clear water. Width of channel variable (about 20 m at sampling site). Depth variable, but no more than 50 cm. Fast flowing current. River cuts off road and there is no bridge to cross it. Some debris present, as well as fine sandy deposits, especially along area where traffic crosses the river. Smaller boulders and rocks are also seen in the river.
Stream 3	17° 33.2" S, 65° 16.2" W 2.650 m.a.s.l.	T 9 °C; pH 6; Cond. 21 µS/cm	Clear water. Width of channel variable (about 2 m at sampling site). Depth variable, but no more than 20 cm. Stream cuts off secondary road, large rocks, pebbles and some isolated sandy deposits compose the bed. Debris present in the form of leaves and twigs. Green and brown algal growths evident on rocks.

For LM analysis, subsamples were digested with 70% nitric acid, 1:1 by volume with the sample, and boiling the solution for 20 min. Repeated rinsing and decanting of samples with distilled water led to a neutral diatom suspension used for the preparation of permanent slides using Cumar R-9 as mounting medium (HOLMES et al. 1981). Slides were analyzed using a Zeiss Universal microscope equipped with DIC and a Spot Insight 2 color digital camera model 18.2. Images were directly captured using SpotSoftware version 4.6 at magnifications ranging between 1250X and 2000X. Measurements of at least 30 valves for the new taxa were taken using the same software and directly on a computer screen. Two slides were analyzed per site to cover as much diatom diversity as possible. Identifications were performed using local floras, frequently checking also floras for Europe and other continents. This literature is cited in the Discussion section below.

In order to assess quickly which were the most common taxa in the samples, counts of 100 valves were performed along randomly placed transects (width determined by the field of view of the microscope) on one slide of each locality and at 1250X magnification. All identification and count data were stored in an EXCEL spreadsheet, version 2003.

SEM work was performed using a Leica® Stereoscan 430i operated at 20 kV. Aluminium stubs were prepared by filtering aliquots of clean sample through polycarbonate filters with a 30 µm mesh, which were then fixed to the stubs using double sided carbon tape. Coating was accomplished using a BAL-TEC MED 020 Modular High Vacuum Coating System. Images were directly captured and stored in the computer.

All images were manipulated and plates made using Adobe Photoshop v. 7.0. Morphological terminology follows ANONYMOUS (1975), ROSS et al. (1979) and ROUND et al. (1990).

Results

A total of 118 taxa at the species and variety levels distributed in 39 genera were encountered. A high percentage of these taxa (36%) could not be identified and were recorded as »sp.« or »cf.« (Tab. 2). The taxa bearing published names were readily identified using South American floras such as METZELTIN and LANGE-BERTALOT (1998), RUMRICH et al. (2000), METZELTIN et al. (2005), METZELTIN and LANGE-BERTALOT (2007). Taxonomic articles by FRENGUELLI (1939), MANGUIN (1964), SERVANT-VILDARY (1986), MORALES and VIS (2007), MORALES et al. 2007 and the annotated checklist by HOHN (1966), also proved useful. Cosmopolitan taxa and taxa characteristic of eutrophic waters (*Diatoma mesodon* Kützing, *Encyonema silesiacum* (Bleisch) Mann, *Melosira varians* Agardh, and others were identified easily using Süsswasserflora von Mitteleuropa series (KRAMMER and LANGE-BERTALOT 1986, 1988, 1991a, 1991b, LANGE-BERTALOT 1993). References specialized in certain genera such as *Navicula* Bory (LANGE-BERTALOT 2001), *Pinnularia* Ehrenberg (KRAMMER 2000), *Brachysira* Kützing (LANGE-BERTALOT and MOSER 1994) and cymbelloids (KRAMMER 1997a, 1997b) were useful, as well. Other books that are frequently used for identification of South American taxa were not useful or did not contain taxa found in the Yungas region.

The epilithic flora in Sehuencas is richer in biraphid taxa (84 spp.), with monoraphids and araphids being represented by 16 and 13 species each. As expected, centric diatoms are

Tab. 2. Relative abundance of diatoms found in counts of 100 valves along random transects. Diatoms marked with »+« were rare and did not appear during counts. 1: Río Ibirizu, 2: Stream 1, 3: Stream 2, 4: Río Sehuencas, 5: Stream 4.

Taxon/Sample	**1**	**2**	**3**	**4**	**5**
Achnanthes inflata (Kützing) Grunow		1	+		
Achnanthidium minutissimum (Kützing) Czarnecki	10	1	+	6	1
Achnanthidium rivulare Potapova et Ponader					+
Achnanthidium sp. 1 SEHUENCAS	7	10	2	6	+
Achnanthidium sp. 2 SEHUENCAS	+			+	
Adlafia cf. *muscora* (Kociolek et Reviers) Moser, Lange-Bertalot et Metzeltin	+	+	6	+	+
Adlafia minuscula (Grunow) Lange-Bertalot	+	+		2	+
Brachysira minor (Krasske) Lange-Bertalot			8		1
Caloneis sp. 1 SEHUENCAS			+		
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	+				
Cymbella naviculiformis Auerswald ex Heiberg		+			
Diadesmis contenta (Grunow ex Van Heurck) Mann			4	+	
Diadesmis gallica Smith				+	
Diatoma mesodon Kützing	1	44	51	4	64
Encyonema minutum (Hilse) Mann	+			+	+
Encyonema silesiacum (Bleisch) Mann	21	6	+	9	3
Encyonema sp. 1 COROICO			+		
Eolimna minima (Grunow) Lange-Bertalot			6	+	+
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot et Metzeltin			+		
Epithemia adnata (Kützing) Brébisson	+			+	
Eunotia cf. *soleirolii* (Kützing) Rabenhorst	+	2	2	1	1
Eunotia cf. *tropico-arcus* Metzeltin et Lange-Bertalot		+	+		
Eunotia fallax var. *groenlandica* (Grunow) Lange-Bertalot et Nörpel			+		
Eunotia rabenhorstii Cleve et Grunow		+			
Eunotia sp. 1 COROICO					+
Eunotia sp. 1 SEHUENCAS					+
Eunotia sp. 2 SEHUENCAS				+	
Eunotia sudetica Müller				+	
Fistulifera pelliculosa (Brébisson ex Kützing) Lange-Bertalot				+	
Fragilaria capucina var. *mesolepta* Rabenhorst				+	
Fragilaria capucina var. *rumpens* (Kützing) Lange-Bertalot				+	
Fragilaria sp. 2 SORATA		1		+	+
Fragilaria vaucheriae (Kützing) Petersen				3	+
Frankophila similioides Lange-Bertalot et Rumrich		+			
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer		+			
Frustulia crassipunctata Metzeltin et Lange-Bertalot		+			
Frustulia kosmolliana Lange-Bertalot et Rumrich		+		+	+
Gomphonema acuminatum Ehrenberg		4			

Tab. 2. – Continued

Taxon/Sample	**1**	**2**	**3**	**4**	**5**
Gomphonema cf. *angustatum* (Kützing) Rabenhorst		+	1	+	1
Gomphonema cf. *gracile* Ehrenberg			+		
Gomphonema cf. *lagenula* Kützing		2	+	+	+
Gomphonema cf. *parvulum* (Kützing) Kützing			+		
Gomphonema coronatum Ehrenberg	+	+		+	
Gomphonema parvulius (Lange-Bertalot et Reichardt) Lange-Bertalot et Reichardt	+	+			
Gomphonema pumilum var. *elegans* Reichardt et Lange-Bertalot	+	+		1	1
Gomphonema pumilum var. *rigidum* Reichardt et Lange-Bertalot		+			
Gomphonema punae Lange-Bertalot et Rumrich	+	+			+
Gomphonema sp. 1 SEHUENCAS			+		+
Gomphonema sp. 2 COROICO	+	2		1	+
Gomphonema sp. 2 SEHUENCAS			+	+	+
Gomphonema sp. 3 SEHUENCAS		+			
Gomphonema sp. 3 SORATA					+
Gomphonema sp. 4 SEHUENCAS			+	+	
Gomphonema sp. 5 SEHUENCAS				+	
Gomphonema sp. 6 SEHUENCAS					+
Gomphonema sp. 7 SEHUENCAS					+
Gomphonema sp. 8 SEHUENCAS			+		
Gomphonema subclavatum (Grunow) Grunow	+	2		+	+
Hannaea arcus (Ehrenberg) Patrick	30	1	+	7	+
Hantzschia abundans Lange-Bertalot			+		+
Luticola acidoclinata Lange-Bertalot			1		
Luticola goeppertiana (Bleisch) Mann				+	
Luticola sp. 1 SEHUENCAS		+			
Melosira varians Agardh	+			7	
Navicula angusta Grunow		+			
Navicula arvensis Hustedt				+	+
Navicula cf. *lundii* Reichardt		4	+		
Navicula cryptocephala Kützing					+
Navicula gregaria Donkin		+		+	
Navicula notha Wallace		2		2	1
Navicula rhynchocephala Kützing				+	
Navicula vaucheriae Petersen			+	2	
Nitzschia acidoclinata Lange-Bertalot		+	+	+	
Nitzschia amphibia Grunow			+		
Nitzschia cf. *tubicola* Grunow				+	
Nitzschia clandestina Manguin	21	+		3	
Nitzschia dissipata (Kützing) Grunow		+		+	1
Nitzschia inconspicua Grunow				+	

Tab. 2. – Continued

Taxon/Sample	**1**	**2**	**3**	**4**	**5**
Nitzschia intermedia Hantzsch ex Cleve et Grunow	+	+		+	
Nitzschia palea (Kützing) Smith		+			
Nitzschia palea fo. *major* Rabenhorst	+				
Nitzschia palea var. *debilis* (Kützing) Grunow				+	
Nitzschia recta Hantzsch ex Rabenhorst				1	1
Nitzschia sp. 1 SEHUENCAS	1	+		1	12
Nitzschia sp. 4 SORATA	+			+	+
Nupela praecipua (Reichardt) Reichardt	+	+	+	+	+
Orthoseira dendroteres (Ehrenberg) Round, Crawford et Mann		+	+	+	
Orthoseira roeseana (Rabenhorst) O'Meara	+		+		
Orthoseira roeseana (Rabenhorst) O'Meara (Morphotype *spiralis*)			+		
Orthoseira sp. 1 SEHUENCAS				+	
Pinnularia biceps Gregory				+	
Pinnularia cf. viridis (Nitzsch) Ehrenberg		+			
Pinnularia parvulissima Krammer				+	+
Pinnularia subcapitata var. *hilseana* (Janisch) Müller		+	+		+
Planothidium biporomum (Hohn et Hellermann) Lange-Bertalot			+		
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot		6	+		
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot	1	8	13	17	2
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	+	+	+	+	+
Planothidium salvadorianum (Hustedt) Lange-Bertalot			+		
Planothidium sp. 1 COROICO			+		1
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round		+		+	+
Pseudostaurosira brevistriata (Grunow) Williams et Round	+				
Pseudostaurosira laucensis (Lange-Bertalot et Rumrich) Morales et Vis	3			2	
Reimeria sinuata (Gregory) Kociolek et Stoermer	4	+	+	11	
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	+	4	6	2	10
Sellaphora seminulum (Grunow) Mann	+	+	+	+	+
Stauroneis kriegerii Patrick			+		
Stauroneis sp. 1 SEHUENCAS				+	
Stephanodiscus sp. 1 SEHUENCAS					+
Surirella angusta Kützing				+	+
Surirella susanae Metzeltin et Lange-Bertalot	+				
Synedra cf. *ulna* var. *andina* Manguin	+				+
Synedra sp. 15 NAWQA MORALES	+			+	
Tabellaria flocculosa (Roth) Kützing	+	+		2	
Ulnaria sp. 1 SEHUENCAS	1	+		+	+
Ulnaria sp. 2 SEHUENCAS	+	+	+	10	
Ulnaria sp. 3 SEHUENCAS				+	
Ulnaria sp. 4 SEHUENCAS	+				
	100	100	100	100	100

less rich (6 spp.) and are mainly represented by *Orthoseira* Thwaites and *Melosira* Agardh, frequently found in benthic collections. Taking compiled relative abundance into account (the sum of relative abundances for all five sites), araphids are by far the most abundant (45%) followed by biraphid diatoms (36%). Monoraphids accounted for 19% and centric diatoms for only 1%.

Of the 39 genera recorded, *Gomphonema* Agardh, *Nitzschia* Hassall, *Eunotia* Ehrenberg and *Navicula* accounted for the most species (23, 13, 8 and 8 spp., respectively) while the remaining genera are represented by 6 species or fewer. The high number of species within *Navicula* is due to the inclusion of several small, unknown entities within this genus for lack of a better placement. The genera with the highest number of unknown taxa are *Gomphonema*, *Eunotia* and *Ulnaria* (Kützing) Compère (14, 5 and 4, respectively).

Compiling the abundance found at all five sites (in order to estimate the overall abundance of each taxon in Sehuencas), *Diatoma* Bory, *Planothidium* Bukhtiyarova et Round, *Nitzschia*, *Encyonema* Kützing and *Hannaea* Patrick are among the most abundant genera. At the species level, *Diatoma mesodon*, *Planothidium haynaldii* (Schaarschmidt) Lange--Bertalot, *Encyonema silesiacum*, *Hannaea arcus* (Ehrenberg) Patrick, *Achnanthidium sehuencoensis* sp. nov. are among the most abundant (Tab. 3). From the list of 10 species with the highest relative abundance presented in table 3, two taxa were undetermined, namely *Achnanthidium sehuencoensis* sp. nov. and *Nitzschia* sp. 1 (from Sehuencas). The *Achnanthidium* representative was found in high proportions under SEM and thus we describe it here as a new taxon. Although less frequent (but easily found under the microscope due to its size), *Fragilaria cochabambina* sp. nov. was also studied under SEM.

Tab. 3. Relative abundance data compiled (summed) for the five sites included in this study. Left two columns: 10 most abundant genera; right two columns: 10 most abundant species. Relative abundance at each site was calculated counting 100 valves along a randomly placed transect. *small *Nitzschia* morphologically related to *N. acidoclinata* Lange-Bertalot, but with no defined central area spacing between fibulae.

Most abundant genera	Relative abundance compiled for 5 sites	Most abundant species	Relative abundance compiled for 5 sites
Diatoma	164	*Diatoma mesodon* Kützing	164
Planothidium	48	*Planothidium haynaldii* (Schaarschmidt) Lange-Bertalot	41
Nitzschia	41	*Encyonema silesiacum* (Bleisch) Mann	39
Encyonema	39	*Hannaea arcus* (Ehrenberg) Patrick	38
Hannaea	38	*Achnanthidium sehuencoensis* SEHUENCAS	25
Rhoicosphenia	22	*Nitzschia clandestina* Manguin	24
Reimeria	15	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	22
Gomphonema	15	*Achnanthidium minutissimum* (Kützing) Czarnecki	18
Navicula	11	*Reimeria sinuata* (Gregory) Kociolek et Stoermer	15
Ulnaria	10	*Nitzschia* sp. 1 SEHUENCAS	14

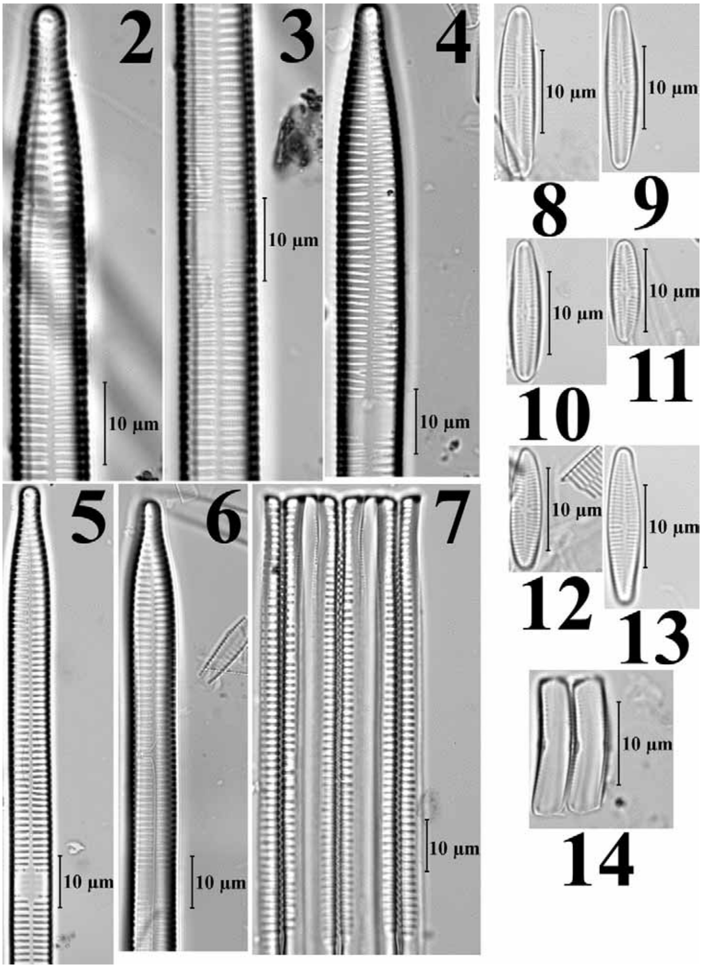
Taxonomic section

***Fragilaria cochabambina* Morales *sp. nov*.** LM Figs. 2–7; SEM Figs. 15–20

Holotype: National Botanical Garden, Meise, Belgium BR-4143

Isotype: Herbario Criptogámico Universidad Católica Boliviana, Cochabamba H.C. UCB D-1

Type locality: Río Ibirizu, Sehuencas, Provincia Carrasco, Cochabamba, Bolivia. 17° 29.8" S, 65° 16.4" W.



Pl. 1. LM images from type material of the two taxa described as new herein (**Figs. 2–14**). ***Fragilaria cochabambina* sp. nov. (Figs. 2–7).** Images 2–4 have been taken at higher magnification to highlight rimoportulae (2 per valve) and striae with a single row of transapically elongated areolae. Notice formation of palisade colonies in figure 7. ***Achnanthidium sehuencoensis* sp. nov. (Figs. 8–14**). Notice clear central area in raphe valve and the absence of such an area in the rapheless valve. Figure 14 shows two contiguous frustules, probably the products of recent vegetative cell division.

Etymology: Epithet makes reference to the Department of Cochabamba, Bolivia.

Descriptio: Frustula longa rectangularia indentata ad apices in margines limbi et valvocopulae junctura. Valvae longae subrostratis nonnunquam subcapitatis late rotundatis apicibus contracti non nisi ad polos, 88–296 µm longae, 8–9 µm latae, striae 8–10 in 10 µm. Area axialis anguste linearis inter strias aequalia ubique valvam. Striae alternae parallelae modice radiantes ad apices. Striae continuae in medio limbis valvae sed interruptae in limbi et valvae junctura per spinas. Costae elevatae aspectu externo. Area centralis fasciis quadratis vel rectangularibus. Aliquot phasma-striae in fasciarum valvaribus marginibus. Spinae spathulatae solidae obfirmans finitimae valvae. Areolae rimiformes apicaliter elongatae in striis plerumque uniseriatis. Parviores rotundatae areolae et rimiformes alternantibus in striis versus polos nonnunquam. Volae ramosae crescentes ex ambitu interno areolae. Area apicalis pororum evoluta typus ocellulimbus constata ex seriebus rotundatis pororum. Duae structurae cornuatae in extremitatibus arearum apicalis pororum. Duae rimoportulae, unum in quoque apicibus valvarum. Valvocopulae clausae cum singulari serie areolarum. Valvocopulae margo ab valva modice curvatus itaque valvocopulae vadosior ad apices. Copulae non visae. Chromatophora incognita.

Description: Frustules long and rectangular with indentations at the apex, where the valve mantle edges meet the valvocopulae. Valves long with subrostrate (subcapitate in some cases), broadly rounded apices that taper only at the end of the valves. Length: 88–296 µm, width: 8–9 µm, striae density 8–10 in 10 µm. Axial area narrow and straight, delimited by striae of equal length throughout the valve. Striae alternate and parallel becoming slightly radiate toward the apices. Each stria continues onto the middle of the valve mantle, but is interrupted at the valve face-mantle junction by spines. Costae are raised in outer view. Central area with clear square or rectangle-shaped fascia. Some ghost striae can be seen on the valve face edges of the fascia. Spines are spatulate and solid, and effectively interlock neighboring valves. Areolae are slit-like, apically elongated, and mostly forming a single row along each stria. Sometimes striae toward the poles exhibit two smaller, round areolae alternating with the regular slit-like openings. Volae are branched and grow from the internal contour of the areola. Apical pore fields well-developed, of the ocellulimbus type, and composed of several rows of round pores. Two terminal horn-like structures are located on top of each apical pore field. Two rimoportulae are present, one on each valve apex. Valvocopulae are closed and bear a single row of areolae of similar characteristics to those along striae. The abvalvar edge of the valvocopula is slightly curved and as consequence, the valvocopula is shallower at the apex. Copulae not observed. Plastids unknown.

***Achnanthidium sehuencoensis* Morales *sp. nov*.** LM Figs. 8–14; SEM Figs. 21–26

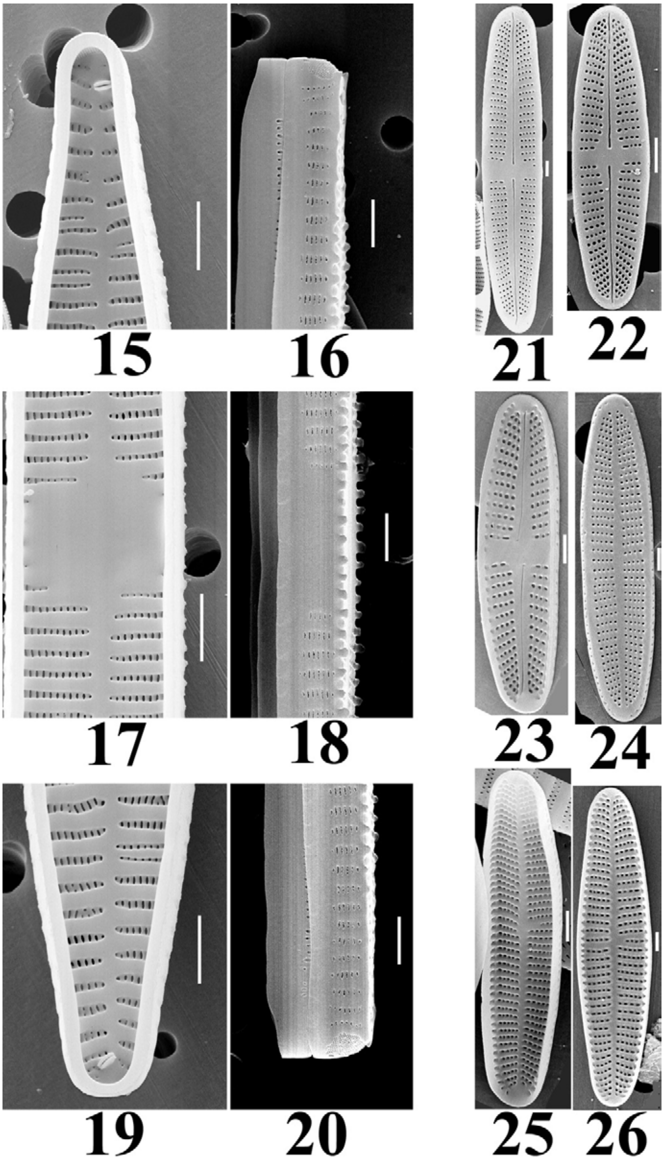
Holotype: National Botanical Garden, Meise, Belgium BR-4144

Isotype: Herbario Criptogámico Universidad Católica Boliviana, Cochabamba H.C. UCB D-2

Type locality: Unnamed stream (Stream 1 in table 1), Sehuencas, Provincia Carrasco, Cochabamba, Bolivia. 17° 31.6" S, 65° 16.2" W

Etymology: Epithet makes reference to the name of the region where the type locality is found.

Descriptio: Frustula rectangularia modice arcuatae itaque raphovalva concava. Raphovalvae lanceolatae vel subellipticae apicibus modice angustatis late rotundatis. Longi-



Pl. 2. SEM images of the new taxa presented in this manuscript (**Figs. 15–26**). ***Fragilaria cochabambina*** **sp. nov.** (**Figs. 15–20**) from type material. Figures 15, 17 and 19, and 16, 18 and 20 are of the same valve. Notice presence of two rimoportulae per valve, the clear central area extending to both sides, the spines located along the striae, and the transapically elongated areolae. Figures 16 and 20 show side views of the apical pore fields bearing two short horn-like spines, and the ends of a closed valvocopula bearing a single row of pores. ***Achnanthidium sehuencoensis*** **sp. nov. (Figs. 21–26)** from type material. Figures 21 and 22 show outer details of the raphe valves. Figure 23 shows an inner view of a raphe valve. Notice clear fusion of the helictoglossa with apical thickenings. Figure 24 depicts an outer view of the rapheless valve. A single row of areolae on mantle can be seen, with more clarity toward valve ends. Figures 25 and 26 show inner details of rapheless valves.

tudo: 12–22 μm, latitudo: 2–4 μm, striae 20–24 in 10 μm. Sternum lanceolatum elevatum interne cum costis nonnunquam area axiali elongata rhombica. Raphe filiformis. Extrema centralia declinata unilateraliter extensa ultra strias in area hyalina affini valva. Interne extrema terminalia helictoglossis elevatis connatis ad condensationem valvae apicis. Externe extrema centralia modice expansis non nisi per microscopium photonicum. Interne extrema centralia uncinata declinata invicem. Striae parallelae sed fortiter radiantes densiores ad apices. Stria curta unilateraliter vel bilateraliter in area centrali. Striae modice separatior in area centrali. Areolae rotundatae interne et externe similares. Extremitas striae in valva procul valvae-limbi marginem pergens ab singulari areola in limbo etiam procul valvae-limbi marginem itaque area hyalina cingenti limbo formans. Hymenes versus aperturam externam areolae. Area centralis stauro conspicuo rectangulari praeditae extenso trans valvam nonnunquam sejunctis areolis. Araphovalvae acutioribus apicibus striationibus similibus quam raphovalvae sed latioribus dispositionibus striarum ad apices stauro inconspicuo. Striae 21–24 in 10 μm. Singularis series pororum in limbo ad apices. Hymenes non visae. Chromatophora incognita.

Description: Frustules rectangular and slightly arched, producing a concave raphe valve. Raphe valves lanceolate to subelliptical with slightly tapering, broadly rounded apices. Length: 12–22 μm, width: 2–4 μm, striae density 20–24 in 10 μm. Sternum lanceolate or sometimes showing an elongated diamond-shaped axial area, and bearing a filiform raphe with terminal endings that are externally only slightly deflected in the same direction. These terminal raphe endings extend beyond the striae and onto a clear area that borders the entire valve face. Internally, the terminal raphe endings are terminated in helictoglossa that are raised and fused to a thickening of the valve apex. Proximal raphe fissures externally slightly expanded, feature visible only under LM. Internally, proximal raphe endings are conspicuously deflected away from each other and are hooked. Striae vary from parallel to strongly radiate toward the apices, where they also increase in density. Areolae round and do not vary much in internal and external views. Each stria ends on the valve face and at a distance from the valve face-mantle edge, then it is continued by a single round areola on the valve mantle, also situated at a distance from the valve mantle-valve face edge, forming as a consequence a clear area that surrounds the entire valve mantle. Hymenes located toward the outer opening of the areolae (not shown here). Central area with a clear, rectangular stauros extending across entire valve face. Sometimes, isolated areolae can be present in the stauros. Rapheless valves with more acute apices than raphe counterparts. Pattern and characteristics of striation are very similar, except that the spacing between striae toward apices is wider and there is no clear stauros. Sometimes a shorter stria can be present at one or both sides of the central area. Striae in the central area are slightly more separated from each other. Striae density 21–24 in 10 μm. Sternum lanceolate and raised together with the costae in internal view. Single row of pores on the mantle stops before the valve apices. Hymenes not observed. Girdle bands not observed. Plastids unknown.

Discussion

The high number of taxa found in only 5 epilithic gatherings from sites located relatively close to each other is remarkable. Sehuencas is only a small part of the Carrasco National Park and therefore, the total number of taxa in the park could be much higher, especially if other compartments within rivers and lakes are included in future sampling efforts. Taking into account that 36% of the recorded flora corresponds to unknown entities, it is possible that a large fraction of these diatoms is endemic to Bolivia and to the region. Conservation of aquatic systems could be easily justified if diatoms and other algae and organisms (for which there are currently no available data) are taken into account.

Traditionally, national parks have been established based on organisms that man has historically considered as »important«, that is, higher plants and vertebrates. However, the recognized value of algae, especially diatoms, as primary producers and in the maintenance of aquatic food webs has been neglected in conservation practices, with adverse affects on applied studies such as environmental impact and bioremediation.

Further studies are needed to assess the true biodiversity potential of Bolivian parks and ecosystems. At present, Bolivia is considered among the 20 countries with most biodiversity in the planet. If the algae were included, the country's ranking could be increased considerably. To assure taxonomic precision and consistency, it is important to recognize the diversity of diatoms in Bolivian water ecosystems by careful taxonomic identification using a combined approach between LM and SEM. It is also of utmost importance that pertinent floras are used as source of names, because the use of floras from parts of the world that are ecologically different from the ecosystems found in tropical South America could lead to serious biodiversity estimation errors (MANN and DROOP 1996, KOCIOLEK and SPAULDING 2000) and to biased assessments of biogeographic patterns (EDLUND and JAHN 2000). Currently, even if all the literature on Bolivian diatoms is combined, an estimation of the species richness of this group would be difficult to determine, precisely because most of that literature is not specialized and because taxa have been force-fitted into European-based concepts (MORALES et al. 2008).

The epilithic flora found in Sehuencas is dominated by cosmopolitan taxa commonly found in waters with high nutrient content (VAN DAM et al. 1994) (Tab. 3). This is evidence of the degree of impact of uncontrolled tourism in the area. It is very common to find bottles, cans, plastic bags, and even piles of organic and other garbage items in the area contiguous to the sampling sites. An added effect comes from cattle and farming practices that would increase the nutrient content in the water favoring the development of taxa common world-wide.

From the list of the most abundant species presented in table 3, eight have been characterized ecologically (VAN DAM et al. 1994). Based on these taxa, the 5 rivers sampled for this study can be determined to be meso to eutrophic, with circumneutral pH, probably with high concentrations of organically bound nitrogen, and oxygen levels above saturation. The two remaining species not found in VAN DAM et al. (1994), namely *Nitzschia clandestina* and *Nitzschia* sp. 1 (from Sehuencas) do not have published ecological information. *Nitzschia clandestina* was originally described from Río Fortaleza near Lima, Peru at 1400 m.a.s.l., where it was found forming epilithic growths (MANGUIN 1964). On the other hand, *N.* sp. 1 (from Sehuencas) was found growing on rocks in Río Ibirizu, Río Sehuencas and Stream 3, which share similar ecological conditions (cold, circumneutral, and low conductivity waters).

Two of the taxa that were found in sufficient abundance during SEM analyses were described above in an initial effort to solve the taxonomy of those 36% of taxa that could not be identified during our study. The rest of the taxa will have to be described at a later date and when developed populations are found in additional field collections.

Fragilaria cochabambina sp. nov. has features similar to other taxa currently included in *Fragilaria* Lyngbye. A recent review by TUJI and WILLIAMS (2006) showed that the presence of a rimoportula at each valve apex is not a restriction for a taxon to be placed in this genus (many *Fragilaria* spp. have only one). *Fragilaria capucina* Desmazières has two rimoportulae per valve, spines interrupting the striae, valvocopulae that are shallower toward the apices and bear a single row of areolae, all features found in *F. cochabambina*. Although the revision of the generitype of *Fragilaria* (*Fragilaria pectinalis* (Müller) Lyngbye) is pending, it is possible that its general structure does not differ too much from that of *F. capucina* and *F. cochabambina*.

One feature that is different in *F. cochabambina* is the closed nature of the girdle bands (open in *F. capucina* and other fragilarioids). *Ulnaria* has closed girdle bands, but consideration of only this feature is insufficient to place *F. cochabambina* in *Ulnaria*. Characters such as striae, areolae, spine structure and position, apical pore fields, and other features of the girdle bands such as arching and presence of a single row of areolae, are all congruent with other taxa in *Fragilaria*.

Fragilaria cochabambina sp. nov. is unique among fragilarioid taxa due to its large size and robust architecture. It shares this characteristic with taxa in *Ulnaria,* especially *U. ungeriana* (Grunow) Compère, which is very similar in gross morphological features. In *U. ungeriana*, however, the degree of tapering of the valve apices is not as pronounced as in *F. cochabambina* and its apices are also more broadly rounded (MORALES 2003, MORALES et al. 2007). The striae in *Ulnaria ungeriana* vary from opposite to alternate on the same valve and are more radiate toward the apices. Also, the areolae in *U. ungeriana* are less elongated and more broadly elliptical. The volae in *U. ungeriana* are conspicuously developed and are composed of a solid plate positioned in the middle of the foramina and attached by several siliceous bridges to the internal contour of the areola. This type of vola has not been observed in *F. cochabambina*, but rather branched volae are present. Another difference lies in the valvocopulae, which are arched in *F. cochabambina*, but straight in *U. ungeriana*. One of the most conspicuous differences between these two taxa is the position of spines, which interrupt the striae in *F. cochabambina*, but are on the costae in *U. ungeriana*.

Achnanthidium sehuencoensis possesses all features currently considered within the genus *Achnanthidium* Kützing. The raphe valves of the new taxon are concave, as in all species currently placed in *Achnanthidium*. The characteristics of the raphe, the progressively less coarse striae toward the apices and the characteristics of areolation are all congruent with other species within the genus. There are a number of taxa with striae interrupted at the central stauros as in *A. sehuencoensis*, but they differ from the latter in valve outline, characteristics of the raphe, striation pattern, and areolae shape and distribution on valve face and mantle of both raphe and rapheless valves (Tab. 4). It is worth noting that the type of the genus, *Achnanthidium minutissimum* (Kützing) Czarnecki also has the clear central stauros, but the valve outline and striation pattern are different from those of *A. sehuencoensis* (compare our SEM material to that of the generitype presented in POTAPOVA and HAMILTON 2007). Additionally, *A. minutissimum* of the type has slits on the valve mantle (and sometimes on the valve face toward the valve face-mantle junction), instead of the round areolae present in *A. sehuencoensis*. It is interesting that the raphe structure is very similar in these two taxa and that the degree of fusion of the helictoglossa with the thickened apex of the valve sets them apart from the rest of *Achnanthidium* spp. for which pub-

Tab. 4. Comparison of relevant morphological features among taxa most similar to *Achnnthidium sehuencoensis* and for which LM and SEM of type material are available.

Taxon	Raphe		Central stauros/central area	Striae
	External view	Internal view		
A. minutissimum	Filamentous, with proximal raphe ends slightly enlarged and ending opposite to each other. Distal ends continue onto valve mantle at apex and curve slightly to the same side. Sometimes at least one of the terminal ends stops before the valve apex, while the other reaches the mantle.	Runs along raised sternum. Branches slightly deviated from each other along apical axis so they end away from each other. Proximal ends slighlty hooked and bent in opposite directions. Helictoglossae raised and fused with the apex by a slight thickening.	Clear in raphe valve, sometimes with a few isolated areolae. No apparent stauros in rapheless valve although some shortened and more spaced striae are present in this area. Raised sternum in internal view lacking.	Radiate throughout in both valves and increasing in density toward apices.
A. sehuencoensis	Filamentous, with proximal raphe ends slightly enlarged and ending opposite to each other. Distal ends touch only slightly the valve mantle at apex and curve slightly to the same side.	Runs along raised sternum. Branches sometimes slightly deviated from each other along apical axis so they end away from each other. Proximal ends slighlty hooked and bent in opposite directions. Helictoglossae raised and fused with the apex by a clearly elevated thickening.	Clear in raphe valve, sometimes with a few isolated areolae. No apparent stauros in rapheless valve although some shortened and more spaced striae are present in this area. Raised sternum in internal view lacking.	Parallel to radiate in raphe valves; slightly to strongly radiate in rapheless valves (sometimes a few striae are parallel at the center of the valve). Increasing in density toward apices in both valves.

lished SEM information is available (see for example KOBAYASI 1997, POTAPOVA and PONADER 2004, CANTONATI and LANGE-BERTALOT 2006, POTAPOVA 2006, PONADER and POTAPOVA 2007, etc.). Other taxa in *Achnanthidium* have the helictoglossa fused to the apex, but the thickening of the latter is not as pronounced (e.g., *A. atomoides* Monnier, Lange-Bertalot et Ector, in MONNIER et al. 2004) (Tab. 4). In other cases, there is a clear depression between the raised termination of the helictoglossa and the apex (as in *A. rivulare* Potapova et Ponader POTAPOVA and PONADER 2004). The significance of the degree of fusion of the helictoglossa to the valve apex is not known, and further assessments are needed to explore its usefulness as a distinguishing character within *Achnanthidium*.

One of the distinguishing features of *A. sehuencoensis* is the considerable deviation of the proximal raphe endings seen in internal view. This feature is also present in *Achnan-*

Tab. 4. – Continued

Taxon	Raphe		Central stauros/central area	Striae
	External view	Internal view		
A. pyrenaicum	Filamentous and running along slightly raised sternum. Proximal raphe ends slightly enlarged and ending opposite to each other. Distal ends never reach valve apex and deflect markedly in the same direction and stop at valve face clear perimeter, never reaching the mantle.	Runs along raised sternum. Branches sometimes slightly deviated from each other along apical axis so they end away from each other. Proximal ends not hooked, but deviate at an open angle and in opposite directions. Helictoglossae raised and not fused with the apex, leaving a clear gap between it and the valve apex internal mantle face.	Sometimes clear in raphe valve, but often with shortened or even complete striae. No apparent stauros in rapheless valve although some shortened and more spaced striae are present in this area. Raised sternum in internal view present.	Mostly parallel or slightly radiate, especially toward apex in both valves. Increase in density toward apices is not conspicuous.
A. atomoides	Filamentous and running along slightly raised sternum with conspicuously swollen central nodule. Proximal raphe ends slightly enlarged and ending opposite to each other. Distal ends never reach the valve apex, but not the mantle. Deflection of the distal ends in the same direction is barely noticeable.	Runs along raised sternum with swollen central nodule. Proximal ends not hooked, but deviate at an open angle and in opposite directions. Helictoglossae slightly raised and with a shallow gap between it and the valve apex internal mantle face.	Clear in raphe valve. No apparent stauros in rapheless valve although some shortened and more spaced striae are present in this area. Raised sternum in internal view lacking.	Strongly radiate in raphe valve and mostly parallel to slightly radiate toward apices in rapheless valve. Increase in density toward apices is more marked in raphe valves.

thidium affine (Grunow) Czarnecki (*sensu* LANGE-BERTALOT and KRAMMER 1989, not the type). The rest of the features are different between *A. sehuencoensis* and *A. affine*. The shape of the valve is predominately lanceolate, the raised helictoglossa is separated from the valve apex, and the row of valve mantle areolae travel around the valve apex in both the raphe and rapheless valves in *A. affine*, which is not the case of *A. sehuencoensis*.

In 1966, HOHN described *Achnanthes kryophiloides* from Río Rondos in the Peruvian Amazon, a taxon that is very similar to *Achnanthidium sehuencoensis*. However, *A. kryophiloides* has slightly wider valves (4.2 µm) and higher average striae density (24 in 10 µm). A preliminary analysis of the type slide deposited by Hohn in Philadelphia (ANSP. G.C. 25858a) yielded no specimens of *Achnanthes kryophiloides* (M. POTAPOVA pers. comm.). Examining the protologue of the species (HOHN 1966) it appears that Hohn based his description on a single specimen and that the range of striae measurements given by him (»22–26/10 µm«) might actually correspond to striae density on the raphe and rapheless valves, respectively. Investigation of type material for *A. kryophiloides* is underway.

Another taxon that is morphologicaly similar to *Achnanthidium sehuencoensis* is *A. pyrenaicum* (Hustedt) Kobayasi (Tab. 3). Both taxa produce rapheless valves that are similar to each other under LM, except that the striae are more dense (up to 38 in 10 µm following POTAPOVA and PONADER 2004) and are mostly parallel becoming radiate only toward the apices in *A. pyrenaicum*. *A. sehuencoensis* has up to 24 striae in 10 µm, only a few are parallel toward the valve center and they quickly become radiate toward the apices. *Achnanthidium pyrenaicum* has more fusiform valves, especially true for the raphe valves (see study of the type presented by POTAPOVA and PONADER 2004); the shape of the valves in *A. sehuencoensis* is subelliptical with more broadly rounded apices. At the SEM level, the differences between *A. sehuencoensis* and *A. pyrenaicum* are more conspicuous. The terminal ends of the raphe are markedly deflected in the same direction in *A. pyrenaicum* while in *A. sehuencoensis* the deflection is slight. The internal view of the raphe valves in *A. pyrenaicum* shows a slightly raised sternum that becomes even more raised toward the valve apices, ending in helictoglossae that are clearly separated from the valve apices (POTAPOVA and PONADER 2004, Fig.107). *A. sehuencoensis* does not have a clearly raised sternum and the helictoglossae are clearly fused with the valve apices.

The two species described in this manuscript as new thrive in circumneutral pH and low conductivity waters (Tab. 1). The temperature of the water is relatively low for both type localities, and both are affected by human-related disturbance, although the waters remain clear. Nutrients were not measured during this investigation due to high costs.

Acknowledgements

We thank the California Academy of Sciences and the Museum of Natural History, The University of Colorado at Boulder, USA for partial financial support of this project. EAM thanks the Academy of Natural Sciences of Philadelphia (especially Dr. D. Charles) for travel support to visit the Gabriel Lippman Research Center in Luxembourg, where the SEM analysis was performed. L. Ector and C. Wetzel made this latter analysis possible, and EAM is greatly indebted to them. Dr. M. Potapova, kindly provided some comments and material from the ANSP Diatom Hebarium included in the discussion. Dr. F.R. Trainor provided useful comments for improvement of the manuscript and Dr. S. Blanco kindly provided Latin translations of the protologues.

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