Diatom Research (2008), Volume 23 (1), 159–169

ULTRASTRUCTURE OF THE FRUSTULE OF *UROSOLENIA* SPECIES FROM THE COLOMBIAN AND PERUVIAN AMAZON: *U. DELICATISSIMA* SPEC. NOV., *U. AMAZONICA* SPEC. NOV. AND *U. BRAUNII* (HUSTEDT) ROTT & KLING

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Fine morphology of the frustule and taxonomy of three *Urosolenia* species are discussed. The analysis with SEM of materials collected in lakes of the Colombian and Peruvian Amazon showed that these taxa differ in valve and girdle bands morphology and in morphometric parameters. One of the species in the conical part of the valve has 1 to 4 buttonhole shaped structures with external more or less developed labiate-like openings. This structure has previously not been described in *Urosolenia* or in the allied genera *Rhizosolenia*, *Prosboscia*, *Neocalyptrella* and *Pseudosolenia*.

Two new species, *Urosolenia delicatissima* and *U. amazonica* are described and new details of the valve morphology of *U. braunii* (Hustedt) Rott & Kling are given.

INTRODUCTION

The genus *Urosolenia,* erected by Round & Crawford (Round *et al.*, 1990), comprises freshwater *Rhizosolenia* species with cylindrical frustules, well developed girdle and conical valves having a long fine hair-like tubular extension called process in the literature. These taxa differ from *Rhizosolenia* species not only in their freshwater habitat but also in the absence of rimoportulae associated with the bases of the valve extension, areolae irregularly distributed on the valve surface and absence of claspers and otarium. When the authors described the new genus, they transferred *Rhizosolenia eriensis* H. L. Smith to *Urosolenia*. Later, Edlund & Stoermer (1993) transferred *R. longiseta* Zacharias, followed by Torgan & Becker (1998) transfering *R. eriensis* var. *morsa* West & West, and then Andresen *et al.* (2000) transferred *R. gracilis* H.L. Smith to *Urosolenia*. Recently, Rott *et al.* (2006) gave an emended diagnosis of the genus, transferring *R. braunii* Hustedt and *R. victoriae* Schröeder and describing *U. brevispinosa* Kling, McGregor & Rott, *U. diademata* Rott & Kling and *U. parva* Kling, Rott & McGregor.

The genus *Urosolenia* has a world wide distribution (Round *et. al.* 1990). In the Amazon River basin, until now the only *Urosolenia* species mentioned was *U. braunii* (Hustedt) Rott & Kling but several species and varieties of *Rhizosolenia* have been reported in studies carried out in Brazil. Uherkovich (1976) reported *Rhizosolenia eriensis* and *R. longiseta*; Uherkovich & Rai (1979) mentioned *R. eriensis* f. *genanensis* Schulz, and *R. eriensis* var. *europaea*

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Hustedt; and Huszar (1996) found *R. eriensis* var. *eriensis*, *R. eriensis* f. *brevispina* Woloszynska and *R. eriensis* var. *morsa*.

In order to explore the biodiversity and to asses productivity and ecological-water quality of aquatic ecosystems of the Colombian and Peruvian Amazon region, numerous lakes, creeks and rivers of the Caquetá-Japurá, Putumayo-Içа and Amazonas-Solimôes river basins were sampled. All sites were characterized by a low mineralization; low trophic level expressed as low chlorophyll-a concentrations and phytoplankton density, and high specific diversity indicating low anthropogenic influence. A preliminary analysis of the collected samples showed that *Urosolenia* species were frequently found in three of the studied lakes.

The aim of this paper is to analyze the ultrastructure of the frustule of this material to improve the knowledge of *Urosolenia* taxa in Amazonian diatom flora and also provide additional information regarding the morphology, taxonomy and distribution of this genus.

MATERIALS AND METHODS

In September 1994 and June 2001, 109 water bodies were sampled, covering the Caquetá-Japurá Putumayo-Iça Amazonas-Solimôes Rivers Basins in Colombia near the Brazilian border and the Putumayo (Iça) River Basin at the frontier between Colombia and Perú. The studied area comprises quaternary alluvial deposits and terraces, the tertiary Pebas formation, and Precambrian rocky outcrops. According to Nuñez-Avellaneda & Duque (2001), the Caquetá and Putumayo Rivers basins comprise water bodies with Black type II waters characterized by lower mineralization and trophic level than the Amazonas River Basin with Black type I waters. Black Waters are considered as Amazon Basin's environment type, originated in poor soil regions related to the Guyanes Shields and the Tertiary Pebas formation, its amber color is due to the high load of organic material of partial decomposition produced by acid waters and low mineralization. The type I is caused by the marine south Colombian Amazon zone influence and therefore the nutrients are higher than those of the northern zone.

The genus *Urosolenia* was present in Lake Taraira (0º 30´15" N / 69º25´36" W), Lake Bufeo (02º18'13" N / 71º31'59 W) and Lake Yahuarcaca (4º11'35" S / 69º57'28" W), belonging to the basins of the Caquetá, Putumayo and Amazonas rivers, respectively.

Horizontal and vertical tows were made with a plankton net (mesh size = 24 µm) at different depths of the photic zone. Metaphitic diatoms were sampled by squeezing macrophytes. At each sampling station physical and chemical parameters including pH, dissolved oxygen, temperature, water transparency (Secchi disc) and conductivity were registered (Table 1).

Table 1. Limnological conditions of the studied sites

Sampling Sites	pH	Specific Conductivity (µS cm^{-1})	Transparency (m)	Temperature (ºC)	Dissolved Oxygen (%)	Species Present
Taraira Lake	4.6	5	1.67	29	61.8	*U. amazonica* *U. delicatissima*
Bufeo Lake	5.5	20	2.16	22.8	89.7	*U. amazonica* *U. delicatissima*
Yahuarcaca III Lake	6.6	99	0.3	29.5	17.5	*U. amazonica* *U. delicatissima* *U. braunii*

Samples were fixed with 6–8% formalin. Part of the samples was treated to eliminate organic matter following the methods described in Hasle & Fryxell (1970) and Prygiel & Coste (2000). Treated and untreated material was mounted in Naphrax for light microscopy (LM) examination. For scanning electron microscopy (SEM) samples were mounted on glass stubs and then coated with gold-palladium. As the material is very delicate, part of the raw samples were treated by critical point drying for SEM examination. Light microscope observations were carried out using a Wild M20 microscope with phase contrast and scanning electron microscopy using a Jeol J.S.M. 6360 LV microscope.

Material has been deposited at the Colección "Ficoteca Amazónica Colombiana" at the Universidad Nacional de Colombia (F.A.). Uncleaned and cleaned subsamples and permanent slides have been deposited at the Herbario of the Departamento Científico Ficología, Museo de Ciencias Naturales de La Plata (LPC) and at the Herbario-COAH del Instituto Amazónico de Investigaciones Científicas - Sinchi, Bogotá, Colombia under the following numbers:

LPC 5514 (F.A. 329): Taraira I Lake, Departamento de Vaupés, Colombia. 9–11–1994, metaphyton.

LPC 5509 (F.A. 300): Yahuarcaca III Lake, Departamento del Amazonas, 3–11–1994, phytoplankton.

LPC 5601 (F.A. 156): Bufeo Lake, Provincia de Maynas, Perú. 10– 2001, phytoplankton.

RESULTS

The analysis with light and electron microscopy of samples collected in lotic and lentic water bodies of the Colombian – Peruvian Amazon, showed that at least three different *Urosolenia* taxa were a frequent component of the plankton and metaphyton of the Lakes Bufeo, Taraira and Yahuarcaca, three lentic water bodies with low pH, low mineralization and low trophic level. These taxa clearly differed in fine morphology of the frustules and dimensions (Table 2). The most striking features that allowed us to differentiate these taxa were: shape of the valve, length and morphology of the ends of the valve extensions (with or without seta), distribution and size of the valve areolae; presence of warts and club-shaped nodules on the valve extension; shape of the girdle bands and their areolae. In the material analyzed the end of the process considered a diagnostic feature by Rott *et al.* (2006) have slight differences within the same species and only for *Urosolenia delicatissima* sp. nov. did all the analyzed specimens have a long seta.

The morphometric parameters that allow differentiation of the species are width and length of the valves, and areola density of the valve and girdle bands. We decided to avoid comparisons of frustules dimensions not only due to the variation related to life cycle but also because the material is extremely delicate and the girdle bands are disrupted even in raw samples prepared by critical point drying.

In relation to the terminology used to describe valve morphology in *Urosolenia*, we adopted the proposal of Rott *et al.* (2006) but suggest that it would be better to use "valve extension" instead of "process" as this extension is part of the valve and not associated with a rimoportula.

Table 2. Dimensions of the studied material and comparison with their allied taxa. * Without extension that are 22–45 µm long.

Taxon	Author	Frustule		Valve		Valve Extension		Girdle	
		Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Teeth	Scales/ 10 µm	Areolae/ 10 µm
***Urosolenia amazonica* sp. nov.**	This study	76–120	6–18	27–48	3–9.5	37	4–6	(8–9) 10–16	73–140
U. eriensis (H.L. Smith) Round & Crawford	Krammer & Lange-Bertalot (1991)	40–150	5–20					3–9	
R. eriensis var. *eriensis* H.L. Smith	Hustedt (1942)	40–150			2–15			3–4	
	Huszar (1996)	48–69	4.7–7.8			26–33		3–4	
R. eriensis var. *tenuis* Hustedt	Hustedt (1942)		6–7			17–24		9–11	
	Simonsen (1987)	42–48	4.7–5.3	12–13.6	1.3–4			14–18	
U. eriensis var. *morsa* (West & West) Torgan & Becker	Torgan & Becker (1998)	40–84*			6–12	22–45	3	5–10 (12)	70–90
R. eriensis var. *morsa* West & West	Hustedt (1942)				5–20			6–9	
	Huszar (1996)	51–61	8–7					9–10	
R. eriensis var. *europea* Hustedt	Simonsen (1987)	93	7	17	2.6			12	
Urosolenia delicatissima **sp. nov.**	This study	179–more than 200	3–10	49–99	2.3–7.5			(3) 4–7	66–95
Rhizosolenia longiseta Zacharias	Krammer & Lange Bertalot (1991)	40–200*	4–10					2–3	
	Hustedt (1929)	70–200*	4–10						
Urosolenia braunii (Hustedt) Rott & Kling	This study			34–53	3.6–4.5			3–4	37–44
R. braunii Hustedt	Rott *et al.* (2006)	90–120	5–8					5–7	70
	Hustedt (1952)		3–4 & 5–8	30–35				5–7	
	Simonsen (1987)	106	10.5	34–36	5–5.5			4.5–8	

***Urosolenia amazonica* spec. nov.** (Figs 1–9)

Cellulae solitariae vel binatae. Frustuli cylindrici. Valvae asymmetricae conicae in extensione longa tubulari recta vel curva terminatae. Valvae pars conica areolis parvis circularibus irregulariter dispositis. Valvae extensio in 4–6 dentibus parvis terminata, ad rimoportulam non consociata. Cingulum bene evolutum, squamis numerosis rectangularibus imbricatis, unaquaeque margine laevi et areolae circularium seriebus irregulariter dispositis. Valvocopula latior quam aliae copulae, duabus dimidiis copulis composita areolis dispositis magis irregulariter quam aliis copulis et parte interiore dentata.

Frustuli: longitudo: 70–120 µm; latitudo: 6–18 µm. Valvae: longitudo: 27–48 µm; latitudo: 3–9.5 µm. Cingulum: 8–16 copulae/10 µm; 73–140 areolae/10 µm.

Cells isolated or joined in pairs (Fig. 1). Frustules cylindrical with asymmetric, conical valves that end in a straight or curved long tubular extension (Figs 1–3). The conical part of the valve has small round areolae (hardly visible with SEM) irregularly distributed, except on one side that is unperforated (Fig. 4). The valve extension (= process), not associated with a rimoportula (Fig. 5), ends in a ring of 4–6 small teeth (Fig. 7). The girdle is well developed, with numerous rectangular imbricate scales, each one with a smooth edge and rows of circular areolae more or less irregularly distributed (Figs 3, 6, 8). The valvocopula is broader than the other bands and composed of two half bands with areolae more irregularly distributed than in the other bands and with a serrated interior edge (Figs 3, 6, 9).

Frustules: length: 70–120 µm; width: 6–18 µm. Valves: length: 27–48 µm; width: 3–9.5 µm. Girdle: 8–16 scales/ 10 µm; 73–140 areolae/ 10 µm.

Type locality: Bufeo Lake, Provincia de Maynas, Perú.

Holotype: sample LPC 5601, Departamento Científico Ficología, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina.

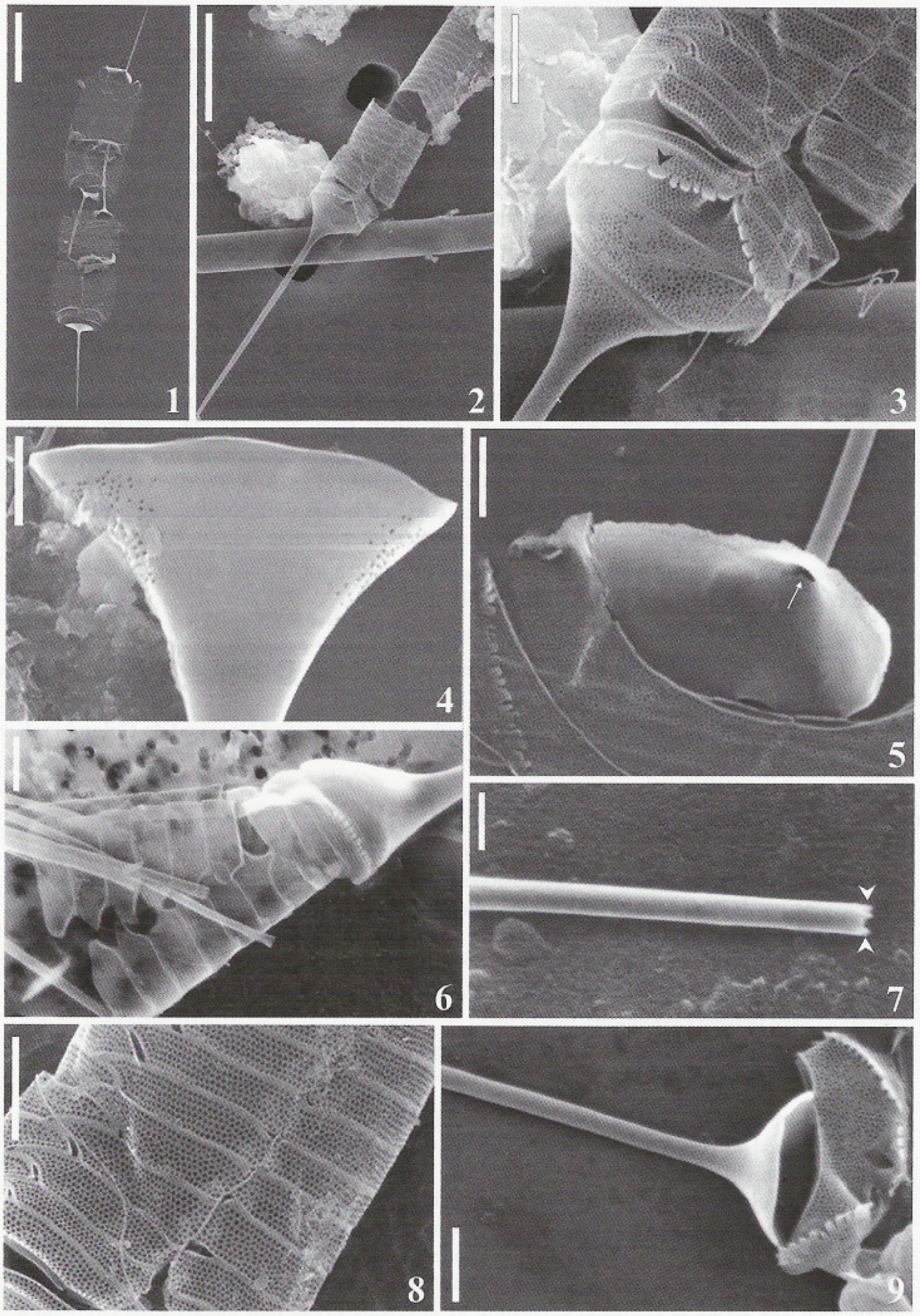
*Isotype***:** sample F.A. 156, Ficoteca Amazónica Colombiana, Universidad Nacional de Colombia, Leticia, Colombia.

***Urosolenia delicatissima* spec. nov.** (Figs 10–17)

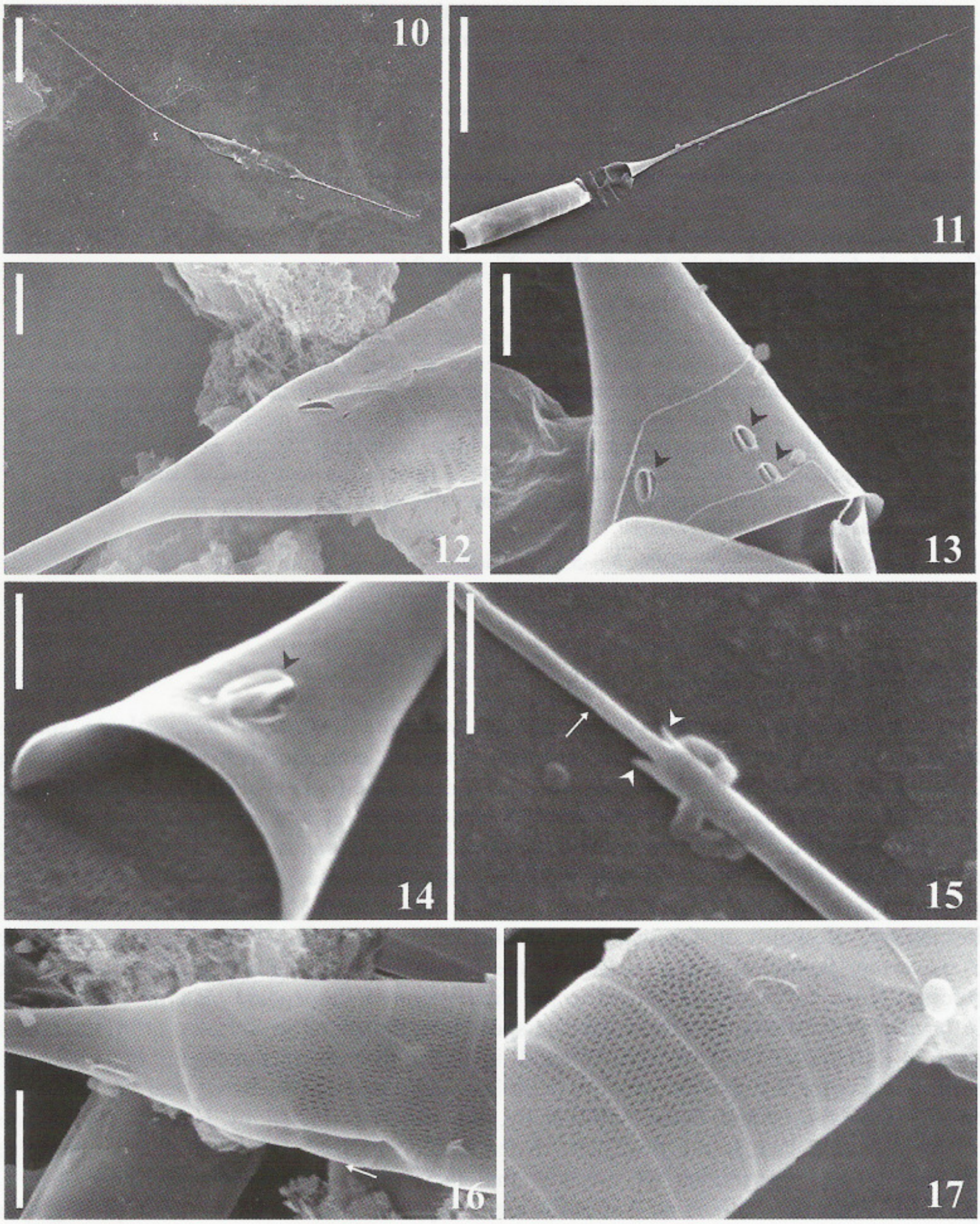
Coloniae paucis cellulis compositae. Frustuli cylindrici depressionem longitudinalem praebentes, valvae adjacentes in depressione insertantes. Valvae asymmetricae conicae, in extensione valde longa recta vel leviter undulata terminatae. Valvae pars conica parvis areolis irregulariter distributis. In uno latere 1 ad 4 aperturae ocelliformes apertura externa labiatiformi. Valvae extensio in longus filiformis seta et 2 dentibus terminata. Cingulum elongatum dimidiis copulis latis imbricatis, uterque margine laevi et areolae elongatae seriebus plus minusve regulariter dispositis.

Frustuli: longitudo: 179-plus quam 200 µm; latitudo: 3–10 µm. Valvae: longitudo: 49–99 µm; latitudo: 2–7.5 µm. Cingulum: (3)4–7 scales/10 µm; 66–95 areolae/10 µm.

Frustules are cylindrical (Fig. 10) with a longitudinal depression where the adjacent valve fits and joined in colonies of a few cells. Valves are asymmetric, conical, continuing in a very long straight or slightly undulated process (Figs 10, 11). The conical part of the valve has small areolae irregularly distributed at one side (Fig. 12). On the other side there are 1 to 4 buttonhole shaped holes with external more or less partially developed labiates like openings (Figs 13, 14, 16). The buttonhole shaped holes are visible even with LM. The valve extension (process) ends in a very long and thread-likes seta with two or three small teeth (Fig. 15). The girdle is elongate, consisting of imbricate broad half bands, each one with a smooth rim and rows of elongated areolae, more or less regularly distributed (Figs 16, 17). Although we examined more than 30 specimens, we did not observed valvocopulae with a serrated interior edge as in *Urosolenia amazonica*. The specimens prepared by critical point drying have a longitudinal depression (Fig. 16). Although we could not see precisely two valves overlap it is possible that this structure is the site where the valve of the sibling cell fits.



Figs 1–9. *Urosolenia amazonica,* SEM. **Fig. 1**. Colony. **Fig. 2**. Frustule in girdle view. **Fig. 3**. Detail of the specimen in Fig. 2, showing the valvocopula with the serrated interior edge (arrowhead). **Fig. 4**. Imperforated part of the valve in external view. **Fig. 5**. Valve in internal view, showing there is no rimoportula associated to the valve extension (arrow). **Fig. 6**. Other specimen in lateral view. **Fig. 7**. Detail of the end of the valve extension, showing the terminal teeth (arrowheads). **Fig. 8**. Detail of the girdle bands. **Fig. 9**. Valvocopula in internal view. Scale bars: 20 µm (Fig. 1); 10 µm (Fig. 2); 2 µm (Figs 3–6, 8, 9); 1 µm (Fig. 7).



Figs 10–17. *Urosolenia delicatissima*, SEM. **Fig. 10.** Frustule in girdle view. **Fig. 11**. Other specimen in external view showing the conical valve and the long valve extension. **Fig. 12**. Detail of the perforated valve portion. **Figs 13–14**. Two specimens in external view showing the conical part of the valve with the buttonhole shaped holes with external labiated openings (arrowheads). **Fig. 15**. Detail of the end of the valve extension, showing the two small teeth (arrowheads) and the seta (arrow). **Fig. 16**. A specimen in lateral view showing the conical part of the valve and the depression of the girdle where the adjacent valve fits (arrow). **Fig. 17**. Detail of the girdle bands, note the elongated areolae. Scale bars: 25 µm (Fig. 10); 20 µm (Fig. 11); 2 µm (Figs 12, 13, 16, 17); 1 µm (Figs 14, 15).

Frustules: length: 179– more than 200 µm; width: 3–10 µm. Valve: length: 49–99 µm; width: 2–7.5 µm. Girdle: (3) 4–7 scales/10 µm; 66–95 areolae/10 µm.

Type locality: Bufeo Lake, Provincia de Maynas, Perú.

Holotype: sample LPC 5601, Departamento Científico Ficología, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina.

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Urosolenia braunii (Hustedt) Rott & Kling (Figs 18–24)

Basionym: *Rhizosolenia braunii* Hustedt

Cells solitary. Valves asymmetric, conical, continued in a long curved extension (Figs 18, 20). Valve basal part perforated with large, circular areolae, arranged in longitudinal rows (Figs 19, 21), while the basal portion of the valve extension has small warts and club-shaped nodules (Fig 24). The extension has variable ends: some specimens are ligulated (Fig. 22) while others have a long tooth and 5–6 little teeth (Fig. 23). The girdle is conposed by imbricate broad half bands, each one with a smooth rim and rows of large circular areolae, more or less irregularly distributed; the valvocopula has the same morphology and width as the other bands (Figs 19, 21, 24).

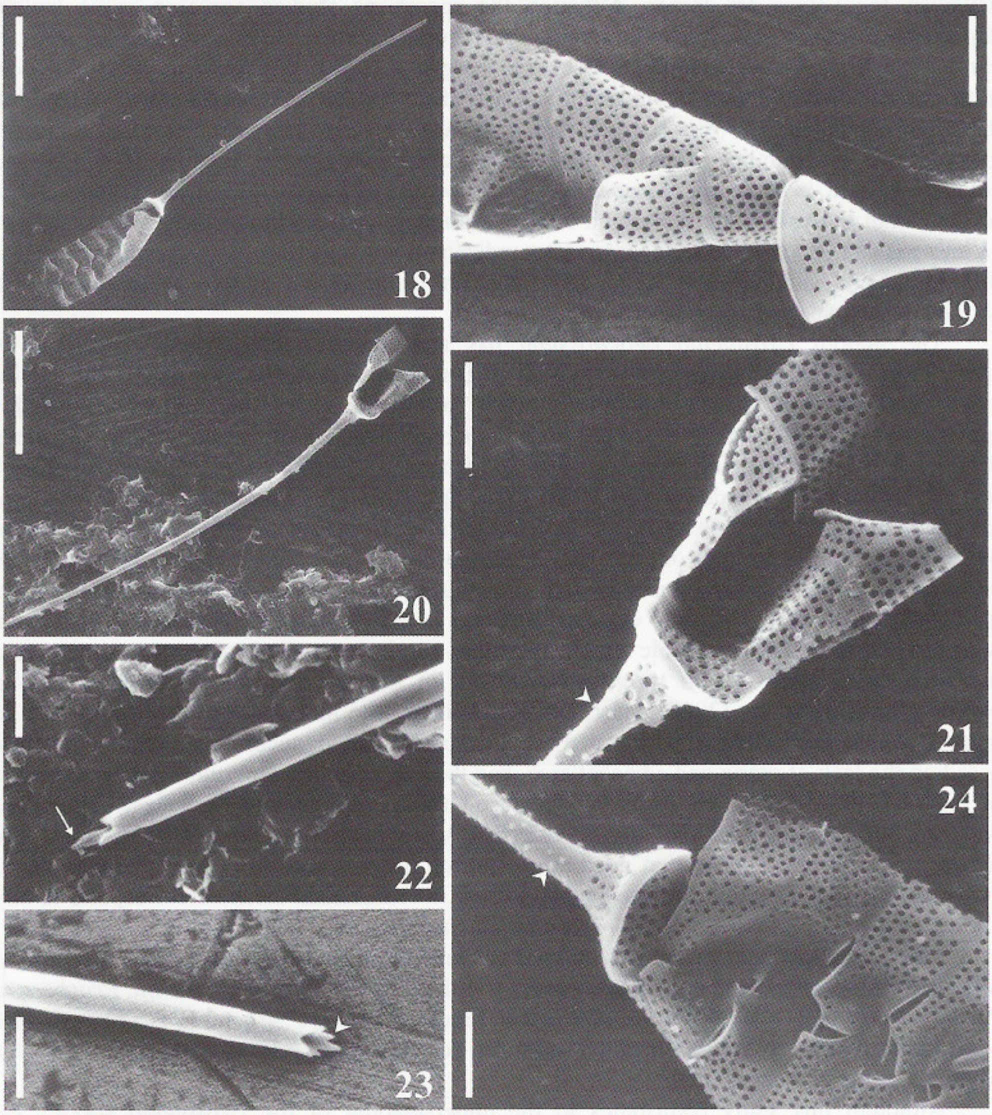
Valvae: length: 30–53 µm; width: 3.6–5.5 µm. Girdle: 3–8 bands/ 10 µm; 37–70 areolae/10 µm.

Distribution: Lake Jurucui, Brasilian Amazon and Lake Taraira Colombian, Amazon.

DISCUSSION AND CONCLUSIONS

Our *Urosolenia* species were compared with the freshwater *Rhizosolenia* species described in the literature and only one was found to coincide with a described species *U. braunii* (Hustedt) Rott & Kling, a species with more silicified valves than other *Urosolenia* species. Valve areolae are larger and valve extension is curved and has small warts and club-like protrusions on its basal portion as shown in SEM photographs of the type material given in Rott *et al.* (2006). Besides, the studied materials lack some structures observed in other tropical heavily silicified taxa (Rott *et al.* 2006). It differs from *U. diademata* Rott & Kling in the absence of spines on the mantle edge and longitudinal ribs of the calyptra, and from *U. victoriae* (Schröder) Kling & Rott in the longitudinal ribs of the basal portion of the process. There are some differences in the dimensions of the studied material and those of *U. braunii* in the literature (Table 2). The specimens illustrated in Simosen (1987) and data given in Rott *et al.* (2006) reveal more bands in 10 µm than our specimens but this difference is attributed to the fact that no complete frustules were found and the bands near the valves are broader than the central ones. Additionally, the material studied by Hustedt (1952) was collected from a lake in the Brazilian Amazonia. On the basis of the similarities in valve morphology and distribution we consider that there is enough evidence to state that the specimens from the Colombian Amazonia are conspecific with *U. braunii*. Our photographs show other details of the structure of the valve extension ends and girdle bands that allow a better understanding of this taxon.

The specimens described as *Urosolenia amazonica* resemble *Urosolenia eriensis* (H.L. Smith) Round & Crawford. Although it is difficult to state morphological differences as there are only a few detailed ultrasturctural studies of this species, the unperforated portion of the calyptra and the serrated interior edge of the valvocopulae have not previously been described neither in *U. eriensis* nor in other related species described in Rott *et al.* (2006). Also differences in morphometric features with var. *eriensis* and other varieties were found (Table 2). The frustule dimensions of our materials agree with dimensions of *Urosolenia eriensis* var. *eriensis* given by Hustedt (1942) and Krammer & Lange-Bertalot (1991), but are



Figs 18–24. *Urosolenia braunii*, SEM. **Fig. 18.** Incomplete frustule in girdle view. **Fig. 19.** Other specimen showing the conical part of the valve and detail of the girdle bands with big circular areolae. **Fig. 20**. Another incomplete specimen showing the long and curved valve extension. **Figs 21, 24**. Detail of two specimens, showing the conical part of the valve with big circular areola and the base of the valve extension with warts and club-shaped nodules (arrowhead). **Fig. 22**. Detail of the ligulated end of a valve extension (arrow). **Fig. 23**. Detail of the end of the valve extension of another specimen with five teeth of different length (arrowhead). Scale bars: 10 µm (Figs 18, 20); 2 µm (Figs 19, 21, 24); 1 µm (Figs 22, 23).

larger than those recorded in Amazonia by Huzsar (1996). The number of girdle bands recorded in the present study, however, is higher than that reported in the literature. In relation to *U. eriensis* var. *tenuis* Hustedt, our material has the same number of scales in 10 µm as that given by Hustedt (1942) and measured in the type material illustrated in Simonsen (1987), however, the valves are larger. Although the specimens studied agree in valve length, however, the valves are larger. Although the specimens studied agree in valve length, depression of the frustule and width of the valvocopula with those described as *U. eriensis* var. *morsa* (West & West) Torgan & Becker in Torgan & Becker (1998), *U. amazonica* differs in the bands density (higher in our material), size of the valve areolae and number of teeth on the valve extension. The material reported from Amazonia by Huzsar (1996) had a similar girdle width and number of girdle bands, but were much shorter. Finally, *U. amazonica* coincides with *R. eriensis* var. *europea* Hustedt in width and length of the frustule and number of scales but the valves are wider and longer. Although studies with electron microscope of *U. eriensis* and its varieties are necessary to understand intraspecific variation, we believe that differences and similarities are enough to support identification of the material described above as a new species.

The diatom *Urosolenia delicatissima* has a structure not previously described in *Urosolenia*: the buttonhole shaped holes with external labiate opening. This material is similar to *U. longiseta* in the conical smooth valve, the long process and frustule dimensions. This species was transferred to the genus *Urosolenia* by Edlund & Stoermer (1993), without a detailed analysis of the fine morphology of the frustule. The only description based on EM referred to the girdle bands (Okuno 1957). Although the *U. longiseta* has wide distribution, information and illustrations are insufficient to provide a comprehensive concept of this taxon. Nevertheless, it differs from our material in the absence of buttonhole shaped holes that are visible with LM and in the number of scales in 10 μm. The majority of the specimens studied have 4–7 scales in 10 μm (only one had 3) while in the literature *U. longiseta* is described with 2–3 scales in 10 μm (Table 2). On the other hand, the studied material is similar to a marine species, *Rhizosolenia setigera* Brightwell in that the conical valves have a long process. Sunesen & Sar (2007) described in detail the fine morphology of this species and pointed out that Sundström (1986) stated that the species had an uncertain taxonomic position based on the lack of otaria and adjacent area, and the presence of a longitudinal groove placed in the first girdle bands where the adjacent cell fits. Although there are clear differences between our material and the marine one that lacks buttonhole shaped holes, there are similarities within the group that need more careful analysis and discussion. As there is no evidence that indicates that the specimens present in Amazonian samples correspond to any known taxa we propose the name, *Urosolenia delicatissima*.

The buttonhole shaped holes with external labiate opening present in *U. delicatissima* were not previously described in *Urosolenia* or in the allied genera *Rhizosolenia*, *Prosboscia*, *Pseudosolenia* and *Neocalyptrella*. The exact function of these structures is unknown but considering that the processes in the species are so long and fragile they could be the sites where the setae of adjacent cells fit.

Although this study contributes to the understanding of the *Urosolenia* species we believe that there are still many questions about morphological variation in relation to size changes and function of some structures that could be solved by studying more natural populations and culturing these materials.

ACKNOWLEDGMENTS

We want to acknowledge specially Dr Frank E. Round who encouraged us with this study, gave us useful advice, kindly helped us with the analyses of the material and also revised the first draft of this manuscript. Also we want to thank Dr Daniel Giuliano who wrote the Latin diagnosis.

This research was held with the financial support of the Universidad Nacional de La Plata, Instituto Amazónico de Investigaciones Científicas–Sinchi and Beca Red Latinoamericana de Botánica RLB-04- P2-Tyler Prize 2004.

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Manuscript accepted for publication: December 2007