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A new species of *Staurosirella* (Bacillariophyta) observed in a spring of the catchment of the Regional Natural Reserve of Jolan and Gazelle Peatlands

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Abstract:

During a survey of headwater springs in France, an unknown *Staurosirella* taxon was observed that could not be identified using the currently available literature. Based on light and scanning electron microscopy observations, the taxon is described as a new species: *Staurosirella lucectoriana*. The new species is characterized by small isopolar, elliptical frustules, connected to each other in girdle view, forming long, ribbon-like colonies via bifurcating, interlocking spines. The new species presents a very small apical pore field, composed of only a few pores on one valve pole. Girdle elements are variable in number, closed and with an open valvocopula presenting fimbriae. *Staurosirella lucectoriana* is typically found in waters with high nitrate concentrations. A detailed comparison with morphologically similar species is added.

Keywords: Europe, French Massif Central, headwaters, new species, *Staurosirella*, ultrastructure

Introduction

For several years, the good ecological state of many natural ecosystems is deteriorated by several negative environmental impacts such as eutrophication, linked to human impact (Solimini et al. 2006). Moreover, natural environments are also affected by climate change as it represents one of the principal threats for freshwater biodiversity (Dudgeon et al. 2006; Woodward et al. 2010). Peatbog ecosystems, and the headwaters feeding them, are strongly impacted by these effects, resulting in physical and chemical alterations, such as an increase in surface water temperature and, most likely, a possible increase of hydrological drought duration (Kaule & Frei 2022). These abiotic modifications influence the species composition and dynamics of biological communities. Anthropogenic pressures, such as agricultural uses exerted on these fragile ecosystems and their watersheds, can also contribute to degrading their quality and altering their biodiversity, further threatening the conservation of their good condition and compromising the ecosystem services provided by these environments (Bernard 2016).

When considering the watershed, springs giving rise to streams flowing through peatland, are classified as unique aquatic habitats, contributing significantly to local and regional biodiversity due to their high habitat complexity" (Cantonati et al. 2012). Springs usually are species-rich habitats and it is well known that abiotic factors such as water chemistry and temperature are important ecological factors determining species distribution and community composition. They also are widely threatened by both regional and global factors, including pollution and climate change leading to changes in biodiversity and species composition (Stevens et al. 2021). As biodiversity is the keystone of the functioning of these ecosystems from which ecosystem services are derived, it is important to protect the water catchment and to increase our knowledge of it.

The Regional Nature Reserve (RNR) of the Jolan and Gazelle peatlands, situated in the French Massif Central, was created in 2018 for the protection of the peatlands inhabiting numerous plant and animal species (including several rare) such as the endangered dragonfly *Somatochlora arctica* Zetterstedt. The area is highly impacted by the eutrophication of the minerotrophic peatlands adjoining the Jolan pond urging for a detailed (impact) study of the Nature Reserve. One of the aspects already studied included the diatom flora together with a physical and chemical analysis of the pond. The study was carried out in the reserve (peatlands and watershed) in June 2022. During this survey, a small-celled species belonging to the genus *Staurosirella* was observed in one of the springs of the watershed that could not be identified using the currently available literature on the genus.

The genus *Staurosirella* D.M.Williams & Round (1987: 274) was originally described in 1987, and further emended in 2006 by Morales & Manoylov. This genus comprises small-celled araphid species showing an oval, elliptical, cruciform, occasionally triangular valve outline (Round et al. 1990). The genus is further characterized by the presence of well-developed marginal spines such as in *S. lapponica* (Grunow) D.M.Williams & Round, allowing the formatting of long chain-like colonies, incipient such as in *S. berolinensis* (Lemmerm.) Bukht. (Bukhtiyarova 1995) or absent as is the case in several species such as *S. lanceolata* (Hust.) E. Morales et al. (Morales et al. 2010). *Staurosirella* species possess uniseriate striae typically composed of areolae that can be circular or elliptical, occasionally transapically elongated, internally occluded by volae (Williams & Round 1987; Round et al. 1990; Morales & Manoylov 2006; Morales et al. 2019a). Apical pore fields are quite variable in size and shape but are always composed of several rows of round areolae disposed in ordered rows (Morales & Manoylov 2006). Finally, the cingulum is composed of several plain, open or closed copulae lacking ligulae (Morales & Manoylov 2006; Van de Vijver et al. 2022), with the valvocopula being considerably larger and possessing fimbriae attached to the costae at the valve interior (Morales & Manoylov 2006; Van de Vijver 2022). *Staurosirella* species occur worldwide in a wide variety of freshwater habitats such as rivers, reservoirs, lakes and pools (Almeida et al. 2015; Guerrero et al. 2019; Morales & Edlund 2003; Morales et al. 2010; Seeligmann et al. 2018; Van de Vijver et al. 2014). Currently, AlgaeBase (Guiry & Guiry 2022) lists almost 60 names are listed under *Staurosirella* (including 53 species and 5 varieties).

Following detailed light microscopy (LM) and scanning electron microscopy (SEM) observations and comparisons with known representatives of this genus, the small-celled *Staurosirella* found in the Jolan and Gazelle peatlands RNR is described as a new species: *Staurosirella lucectoriana* Beauger, C.E.Wetzel & Van de Vijver, sp. nov. The morphology of the new species is compared with the most similar *Staurosirella* taxa occurring worldwide. Notes on its ecological preferences are added.

Materials and Methods

The Jolan and Gazelle peatlands RNR is located at the head of the Adour-Garonne watershed (Rhue catchment) near the city of Ségur-les-Villas (Cantal, Auvergne, France) (Fig. 1). The RNR, located at 1,130 m a.s.l. on the volcanic plateau of Cézallier, is part of the French Massif Central and influenced by oceanic, continental, and Mediterranean climate. The total area of 155 ha is essentially occupied by habitats of regional or even national interest. This protected area shelters peat bogs of major interest for the Auvergne region and the massif. Part of the meadows and grasses in the watershed of these wetlands are also included in the protection perimeter. The studied spring, situated in pastures, is located at 1,143 m a.s.l., and emerges in a concrete drinking trough (Fig. 1d).

On the 15th June 2022, an epilithic sample was taken using a toothbrush, brushing, the drinking trough. Sample was preserved with an ethanol solution to a final concentration of 70%. *In-situ*, pH, conductivity (µS cm^{-1}) and water temperature (°C) were measured using a WTW Multiline P4. For dissolved oxygen (% saturation and mg L^{-1}), a ProODO oxygen probe was used. Two water samples were collected for further chemical analysis in the laboratory and were analysed using high pressure ion chromatography methods. On one sample, carbonate concentration (HCO_3^-) (mg L^{-1}) was measured using a HACH Digital Titrator, sulfuric acid (0.1600 N and 1.600 N) and the Bromocresol Green-Methyl Red Indicator (Hach method 8203). The second sample was filtered using Whatmann GF/C filters prior to analysis. A Thermo Scientific Dionex ICS1100 system was used for the cation analysis and, for the anions, the Thermo Scientific Dionex Aquion system was applied. The concentrations of lithium, sodium, ammonium, potassium, magnesium, calcium, fluoride, chloride, nitrite, nitrate, phosphate and sulphate were measured (mg L^{-1}) (Table 1).

Samples were prepared for LM and SEM observations following the method described in Prygiel & Coste (2000) cleaning a small sub-sample of epilithic raw material with hydrogen peroxide (H_2O_2, 35% v/v;) and hydrochloric acid (HCl 37% v/v). The sample was then rinsed several times and subsequently diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Finally, a drop of the diluted cleaned material was dried on coverslips and mounted in Naphrax®. LM observations and morphometric measurements were performed using a Leica® DM2700M at 1000X magnification (N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics. Light micrographs were taken with a Leica® DMC2900 camera. At least 400 diatom valves were enumerated on random transects to get an overview of the composing diatom flora, and converted into percentage relative abundance.

For SEM, parts of the oxidized suspensions weandinore filtered with additional deionized water through a 0.2 µm Isopore polycarbonate membrane filter. Pieces of which were fixed on aluminum stubs after air–drying and coated with a 2 nm layer of chrome, using a high vacuum coating with a 5nm Chrome layer using 100mA (1 minute) in a Quorum sputter coater (Q150 TES Plus). Then, it was studied using a Hitachi Regulus 8230 ultrahigh-resolution analytical field emission (FE) scanning electron microscope (Hitachi High-Technologies Corporation, Japan), operated at 2 kV and 10 mm working distance.

Plates were prepared using Adobe InDesign 16.4. Samples and slides are stored at the Herbiers Universitaires de Clermont-Ferrand (CLF) (France) and Meise Botanic Garden (BR) (Belgium). Diatom terminology follows Ross et al. (1979), Barber & Haworth (1981) for terminology related to valve shape and striae orientation (valve shape, stria/areola structure) and Round et al. (1990) for terminology on areolar substructures and girdle band features.

Taxonomic comparisons referred to the following publications: Guerrero et al. (2019), Morales et al. (2015; 2019b), Osório et al. (2021) and Van de Vijver et al. (2022).

Results

Division Bacillariophyta

Class Bacillariophyceae

Subclass Fragilariophycidae

Order Fragilariales

Family Staurosiraceae

Genus *Staurosirella* D.M.Williams & Round 1987

***Staurosirella lucectoriana* Beauger, C.E.Wetzel & Van de Vijver sp. nov.**

PhycoBank registration: http://phycobank.org/103828

Light microscopy (Figs 2–37): frustules rectangular in girdle view (Fig. 35), connected to each other to form long, ribbon-like colonies (Figs 36–37). Valves isopolar, elliptical; longer valves with weakly parallel margins and shorter valves with convex margins. Apices broadly rounded. Valves dimensions (n=45): length 5.5–8.5, width 3–4.5. Very little size variation observed. Sternum narrow. Central area absent. Striae equidistant, running continuously from apex to apex, broad, 10–12 in 10 µm. Striae parallel in the middle becoming radiate near the apices. Areolae not discernible in LM.

Scanning electron microscopy (Figs 38–47): Frustules linked by marginal bifurcating and interlocking spines (Figs 38–39). Spines, originating from a single point, well-developed, and dichotomously branched, with a circular columnar base (Figs 39–40), located on the virgae between the striae at the valve face/mantle junction (Figs 40–41). Valve face externally uneven with raised virgae and sternum and adjacent striae slightly sunken in 'punch hole-like' depressions. Striae uniseriate, composed of long, slit-like, linear areolae, running almost parallel to the apical axis (Figs 40–41) or with two terminal striae located on top of the continuation of the axial area onto the mantle (Fig. 42), with areolae in size gradually narrowing at both ends (Figs 40, 44–45). Volae projected towards the valve interior (Fig. 41). Externally, volae intertwining at the same level as their points of origin (Fig. 41). Vimines long and narrow becoming longer toward the valve face edge (Fig. 41). Sternum narrowly lanceolate (Figs 40–41, 44). Mantle rather deep (Fig. 44) with small siliceous plaques located at the mantle edge (Fig. 39, arrow). Apical pore field (APF) present on one apex, occasionally absent, located on the valve face/mantle junction, very small, composed of only one pore or a possibly reduced lineola (Figs 41, 45, arrows). On the other apex, apical pore field replaced by vestiges of a stria (Fig. 44). Internally, striae clearly sunken between flattened virgae and sternum (Figs 42–43). Girdle elements variable in number, closed (Figs 38, 46–47). Open alvocopula showing reduced fimbriae (Fig. 46).

Holotype: CLF121600 (Herbiers Universitaires de Clermont-Ferrand, France)
Isotype: BR-4802 (Meise Botanic Garden, Belgium)
Type locality: FRANCE, spring of the Regional Nature Reserve of the Jolan and Gazelle peatlands at Ségur-les-Villas, E688741.423 and N6455250.009 (Lambert 93)
Etymology: The new species is named in honour to our friend Luc Ector (1962–2022).
Ecology: The well-oxygenated spring was characterized by low conductivity and slightly acidic waters (Table 2). The concentration in nitrates was high with 22 mg L^{-1}.
The diatom flora in the spring was dominated by *S. lucectoriana* (44% of all counted diatoms), *Nitzschia fonticola* (Grunow) Grunow (28%) and *N. soratensis* E.Morales & Vis (7.5%). Other, less frequent (<5%) taxa include *Amphora indistincta* Levkov (3.7%), *Navicula veneta* Kütz. (3%), *Staurosira* cf. *sviridae* Kulikovskiy et al. (3%), *Sellaphora atomoides* C.E.Wetzel & Van de Vijver (2%), *Encyonema minutum* (Hilse) D.G.Mann (1.5%), *Planothidium lanceolatum* (Bréb. ex Kütz.) Lange-Bert. (1.4%), *Nitzschia linearis* (C.Agardh) W.Sm. (1.1%). At last, *Achnanthidium eutrophilum* (Lange-Bert.) Lange-Bert., *Adlafia minuscula* (Grunow) Lange-Bert., *Amphora copulata* (Kütz.) Schoeman & R.E.M.Archibald, *Cocconeis rouxii* Hérib. & Brun, *Diatoma mesodon* (Ehrenb.) Kütz., *Encyonema ventricosum* (Kütz.) Grunow, *Meridion circulare* (Grev.) C.Agardh, *Navicula cryptocephala* Kütz., *Navicula gregaria* Donkin, *Nitzschia alpina* Hust., *Nitzschia palea* var. *tenuirostris* Grunow, *Planothidium curtistriatum* C.E.Wetzel et al., *Planothidium frequentissimum* (Lange-Bert.) Lange-Bert., *Psammothidium lauenburgianum* (Hust.) Bukhtiyarova & Round and *Sellaphora crassulexigua* (E.Reichardt) C.E.Wetzel & Ector represented each one less than 1% of the whole community.

Discussion

Staurosirella lucectoriana is one of the few *Staurosirella* species known to form long, chain-like colonies. At present, only two other *Staurosirella* species form similar colonies: *S. lapponica* (Grunow) D.M.Williams & Round and *S. mutabilis* (W.Sm.) E.Morales & Van de Vijver. These three species share some morphological features such as the typical interlocking, bifurcating spines that can also be seen in for instance *S. lapponica* (Van de Vijver et al. 2022), and the reduced apical pore fields. Both *S. lapponica* and *S. mutabilis*, however, possess a very broad sternum, compared to *Staurosirella lucectoriana*, formed by reduced marginal striae (Morales et al. 2015; Van de Vijver et al. 2022). Contrary, *S. lucectoriana* presents striae that almost reach each other on the sternum.

It appears that the type population observed of *Staurosirella lucectoriana* present very little size variation. Thus, based on valve outline and morphometric dimensions, only three *Staurosirella* species show some resemblance, although they all lack the typical colony formation and bifurcating spines (Table 2): *S. andinopatagonica* J.M.Guerrero et al., *S. neopinnata* E.Morales et al. and *S. paranaensis* N.C.Osório et al. (Guerrero et al. 2019; Morales et al. 2019b; Osório et al. 2021). *Staurosirella neopinnata* often has longer, more elongated valves with almost parallel margins, a pattern not observed in *S. lucectoriana*, where only rounded to elliptical valves were observed with convex margins. In outer view, the striae in *S. paranaensis* are narrower due to more heavily build, clearly raised virgae, contrary to *S. lucectoriana* that possesses striae almost equal in width as the virgae (Osório et al. 2021, see for instance figs 35 & 38). *Staurosirella andino-patagonica* has two thin spines per virga, and lacks the robust bifurcating spines of *S. lucectoriana* (Guerrero et al. 2019). Moreover, *S. andino-patagonica* has less raised external virgae with the striae therefore less sunken between them.

The reduced apical pore field, observed in *S. lucectoriana,* is typical for colony-forming *Staurosirella* species and was also encountered in *S. lapponica* and *S. mutabilis* (Morales et al. 2015, see fig. 45; Van de Vijver et al. 2022, see fig. 30). The ability to form colonies by linking spines reduced the need to connect cells via the apices and therefore larger apical pore fields seem less necessary. Apart from the colony-forming species, several solitary *Staurosirella* species also have reduced apical pore fields, such as *S. andinopatagonica*, *S. krammeri* E.Morales et al., *S. neopinnata* and *S. paranaensis*, that all have an apical pore field composed of only a few small pores. This is in clear contrast with other *Staurosirella* species showing large pore fields with long, regular rows of small pores such as *S. baicalensis* with an APF widely extended on the foot poles and less on the head poles Kulikovskiy et al. 2015), *S.*

dubia (Grunow) E.Morales & Manoylov (Morales & Manoylov 2006) and *S. minuta* E.Morales & Edlund (Morales & Edlund 2003) with APF composed of several discernible rows of round poroids at both apices. Morales et al. (2010) discussed several small-celled *Staurosirella* species that, despite their reduced valve dimensions, still possess well-developed apical porefields, making the APF of the new species rather unique.
Compared to other small round-celled species, the valvocopula of the new species is also closed andthe other girdle bands are closed.
Considering the internal view, it appears that no feature differs between the species except that for *S. andinopatagonica*, areolae are occluded by delicate and dichotomously branched volae while for the other including the new species, volae are projected towards the valve interior. At last, for all species, the striae are in depressions.
When considering the ecology of the new species, *Staurosirella lucectoriana* was observed in a headwater spring that emerges in a concrete drinking trough. The high nitrate concentration reflected the impact of agricultural activities in the catchment (Taboada-Castro et al. 2004). The new species was associated with two freshwater species: *Nitzschia fonticola*, known to live in oligo- to beta-mesosaprobic and meso-eutrophic environments and *Nitzschia soratensis*, prefering slightly eutrophic to eutrophic waters pointing out the presence of nutrients in the spring (Van Dam et al. 1994; Lange-Bertalot et al. 2017).
When considering the other species compared to the new one, they were encountered in different environments: *S. andinopatagonica* and *S. neopinnata* were described in lake samples and sediment core and, *S. paranaensis* was first observed in a large river. *Staurosirella mutabilis* was observed in fresh water but there was no information on the physical and chemical parameters of the habitat. Likewise, for *S. lapponica*, information on the ecological characteristics of its type habitat is lacking.
This study underlined the presence of a particular diatom biodiversity in the RNR of the Jolan and Gazelle peatlands, with the presence of new species and others are under review. This confirms the interest to have created a reserve in this area and the necessary to protect it and to continue care. It will be interested to to increase our knowledge on the diatom biodiversity and to do other surveys done in other mountainous areas of the French Massif Central.

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Figure legends

Figure 1. Map showing the location of the study spring. a and b: general location of the spring in France and in the Auvergne region; c: location of the spring (blue circle) in Regional Nature Reserve (RNR) of the Jolan and Gazelle peatlands; d: photography of the studied spring.

Figures 2–43. *Staurosirella lucectoriana* sp. nov. Figs 2–37. LM. Type population of a spring of the RNR of the Jolan and Gazelle peatlands, France. Scale bar = 10 µm. Figs 38–43. SEM. Figs 38. External view of two frustules and one valve connected to each other with dichotomously branched and interlocking spines. Fig. 39. External detail of the linking spines. The arrow indicates the presence of mantle plaques. Fig. 40. External view of an entire valve. Fig. 41. External detail of the apex. The arrow indicates the reduced APF. Fig. 42. Internal view of an entire valve. Fig. 43. Internal detail of the apex. Scale bar = 5 µm (Fig. 38), 4 µm (Figs 40, 42), 3 µm (Fig. 39) and 1 µm (Figs 41, 43).

Figures 44–47. Fig 44. External view of a tilted valve. Apical pore field replaced by vestiges of a stria, reduced to rounded areolae. Fig. 45. External view of the apex. The arrow indicates the reduced APF composed of only one pore. The isolated pore subtends an apical spine and always seem to be off the apical axis. Figs 46–47. Valvocopula bearing fimbriae. Scale bar = 5 µm (Fig. 44), 4 µm (Figs 46, 47) and 2 µm (Fig. 45).

Table 1: Physical variables and chemical variables measured in the spring.

Date	15/06/2022
Conductivity ($\mu S\ cm^{-1}$)	127.5
pH (pH units)	6.46
Temperature (°C)	12.4
Dissolved oxygen (%)	93.4
Li^+ ($mg\ L^{-1}$)	<0.005
Na^+ ($mg\ L^{-1}$)	5.20
NH_4^+ ($mg\ L^{-1}$)	<0.005
K^+ ($mg\ L^{-1}$)	2.72
Mg^{2+} ($mg\ L^{-1}$)	5.01
Ca^{2+} ($mg\ L^{-1}$)	9.89
F^- ($mg\ L^{-1}$)	0.05
Cl^- ($mg\ L^{-1}$)	6.83
NO_2^- ($mg\ L^{-1}$)	0.01
NO_3^- ($mg\ L^{-1}$)	**22.05**
PO_4^{3-} ($mg\ L^{-1}$)	0.05
HCO^{3-} ($mg\ L^{-1}$)	35.8
SO_4^{2-} ($mg\ L^{-1}$)	1.89

Table 2: Main characteristics of *Staurosirella lucectoriana* and 5 similar *Staurosirella* species.

	Reference	Valve length (µm)	Valve width (µm)	Number of striae (in 10µm)	Valve outline	Striae	Spines	Axial area	Apical pore fields	V
Staurosirella lucectoriana	This study	5.5–8.5	3.0–4.5	10.0–12.0	Isopolar, elliptical; longer valves with weakly parallel margins and shorter valves with convex margins	Uniseriate, composed of long, slit-like, linear areolae, running parallel to the apical axis, with areolae in size gradually narrowing at both ends	Originating from a single point, well-developed, dichotomously branched, with a circular columnar base, located on the virgae between the striae at the valve face/mantle junction	Narrowly lanceolate	Present on at least one apex, located on the valve face/mantle junction, very small, composed of only one pore. On the other apex, APF replaced by vestiges of a stria, reduced to rounded areolae	C
Staurosirella andinopatagonica	Guerrero et al. (2019)	4.4–6.5	2.4–3.7	(8)11.0–13.0(15)	Isopolar to slightly heteropolar, broadly elliptic	Uniseriate, uninterrupted from valve face to valve mantle	2 spines, exceptionally 1 or 3, located on virgae, conical	Narrow, linear to slightly lanceolate	More developed at one pole, composed of 2–3	C p

						and composed of slit-like areolae oriented parallel to the apical axis	and parallel, anastomosing at the base		rows of small, round poroids	
Staurosirella lapponica	Van de Vijver et al. (2022)	7.0–35.0(40)	4.0–6.0	6.0–7.0	Linear to linear-elliptic, isopolar	Short, extending to about 1/3 of the valve mantle	Present, hollow, circular base, upper portion a thick V-shaped with birfurcate lateral extensions	Wide	Reduced on both poles	N
Staurosirella mutabilis	Morales et al. (2015)	8.5–26.0	4.0–25.0	8.0–9.0	Elliptic, isopolar	Short, extending to mid valve mantle	Present, hollow, ellitpic base, upper portion diapason shape with pointy lateral extensions.	Wide	Reduced on both poles	C
Staurosirella neopinnata	Morales et al. (2019b)	4.0–25.0	4.0–4.7	8.0–9.5	Elliptical, most frequently isopolar to rarely slightly heteropolar	Uninterrupted from valve face to mantle	Originating from two (rarely three) points on each virgae at the valve face-mantle	Narrowly lanceolate	Reduced or developed, equal size at both poles	C

							junction, initially hollow, tip spatulate			
Staurosirella paranaensis	Osório et al. (2021)	6.0–10.0	3.0–4.5	8.0–11.0	Elliptical, most frequently isopolar to rarely slightly heteropolar	Uninterrupted from valve face to mantle	Solid and thin; one per costa, originating from one point on each virgae at the valve face-mantle junction, initially hollow, tip spatulate	Narrowly lanceolate	Usually equally developed on both valve poles composed of round poroids	

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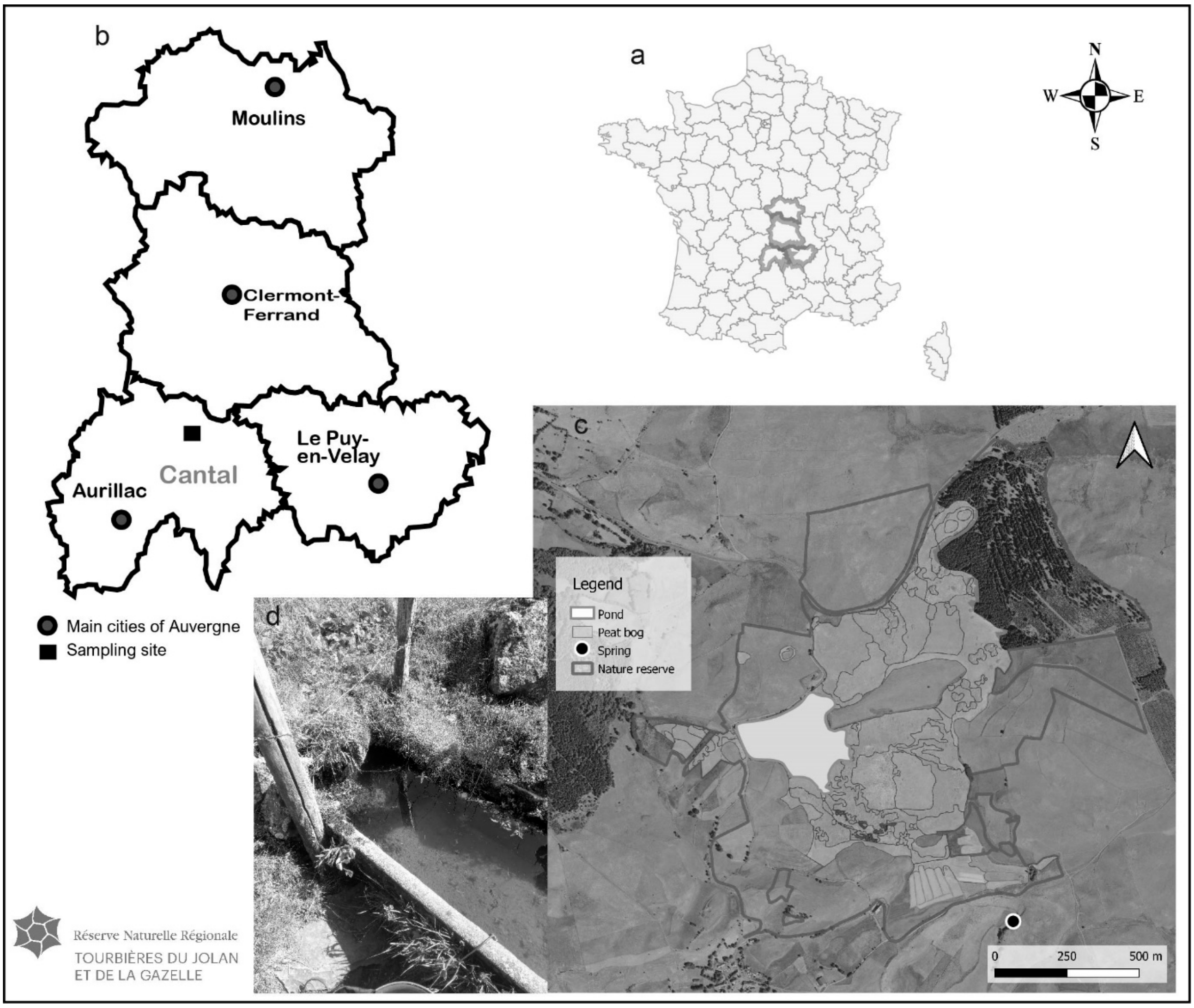

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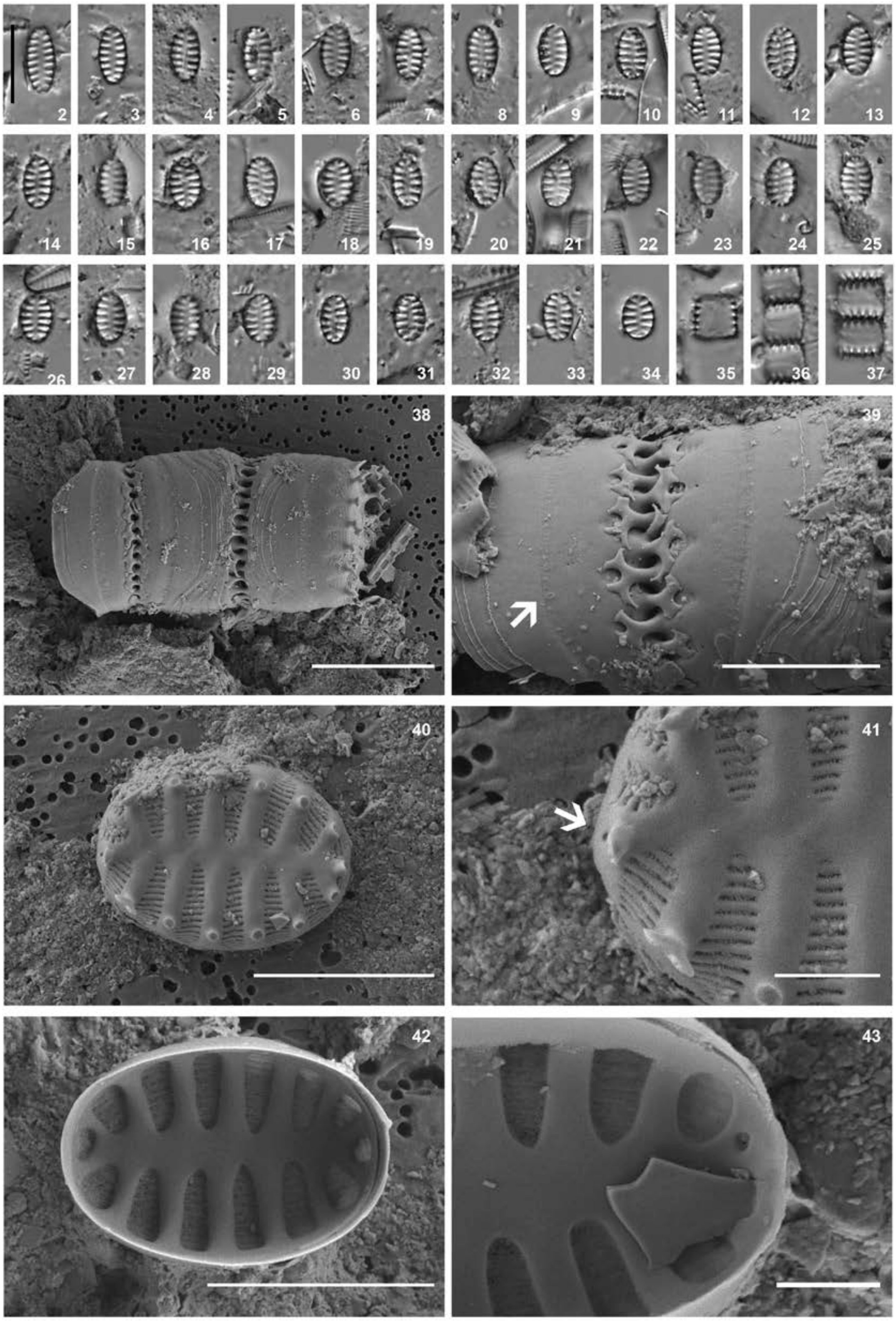
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