Gomphosphenia vallei (Bacillariophyta), a new diatom species from a stream in the “Réserve Naturelle Nationale de la Vallée de Chaudefour”, Massif Central (France)

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Abstract

A new *Gomphosphenia* species (Bacillariophyceae) was found during a diatom survey in the "Réserve Naturelle Nationale de la Vallée de Chaudefour" (RNN VC) , in the Massif Central (France),. *Gomphosphenia vallei* sp. nov. is described from stone surfaces in Pérouse Stream, Auvergne, France. A detailed description of morphology of *G. vallei* is presented, based on light and scanning electron microscopy. This new small-sized species is characterized by linear-clavate to elliptical valves. Striae are uniseriate and composed of single slit-like areolae, an axial area linear and narrow and a low number of striae. *Gomphosphenia vallei* seems to inhabit headwater stream.

Keywords: crystalline area, Europe, *Gomphosphenia*, headwaters, new species, ultrastructure

Introduction

The genus *Gomphosphenia* Lange-Bertalot (1995: 243) was separated from *Gomphonema* Ehrenberg (1832: 87) by several ultrastructural features including transapically elongated areolae and T-shaped internal central raphe fissures, while lacking an apical pore field, isolated stigma, and the deflected terminal raphe fissures found in *Gomphonema*. The type species of the genus is *Gomphosphenia lingulatiformis* (Lange-Bertalot & E.Reichardt in Lange-Bertalot 1993: 60) Lange-Bertalot (1995: 242).

Gomphosphenia species have been observed in diverse types of habitats such as rivers (Van de Vijver *et al*. 2012, Saber *et al.* 2021), lakes (Kociolek *et al.* 2014, Genkal & Yarushina 2016, Gligora Udovič *et al*. 2018, Ohtsuka e*t al.* 2018), carbonate springs in karst landscapes (Werum & Lange-Bertalot 2004, Straub 2013, Dedić *et al*. 2015, Peeters *et al*. 2019), and artificial habitats like drainage channels of dams (Noga *et al.* 2016). For many species of *Gomphosphenia*, individuals were observed from cold oligotrophic and alkaline waters such as *G. stoermeri* Kociolek & E.W.Thomas in Thomas *et al.* (2009: 230). *Gomphosphenia fontinalis* Lange-Bertalot, Ector & Werum in Werum & Lange-Bertalot (2004: 163) is apparently restricted to calcium-bicarbonate-rich springs, eutrophic and even α-mesosaprobic, with moderate electric conductivity (Werum & Lange-Bertalot 2004, Lange-Bertalot *et al.* 2017). *Gomphosphenia lingulatiformis* might occur quite common in alkaline, eutrophic to eu-polytrophic rivers, canals, and lakes (Krammer & Lange-Bertalot 1986, Lange-Bertalot *et al.* 2017).

Nowadays new species are still observed and described such as *G. plenkoviciae* Gligora Udovič & Zutinić in Gligora Udovič *et al*. (2018: 231) in a karstic sinkhole lake and *G. patrickiana* Cantonati, Lange-Bertalot, Kociolek & A.A.Saber in Saber *et al.* (2021: 222) in rivers from Puerto Rico. According to AlgaeBase (Guiry & Guiry 2021) and DiatomBase (Kociolek *et al.* 2021), the genus *Gomphosphenia* holds 18 species and 3 varieties (Annex).

In the Massif Central different surveys on diatoms are regularly conducted, and new species are observed and described (Beauger *et al*. 2015, 2016, 2017, 2019). In summer 2021, during a study on the diatom biodiversity of the "Réserve Naturelle Nationale de la Vallée de Chaudefour" (RNN VC), different samples were taken in headwater and mineral springs. A small-sized *Gomphosphenia* was observed in the Pérouse Stream. Following detailed light microscopy (LM) and scanning electron microscopy (SEM) observations and comparisons with known representatives of this genus, this *Gomphosphenia* is described as new. A morphological comparison is made with the most similar *Gomphosphenia* taxa. Moreover, ecological information about the new species is also provided.

Material & Methods

Study site:—The Pérouse Stream is located in the French Massif Central region, in the department of Puy-de-Dôme (63) and more precisely in the Chaudefour Valley (Fig. 1).

Nowadays this valley is protected with the establishment of the RNN VC that covers 820.50 hectares. Located in the heart of the Monts Dore, on the Sancy massif, which highest point is at an elevation of 1,886 m high (Réserves naturelles de France 2021; Rossi 2021).
This valley appeared around 600,000 years ago and was created by the partial destruction of the Sancy's volcanic massif (Lavina 1995). The lateral collapse of the volcano was caused by a violent eruption. This explosion created a glacial cirque where about thirty volcanoes appeared that constitute the current structure of the ridges (Lavina 1995; Audubert *et al.* 2013; Réserves naturelles de France 2021). The typical shape of the valley is caused to glacial erosion. Lava needles and dykes (such as the "dent de la Rancune") have appeared as a result of fluvio-torrential erosion.
A multitude of headwater streams, among them Pérouse Stream, coming from the glacial valley confluence to form the river called "Couze de Chaudefour". The Pérouse Stream has its source in the upper part of the valley, in subalpine bogs of the reserve. It flows into the valley at the "Cascade de Pérouse" that is present at the entrance of the cirque. The sampling point was taken at the coordinates 2°51'11"E, 45°32'12"N and at 1,184 meters a.s.l.

Diatom sampling and physical and chemical analysis:— Five stones were removed from a riffle to make a composite epilithic sample. While in the field, the stones were brushed using a toothbrooth on 20th August 2021. The sample was fixed with an ethanol solution to a final concentration of 70%. *In-situ*, pH (pH unit), conductivity ($\mu S.cm^{-1}$) and water temperature (°C) were measured at the sampling site using a WTW Multiline P4. For Dissolved oxygen (% and mg L^{-1}), a ProODO oxygen probe was used. A water sample was collected for further chemical analysis in the laboratory This sample was first filtered using Whatmann GF/C filters. Then, it was analyzed with the high-pressure ion chromatography technic to obtain ionic concentrations of lithium, sodium, ammonium, potassium, magnesium, calcium, bromine, fluoride, chloride, nitrite, nitrate, phosphate and sulphate (in mg L^{-1}). For the cations analysis a Thermo Scientific Dionex ICS1100 system was used, while for the anions a Thermo Scientific Dionex Aquion system was used. .

Slide preparation and microscopy: — Two milliliters of preserved material were prepared for LM observation following the method described in Prygiel & Coste (2000): cleaning using H_2O_2 and dilute hydrochloric acid. After several rinses in distilled water, a portion of the cleaned diatom material was air-dried onto a cover slips and then mounted in Naphrax®. LM observations were done using a Leica® DM2700M microscope with 100x oil immersion objective using a differential interference contrast.

The species present were identified according to different taxonomical works (Krammer 2000, 2002, 2003, Lange-Bertalot 1993, Lange-Bertalot & Metzeltin 1996, Wetzel *et al*. 2015, Levkov *et al*. 2016, Lange-Bertalot *et al*. 2017) and the count data were converted into percentage relative abundance in respect to the total count (424 valves) for each sample.

Morphometric measurements were performed using a Leica® DMRX brightfield microscope with 100x oil immersion objective, and light micrographs were taken with a Leica® DMC 2900 camera.

For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 0.2 µm Isopore polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminum stubs and coated with carbon. An ultrahigh-resolution analytical field emission (FE) scanning electron microscope, Hitachi Regulus 8230 (Hitachi High-Technologies Corporation, Japan), was operated at 2 kV and 10 mm distance for image analysis.

Images were digitally manipulated and plates containing light and scanning electron microscopy micrographs were created using Adobe InDesign 16.4. Samples and slides are stored at the Herbiers Universitaires de Clermont-Ferrand (France) and Botanic Garden Meise (Belgium). Diatom terminology follows Ross *et al.* (1979), Barber & Haworth (1981) (valve shape, stria/areola structure) and Round *et al.* (1990) (raphe characteristics).

Results

Detailed morphological description of *Gomphosphenia vallei* Beauger, C.E.Wetzel, Allain & Ector sp. nov. (Figs 2–45, Tab. 2) are presented in this paper from the sample taken in Pérouse Stream.

Class: **Bacillariophyceae** Haeckel emend. Medlin & Kaczmarska 2004: 267
Subclass: **Bacillariophycidae** D.G.Mann in Round *et al.* 1990: 128
Order: **Cymbellales** D.G.Mann in Round *et al*. 1990: 128
Family: **Rhoicospheniaceae** J.Y.Chen & H.Z.Zhu 1983: 453
Genus: ***Gomphosphenia*** Lange-Bertalot 1995: 243

Gomphosphenia vallei **Beauger, C.E.Wetzel, Allain & Ector *sp. nov*. (Figs 2–45)**

Light microscopy observations (Figs 2–25): Valves linear-clavate in larger specimens to clavate in smaller ones and elliptical in the smallest individuals. Headpoles rounded, footpoles narrowly rounded. Valve dimensions (n = 30): length 4.2–13.1 μm, width 1.5–2.5 μm. Raphe straight, filiform. Striae visible in LM, almost parallel throughout the valve to slightly radiate in the mid-valve, 23–29 in 10 μm. Axial area linear and narrow. Central area from absent to asymmetrical with round and distinct central nodule. In girdle view, frustules almost rectangular to slightly wedge-shaped.

Scanning electron microscopy observations (Figs 26–33): Valve face flat. Externally, striae composed of single slit-like areolae, becoming very short slit near the poles (Figs 26–30). Striae slightly radiate in central part of valve, becoming convergent towards both head and footpoles. Raphe straight, filiform, with slightly dilated distal and proximal raphe endings (Figs 26–30). Distal raphe fissures terminating on the valve face, not extending onto the valve mantle (Fig. 28). Internally, proximal raphe endings bent in the same direction (Fig. 31). Internal terminal raphe fissures ending in helictoglossae (Fig. 31). Single row of slit-like areolae located on the valve mantle (Figs 26, 28, 30, 32), sometimes rounded in shape near the mid-valve and towards the footpole (Figs 27, 33). Central area with a unilateral fascia, with a short areola on one side and absence of areola on the other side (Figs 26–29). Central nodule slightly raised (Fig. 31). Apical pore fields absent (Fig. 28). In girdle view, frustules with cingulum composed of open bands, each bearing one row of small round pores (Figs 28, 30, 32–33).

Type locality:**—**FRANCE. Chambon-sur-Lac, “Réserve Naturelle Nationale de la Vallée de Chaudefour”: Pérouse Stream, sample PER_200821, 1,184 m a.s.l., E688534.487, N6493049.143 (Lambert 93), collection date *20 August 2021* (assigned here, holotype: CLF!, slide no. CLF310234; isotype: BR! slide no. BR-4707. The holotype is represented by Fig. 12).

Etymology:**—**This species is named for Eric Vallé, curator of the National Nature Reserve and passionate about botany.

Ecology and associated diatom taxa:**—** The Pérouse Stream, situated in the glacial cirque of Chaudefour Valley, is characterized by a low conductivity (35 μS cm^{-1}), a low temperature (11.9 °C) and is well oxygenated (92.9% of dissolved oxygen). The pH is 7.09. As underlined the conductivity, the concentrations of the measured ions are low with a maximum for the sodium concentration (2.53 mg L^{-1}).

The type population of *Gomphosphenia vallei* was found in Pérouse Stream associated with other taxa. In August 2021, the two co-dominant taxa were *Planothidium lanceolatum* (Brébisson ex Kützing 1846: 247) Lange-Bertalot (1999: 287) (36%) and *Rhoicosphenia abbreviata* (C.Agardh 1831: 34) Lange-Bertalot (1980: 586) (30%). The other species well represented (<10%) were *Achnanthidium subatomus* (Hustedt 1939: 554) Lange-Bertalot (1999: 273) (7.1%), *Gomphonema* sp. cf. *minutum* C.Agardh (1831: 34) (6.4%), *Navicula reichardtiana* Lange-Bertalot in Lange-Bertalot & Krammer (1989: 163) (2.3%), *Odontidium mesodon* (Ehrenberg 1839: 57) Kützing (1849: 12) (2.3%), *Gomphosphenia vallei* (2.3%), *Reimeria sinuata* (W.Gregory 1856: 4) Kociolek & Stoermer (1987: 457) (1.9%), *Planothidium curtistriatum* C.E.Wetzel, Van de Vijver & Ector in Wetzel *et al*. (2019: 61) (1.9%), *Cocconeis rouxii* Héribaud & Brun in Héribaud (1893: 45) (1.9%) and *Encyonema minutum* (Hilse in Rabenhorst 1862: No. 1261) D.G.Mann in Round *et al.* (1990: 667) (1.1%). At last, *Chamaepinnularia obsoleta* (Hustedt 1942a: 69) C.E.Wetzel & Ector in Wetzel *et al.* (2013: 158), *Cyclotella antiqua* W.Smith (1853: 28), *Geissleria acceptata* (Hustedt 1950: 398) Lange-Bertalot & Metzeltin (1996: 64), *Gomphonema clavatum* Ehrenberg (1832: 88), *Gomphonema exilissimum* (Grunow in Van Heurck 1880: pl. 25, fig. 12) Lange-Bertalot & E.Reichardt in Lange-Bertalot & Metzeltin (1996: 70), *Humidophila perpusilla* (Grunow 1860: 552) R.L.Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová (2014: 358), *Mayamaea permitis* (Hustedt 1945: 919) Bruder & Medlin (2008: 327), *Meridion constrictum* Ralfs (1843: 458), *Nitzschia hantzschiana* Rabenhorst (1860: 40), *Nitzschia soratensis* E.Morales & M.L.Vis (2007: 128), *Nupela lapidosa* (Krasske 1929: 350) Lange-Bertalot (1999: 274), *Pantocsekiella ocellata* (Pantocsek 1901: 134) K.T.Kiss & Ács in Ács *et al.* (2016: 62), *Pinnularia sinistra* Krammer (1992: 105), *Psammothidium helveticum* (Hustedt 1933: 385) Bukhtiyarova & Round (1996: 8), *Psammothidium subatomoides* (Hustedt in Schmidt *et al*. 1936: pl. 404) Bukhtiyarova & Round (1996: 13) and *Sellaphora atomoides* (Grunow in Van Heurck 1880: 107) C.E.Wetzel & Van de Vijver in Wetzel *et al.* (2015: 219) represented less than 1% of the whole community.

Discussion

The "Réserve Naturelle Nationale de la Vallée de Chaudefour" is a glacial valley which shelters a great diversity of protected species of flora and fauna, typical of the Alps and of great scenic and scientific interest. To complete the knowledge on the reserve, a diatom survey done in summer 2021 has highlighted the presence of a new species of *Gomphosphenia.*

Among the 18 *Gomphosphenia* species, *G. vallei* present similarities with only four *Gomphosphenia* species (Table 1): *G. americana* Kociolek & E.W.Thomas in Kociolek *et al.* (2014: 45), *G. fontinalis* Lange-Bertalot, Ector & Werum in Werum & Lange-Bertalot (2004: 163), *G. indistincta* Kociolek & E.W.Thomas in Kociolek *et al*. (2014: 46) and *G. stoermeri* Kociolek & E.W.Thomas in Thomas *et al.* (2009: 230).

Indeed, the shape of *G. vallei* (linear-clavate to elliptical for the smallest individuals) allowed to separate it from species presenting: 1) clavate outline with broadly rounded headpole such as *G. ryukyuensis* Tuji & Ohtsuka in Tuji (2016: 2), 2) linear-clavate with headpole obtusely cuneate, gradually tapered from the central portion to the footpole such as *G. biwaensis* Ohtsuka & D.Nakai in Ohtsuka *et al.* (2018: 108) and *G. grovei* (M.Schmidt 1899: 214) Lange-Bertalot (1995: 243), 3) broadly lanceolate-clavate with protracted rostrate head and footpoles such as *G. praegnans* Ress, E.W.Thomas & Kociolek (2016: 56, 57), 4) linear lanceolate outline such as *G. oahuensis* (Hustedt 1942a: 98) Lange-Bertalot in Moser *et al.* (1998: 42).

The striae was another characteristic to separate the new species from other ones. Indeed, *G. grovei*, *G. pfannkucheae* (Cholnoky 1966: 25, 26) Lange-Bertalot (1995: 244), *G. reicheltii* (M.Schmidt 1899: 214) Lange-Bertalot (1995: 244), *G. ryukyuensis, G. tackei* (Hustedt 1942b: 205) Lange-Bertalot (1995: 244) and *G. tenerrima* (Hustedt 1938: 444) E.Reichardt (1999: 57) are characterized by striae composed of a few areolae, unlike to the new species that are composed of a single long radiate slit-like areolae. Moreover, the internal proximal raphe endings of the new species are not T-shaped such as the generitype *G. lingulatiformis* and also *G. biwaensis*, *G. holmquistii* (Foged 1968: 8) Lange-Bertalot (1995: 244), *G. plenkoviciae*, *G. patrickiana*, *G. ryukyuensis*, *G. tackei* and *G. tenuis* Levkov & D.M.Williams (2011: 20) but they are deflected in the same direction.

Thus, only the four species, previously named, can be compared to the new species. *Gomphosphenia americana* has valves lanceolate-clavate (Kociolek *et al*. 2014). The number of striae is higher than the new species with 29 to 32 striae in 10 µm. Moreover, in girdle view, valves are cuneate and flexed about the transapical axis differing in this pattern from the new species. When considering *G. fontinalis,* the outline is clavate while *G. vallei* is linear-clavate (and clavate to elliptical for smaller individuals). The valve dimensions are comparable to the new species (Werum & Lange-Bertalot 2004). However, less striae are observed in the new species (23–29 in 10 µm). Indeed *G. fontinalis* has 25 to 32 striae in 10 µm for Werum & Lange-Bertalot (2004) and, 32 to 36 striae in 10 µm according to the observations of Noga *et al*. (2016).

The new species is longer (4.2 to 13.1 µm) compared to *G. fontinalis* (5.0–8.0 µm for Werum & Lange-Bertalot 2004 and 3.2–7.9 µm for Noga *et al*. 2016). Moreover for *G. fontinalis*, transapical striae are very short and subparallel in marginal position (Werum & Lange-Bertalot 2004). Striae of *G. fontinalis* can be longer in some individuals related to the observations of Noga *et al*. (2016) while for *G. vallei* the striae are longer, composed of single slit-like areolae. *Gomphosphenia fontinalis* is characterized by a wide central area, not distinctly separated from the axial area. For *G. vallei*, the central area is absent to asymmetrical and appeared as a unilateral fascia in SEM, with a short areola on one side and absence of areola on the other side, and the axial area is linear and narrow. Internally, the proximal raphe endings are deflected in the same direction for *G. vallei* while they "declined unilaterally as small hooks" in *G. fontinalis*. At last, in girdle view, the frustules of *G. vallei* are almost rectangular to slightly wedge-shaped while for *G. fontinalis* they are club-shaped.

Gomphosphenia indistincta present valves linear-lanceolate clavate with margins with undulations in larger specimens (Kociolek *et al*. 2014), a pattern never observed in *G. vallei*. This species is longer (7.5–25 µm) and larger (2–3 µm) than the new species. At last, *G. stoermeri* present valves linear-clavate with rounded to narrowly rounded poles. This species is longer (10–21.1 µm) and the density of striae is higher (28–35 striae in 10 µm) (Thomas *et al.* 2009) than for *G. valley* (length: 4.2–13.1 µm; number of striae: 24–29 in 10 µm). Moreover internal proximal raphe endings are tightly hooked in one direction while for *G. vallei* they are deflected in one direction. In girdle view, frustules are slightly wedge-shaped for both species.

Furthermore, the other comparable species have a different ecology (Table 2). Indeed, *G. vallei* was collected in a headwater stream in running waters of low conductivity in a granitic and volcanic context while *G. americana* and *G. indistincta* were found in lakes (Kociolek *et al*. 2014). For *G. fontinalis*, this species is apparently typical of carbonate springs (Werum & Lange-Bertalot 2004, Straub 2013, Noga *et al*. 2016, Peeters *et al*. 2019). At last, *G. stoermeri* was observed as epilithic, subaerial in Cataract Falls (Thomas *et al.* 2009) and also in rivers in northern Sweden (Van de Vijver *et al.* 2012).

In conclusion, this study of the diatom community done in the "Réserve Naturelle Nationale de la Vallée de Chaudefour" revealed a small-sized species of *Gomphosphenia* with a previously unknown combination of the morphological characteristics (shape, size, internal proximal raphe endings) leading to the description of *G. vallei*. This species was observed in a small stream of the reserve. Additional investigations are needed to identify other sites with the presence of this species.

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TABLE 1. Physical and chemical variables measured in the spring (below detection limit: Bdl).

	Pérouse Stream
Conductivity (µS cm^{-1})	35
pH (pH units)	7.09
Temperature (°C)	11.90
Dissolved oxygen (%)	92.90
Li^{+} (mg L^{-1})	Bdl
Na^{+} (mg L^{-1})	2.53
NH_4^{+} (mg L^{-1})	Bdl
K^{+} (mg L^{-1})	1.93
Mg^{2+} (mg L^{-1})	0.84
Ca^{2+} (mg L^{-1})	2.38
F^{-} (mg L^{-1})	0.05
Cl^{-} (mg L^{-1})	1.04
NO_3^{-} (mg L^{-1})	0.57
NO_2^{-} (mg L^{-1})	0.01
Br^{-} (mg L^{-1})	Bdl
PO_4^{3-} (mg L^{-1})	0.11
HCO^{3-} (mg L^{-1})	16.50
SO_4^{2-} (mg L^{-1})	0.67

TABLE 2. Main characteristics of *Gomphosphenia vallei* and 4 similar *Gomphosphenia* species.

Species	Valve length (µm)	Valve width (µm)	Number of striae (in 10 µm)	Valve outline	Areolae	Axial area	Central area	Internal proximal raphe endings	Girdle view	Ecology
Gomphosphenia vallei	4.2–13.1	1.5–2.5	23–29	linear-clavate in larger specimens to clavate in smaller ones and elliptical in the smallest individuals	striae uniseriate, composed of single slit-like areolae, becoming almost round poroids near the poles	linear and narrow	absent to asymmetrical	deflected in one direction	almost rectangular to slightly wedge-shaped	in running waters (streams) with low conductivity
Gomphosphenia americana Kociolek & E.W.Thomas in Kociolek *et al.* (2014: 45)	9.0–13.0	2.0–3.0	29–32	lanceolate-clavate	single elongated areola	linear and narrow	linear to circular, sometimes asymmetrical	recurved to one side	cuneate and flexed about the transapical axis	in lakes
Gomphosphenia fontinalis Lange-Bertalot, Ector & Werum in Werum & Lange-Bertalot (2004: 163)	5.0–8.0	1.5–2.3	25–32(36)	club-shaped	single elongated areola	lanceolate	wide, not distinctly separated from the axial area	unilaterally deflected as small hooks	wedge-shaped	species of carbonate water, apparently strictly dependent on karstic springs
Gomphosphenia indistincta Kociolek & E.W.Thomas in Kociolek *et al.* (2014: 46)	7.5–25.0	2.0–3.0	almost 24	linear-lanceolate clavate with margins with undulations in larger specimens	single elongated areola	narrow straight	asymmetrical-expanded	recurved in one direction	/	in lakes
Gomphosphenia stoermeri Kociolek & E.W.Thomas in Thomas *et al.* (2009: 230)	10.0–21.1	1.5–3.1	28–35	linear-clavate	single elongated areola	rather narrow, linear	absent to only weakly developed	tightly hooked in one direction	slightly wedge-shaped	epilithic, subaerial in Cataract Falls, on rivers of northern Sweden

ANNEX

Gomphosphenia americana Kociolek & E.W.Thomas in Kociolek *et al.* (2014: 45)
Gomphosphenia biwaensis Ohtsuka & D.Nakai in Ohtsuka *et al.* (2018: 108)
Gomphosphenia fontinalis Lange-Bertalot, Ector & Werum in Werum & Lange-Bertalot (2004: 163)
Gomphosphenia grovei (M.Schmidt 1899: 214) Lange-Bertalot (1995: 243)
Gomphosphenia grovei var. *lingulata* (Hustedt 1927: 166) Lange-Bertalot (1995: 243)
Gomphosphenia holmquistii (Foged 1968: 8) Lange-Bertalot (1995: 244)
Gomphosphenia holmquistii var. *alaskaensis* (Foged 1968: 8) Lange-Bertalot (1995: 244)
Gomphosphenia indistincta Kociolek & E.W.Thomas in Kociolek *et al*. (2014: 46)
Gomphosphenia lingulatiformis (Lange-Bertalot & E.Reichardt in Lange-Bertalot 1993: 60) Lange-Bertalot (1995: 242)
Gomphosphenia oahuensis (Hustedt 1942a: 98) Lange-Bertalot in Moser *et al.* (1998: 42)
Gomphosphenia patrickiana Cantonati, Lange-Bertalot, Kociolek & A.A.Saber in Saber *et al.* (2021: 222)
Gomphosphenia pfannkucheae (Cholnoky 1966: 25, 26) Lange-Bertalot (1995: 244)
Gomphosphenia plenkoviciae Gligora Udovič & Zutinić in Gligora Udovič *et al*. (2018: 231)
Gomphosphenia praegnans Ress, E.W.Thomas & Kociolek (2016: 56, 57)
Gomphosphenia reicheltii (M.Schmidt 1899: 214) Lange-Bertalot (1995: 244)
Gomphosphenia ryukyuensis Tuji & Ohtsuka in Tuji (2016: 2)
Gomphosphenia stoermeri Kociolek & E.W.Thomas in Thomas et al. (2009: 230)
Gomphosphenia tackei (Hustedt 1942b: 205) Lange-Bertalot (1995: 244)
Gomphosphenia tenerrima (Hustedt 1938: 444) E.Reichardt (1999: 57)
Gomphosphenia tenerrima var. *nunguaensis* (Foged 1966: 109, 146) M.A.Harper (2012: 162)
Gomphosphenia tenuis Levkov & D.M.Williams (2011: 20)

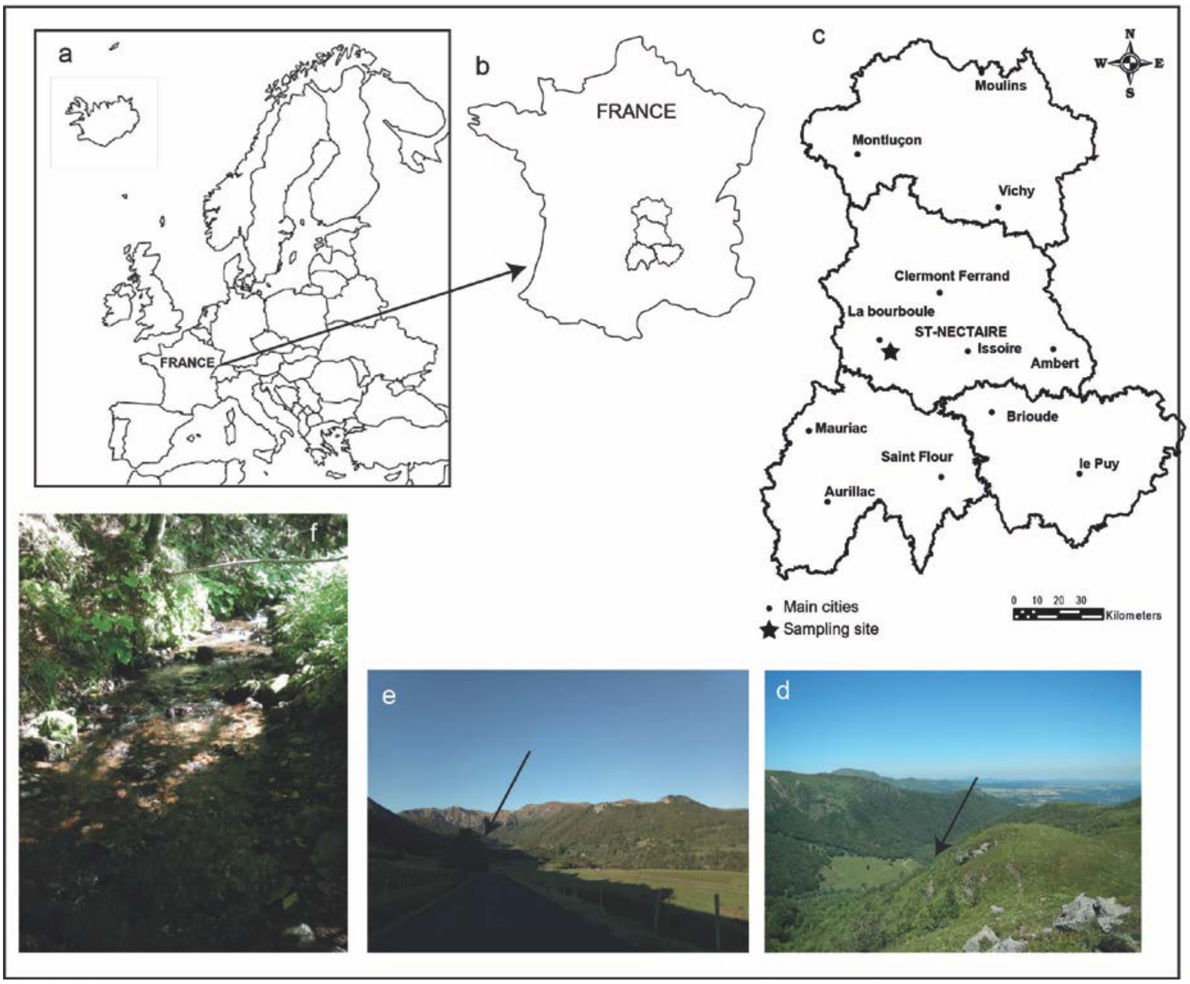
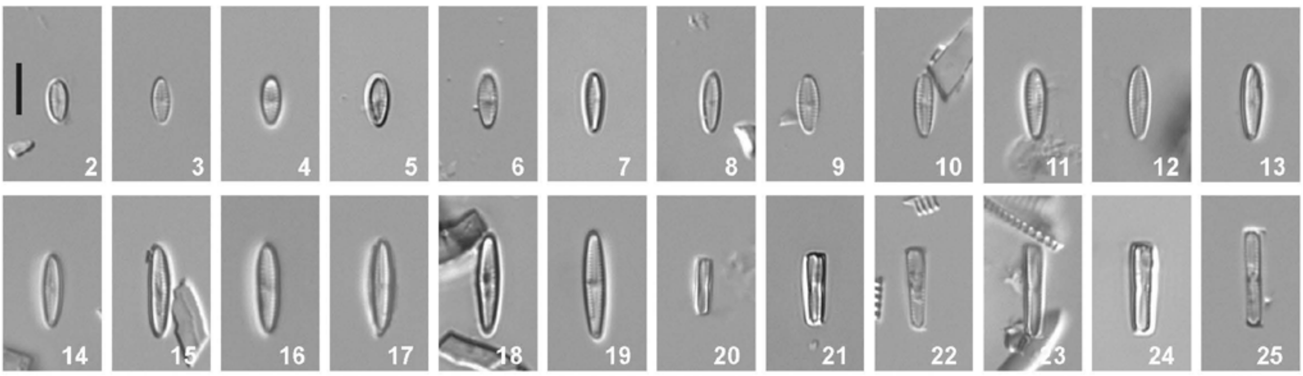
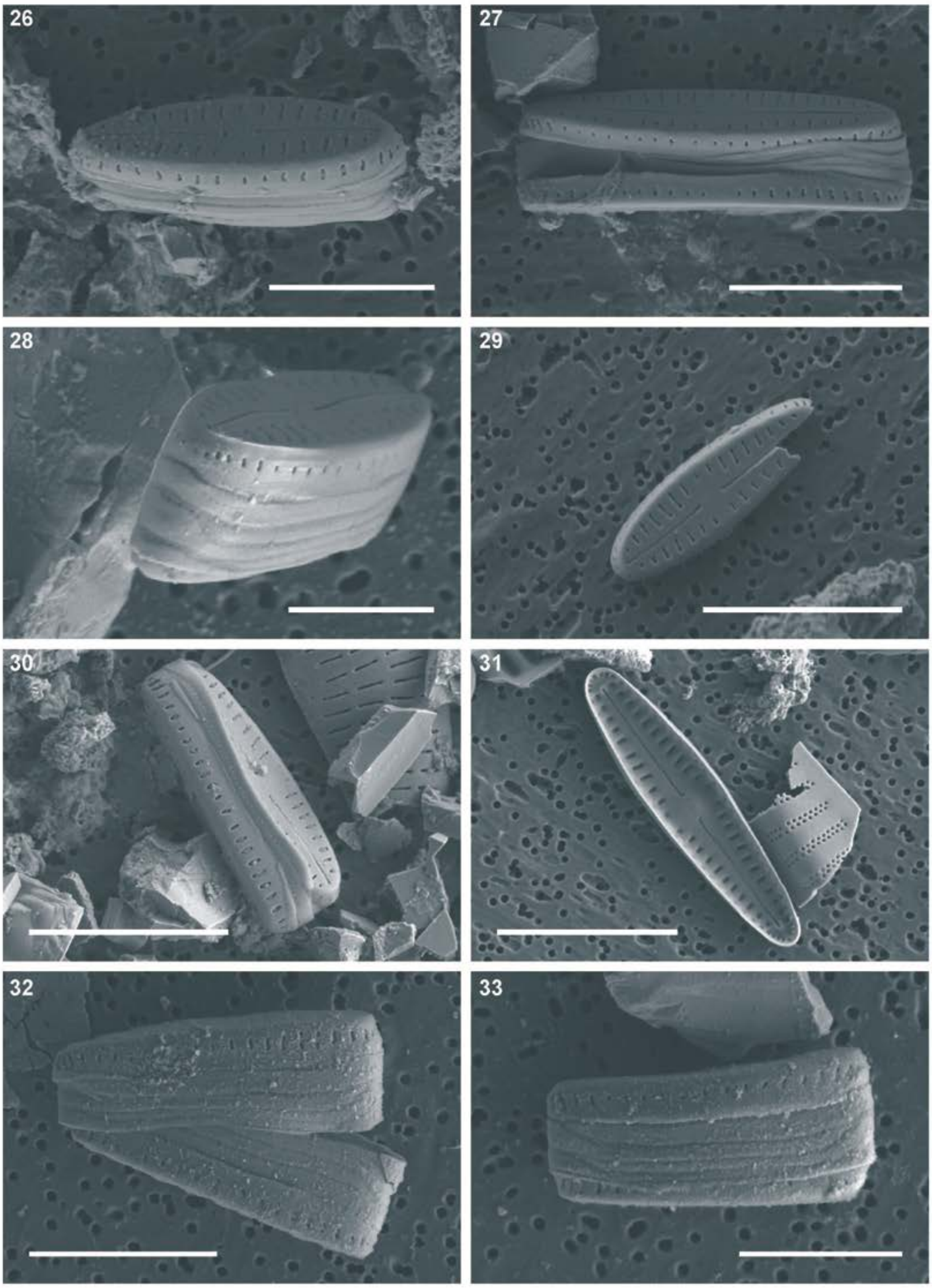


FIGURE 1. Map of the studied stream. a: map of Europe; b: map of France; c: location of the sampling site (star); d: photograph of the sampling site in the Chaudefour Valley taken from the upper part of the cirque; e: photograph of the sampling site in the Chaudefour Valley taken from the access road to the Reserve; f: photograph of the Pérouse Stream taken in July 2021.



FIGURES 2–25. LM images of Gomphosphenia vallei. Type population from Pérouse Stream. 2–19: Valve view; 20–25: Girdle view. Scale bar = 5 µm.



FIGURES 26–33. SEM images of Gomphosphenia vallei. Type population from Pérouse Stream. 26–30: External view of entire valve showing the raphe structure and the striae. 31: Internal view of an entire valve showing the raphe structure and the striae: 32–33: Girdle view of the frustule. Scale bar = 5 μm (Figs 29–31), 4 μm (Figs 27, 32), 3 μm (Figs 26, 33) and 2 μm (Fig. 28).