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ACHNANTHIDIUM ATOMUS COMB. NOV.
DIATOMS
ECOLOGY
LUXEMBOURG
TAXONOMY

SUMMARY. – *Achnanthidium atomoides* Monnier, Lange-Bertalot et Ector, a new species of the family Achnanthidiaceae D.G. Mann is described on the basis of light and scanning electron microscopy from rivers of the Grand-Duchy of Luxembourg, together with data on its distribution and ecology. It is compared with the very closely related species *Achnanthes atomus* Hustedt, recombined in the genus *Achnanthidium* Kützing as *Achnanthidium atomus* (Hustedt) Monnier, Lange-Bertalot et Ector.

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RÉSUMÉ. – *Achnanthidium atomoides* Monnier, Lange-Bertalot et Ector, une nouvelle espèce de la famille des Achnanthidiaceae D.G. Mann vivant dans certaines rivières du Grand-Duché du Luxembourg, est décrite en microscopie optique et électronique à balayage. Sa distribution et son écologie sont précisées. Elle est comparée à l'espèce très proche *Achnanthes atomus* Hustedt, recombinée dans le genre *Achnanthidium* Kützing comme *Achnanthidium atomus* (Hustedt) Monnier, Lange-Bertalot et Ector.

INTRODUCTION

The only extensive work on the diatom flora of the Grand-Duchy of Luxembourg was carried out by Weckering (1953), who investigated different freshwater bodies of the south of the country (Gutland region). Since then, a single taxonomical study on Luxembourg diatoms has been published by Kiss *et al.* (2002) concerning the genus *Thalassiosira* Cleve. Besides, numerous diatom inventories of rivers and brooks of Luxembourg exist as a result of studies using diatoms for water quality assessment (Leclercq & Vandevenne 1987, Ector *et al*. 1996, Descy & Ector 1997, Rimet *et al*. 2004a, 2004b, Gevrey *et al*. 2004).

In the framework of a study on the flora and fauna of Luxembourgish running waters, 421 epilithic samples were taken over the whole territory, from 1994 to 2003. A total of 454 diatom taxa were identified by light microscopy, among which 39 taxa belong to the family Achnanthidiaceae D.G. Mann. In view of the unresolved taxonomy of this family, the taxa found in Luxembourg were subject to a detailed taxonomical investigation. This study led to the description of a new taxon, belonging to the genus *Achnanthidium* Kützing. This new taxon is compared to the closely related species *Achnanthes atomus* Hustedt.

Formerly considered as a subgenus of *Achnanthes* Bory, *Achnanthidium* (Kützing) Reimer (Patrick & Reimer 1966) was erected to the generic level by Mann (in Round *et al*. 1990). But since, this genus was split into numerous genera and *Achnanthidium* was confined to the taxa around *A. minutissimum* (Kützing) Czarnecki by Round & Bukhtiyarova (1996), corresponding *pro parte* to the "*minutissima*" species complex of Krammer & Lange-Bertalot (1991b). Round & Bukhtiyarova (1996) redefined this genus considering morphological aspects as elongate concave cells, striae composed of simple rows of areolae, interrupted or shorter and less close in the centre, simple raphe fissures with straight or sometimes turned distal ends on the valve plan and presence of a separated row of areolae on the mantle.

MATERIAL AND METHODS

Epilithic material from the rivers and brooks of Luxembourg and the original material of the type of *Achnanthes atomus* from Java were investigated. The samples were treated with H_2O_2 and HCl, rinsed three times, then mounted on permanent slides with Naphrax(r) for light microscopy (Iserentant *et al*. 1999). Optical observations, identifications, measurements and micrographs were performed with a Leica(r) DMRB with differential interference contrast. Treated samples were also mounted on stubs and sputtered with gold (40 nm) for scanning electron microscopy (SEM) observations, measurements and photographs, performed with a Leica(r) Stereoscan 430i, operated at 20 kV. Both optical and SEM measurements were used in the diagnosis and description of *Achnanthidium atomoides*. The "Süßwasserflora von Mitteleuropa" (Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b) was used as the basis for identification of diatoms. In order to describe the diatom community in which the new taxon lives, up to 400 valves were counted in each sample. Environmental parameters (altitude, distance to the source, river width, discharge, water temperature, conductivity, total hardness, carbonate hardness, pH, dissolved oxygen, biological oxygen demand, NO_2^-, NH_4^+, NO_3^-, total phosphorus, PO_4^{3-}, SO_4^{2-}, Cl^-, K^+, Na^+) were measured at the time and two months before the diatom sampling for major ions. Lowest and highest values are given in the text for the different parameters.

OBSERVATIONS

Achnanthidium atomoides Monnier, Lange-Bertalot et Ector spec. nov. (Figs 1-41, 117-123).

Frustula cingulorum aspectu circiter 2 µm lata et leviter flexa. Raphovalvae concavae cum nodulo centrali etiam aspectabili microscopio photonico. Areovalvae convexae. Etiam sectio transversa apicalis demonstrat raphovalvas concavas et areovalvas convexas. Valvae lineari-ellipticae, ellipticae quoad specimina minima sed fere lineares apicibus late rotundatis quoad specimina maiora. Longitudo 4.3-11.6 µm, latitudo 2.2-3.2 µm. Striae transapicales semper uniseriatae. Areolarum foramina externe circularia et parva sed areolae dilatantes ad faciem internam. Raphovalva cum area axiali angusta. Area centralis circiter rectangulata margines valvae attingens. Striae 25-32 in 10 µm parum radiantes in media parte valvae fortiter radiantes ad apices plus minusve curvatae. Areovalva: Area axialis angustissima linearis, area centralis non formata. Striae transapicales distantius sitae enim 23-28 in 10 µm subparallelae et irregulariter positae inter se magis dense versus apices. Particulariter in media parte valvae striae aliquid alternantes utrimque ita ut in speciebus nonnullis generis Fragilaria *H.C. Lyngbye. Aspectus ultramicroscopicus (Figs 117-123): Raphis rami paene recti extremis centralibus terminalibusque non dilatatis externe. Raphis extrema centralia interne non coaxialia sed divergentia ut plerumque in familia Achnanthidiaceae, inter se 0.4 µm distantia externe et 0.6 µm interne. Striarum transapicalium areolae circiter 65 in 10 µm in raphovalva et 50 in 10 µm in areovalva. Valvae limbus areolis elongatis separatis praeditus.*

Species similissima est Achnanthes atomus *Hustedt, habitat insulis Java et Sumatra, Indonesia, sed differt dimensionibus inferioribus et proprie 19-21 striis in 10 µm transapicalibus in areovalva et 22-26 striis in 10 µm in raphovalva id est distinctissime plus quam 22-28 et 25-32 in specie* Achnanthidium atomoides (Table I).

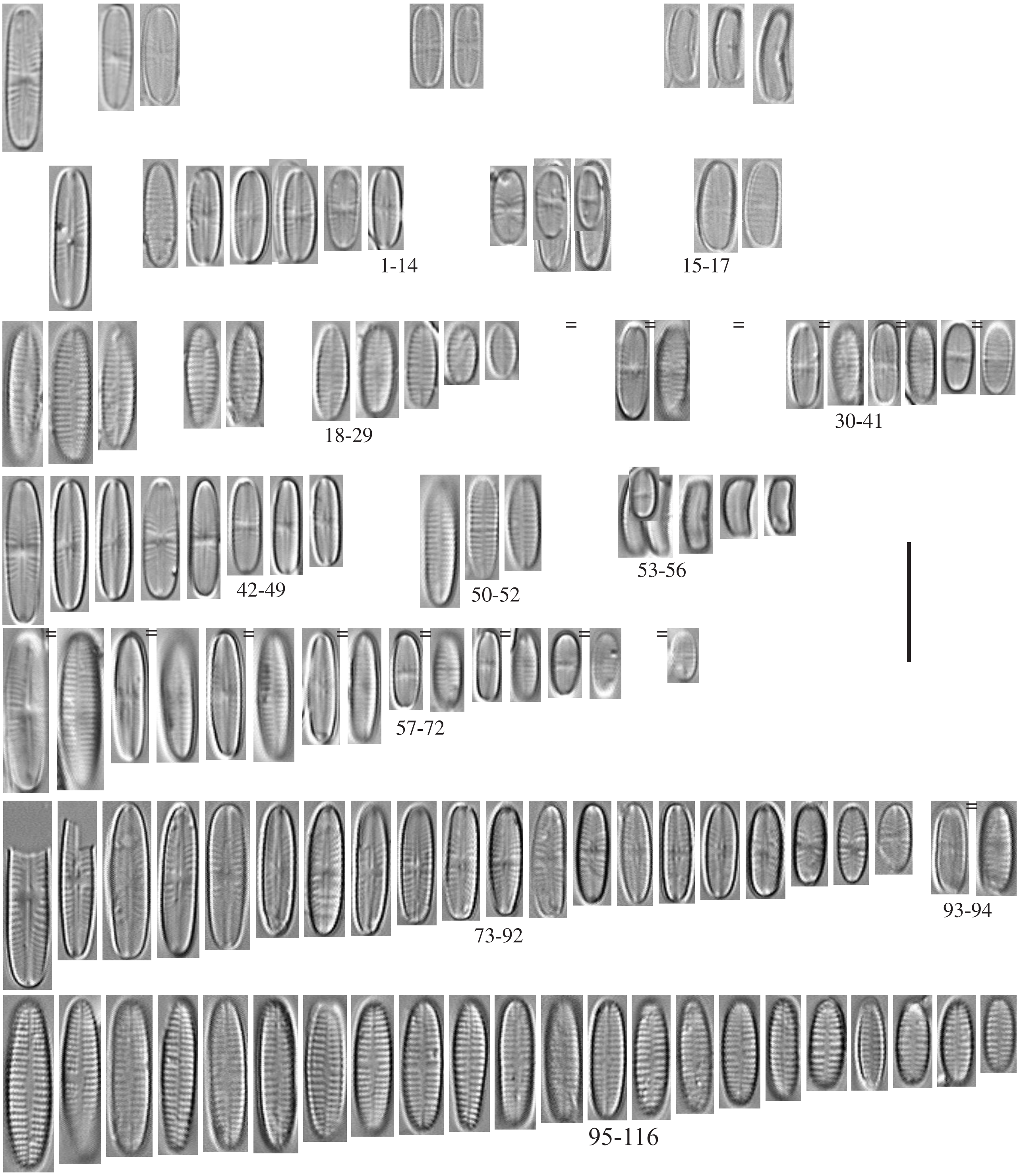
Holotypus: *Alfred Wegener Institut für Polar-und Meeresforschung, Bremerhaven, Germany.*

Locus typicus: *Ernz Blanche riparia prope urbem Eppeldorf (Hessemillen), Luxembourg, 49°51'05"N, 6°14'28"E.*

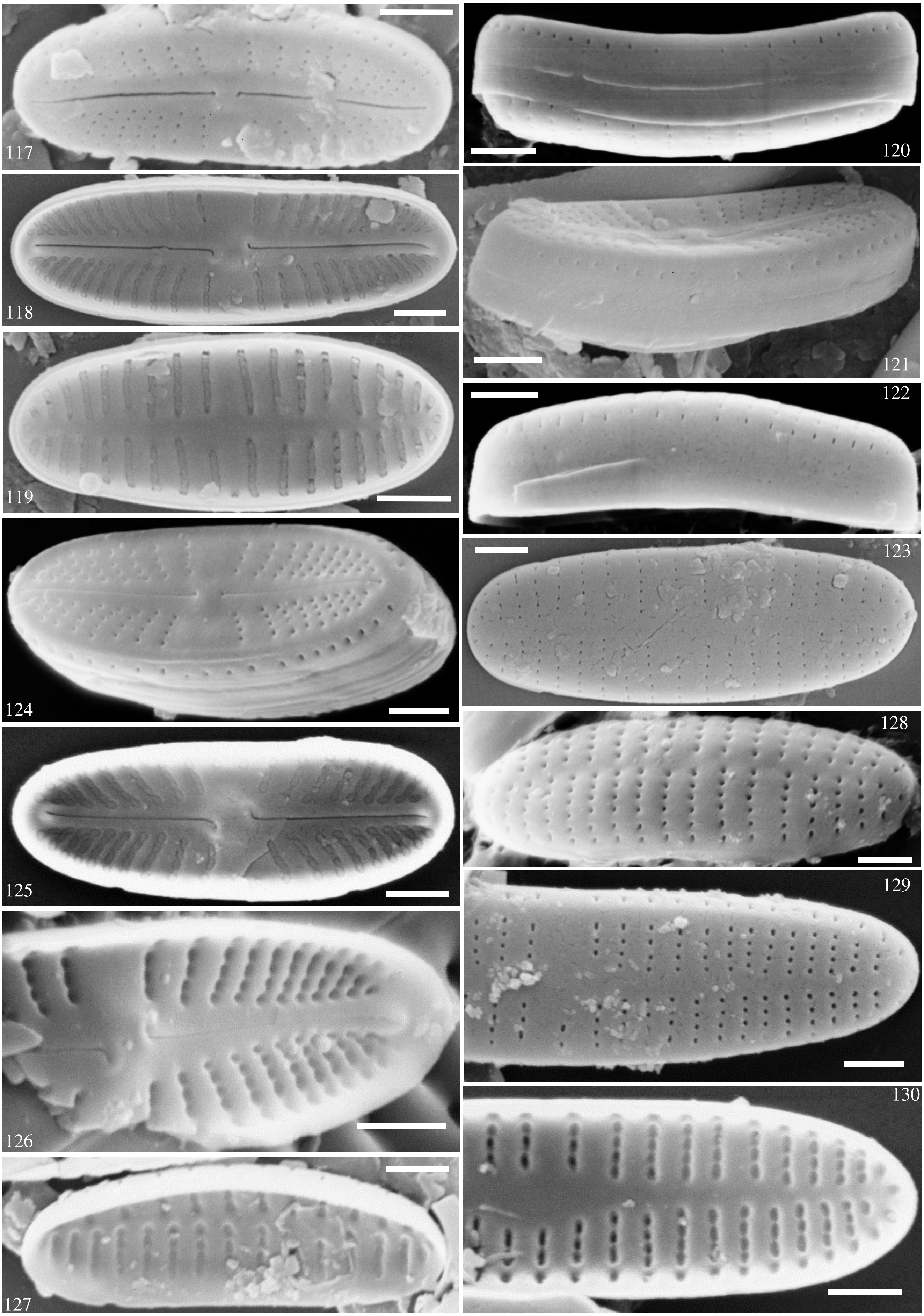
Isotypus: *National Botanical Garden, Meise, Belgium; Academy of Natural Sciences, Philadelphia, USA; British Museum of Natural History, London, UK.*

Description

Cells in girdle view narrow, circa 2 µm, slightly bent along the apical axis, central nodule visible in LM (Figs 16, 17). Raphe valve concave (Figs 15-17, 121) and rapheless valve convex (Figs 15-17, 120, 122) along transapical axis. Valves linear-elliptic, small valves more elliptic and large valves more linear with round ends. Length 4.3 to 11.6 µm. Width 2.2 to 3.2 µm. Length/width ratio 1.7 to 4.1. Striae always uniseriate. Foramen of the areolae small and circular on the outside of valves, expanding internally, closed by discoid hymens (Figs 118, 119). Raphe valve striae between 25 to 32 in 10 µm, denser at valve ends, diversely but slightly radiate near the centre, becoming strongly radiate towards the ends and slightly to strongly curved with a maximum halfway between the centre and distal ends of the valve (Figs 117, 118). Striae interrupted at the valve face/mantle junction by a weakly thickened hyaline area finishing close to the margins of the valve (Figs 117, 120, 121). Striae interrupted at the terminal nodules (Figs 117, 121) forming a bow-tie shape. Rapheless valve striae very different (Figs 119, 123), between 23 to 28 in 10 µm, parallel through almost the valve length, terminal short striae slightly radial. Striae irregularly spaced with increasing density from the centre towards the ends of the valve and slightly passing over onto the valve mantle (Figs 120, 122). Some striae alternating in the manner of certain taxa of *Fragilaria*. No obvious break occurs outside of the valve in the striae at the smooth and round valve plane/mantle junction, just a small interruption existing externally between the two last areolae (Fig. 122). Areolae small and round in valve view (Fig. 123) and in girdle view (Figs 120, 122). Valve interior: areolae foramina widen to form an alveolus furrow. Hyaline area (pseudoraphe) of the rapheless valve very narrow and linear to linear-lanceolate (Figs 119, 123), often slightly displaced towards one margin. Externally, number of areolae approximately 65 in 10 µm on the raphe valve and 50 in 10 µm on the rapheless valve. Costae broad and weakly prominent on the internal valve plane. The raphe is a simple slit in the middle of the sternum (Figs 117, 118). Raphe branches filiform, straight, with very small pin-hole ends in external view, slightly coarser at proximal ends (Fig. 117). On the



Figs 1-116. – LM. Scale bar = 10 µm. Figs 1-41: *Achnanthidium atomoides* (type material from river Ernz Blanche at Hessemillen, Luxembourg). Figs 1-14: raphe valves. Figs 15-17: girdle views. Figs 18-29: rapheless valves. Figs 30-41: two valves of the same individual. Figs 42-72: *A. atomoides* (material from river Nalón, Spain). Figs 42-49: raphe valves. Figs 50-52: rapheless valves. Figs 53-56: girdle views. Figs 57-72: two valves of the same individual. Figs 73-116: *A. atomus* (original material from type locality, Java). Figs 73-92: raphe valves. Figs 93, 94: two valves of the same individual. Figs 95-116: rapheless valves.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internal side of the valve, proximal ends deflected to opposite directions (Fig. 118). Proximal raphe endings separated by 0.4 µm outside (Fig. 117) and 0.5-0.6 µm inside (Fig. 118). Axial area of the raphe valve narrow, linear-lanceolate. Central nodule slightly prominent between proximal raphe endings.

Taxonomical notes

Achnanthidium atomoides is closely related to *Achnanthes atomus* (Hustedt 1937-1939) described from Java and Sumatra (Indonesia). It differs from this last one by consistently smaller valve sizes and by lesser spaced striae and areolae: 19-21 striae in 10 µm in rapheless valve and 22-26 striae in 10 µm in raphe valve conversely to 22-28 and 25-32 in *Achnanthidium atomoides*; averages of 37 areolae in 10 µm in rapheless valve and 45 striae in 10 µm in raphe valve conversely to 50 and 65 in *A. atomoides* (Table I).

The more accentuate transapical concavity of the raphe valve in *A. atomoides* (Fig. 121) explains a greater density of areolae in the outside view comparatively to the inside view in *A. atomoides* relatively to *Achnanthes atomus*.

Furthermore, observations on the original material of the type of *A. atomus* from Java revealed 22-26 striae in 10 µm on the raphe valve and 19-21 striae on the rapheless valve (Table I), whereas Hustedt (1937-1939) stated 28-30 striae on the raphe valve and 22-25 on the rapheless valve. However, only 22 striae on the raphe valve and 20 striae on the rapheless valve can be counted on Simonsen's (1987) photographs (pl. 325, Figs 32-38) of Hustedt's type material from Java, in accordance with our own data (Table I). SEM observations of the original material of *A. atomus* showed *A. crassa* Hustedt as fairly frequent, although Hustedt (1937-1939) did not note it in this sample. It is possible that at that moment Hustedt considered *A. crassa* conspecific with *A. atomus*, which would explain the difference in measurements on the same sample between Hustedt, Simonsen and the present study. Striae on Hustedt's (1937-1939)

Table I. – Top, Comparison of morphometric features of the type material of *Achnanthidium atomoides* (Luxembourg, Eb87a) and the original material from the type locality of *Achnanthes atomus* (Java, As 453). First and last numbers are minimal and maximal values; bold numbers are averages. 30 valves of each taxon have been measured with SEM. Table II. – Bottom, Environmental parameters of the type locality of *Achnanthidium atomoides* on the river Ernz Blanche (Luxembourg, Eb 87a). Samplings of the 27/09/2002 and the 16/07/2002(*). Th °f = total hardness in French degrees, Tac °f = carbonate hardness in French degrees, OPI = Organic Pollution Index (Leclercq & Maquet 1987), SPI = Specific Polluosensitivity Index (Cemagref 1982).

			Achnanthidium atomoides	***Achnanthidium atomus***
Length (µm)			4.3-**7.1**-9.7	6.6-**9.1**-13.4
Width (µm)			2.2-**2.6**-3.2	2.7-**3.1**-3.7
Length/width ratio			1.8-**2.6**-3.1	2.2-**3.0**-4.0
Distance between central raphe ends	Externally (µm)		**0.43**	0.54-**0.56**-0.65
	Internally (µm)		0.54-**0.63**-0.65	0.54-**0.69**-0.87
Number of striae in 10 µm	Raphe valve		25.3-**29.0**-32.0	21.6-**23.6**-26.2
	Rapheless valve		22.8-**25.8**-28.1	18.5-**19.9**-21.2
Number of areolae in 10 µm	Raphe valve	Externally	60-**65**-69	42-**45**-46
		Internally	60-**62**-65	51-**54**-65
	Rapheless valve	Externally	46-**50**-55	**37**
		Internally	55-**58**-60	37-**44**-46

Altitude (m)	*Distance to source (km)*	*Temp (°C)*	*pH*	*Conductivity (µS.cm^{-1})*	**Th (°f)*	**Tac (°f)*	**NO_3^- (mg.l^{-1})*	**NO_2^- (mg.l^{-1})*	**NH_4^+ (mg.l^{-1})*	*OPI*
220	27.8	10.2	8.3	664	35.6	23.3	23	0.14	0.05	4
Discharge (m^3.s^{-1})	*River width (m)*	*O_2 (mg.l^{-1})*	**PO_4^{3-} (mg.l^{-1})*	**Cl^- (mg.l^{-1})*	**K^+ (mg.l^{-1})*	**SO_4^{2-} (mg.l^{-1})*	**Na^+ (mg.l^{-1})*	**Ptot (mg.l^{-1})*	**BOD_5 (mg.l^{-1})*	*SPI*
0.4	5.9	10.7	0.15	19	2.7	106	8.7	0.16	0.9	15.9

◄

Figs 117-130. – SEM. Scale bars = 1 µm. Figs 117-123: *Achnanthidium atomoides* (original material from river Ernz Blanche at Hessemillen, Luxembourg). Fig. 117: raphe valve, outside view. Fig. 118: raphe valve, inside view. Fig. 119: rapheless valve, inside view. Fig. 120: frustule, girdle view. Fig. 121: frustule, raphe valve and girdle, outside view. Fig. 122: rapheless valve, girdle view. Fig. 123: rapheless valve, outside view. Figs 124-130: *A. atomus* (original material from type locality, Java). Fig. 124: frustule, raphe valve and girdle, outside view. Figs 125, 126: raphe valve, inside views. Fig. 127: rapheless valve, inside view. Figs 128, 129: rapheless valve, outside views. Fig. 130: rapheless valve, inside view.

and Cholnoky's (1957) drawings of *A. atomus* raphe valves are straight, but the photographs of Hustedt's type slide by Simonsen (1987) and Lange-Bertalot & Krammer (1989) are showing curved striae. Our observations of the original material of *A. atomus* (Figs 124-130) are showing slightly curved striae (Figs 124, 125). On the other hand, rapheless valves of *A. atomus* present less radiate short distal striae (Figs 127-130) than *Achnanthidium atomoides* (Figs 119, 123), and the central part of the hyaline area is less strictly shaped in *Achnanthes atomus*, especially in larger individuals (Figs 129, 130), flatter with two, sometimes coalescent, separated rows of areolae on the mantle, where it becomes slightly rhombic. *A. atomus* can be also differentiated from *Achnanthidium atomoides* by the presence on the central part of the raphe valve of an acute external marginal hyaline rim with a rib (Fig. 124) at the intersection of the valve face and the valve mantle. This rib is absent in *A. atomoides*.

Distribution and ecology

A. atomoides is rarely observed in Luxembourg. It is present in the epilithon of rivers Ernz Blanche and Ernz Noire lower basins (river width from 5.9 to 10.4 m; distance from the source 20 to 30 km) of the sandy-sedimentary Gutland region of Luxembourg (Fig. 131). *A. atomoides* was also observed, but rarely, at the confluence of the river Enz into the river Prüm in the Eifel region (Germany), only ten km from the type locality in Luxembourg, among *A. kranzii* (Lange-Bertalot) Round et Bukhtiyarova and *A. minutissimum.* In Luxembourg (Table I, bottom), this taxon was present in fairly cold (8.7 to 10.2 °C) well-oxygenated waters (8.4 to 11.6 mg.l^{-1} O_2) with conductivities from 587 to 771 µS.cm^{-1} and basic pH (from 8.1 to 8.5). Its occurrence could be related with high concentrations of sulphates (from 105 to 110 mg.l^{-1} SO_4^{2-}), which are among the highest observed in Luxembourg rivers. This taxon is rather tolerant to high levels of nutrients and organic load, it was observed at different levels of total phosphorus concentrations (from 0.16 to 0.81 mg.l^{-1} Ptot) and biological oxygen demand (BOD_5 from 0.9 to 11 mg.l^{-1} O_2). Nitrate concentrations were rather homogeneous (from 20 to 25 mg.l^{-1} NO_3). The most frequent taxa accompanying *A. atomoides* in the different localities in Luxembourg are listed in Table III. The main diatoms are the same than in the German sample, except *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot. Nevertheless new samplings should be carried out to characterize with more accuracy its ecology, since this taxon was observed in only four samples in Luxembourg and one in Germany. Furthermore, *Achnanthidium atomoides* was observed in a sample recently (29 September 2003) collected from the mountain river Nalón in Spain (Figs 42-72). The most frequent species accompanying *A. atomoides* were different from those observed in Luxembourg: *Nitzschia fonticola* Grunow, *Achnanthidium*

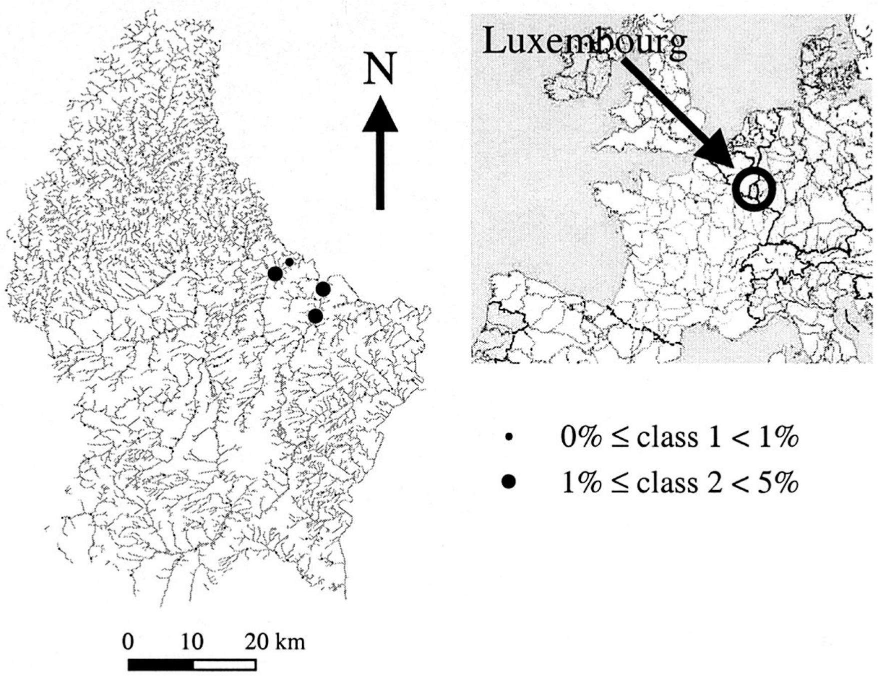

Fig. 131. – Distribution and relative abundance (%) of *Achnanthidium atomoides* in rivers and streams of Luxembourg.

Table III. – Relative abundances (%) of the main diatoms from the four sites where *Achnanthidium atomoides* was observed in Luxembourg. 1 = Type locality, river Ernz Blanche in Hessemillen; 2 = river Ernz Noire in Grundhof; 3 = river Ernz Noire in Vugelsmillen; 4 = river Ernz Blanche in Reisdorf; A = Average of the four localities.

Taxa \ Sites	1	2	3	4	A
Amphora pediculus (Kützing) Grunow	80.8	68.5	62.0	27.3	59.7
Navicula cryptotenella Lange-Bertalot	1.0	2.3	11.5	18.5	8.3
Achnanthidium minutissimum (Kützing) Czarnecki	5.1	7.9	2.5	7.5	5.6
Navicula tripunctata (O.F. Müller) Bory	0.7	3.2	2.0	13.3	4.8
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	2.9	2.3	7.6	5.1	4.5
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	3.2	2.9	3.4	3.0	3.1
Nitzschia dissipata (Kützing) Grunow	0	0.9	1.7	6.1	2.2
Navicula gregaria Donkin	0	1.6	1.5	5.4	2.1
Achnanthidium atomoides Monnier, Lange-Bertalot et Ector	**2.9**	**1.4**	**1.2**	**0.5**	**1.5**
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	0	1.4	0	4.0	1.4
Other taxa	3.4	7.6	6.6	9.3	6.7

pyrenaicum (Hustedt) Kobayasi, *A. minutissimum* and *Gomphonema pumilum* (Grunow) Reichardt et Lange-Bertalot.

Achnanthidium atomus (Hustedt) Monnier, Lange-Bertalot et Ector nov. comb. (Figs 73-116, 124-130).

Basionym: *Achnanthes atomus* Hustedt 1937, *Arch. Hydrobiol.* Suppl Band 15, Teil II, p. 194, pl. 13, Figs 33-36.
References: Lange-Bertalot & Krammer 1989, p. 24, pl. 59, Figs 15-18; Simonsen 1987, p. 211, pl. 325, Figs 32-38; Lange-Bertalot & Ruppel 1980, p. 13, Figs 190-196; Cholnoky 1962, p. 59; Cholnoky 1957, p. 38, pl. 1, Figs 1, 2; Cholnoky 1954, p. 410, pl. 1, Figs 1, 2; Hustedt 1939, p. 554; Hustedt 1937 in Schmidt *et al.* 1874-1959, pl. 412, Figs 42-54.

Taxonomical notes

Morphological features of *A. atomus*, as linear-elliptic cells, slightly bent in girdle view, concave raphe valve, uniseriate striae and small and round areolae ..., are relevant to the genus *Achnanthidium* Kützing sensu Mann in Round *et al.* (1990) emend. Round & Bukhtiyarova (1996), and justify its combination in the latter genus. Some comments on this taxon and comparison with *A. atomoides* are given under this species and in Table I.

On the basis of the reinvestigation of the original material from Java, the diagnosis of *A. atomus* should be emended as follows: raphe valve with 22-26 striae in 10 µm, rapheless valve with 19-21 striae in 10 µm, raphe valve with some curved striae.

The taxon presented by Kaczmarska & Rushforth (1983), possibly related to *Achnanthes* (*atomus* var.?) *congolensis* Hustedt, does not show characteristics similar to *Achnanthidium atomus*. As supposed by Hustedt (1949) and illustrated by Simonsen (1987), *Achnanthes* (*atomus* var.?) *congolensis* is quite different from *Achnanthidium atomus* and is certainly a different species.

Distribution and ecology

A. atomus was originally described from Java and Sumatra (Hustedt 1937-1939). This taxon was recorded from a great variety of running water environments and substrates in Indonesia: waterfalls, rivers, stones, wood, mosses, especially on sprinkling waters, then considered rheophilous, living at temperatures between 10 and 25°C, but rarely above 20°C (type locality: 14.2°C), in waters with a fairly high mineral content and slightly alkaline pH (7-8, type locality: 8.1), but rather poor in calcium carbonate (volcanic substrate). In the type locality, the richest sample in diatom taxa came from an embedded shady ravine. Some of the most frequent species accompanying *A. atomus* were *Achnanthes subhudsonis* Hustedt, *A. cataractarum* (Hustedt) Lange-Bertalot et Krammer, *A. crenulata* Grunow, *A. minutissima* var. *cryptocephala* Grunow, *Eolimna ruttneri* (Hustedt) Lange-Bertalot et Monnier (Monnier *et al.* 2003), *Fragilaria capucina* Desmazières var. *vaucheriae* (Kützing) Lange-Bertalot, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Ulnaria ulna* (Nitzsch) Compère. Cholnoky (1954) considered *Achnanthidium atomus* as widely distributed in Africa, in neutral (Cholnoky 1957) to slightly alkaline (Cholnoky 1962) river waters. *A. atomus* was also found in Europe, but rarely, in alkaline waters of the Gave d'Ossau in the Pyrenees mountains (Hustedt 1939). In the same area, *A. atomus* was observed in the framework of water quality assessment studies in several sites on both sides of the Pyrenees, in the Ebro basin in Spain (J Goma & R Ortiz pers comm) and in the Garonne basin in France (M Coste pers comm), and it was also observed in the French Alps (R Chaveau pers comm). According to these rare data about *A. atomus* sensu lato, it should be considered as tolerant to a wide range of ecological conditions and as almost cosmopolitan, if the different populations really belong to *A. atomus* and not to *A. atomoides*. However, the relationship among the Asian, African and European populations should be checked, in accordance with a precise treatment of their ecological requirements.

The distribution and ecology of *A. atomus* from Indonesia is quite different from that of *A. atomoides* from Luxembourg, despite their morphological resemblance, since they were found in different geographical areas, with quite different water characteristics, although both were observed in waters with fairly high electrolytic content and basic pH.

DISCUSSION AND CONCLUSION

Considering the morphological attributes of *A. atomus*, this taxon as well as the new species *A. atomoides* belong to the genus *Achnanthidium* within the family Achnanthidiaceae. In contrast to most *Achnanthidium* species, however, the raphe valve is concave along the transapical axis (Fig. 121) and the rapheless valve is convex (Figs 120, 122, 128). The parallel and irregularly spaced striae of the rapheless valve (Figs 119, 123, 128-130) are also markedly different from many *Achnanthidium* taxa. Conversely to other Achnanthidiaceae genera, the inversion of the transapical flexure of the valves probably represents an important evolutionary feature and could justify the separation of *A. atomus* and related taxa from the *A. minutissimum* group. In this context, a certain variability in the transapical axis flexure of the valves within the genus *Achnanthidium* should be considered, for which *A. minutissimum* and *A. atomoides* or *A. atomus* are showing two opposite tendencies. Full evidence of a discontinuity of this morphological feature in species related to *A. minutissimum* requires further investigations. For instance, there is actually no other SEM documented species with precisely this combination of characters. However, some *Achnanthidium* species show a tendency towards *A. atomus* and *A. atomoides*, as *A. pyrenaicum* (Table IV) and *A. japonicum* (Kobayasi) Kobayasi (Kobayasi 1997), although not as markedly concave about the transapical axis of the raphe valve. Phylogenetical links between *A. atomoides* and *A. atomus* must be investigated, also with *A. japonicum*, *A. pyrenaicum*, *A. convergens* (Kobayasi) Kobayasi, *A. subatomus* (Hustedt) Lange-Bertalot (Table III), *A. alteragracillima* (Lange-Bertalot) Round et Bukhtiyarova and *Achnanthes biasolettiana* Grunow var. *thienemannii* (Hustedt) Lange-Bertalot.

As interbreeding of morphodemes and clonal variability have not been investigated in the laboratory, only phenetic arguments permit to separate *Achnanthidium atomoides* and *A. atomus*. The number of areolae in 10 µm is the main diacritical feature to separate these two taxa. The density of areolae is independent of the valve size in the two taxa. On small valves, there are less areolae per striae than on large ones. The variability of areolae density observed in other species of this genus (Table IV) is not observed in *A. atomoides*, except when the two valves of a same frustule are compared. In practice, this criterion of areolae density is not commonly considered in phenetic circumscriptions of taxa. The areolae were, however, already used for other taxa as a valid morphological criterion to separate taxa not clearly different on the basis of the valve physiognomy (shape), furthermore variable inside a same taxon (Mann 1984, Cox 1986), e.g. *Cymbella tropica* var. *tenuipunctata* Krammer and many other *Cymbella* CA Agardh taxa (Krammer 2002), or *Achnanthes rupestoides*

Table IV. – Comparison of morphological characters of *Achnanthidium atomoides* with other observed *Achnanthidium* taxa from rivers of Luxembourg. RV = raphe valve, RLV = rapheless valve.

Characters \ Taxa	*Achnanthidium atomoides*	*Achnanthidium pyrenaicum*	*Achnanthidium subatomus*	*Achnanthidium minutissimum*
Raphe valve apical axis	Concave	Slightly concave	Slightly concave	Concave
Raphe valve transapical axis	Concave	Slightly convex	Convex	Convex
Valve shape	Linear-elliptic	Elliptic to linear-lanceolate	Elliptic to linear-elliptic	Elliptic to linear-lanceolate
Apices shape	Round	Slightly subrostrate	Round	Diversely subrostrate
Length (µm)	4.3-11.6	7.9-22.4	7.0-18.0	5.0-17
Width (µm)	2.2-3.2	3.2-4.7	3.2-5.1	2.2-3.2
Length/Width ratio	1.7-4.1	2.4-4.8	2.2-4.0	2.4-5.6
Distance between central raphe ends	0.4-0.6	0.4-0.7	0.4-0.6	0.4-0.7
External central raphe ends	Pin-hole	Drop-shaped	Pin-hole	Slit
External distal raphe ends	Straight slit	Hooked	Curved	Straight to curved
Raphe valve axial area	Linear-lanceolate	Linear-lanceolate	Lanceolate	Almost linear
Raphe valve central area	Rectangular	Narrow rectangular	Rounded	Round to rectangular
Central striae	Absent	Absent to long	Long	Short or absent
Rapheless valve hyaline area	Linear	Linear-lanceolate	Almost linear	Linear-lanceolate
Raphe valve striae (RV)	Curved	Straight, irregular	Straight	Straight to curved
Number of RV striae in 10 µm	25-32	24-30	21-23	25-33
Rapheless valve striae (RLV)	Parallel, alternate	Straight, irregular	Straight	Straight to curved
Number of RLV striae in 10 µm	23-28	20-24	20-22	25-33
Foramina	Round	Round to elongate	Often coalescent	Round to elongate
Number of areolae in 10 µm	RV 60-69, RLV 46-55	(30)40-50	(20)35-45	40-60
Mantle areolae	RV short, RLV round	Elongate	Elongate	Elongate

Hohn var. *uniseriata* Lange-Bertalot et Monnier (Monnier *et al.* 2003). In these examples, the density of areolae is used to separate taxa at the variety level. Nevertheless, arguments to consider areolae as a variety criterion are only hierarchical (Krammer 2002) and not obviously supported by ontogenetic studies (Mc Bride Edgar & Theriot 2003, Mann 1999, Cox 1995, Schmid 1994, Mizuno 1987).

In separating *Achnanthidium atomus* and *A. atomoides*, our purpose is to fix the latter deme, varying from the type and phenetic concept of *A. atomus*, until this nomenclatural choice can be confirmed or refuted by new results. The question is still open to know if the differences observed are due to an intraspecific variability or if they are the mark of two independent species. The choice of the specific level is only formal, as no consensus exists in the use of the different taxonomical ranks (Cox 1997, Round 1997), but tending to a narrower species concept (Behnke *et al.* 2004, Mann 1997, 1999, 2001). Pragmatically and especially concerning applied ecological studies, the use of the specific level avoids any temptation to consider forms or varieties as ecologically similar and to group different ecodemes or ecotypes under a same taxonomical entity. It cannot be actually substantiated that *A. atomus* and *A. atomoides*, considering their different habitat conditions in a temperate and a tropical region, are not simply ecodemes or growth stages of a same biological entity (Cox 1986). However, the separation of ecologically distinct demes is strongly recommendable for ecological and applied studies, even if it requires more experiments (Cox 1997).

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