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***Pseudostaurosira bardii* (Fragilariaceae, Bacillariophyta), a new species from a saline hydrothermal spring of the Massif Central (France)**

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**ABSTRACT**

Light (LM) and scanning electron microscopy (SEM) observations on a new species of the genus *Pseudostaurosira* from a spring of the Massif Central (France) are presented. The new species with very small dimensions presents similarities with other species previously observed in other parts of Europe. However, based on its morphology, the new species presents striae sometimes composed of only two areolae on the valvar surface: one big, round or elliptic near the mantle edge and the second smaller, slit-like or round (sometimes completely occluded externally) situated further toward the interior. Moreover, the size, the density of striae and the presence of volae allow the separation of *Pseudostaurosira bardii* from the other species. Morphologically similar species were also re-investigated in the current project, including the type material of *Pseudostaurosira trainorii* E. Morales, *Pseudostaurosira connecticutensis* E. Morales and *Pseudostaurosira cataractarum* (Hustedt) C.E. Wetzel, E. Morales et Ector. They were also investigated and illustrated using SEM. The ecological preferences of *Pseudostaurosira bardii* are briefly discussed.

**KEYWORDS**

Araphid diatom; epipelon; mineral spring; scanning electron microscopy; type material; ultrastructure

## Introduction

The taxonomy of genera belonging to the Fragilariaceae have long been problematic despite major taxonomic revisions occurred in the 1980s (Round 1984; Williams 1986; Williams and Round 1987). The genus *Fragilaria* Lyngbye was originally conceived for species that form linear colonies (Lyngbye 1819). Different taxa were described and associated to *Fragilaria* as *F. pectinalis*; this last species has made the lectotype (Tuji and Williams 2006). Later, Ehrenberg (1832) described the genus *Synedra* using *S. ulna* (Nitzsch) Ehrenberg as type material. However, the distinction between *Synedra* and *Fragilaria* was never clearly defined and many different authors have worked on them (Hustedt 1930, Patrick and Reimer 1966; Lange-Bertalot 1980; Krammer and Lange-Bertalot 1991). Since then, studies have worked out morphological details on many new genera. The subdivision of both *Synedra* and *Fragilaria* began with Round (1979, 1984), and later Williams and Round (1987) described a series of new genera as *Neofragilaria*, *Pseudostaurosira*, *Punctastriata* and *Staurosirella*. The type of *Pseudostaurosira* is *Fragilaria brevistriata* Grunow in Van Heurck (Williams and Round 1987; Morales et al. 2015). This genus was described as presenting frustules rectangular, forming chains. For Williams and Round (1987), the valves are elliptical to linear often undulate and occasionally cruciform. As defined by Round, Crawford, and Mann (1990), the striae are uniseriate consisting of a few large elliptical areolae (often smaller round areolae) and rarely more than four areolae per stria. The closing plate is delicate and highly branched and the sternum is wide. Apical pore fields are not always present; if present they usually consist of a few pores. Rimoportulae is absent and mantle plaques are present along the mantle edge. Spathulate or spathulate/branched spines sometimes branched at the tips are situated along the valve edge usually in a somewhat plain region of the valve. The cingulum consists of several open, plain, ligulate copulae and the valvocopula is large and non-areolate. The plastids are probably parietal and plate-like. However, when considering more recent studies, the areolae could be square with rounded corners as for *P. alvareziae* Cejudo-Figueiras, E. Morales et Ector (Cejudo-Figueiras et al. 2011), volae are also observed (Morales 2005; Cejudo-Figueiras et al. 2011) as in *P. alvareziae* and *P. subsalina*. More than four areolae were observed on the valve face for *P. trainorii* (number of areolae between 1 and 6) (Morales 2001). Another interesting feature observed was the presence of quasifract bands (presenting a general appearance of being broken in small units), a character also observed in *Pseudostaurosira cataractarum* and *P. (Nanofrustulum) shiloi* (Round, Hallsteinsen, and Paasche 1999) and also found in members of the Cymatosiraceae family (Hasle, Stosch Von, and Syvertsen 1983).

To date, the genus *Pseudostaurosira* currently holds 35 species and 8 varieties (Fourtanier and Kocielek 2011; Kulikovskiy, Genkal, and Mikheyeva 2011; Wetzal et al. 2013; Morales et al. 2015; Guiry and Guiry 2017). Most species are from freshwater environments although some of them are brackish species, such as the recently described by Seddon and Witkowski *Pseudostaurosira diablarum* observed in a mangrove ecosystem in the Galápagos Islands (Seddon et al. 2014).

A new *Pseudostaurosira* was observed in a mineral spring found in the French Massif Central, more precisely in the Puy-de-Dôme department. Truchot (1878) described more than 200 springs linked to the geology and past volcanic activities. Due to the history of the massif (over 85,000 km<sup>2</sup>), a high variety of habitat conditions is present with high altitude springs (>1000 m a.s.l.), salt spring and also the hottest spring of Europe (80°C). The first studies conducted on springs from the Massif Central underlined the high algae biodiversity (Héribaud 1893; Chaouite 1987; Chaouite and Romagoux 1989; Tudesque 1996; Cordonnier 2008). The first survey performed in 2014–2015 in order to estimate the biodiversity in different mineral springs more or less impacted by human activities, revealed the presence of several new taxa, including some recently described such as *Navicula sanctamargaritae* Beauger in Beauger et al. (2015) and *Sellaphora labernardierei* Beauger, C.E. Wetzal et Ector in Beauger et al. (2016). In the present paper a new species of *Pseudostaurosira* is described using light (LM) and scanning electron microscopy (SEM). A morphological comparison is made with the most similar *Pseudostaurosira* taxa and its ecology is also addressed. Moreover, the type material of similar small elliptic araphid species: *P. trainorii* E. Morales (Avery Pond, Connecticut USA), *Pseudostaurosira connecticutensis* E. Morales (Avery Pond, Connecticut USA) and *Pseudostaurosira cataractarum* (Hustedt) C.E. Wetzal, E. Morales et Ector (Java, Indonesia) were obtained, investigated and illustrated by SEM in this article. *Pseudostaurosira cataractarum* observed in the French Massif Central was also investigated and illustrated by SEM.

## Materials and Methods

**Study site.** The Cézallier area belongs to an active natural hydrothermal system located in the northern section of the French Massif Central (Figure 1). The Cézallier area basement is primarily composed of Variscan metamorphic rocks, which are partially covered by bedrock or else a Quaternary alkaline basalt (Feuga 1987). In the Cézallier region, located south of Clermont-Ferrand, some fifty cold mineral springs have been identified over the last 15 years (Fouillac 1983). These springs are saline and carbo-gaseous hydrothermal waters. The waters are neutral (pH 6–7) and not in equilibrium with atmospheric CO<sub>2</sub> pressure (Cornu, Négrel, and Brach 2001). Their chemical composition is dominated by Cl, Na and HCO<sub>3</sub>, and they are commonly rich in calcium, iron, aluminium, phosphate and in trace elements (arsenic, selenium, etc.) (Fouillac 1983; Beaucaire, Criaud, and Michard 1987; Criaud and Fouillac 1989; Négrel et al. 2000). According to Criaud and Fouillac (1989) and Négrel et al. (2000), these waters are derived from the mixing of meteoric water with deep water during their convective cycle and final rise through the fractured Hercynian metamorphic basement. Arriving at the surface, CO degassing and changes in the oxidation state of these waters give rise to the formation of carbonates and iron oxides that trap a certain number of trace elements (Beaucaire, Criaud, and Michard 1987). The spring outlets are, thus, commonly associated with travertine extending over a surface of one to several meters in length (Cornu, Négrel, and Brach 2001). The present study focuses on an area comprising several spring outlets located nearby the hamlet of Bard (Boudes, Puy-de-Dôme department, Auvergne-Rhône-Alpes region). The springs (included the studied ones: Bard and Bard 2) lie along a fault and rise through heterogeneous anatexite (Berthier et al. 1982). Bard spring is characterized by the presence of a small roman basin in stone and is situated next to pastures (elevation of 489 m a.s.l.) while Bard 2 spring is located within an oak grove (elevation of 496 m a.s.l.). The new *Pseudostaurosira* species was observed only in Bard 2.

**Diatom sampling and chemical analyses.** An epipelagic sample coming from the Bard 2 spring was taken by picking up sediment (mainly silt) in the basin on 11 December 2015. The geographical position of the sampling site was taken using a DGPS Trimble Geo7x, in Lambert 93. Conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), pH (pH units), and water temperature ( $^{\circ}\text{C}$ ) were measured at the sampling site, using a WTW Multiline P4. The oxygen was measured using an oximeter Ysi ProODO A water sample was collected for further chemical analysis in the laboratory and was analysed using the high pressure ion chromatography technic. First, these samples were filtered using Whatmann GF/C filters. For the cation analysis, a Thermo Scientific Dionex ICS1100 system was used; and for the anions, a Thermo Scientific Dionex DX120 system. The concentrations ( $\text{mg L}^{-1}$ ) of lithium, sodium, ammonium, potassium, magnesium, calcium, fluoride, chloride, nitrite, nitrate, phosphate, sulphate and carbonate were measured in the laboratory.

**Type material.** We obtained the type material of the three more similar taxa to the new species belonging the genus *Pseudostaurosira*. For *P. trainorii* E.Morales (2001) and *Pseudostaurosira connecticutensis* E.Morales (2001), we worked on the material #4198 of the General Collection, Diatom Herbarium, Academy of Natural Sciences of Philadelphia collected from the Avery Pond, Connecticut (U.S.A). For *Pseudostaurosira cataractarum* (Hustedt) C.E.Wetzel, E.Morales et Ector in Wetzel et al. (2013), we used the type material AS1524 from the Hustedt Collection (Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany) collected from Tjurug Tjibeureum waterfalls, Java (Indonesia). The material corresponds to the holotype slide no. A2/27.

**Diatom analyses.** Small sub-samples of raw material were prepared for LM observation following the method described in Prygiel and Coste (2000). Samples were cleaned using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 35%) and hydrochloric acid (HCl), and rinsed several times. Cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides and mounted in Naphrax®. LM observations were done using a Leica® DM2700M microscope with 100x oil immersion objective using a differential interference contrast. Morphometric measurements were performed using a Leica® DMRX bright field microscope with 100x oil immersion objective, and light photographs were taken with a Leica® DC500 camera. For the scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3- $\mu\text{m}$  Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum using a Modular High Vacuum Coating System BAL-TEC MED 020 (BAL-TEC AG, Balzers, Liechtenstein). An ultrahigh-resolution analytical field emission (FE) scanning electron microscope Hitachi SU-70 (Hitachi High-Technologies Corporation, Japan) operated at 5 kV and 10 mm distance

was used for the analysis. SEM images were taken using the lower (SE-L) and upper (SE-U) detector signal and sometimes tilted up to an angle of 28 degrees. Photographs were digitally manipulated and plates containing light and scanning electron microscopy images were created using CorelDraw X6. Samples and slides are stored at the Herbiers Universitaires de Clermont-Ferrand, France (CLF) and Botanic Garden Meise, Belgium (BR).

Morphological terminology follows Ross et al. (1979) (striae, areolae and spines), Barber and Haworth (1981) (valve shape and striae orientation) and Round, Crawford, and Mann (1990) (areolar substructures, girdle band features and apical pore field characteristics). To compare the new species with the other small-celled fragilarioids retained we used the previous taxonomic literature (Ross et al. 1979; Barber and Haworth 1981; Round, Crawford, and Mann 1990) and Hasle, von Stosch, and Syvertsen (1983).

## Results

*Pseudostaurosira bardii* Beauger, C.E. Wetzel et Ector, *sp. nov.* (Figures 2–56)

**Description:** LM: Frustules rectangular in girdle view, forming chains with the aid of spines (Figures 43–46, 51–53). Valves isopolar, elliptical to round with broadly rounded ends; 4.0–6.5  $\mu\text{m}$  long, 3.0–5.5  $\mu\text{m}$  wide ( $n=50$ ). Sternum generally wide. Striae alternate, parallel or slightly radial towards the apices (Figures 2–42, 47–50); stria density 12–16 in 10  $\mu\text{m}$ . Plastids not observed.

SEM: Siliceous plaques present along valve mantle edge (Figures 51–54). Striae uniseriate, composed of mainly 1–2 areolae and sometimes 3 areolae on valve face and 1–2 areolae on valve mantle (Figures 47–50, 54–56). Striae distinct, composed of round to elliptical (transapically elongated) areolae decreasing in size from the valve face/mantle edge to both the central sternum and the valve mantle (Figures 47–50). Striae sometimes composed of only two areolae: one round and the second long and elliptical (Figure 47). Volae generally robust and arising from the inner periphery of each areola (Figure 56). Spines variable in shape, from spatulate (Figure 51) to lanceolate (Figure 53), always with serrate margins near the apices (Figures 51–53), interrupting the striae at the valve face/mantle junction (Figures 47–50). Costae slightly raised with respect to striae (Figures 48, 54). Apical pore fields absent or very weakly developed, composed of few round poroids (Figure 55 partially occluded). Valves present ‘discrete’ apical pore fields mainly detected internally (Figure 56). Rimoportula absent. Copulae and girdle bands are open, segmented (quasifract) and not perforated (Figure 54).

**Holotype:** Holotype population deposited in Herbiers Universitaires de Clermont-Ferrand, France (CLF 104885).

**Isotype:** Populations deposited in Herbiers Universitaires de Clermont-Ferrand, France (CLF 104886) and in Botanic Garden Meise, Belgium (BR-4469).

**Type locality:** Bard 2 spring at Boudes (Puy-de-Dôme department, Auvergne-Rhône-Alpes region, France), E713631.82 and N6483209.55.

**Etymology:** The term “*bardii*” refers to the Bard spring in the Massif Central.

**Associated diatom species:** Among the benthic species found in the holotype slide, the dominant taxa were *Crenotia thermalis* (Rabenhorst) Wojtal (50%), *Navicula sanctamargaritae* (12%) and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot (10%). The other associated taxa (<5%) were *Diploneis krammeri* Lange-Bertalot et E.Reichardt, *D. fontium* E.Reichardt et Lange-Bertalot, *Fallacia pygmaea* (Kützing) Stickle et D.G. Mann, *Navicula veneta* Kützing, *Nitzschia supralitorea* Lange-Bertalot, *Rhopalodia acuminata* Krammer, *Sellaphora atomoides* C.E.Wetzel et Van de Vijver, *Sellaphora nigri* (De Notaris in De Notaris & Baglietto) C.E.Wetzel et Ector.

**Physical and chemical character of the spring water:** The spring has a slightly acidic pH (6.53), an elevated conductivity level (6510  $\mu\text{S cm}^{-1}$ ) and is enriched with sodium-chloride and bicarbonate (Table 1). The concentrations of the different ions (potassium, calcium and magnesium) are also

elevated ( $>100 \text{ mg L}^{-1}$ ). The water temperature is about  $15^{\circ}\text{C}$  throughout the year. The concentration of lithium ions ( $4.8 \text{ mg L}^{-1}$ ) denote the deep water origin (Michard 1990).

### **Additional type materials investigated**

In order to compare the new species *Pseudostaurosira bardii*, three similar species were examined (Figures 87–94; Table 2) and their type materials were examined. The original descriptions (valve dimensions and number of striae) are summarized in Table 2. The species are already well illustrated in LM in previous publications (Morales 2001; Wetzel et al. 2013). However, LM pictures of *P. cataractarum* were presented because living populations were observed for the first time in the mineral springs from France and Europe.

*Pseudostaurosira trainorii* E.Morales (2001, p. 113, fig. 6a-l)

Figures 87–90 (SEM)

Holotype: Material #4198.

Type locality: Avery Pond, Connecticut, U.S.A.

Description: Valves elliptical to round. Number of striae only very rarely as low as 15 or as high as 30 per  $10 \mu\text{m}$ . Number of areolae on valve face varies between 1 and 5, whereas at the valve mantle this number ranges from 2 to 4 (Figure 87). Sternum generally wide, although in the morphs possessing a high number of areolae on the valve face, the sternum can be substantially reduced in width (Figures 88, 89). Apical pore fields generally absent, but sometimes reduced pore fields composed of 2 or 3 poroids are present on the valve mantle (Figure 87).

Ecology: Found in lentic ecosystems (Morales 2001; Morales et al. 2010) and large rivers as the Loire River (Ector et al. 2015).

*Pseudostaurosira connecticutensis* E.Morales (2001, p. 117, fig. 7a-l)

Figures 91, 92 (SEM)

Holotype: Material # 4198.

Type locality: Avery Pond, Connecticut, U.S.A.

Description: Valves round to narrowly elliptical in shape. Number of areolae per stria varies between 2 and 6 and number of areolae on either valve face or valve mantle between 1 and 3. Apical pore fields generally absent, but sometimes reduced pore fields composed of 2 or 3 poroids are present on the valve mantle (Figure 91). The girdle bands seem to be open and not segmented.

Ecology: Found in small, mesotrophic to eutrophic, shallow lakes.

*Pseudostaurosira cataractarum* (Hustedt) C.E. Wetzel, E. Morales et Ector in Wetzel et al. (2013, p. 56, figs 1A-AB, 2A-G)

Figures 93, 94 (SEM)

Type material: Material AS1524.

Type locality: Tjibeureum Wasserfall, Java TJ2.III.c.

Description: Valves are round to slightly elliptical. Striae radiate throughout the entire valve and extending onto the valve mantle. The striae are composed of round to oval areolae with the oval areola at the center; the number varies between 1 and 5 areolae per stria. The girdle bands are open, segmented (quasifract) and not perforated.

Ecology: Found in different countries and ecosystems such as hot springs (Wetzel et al. 2013). At the Massif Central, this taxa was observed in two different mineral springs: Puits spring at Courpière (E 706546.54 and N 6475917.38) and Zagat spring at Ardes-sur-Couze (E 744209.91 and N 6516138.53 (Figures 57-86). The dimensions were those reported by Grana et al. (2015). In the Massif Central, the springs were temperate and a difference of mineralization was observed between the two sites (Table 2).

## Discussion

*Pseudostaurosira bardii* belongs to the genus defined by Williams and Round (1987). The taxa currently ascribed to this genus share many features that were observed on the type *P. brevistriata*, such as the type of striae and areolae (short striae and wide areolae bearing well-developed volae), the position of the spines (interrupting the striae) and the characteristics of the apical pore fields (Williams and Round 1987; Morales et al. 2015). In LM, the areolae could be resolvable (Fig. 12) but sometimes the observation is difficult and, thus, the new taxon could not be confused with other taxa such as *Staurosira venter* (Ehrenberg) Cleve et J.D.Möller), *Staurosirella ovata* E.Morales et Manoylov or *Punctastriata ovalis* D.M. Williams et Round (Williams and Round 1987; Morales and Manoylov 2006).

An extensive literature search was conducted and revealed some taxa that appear morphologically similar under the LM to *Pseudostaurosira bardii* and could therefore be confused with this new species. Three species were retained based on their LM morphology (Table 3). We eliminated after comparison and literature analysis, *P. shiloi* (J.J. Lee, Reimer et McEnery) Hallegraef et Burford as it is smaller (2.8–7.5  $\mu\text{m}$  long) and has a lower number of striae (10–12 per 10  $\mu\text{m}$ ) than *P. bardii*, the narrow *P. sajamaensis* E. Morales et Ector in Morales et al. (2012) (2–4  $\mu\text{m}$  wide) has only 1 or 2 areolae round or trapezoid per striae. We also eliminated *P. sopotensis* (Witkowski et Lange-Bertalot) E.Morales, C.E.Wetzel et Ector in Wetzel et al. (2013) even if this taxon inhabits brackish waters, because the valve face striae are composed of very fine poroids (ca. 40 in 10  $\mu\text{m}$ ) prolonged by several (up to 4) larger areolae on the mantle avoiding confusion with the new taxon *P. bardii*. At last, *Staurosira altiplanensis* Lange-Bertalot and Rumrich in Rumrich et al. (2000), is more elliptical and narrower (2.8–3.6  $\mu\text{m}$  wide) than the new species as was not retained.

Actually, the areolae were never observed as transapically elongated with no more than two areolae. Based on LM and SEM morphology, the new species presents particular features that have not been described previously in the genus such as the special pattern of the areolae: the striae are composed of mainly 1–2 areolae and sometimes 3 areolae on valve face and 1–2 areolae on valve mantle, that are round to elliptical (transapically elongated); the areolae decrease in size from the valve face/mantle edge to both the central sternum and the valve mantle. Sometimes the striae are composed of only two areolae (one round and the second long and elliptical) and the presence of the slit like areolae could be related to the cell life cycle. Even if the last characteristic is common to taxa such as *Pseudostaurosira alvareziae*, *P. americana* and *P. subsalina* (Cejudo-Figueiras et al. 2011), the association of all the characteristics: the size, the areola pattern, the density of striae and the presence of volae allow to separate *P. bardii* from the other described species of the genus.

Confusion in LM could occur with *P. trainorii*, but this species has a higher number of areolae per stria (up to six) than *P. bardii* (Morales 2001). In SEM, even if similarities occur between both species such as the presence of serrate spines, differences allow to separate them. The first difference is the pattern of striae: they consisted of rows of circular to elliptical areolae, which are occluded with complex vela and extended from the valve face onto the mantle (Morales 2001; Morales et al. 2010; Sato et al. 2011). Moreover, *P. trainorii* is mainly observed in lentic habitat with low conductivity (ca. 125  $\mu\text{S cm}^{-1}$ ). For *Pseudostaurosira connecticutensis*, only the smaller representatives have similarities with the new species; *P. connecticutensis*, a freshwater species, has a higher stria density (17–20 per 10  $\mu\text{m}$ ) and, observed using SEM, the closing plate consists of a disk-like structure that covers most of the areolar opening, a characteristic typical of the genus *Pseudostaurosira* (Morales 2001) (Figure 91). In *Pseudostaurosira* species, even if this kind of

flaps could be observed, underneath them there are developed volae as in *P. bardii*. When considering *P. cataractarum*, confusion could occur with *P. bardii* based on the areola pattern and the presence in springs (Wetzel et al. 2013), even if the striae appear to be thinner and the valve shape more rounded in LM (Figures 59-77) for *P. cataractarum*. Indeed, the oval and long areola near the sternum is similar in both species when this kind of areola is present for the new species. However, *P. cataractarum* is larger (5.8–8.2  $\mu\text{m}$ ) than the new species, the apical pore fields are absent and vola are not present. Moreover, for this species the number of areolae on the valve mantle is high (2 to 5) (Figures 78, 79, 84, 86, 94) instead of one large areola and sometimes a second smaller for *P. bardii*. When we compared the two species that were observed in Massif Central (*P. bardii* from Bard spring and *P. cataractarum* from Puits and Zagat springs), the differences in LM and SEM are clear.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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Aude Beauger is an ecologist and PhD at the Centre national de la recherche scientifique. She has written over 20 articles and he has been working on diatoms in rivers, lakes and springs. She has been teaching on bioassessment using diatoms. She is the treasurer of the "Association des Diatomistes de Langue Française (ADLaF)" and have organized the annual meeting on diatom taxonomy, ecology and related subjects in 2014. *Contribution*: create the research program "Biodiversity study of the diatoms of mineral springs of the French Massif Central" and write the article.

Carlos Wetzel is a botanist and a researcher at the Luxembourg Institute of Science and Technology. He has written over 90 articles and he has been working on diatoms in rivers, lakes and soils. Carlos Wetzel has been teaching numerous training courses on diatom ecology and taxonomy designed for biologists, technicians and ecologists, contributing to the continuous improvement in the Water Framework Directive implementation in Europe. *Contribution*: plates, discussion of results and development of manuscript.

Olivier Voltaire is engineer assistant at the CNRS. He has participated in more than 20 articles. He is specialized in scientific instrumentation and particularly in physical and chemical analyses. He participate in the research program "Biodiversity study of the diatoms of mineral springs of the French Massif Central". *Contribution*: write of the Tables 1 and 2 and discussion of the physical and chemical results.

Luc Ector is a botanist and senior researcher at the Luxembourg Institute of Science and Technology. He has written over 185 articles and has been working on diatoms in rivers, lakes and soils for the last 30 years. He was the President of the "Association des Diatomistes de Langue Française (ADLaF)", which organizes annual meetings on diatom taxonomy, ecology and related subjects. Over the last 20 years, he has been organizing and teaching numerous training courses on diatom ecology and taxonomy designed for biologists, technicians and ecologists, contributing to the continuous



improvement in the Water Framework Directive implementation in Europe. *Contribution*: discussion of results and development of manuscript.

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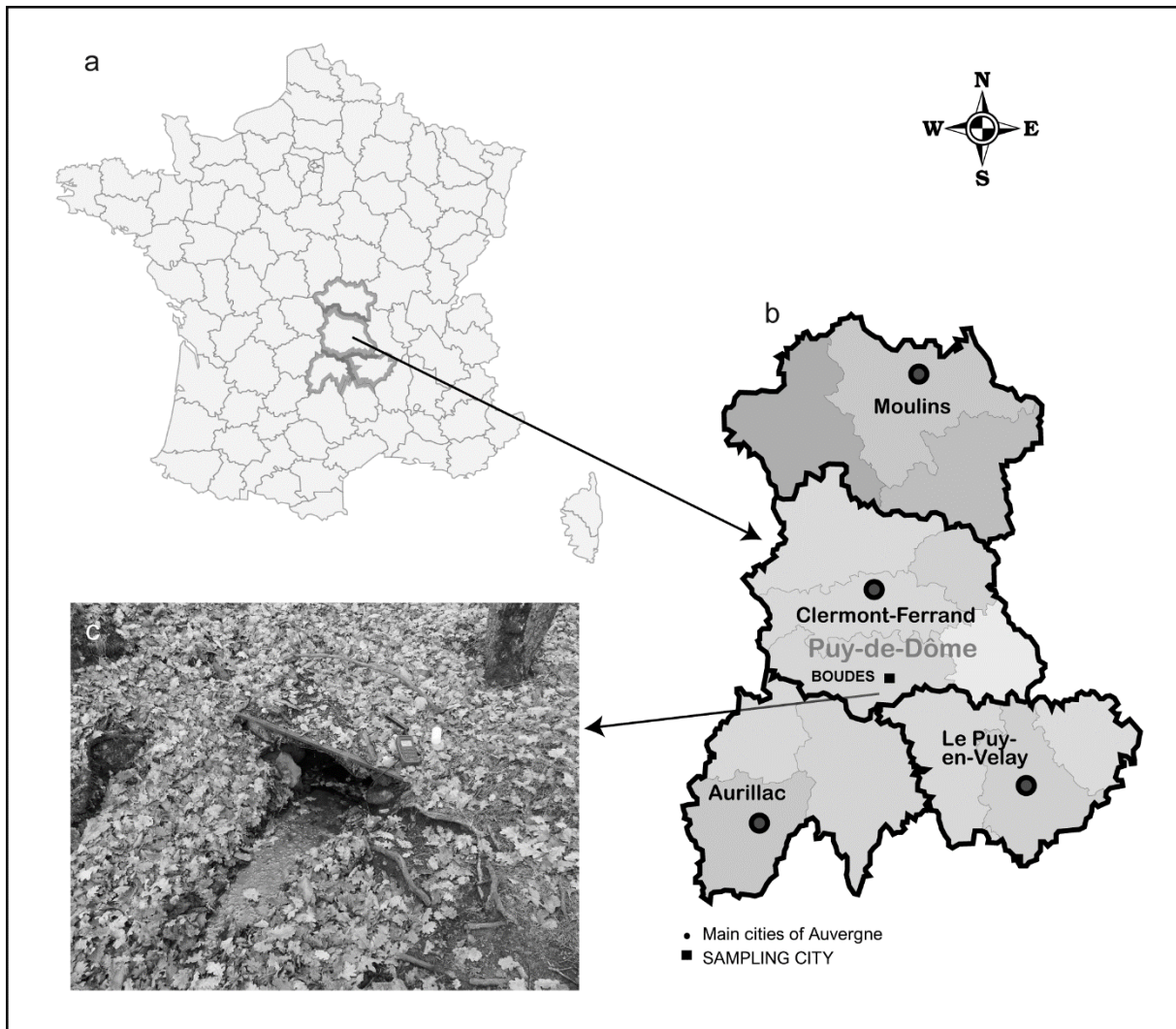
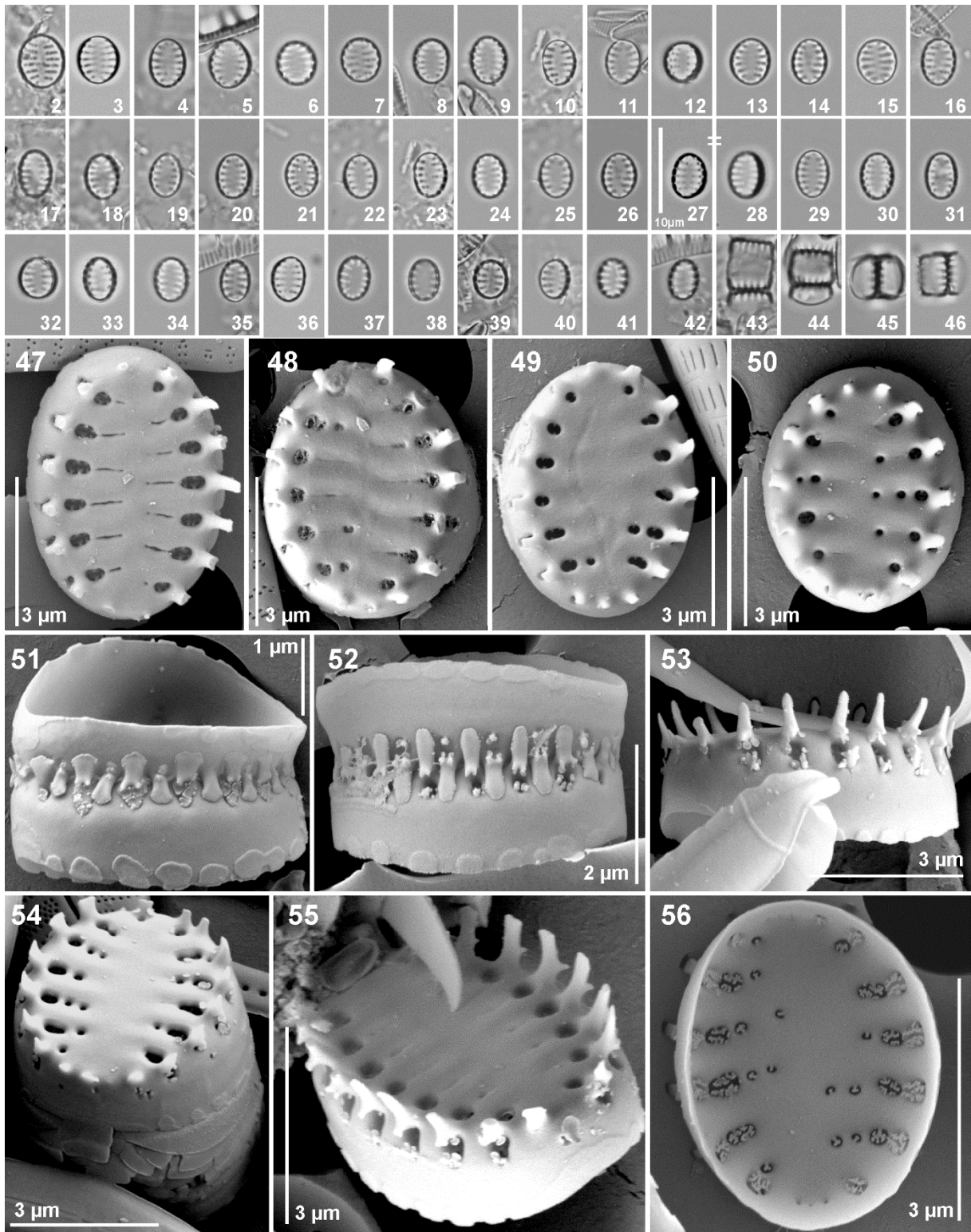
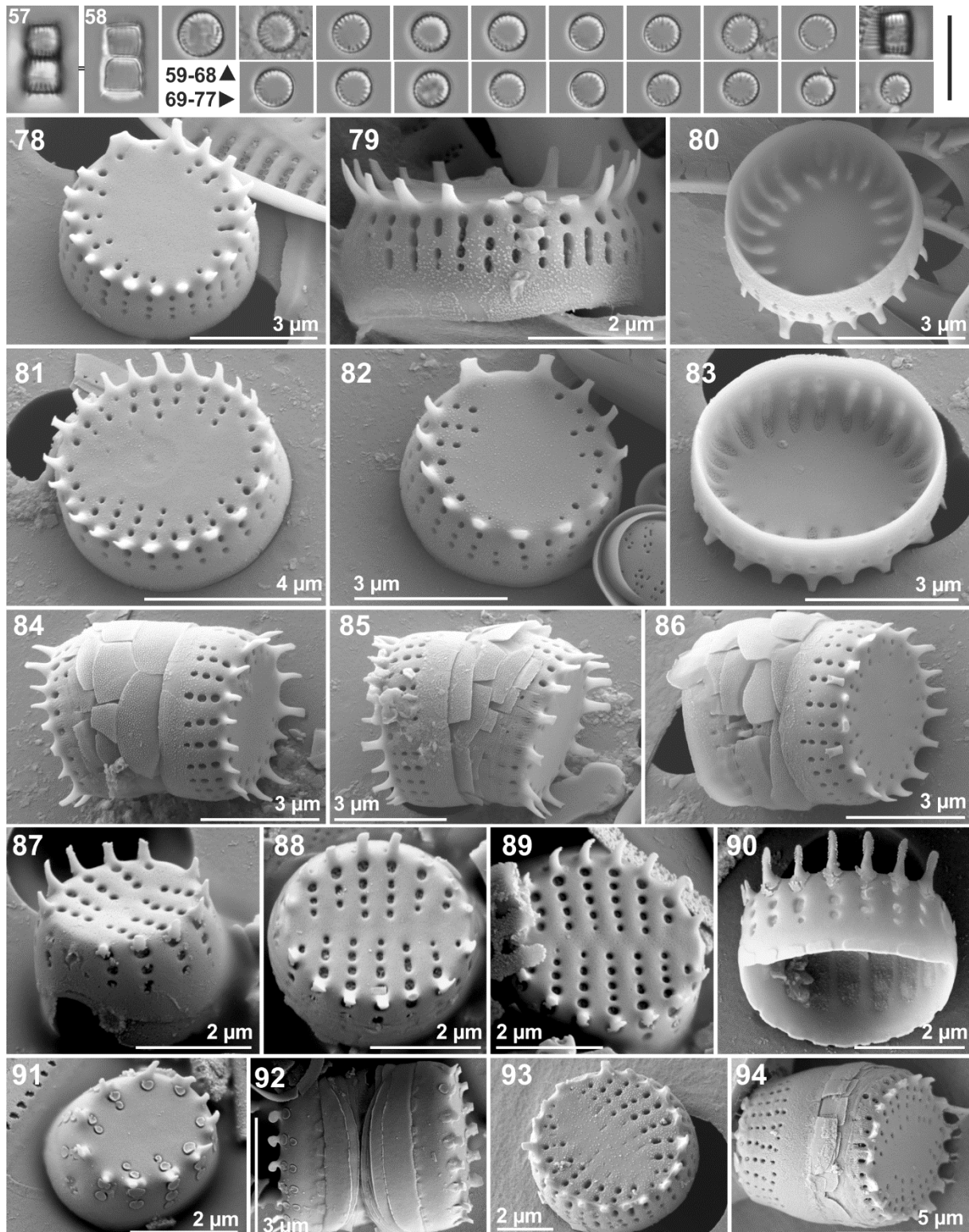


Figure 1. Map of the studied springs. a: map of Europe; b and c: general location map of the French Massif Central and of the study area where Bard springs are located (square); d: photography of the Bard 2 spring.



Figures 2–56. *Pseudostaurosira bardii* Beauger, C.E. Wetzel et Ector *sp. nov.* LM (Figures 2–46). Type population from Bard 2 spring. Figures 43–46 show a short chain with frustules in girdle view. SEM (Figures 47–56). Type population from Bard 2 spring. (47–50) Overall characteristics of the outer surface of the valve face. Notice the areola pattern that differs from one photo to the others (no more than three areolae per stria) and the presence of volae. (51–53) Girdle view of a frustule and neighbouring valve still attached (51, 52). Notice the siliceous plaques present along valve mantle edge and interlocking spines with developing ligulae. (54) Tilted valve showing the opened girdle bands. (55) Tilted valve showing characteristics of the external valve surface and the valve mantle. Notice the position of spines and pore fields weakly developed. (56) Inner view of valve. Inner opening

of the areolae present also finely branched closing plates. Scale bars 10  $\mu\text{m}$  (Figures 2–46); 3  $\mu\text{m}$  (Figures 47–50, 53–56); 2  $\mu\text{m}$  (Figure 52); 1  $\mu\text{m}$  (Figure 51).



Figures 57–94. Similar small araphid species in LM and SEM. Figures 57–86. Population of *Pseudostaurosira cataractarum* observed in two springs of the French Massif Central (Puits and Zagat springs). Figures 87–94. SEM type materials of similar small elliptic 'araphid' species. Figures 87–90. *P. trainorii* E. Morales (Avery Pond, Connecticut USA); Figures 91, 92. *Pseudostaurosira connecticutensis* E. Morales (Avery Pond, Connecticut USA); Figures 93, 94: *Pseudostaurosira*

*cataractarum* (Hustedt) C.E. Wetzel, E. Morales et Ector (Java, Indonesia). Scale bars 10  $\mu\text{m}$  (Figures 57–77); 5  $\mu\text{m}$  (Figure 94); 4  $\mu\text{m}$  (Figure 81); 3  $\mu\text{m}$  (Figures 78, 80, 82–86, 92); 2  $\mu\text{m}$  (Figures 79, 87–91, 93).

Table 1: Physical and chemical variables measured in the Bard 2 spring at Boudes on December 11, 2015.

Conductivity ( $\mu\text{S cm}^{-1}$ )	6350
pH (pH units)	6.57
Temperature ( $^{\circ}\text{C}$ )	15.1
Oxygen (%)	0.34
$\text{Li}^+$ ( $\text{mg L}^{-1}$ )	4.80
$\text{Na}^+$ ( $\text{mg L}^{-1}$ )	911.15
$\text{NH}_4^+$ ( $\text{mg L}^{-1}$ )	1.35
$\text{K}^+$ ( $\text{mg L}^{-1}$ )	200.72
$\text{Mg}^{2+}$ ( $\text{mg L}^{-1}$ )	135.46
$\text{Ca}^{2+}$ ( $\text{mg L}^{-1}$ )	229.36
$\text{F}^-$ ( $\text{mg L}^{-1}$ )	1.24
$\text{Cl}^-$ ( $\text{mg L}^{-1}$ )	782.80
$\text{NO}_3^-$ ( $\text{mg L}^{-1}$ )	0.13
$\text{NO}_2^-$ ( $\text{mg L}^{-1}$ )	<0.05
$\text{PO}_4^{3-}$ ( $\text{mg L}^{-1}$ )	0.08
$\text{HCO}_3^-$ ( $\text{mg L}^{-1}$ )	2475.00
$\text{SO}_4^{2-}$ ( $\text{mg L}^{-1}$ )	37.77

Table 2: Physical and chemical variables measured in the Puits and Zagat springs on December 28, 2016 and January 30, 2017 respectively.

	Puits spring	Zagat spring
Conductivity ( $\mu\text{S cm}^{-1}$ )	1464	4960
pH (pH units)	7.06	6.71
Temperature ( $^{\circ}\text{C}$ )	8.1	8.9
Oxygen (%)	33.6	10.3
$\text{Li}^+$ ( $\text{mg L}^{-1}$ )	5.99	1.51
$\text{Na}^+$ ( $\text{mg L}^{-1}$ )	920.82	227.33
$\text{NH}_4^+$ ( $\text{mg L}^{-1}$ )	0.75	0.54
$\text{K}^+$ ( $\text{mg L}^{-1}$ )	112.61	41.79
$\text{Mg}^{2+}$ ( $\text{mg L}^{-1}$ )	82.23	48.45
$\text{Ca}^{2+}$ ( $\text{mg L}^{-1}$ )	79.33	89.91
$\text{F}^-$ ( $\text{mg L}^{-1}$ )	0.90	0.41
$\text{Cl}^-$ ( $\text{mg L}^{-1}$ )	419.38	39.51
$\text{NO}_3^-$ ( $\text{mg L}^{-1}$ )	0.62	0.45
$\text{NO}_2^-$ ( $\text{mg L}^{-1}$ )	0.01	0.01
$\text{PO}_4^{3-}$ ( $\text{mg L}^{-1}$ )	0.08	0.08
$\text{HCO}_3^-$ ( $\text{mg L}^{-1}$ )	920	2570
$\text{SO}_4^{2-}$ ( $\text{mg L}^{-1}$ )	59.93	39.70



Table 3. Comparison of morphological characteristics of taxa similar to *Pseudostaurosira bardii* sp. nov. based on the reexamination of the material types.

Species	Valve length and width ( $\mu\text{m}$ )	Striae density (in 10 $\mu\text{m}$ ) and morphology	Valve shape; sternum	Girdle bands	Closing plates
<i>Pseudostaurosira bardii</i> sp. nov.	4.0–6.5; 3.0–5.5	12–16; uniseriate, composed of 1–2 (3) areolae, round to elliptical	elliptical to rounded; generally wide	open, segmented (quasifract), not perforated	volae robust, arising from the inner periphery of each areola
<i>Pseudostaurosira cataractarum</i> (Hustedt) C.E. Wetzel, E. Morales et Ector	5.8–8.2; 5.4–7.2	15–18; uniseriate, composed of round to oval areolae decreasing in size from the valve face/mantle edge to both the central sternum and the valve mantle	round to slightly elliptical; broad	open, segmented (quasifract) and not perforated	not observed
<i>Pseudostaurosira trainorii</i> E. Morales	1.9–8.6; 1.7–4.4	20–25; uniseriate, composed of mainly 1 to 6 round or slightly rectangular areolae	elliptical to rounded; generally wide	open	volae finely branched closing plates
<i>Pseudostaurosira connecticutensis</i> E. Morales	1.9–13.5; 1.5–4.7	(12)17–20(28); uniseriate, composed of mainly 1 to 3 round or slightly elliptical areolae	round to narrowly elliptical; wide, but narrower as the number of areolae on the valve face increases	open, not segmented	circular, flattened, plate-like structures almost entirely occluding the areolar opening