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***Chamaepinnularia salina* (Bacillariophyta), a new diatom species from French mineral springs (Massif Central)**

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Abstract

During a survey done on the mineral springs in the French Massif Central region, a new *Chamaepinnularia* species (Bacillariophyceae) was found and is hereby described: *Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector *sp. nov.* This new small species, with valves linear with almost parallel margins, is formally described using light and scanning electron microscopy. The central area is rectangular, forming a rather broad fascia and the raphe is straight, filiform located on an elevated sternum. Virgae between striae are developed. The ecological preferences of *Chamaepinnularia salina* are presented. This species is mainly in mineral springs with Na-K-HCO₃ water type.

Keywords:

Europe, mineral spring, natural radioactivity, small naviculoid diatoms, ultrastructure

Introduction

Mineral springs are influenced by their deep circulation in the basement. Indeed, groundwater chemistry depends mainly on water-rocks interactions. Rainwaters infiltrate rocks and supply deep aquifers using faults up to several kilometers. In depth, hot temperatures, long residence times within aquifers and CO₂ lead to very mineralized waters with specific geochemical signatures. During the rise of deep waters to the surface, different processes occur such as dissolution, ion exchange, precipitation, leading to change chemical composition of the fluids. Mixing with poorly mineralized surface waters are frequently observed and induced dilution of deep waters (Boineau & Maisonneuve 1972, Fouillac 1983, Negrel *et al.* 1997a, Pauwels *et al.* 1997, Brenot *et al.* 2008, Cantonati *et al.* 2012, Petelet-Giraud *et al.* 2018).

Among the communities thriving in these habitats are diatoms and it is well known that mineral springs host important biodiversity and specific biocenoses (species adapted to the constraints imposed by these ecosystems) (Wojtal 2013, Beauger *et al.* 2015, 2016, 2020, Lai *et al.* 2019a). Thus, living organisms in mineral springs are often unique, with very specific traits and distribution patterns (Wojtal 2013, Segadelli *et al.* 2015, Lai *et al.* 2019b, Beauger *et al.* 2020). Diatomists have studied these algae all over the world and new species are regularly identified in springs (e.g. Ector & Iserentant 1988, Sabater & Roca 1992, Werum & Lange-Bertalot 2004, Reichardt 2006, Potapova & Ponader 2008, Wojtal 2009, 2013, Angeli *et al.* 2010, Cantonati & Lange-Bertalot 2011, Źelazna-Wieczorek 2011, Solak & Wojtal 2012, Beauger *et al.* 2015, 2016, 2017, 2019, Lai *et al.* 2018).

In the French Massif Central, few studies have been conducted on diatoms in mineral springs and the first was done by Héribaud (1893, 1920). Then, other studies were conducted during the 20th century (Chaouite 1987, Chaouite & Romagoux 1989, Tudesque 1996) on a limited number of emergences that revealed a high biodiversity in the springs. As a consequence, a recent survey has been initiated and new taxa have been recently discovered in Massif Central springs, such as *Navicula sanctamargaritae* Beauger in Beauger *et al.* (2015: 174), *Sellaphora labernardierei* Beauger, C.E.Wetzel & Ector in Beauger *et al.* (2016: 238), *Craticula lecohui* Beauger, C.E.Wetzel & Ector in Beauger *et al.* (2017: 10) and *Pseudostaurosira bardii* Beauger, C.E.Wetzel & Ector in Beauger *et al.* (2019: 6).

Amongst the diatom genera found in these habitats, *Chamaepinnularia* Lange-Bertalot & Krammer is present in communities. This genus was originally described by Lange-Bertalot & Krammer (in Lange-Bertalot & Metzeltin 1996: 32) and comprises several taxa initially allocated to the genera *Navicula* Bory or *Pinnularia* Ehrenberg (Lange-Bertalot & Metzeltin

1996) by previous authors (e.g., Petersen 1924, 1928, Krasske 1929, Hustedt 1934, 1942, 1962). Species have linear-elliptic to linear-lanceolate valve outlines and are relatively small. In general striae are arranged in a single row and composed by a large areola interrupted by a silica line near the valve face-mantle junction along the apical plane. Moreover, the external apertures of the areolae are occluded by thin unstructured hymenes and internally, the openings are divided by plates or silica bridges (Lange-Bertalot & Metzeltin 1996, Wetzel *et al.* 2013). The representatives of this genus are isolated cells, have a cosmopolitan distribution and are present in different habitats such as springs (Wetzel *et al.* 2013, Źelazna-Wieczorek & Olszyński 2016, Lai *et al.* 2019c). Some of these species are typical of this kind of ecosystem such as *Chamaepinnularia submuscicola* (Werum & Lange-Bertalot 2004) and *C. thermophila* (Lai *et al.* 2019c). According to AlgaeBase (Guiry & Guiry 2021) and DiatomBase (Kociolek *et al.* 2021), the genus *Chamaepinnularia* holds 62 species, 1 subspecies, 4 varieties and 1 form.

Pressures caused by various human activities affect mineral springs and degrade biodiversity (Cantonati & Lange-Bertalot 2009, Strayer & Dudgeon 2010, Nowicka-Krawczyk & Źelazna-Wieczorek 2017, Beauger *et al.* 2021) while it is the keystone of the functioning of the ecosystems from which human societies derive ecosystemic services. It is therefore important to study particularly these peculiar ecosystems in order to have a better knowledge of their biodiversity. For this purpose, a floristic survey was started in 2014 on mineral springs in the French Massif Central and a *Chamaepinnularia* could not be identified using the currently available literature. Following detailed light microscopy (LM) and scanning electron microscopy (SEM) observations and comparisons with known representatives of the genus, this *Chamaepinnularia* is described as new. A morphological comparison is made with the most similar taxa, as well as observations concerning its ecology.

Material & Methods

Study site:—The area is located in the southern part of the Limagne graben of the French Massif Central. This highland region in the middle of Southern France is influenced by oceanic, continental and Mediterranean climates. On each bank of the Allier River, between the cities of Vic-le-Comte and Mirefleurs (Puy-de-Dôme, Auvergne, Massif Central, France), several well-known springs (Beauger *et al.* 2016, 2020) are located just above two principal fault systems, which are certainly inherited from the Tardi-Hercynian period (Michon 2000). The mineral and thermal waters are either influenced by their deep circulation in the crystalline basement (Fouillac 1983), or have a more superficial origin (Surmely 2004,

Labernardière, personal communication). The small Sail spring (elevation of 334 m a.s.l.) emerges near the city of Mirefleurs on the right side of the Allier River (Fig. 1), at the contact between the Hercynian basement and the transgressive Oligocene marl, clay and limestone (Battani *et al.* 2010, Mohammed *et al.* 2014). About 10 meters of recent alluvial deposits (gravel, sand, clay) overlie the Oligocene sediments. An unconfined aquifer, 5 to 7 m of saturated thickness, take place in these deposits. The alluvial aquifer is connected to Allier River and in natural conditions the flow path is from the alluvial aquifer to the river (BRGM 1975).

Partly covered by a small stone construction, the spring is located near a salt meadow that features several varieties of halophilic plants. The site is managed and protected by the “Conservatoire des Espèces Naturelles d’Auvergne”. In addition to having high conductivity, which classifies it in so-called “mineral springs”, it also has a noticeable level of natural radioactivity: according to Jacquet (1929), Sail spring is the second most radioactive spring in Auvergne (after La Montagne spring) with activities reaching 3145 Becquerel per liter (Bq L⁻¹).

Diatom sampling and physical and chemical analysis:—In the context of a survey of 150 mineral springs in the French Massif Central, an epilithic sample from the Sail spring was collected by brushing stones inside the spring on 20th December 2014. Samples were fixed *in situ* with a formaldehyde solution to a final concentration of 4%. pH (pH unit), conductivity ($\mu\text{S cm}^{-1}$) and water temperature ($^{\circ}\text{C}$) were measured at the sampling site using a WTW Multiline P4 and dissolved oxygen (%) with a ProODO oxygen probe. A water sample was collected for further chemical analysis in the laboratory and was analyzed with the high pressure ion chromatography technic. This sample was first filtered using Whatmann GF/C filters. For the cations analysis, a Thermo Scientific Dionex ICS1100 system was used; and for the anions, a Thermo Scientific Dionex DX120 system. The concentrations in lithium, sodium, ammonium, potassium, magnesium, calcium, bromine, fluoride, chloride, nitrite, nitrate, phosphate, sulphate and carbonate were measured (mg L^{-1}) (Table 1). The same analysis was repeated on 23rd November 2018, on 15th October 2019 and on the 26th May 2020 by taking the first millimetres of mud present in the emergence. In May 2020, diatoms were collected both on mud and stones. In November 2018, to have a better knowledge on the chemistry of the spring, major and trace elements characterizations were done together with strontium isotopes concentrations (ICP-MS analyses) (Table 2).

Regarding radioactivity, Sail spring displays a quite peculiar pattern with significant variations over the course of the year. In October 2019, *in situ* ambient gamma-ray dosimetry measurements were conducted during the sampling using a Colibri radiometer (MIRION Technologies). Moreover, one month later, a Marinelli beaker was filled with water and sealed *in situ*, and got measured in the well-type Germanium detector (Courtine *et al.* 2008) at LPC. The determination of the ²²²Radon activity was achieved using the γ -rays of ²¹⁴Pb, ²²²Radon daughter nucleus. The presence of dissolved Radon in mineral spring waters stems mainly from the ²³⁸U decay chain.

Slide preparation and microscopy: — Small sub-samples of raw material were prepared for LM observation following the method described in Prygiel & Coste (2000): cleaning using H₂O₂ and dilute hydrochloric acid, and rinsing 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 brightfield microscope with 100x oil immersion objective, and light photographs were taken with a Leica® DC500 camera. For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered with additional deionized water through a 3- μ m Isopore™ polycarbonate membrane filter (Merck Millipore). SEM images were taken using a Hitachi SU-70 electron microscope (Hitachi High-Tech Corporation, Tokyo, Japan) at 5 kV and 10 mm working distance, using the lower detector. The oxidized samples were coated with platinum (10 nm film thickness) using a Leica sputter coater EM ACE600 (Leica Microsystems GmbH, Wetzlar, Germany and up to 28° tilted. 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) and Botanic Garden Meise (Belgium). Diatom terminology follows Ross *et al.* (1979), Barber & Haworth (1981) (stria/areola structure) and Round *et al.* (1990) (raphe structure).

Results

Class: **Bacillariophyceae** Haeckel emend. Medlin & Kaczmarska 2004: 267

Subclass: **Bacillariophycidae** D.G.Mann in Round *et al.* 1990: 128

Order: **Naviculales** Bessey 1907: 284

Family: Naviculales incertae sedis

Genus: *Chamaepinnularia* Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin 1996:

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***Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector sp. nov. (Figs 2–40)**

Light microscopy observations (Figs 2–28): Valves linear with almost parallel margins (the longest individuals slightly inflated in the middle). Broadly rounded to slightly rostrate–capitate apices (Figs 2–28). Valve dimensions (n = 35): length 7–16 µm, width 2–3.2 µm. Axial area lanceolate, central area rectangular, forming a rather broad fascia. Striae parallel to weakly radiate near the central area, 30–40 in 10 µm.

Scanning electron microscopy observations (Figs 29–40): Raphe straight (exceptionally slightly curved in the middle), filiform located on an elevated sternum and central area a rectangular fascia (Figs 29–35). Axial area narrow. Virgae between striae developed (Fig. 36). Raphe with straight, slightly expanded central endings and terminal fissures hooked in the same direction, continuing onto the mantle. Central endings expanded, drop-like (Fig. 29). Striae composed of two large areolae, one located on the valve face and a second on the mantle, separated by a silica line at the valve face/mantle junction, covered externally by porous hymenes, visible also internally (Figs 36–39). Striae sometimes slightly depressed (Figs 36–37). Around the valve apices, areolae on the mantle continuous externally and internally (Figs 38–39). Internally, terminal fissures slightly deflected, terminating in a slightly swollen hyaline area (Fig. 39).

Type:—FRANCE. Mirefleurs: Sail spring, sample S10_BA_201214, 334 m a.s.l., E716182.68, N6509572.63 (Lambert 93), collection date 20 December 2014 (designated here, holotype: CLF!, slide no. CLF103304, here depicted in Figs 7–8; isotypes: BR! slide no. 4649).

Etymology:—The new species is named after the mineral and salty spring where it was discovered, Sail spring, situated in the French Massif Central.

Associated diatom taxa:—The type population of *Chamaepinnularia salina* was found in Sail spring associated with other taxa. In December 2014, the two co-dominant taxa were *Planothidium frequentissimum* (Lange-Bertalot 1993: 4) Lange-Bertalot (1999: 282) (31.6%) and *Chamaepinnularia salina* (13.2 %). *Crenotia thermalis* (Rabenhorst 1864: 107) Wojtal (2013: 81) represented 9.4 % of the community with *Navicula veneta* Kützing (1844: 95)

(9.1%), *Navicula sanctamargaritae* (5.1%), *Microcostatus* sp. (4.6 %), *Nitzschia* cf. *liebetruthii* (3.8 %), *Gomphonema parvulum* Kützing (1849: 65) (3.8 %), *Pinnularia kuetzingii* Krammer (1992: 104, 173) (3.8%), *Nitzschia vitrea* G.Norman (1861: 7) (2.3 %), *Surirella brebissonii* Krammer & Lange-Bertalot (1987: 82) (2.0 %), *Nitzschia inconspicua* Grunow (1862: 579, 580) (1.3 %). The other taxa (<1%) were *Fallacia pygmaea* (Kützing 1849: 77) Stickle & D.G.Mann in Round *et al.* (1990: 668), *Fragilaria famelica* (Kützing 1844: 64) Lange-Bertalot (1980: 749), *Nitzschia soratensis* E.Morales & M.L.Vis (2007: 128), *Sellaphora labernardierei* and lastly *Pseudostaurosira* sp.

Physical and chemical environment of the species:— The Sail spring has a slightly acidic pH (6.8) and an elevated electrical conductivity (EC) level (more than 5000 $\mu\text{S cm}^{-1}$). Water temperature is not high, in the range of 12.9 to 16.6°C for our measurements. The spring is rich in several major elements e.g. sodium, calcium and, the concentration in nitrate is lower less than 10 mg L⁻¹ (Table 1). High trace elements concentrations such as boron, strontium, barium, manganese and arsenic are found in the 2018 sample (Table 2). In October 2019, physical parameters and ionic concentrations are significantly different than the other periods of sampling, e.g. electrical conductivity, usually about 7600 $\mu\text{S.cm}^{-1}$, has dropped to 5390 $\mu\text{S.cm}^{-1}$ and chloride content has divided by two.

The Sail spring displays a very peculiar pattern regarding radioactivity: radon concentration in water varies significantly along the year and can reach significant levels up to 1000 Becquerels per liter. Indeed, the equivalent doses recorded (in nanoSieverts per hour) were on average 150 nSv h⁻¹ and 270 nSv h⁻¹ when measuring at 1-meter height above the spring and at the water level respectively. These values are in agreement with the mean background values of Auvergne region (150–200 nSv h⁻¹) without indicating any significant radioactive signature. The water sample collected for gamma-spectroscopy measurements showed a significant Radon activity of 1006.1 ± 1.6 Bq L⁻¹.

Chamaepinnularia salina was also present in each sample taken in 11 other springs of the French Massif Central (Figs 41–44; 45). In these springs, the relative abundance of *C. salina* varied between 0.2% (one individual in Zagat and Tete de Lion springs) and 50.6% (Camuse spring). All the springs were characterized by high conductivity (between 1464 $\mu\text{S.cm}^{-1}$ and 7240 $\mu\text{S.cm}^{-1}$) and by high sodium and potassium contents (Fig. 45). Major anions are bicarbonates except for the Poix spring enriched in sulphate. For Camuse spring, where rock was brushed, *C. salina* was dominant (50.6%) associated with *Humidophila gallica* (W.Smith

1857: 11) R.L.Lowe, Kociolek, Q.You, Q.Wang & Stepanek (2017: 281) (30.8%). For Camuse spring, EC was less than 5000 $\mu\text{S cm}^{-1}$ and ionic concentrations were lower than in Sail spring. When collecting diatoms on mud, in November 2018, in Sail spring, only two individuals were observed and in October 2019, seven individuals were present. In June 2020, there were as many individuals (1.5%) in both samples (mud and stones). In the 11 other springs, *C. salina* was present both on stone and sediment substrates (the dominant substrates taken at each spring).

Discussion

Among the *Chamaepinnularia*, species differs related to different characteristics. Among them the shape of the apices allows to identify two groups. Different species present capitate and sub-capitate apices such as *C. calida* (Hendey 1964: 198) Lange-Bertalot in Lange-Bertalot & Genkal (1999: 35), *C. hassiaca* (Krasske 1925: 47) Cantonati & Lange-Bertalot (2009: 228), *C. krookiformis* (Krammer 1992: 79, 173) Lange-Bertalot & Krammer in Lange-Bertalot & Genkal (1999: 37), *C. margaritacea* (Hustedt 1937: 253) Lange-Bertalot in Lange-Bertalot & Metzeltin (1996: 34), *C. plinskii* Żelazna-Wieczorek & Olszyński (2016: 114), *C. soehrensis* var. *capitata* (Krasske 1925: 47) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin (1996: 37), *C. thermophila* (Manguin in Bourrelly & Manguin 1952: 60) C.E.Wetzel & Ector (2016: 96). Others have cuneiform or obtuse rounded ends such as *C. parsura* (Hustedt 1962: 240) C.E.Wetzel & Ector in Wetzel *et al.* (2013: 156) and *C. evanida* (Hustedt 1942: 66) Lange-Bertalot in Lange-Bertalot & Metzeltin (1996: 34). The new species *C. salina* is associated with the representatives of this genus with rounded ends (to slightly rostrate-capitate).

Chamaepinnularia presents unique morphological characters such as the presence of an elevated sternum, developed virgae between striae, and a central area rectangular, forming a rather broad fascia. The species shows similarities to *Chamaepinnularia gandrupii* (J.B.Petersen 1924: 16) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin (1996: 34), *C. gerlachei* Van de Vijver & Sterken in Van de Vijver *et al.* (2010: 432), *C. krasskei* Lange-Bertalot in Lange-Bertalot & Genkal (1999: 36), *C. minutissima* (Manguin in Bourrelly & Manguin 1952: 81) Metzeltin & Lange-Bertalot (2007: 64) and *C. rupicola* (Hustedt 1937: 242) Lange-Bertalot in Lange-Bertalot & Metzeltin (1996: 35) (Table 3). The new species present also similarities with *Microcostatus turritus* P.B.Hamilton & D.Antoniades in Antoniades *et al.* (2009: 65) (Table 3).

Considering the valve sizes, the valve length allowed to separate the new species from *C. gerlachei* and *C. krasskei* that are longer (12–30 μm and 15–27 μm respectively). It is the same for the valve width. Indeed *C. salina* is narrower than *C. gerlachei* (3.3–4.9 μm) and *C. krasskei* (3.3–4.0 μm). The striae density is the main characteristic separating the new species from the other taxa. Indeed *C. salina*, with 30 to 40 striae per 10 μm , has a higher striae density than the other more similar species.

The valve shape of *C. salina* is linear with almost parallel margins and the longest individuals are slightly inflated in the middle. Similar variability in the valve shape is reported for *C. gandrupii*. For this species, several morphotypes are observed with, for example, three slight undulations (Lange-Bertalot & Metzeltin 1996, Genkal & Yarushina 2018). For *Microcostatus turritus*, valves are elliptical to elliptical-lanceolate (Figs 46–58).

When considering the striae, for the new species, they are composed of two large areolae, one located on the valve face and a second on the mantle, separated by a silica line at the valve face/mantle junction, covered externally by porous hymenes. Around the valve apices, areolae on the mantle are continuous externally and internally. In *C. gandrupii*, striae are composed of a multiserial pore field, located internally between thickened costae and continue without interruption around the apices (Genkal & Yarushina 2018). In *C. gerlackei*, striae are interrupted by a hyaline line starting from the central area and terminating several striae below the valve apices, and internally, this line is visible as a rib-like structure inside the alveoli. In *C. krasskei*, striae are composed by a large areola occluded by thin unstructured hymenes (Lange-Bertalot & Genkal 1999). For *C. minutissima* (Bourelly & Manguin 1952) and *C. rupicola* (Hustedt 1937, Simonsen 1987), no SEM pictures are available. At last, for *Microcostatus turritus*, microcostae separated the axial area and the two-segmented striae (Fig. 64). The microcostae associated with depressed striae appear “as “towers” adjacent the axial area from the center to the apex” (Antoniades *et al.* 2009).

Focusing on other patterns, *C. salina* present a raphe straight, filiform, located on an elevated sternum that is a particular characteristic. The central area shape of the new species is also different as it is rectangular. Thus, for *C. gandrupii*, *C. krasskei* and *C. minutissima*, it is elliptical and rhombic-lanceolate while *C. gerlachei* and *C. rupicola* have a rhomboid to

rectangular central area. *Microcostatus turritus* is characterized by the presence of a stauros fascia so with a bow-tie shape (Antoniades *et al.* 2009; Figs 59–63). At last, none of the *Chamaepinnularia* species present virgae between striae developed as for *C. salina* contrary to *Microcostatus turritus*. Indeed, for this species, axial area is elevated but separated from striae by microcostae (Antoniades *et al.* 2009; Figs 59–63).

In addition to the morphological characteristics, the new species differs also when considering its ecology. Indeed, the species was present in mineral springs such as Sail and Camuse springs characterized by a high electrolyte content unlike the other comparable *Chamaepinnularia* species (Table 3). Indeed, *C. gandrupii* is a freshwater species present in oligotrophic waterbodies and watercourses (Genkal & Yarushina 2018). *Chamaepinnularia gerlackei* was observed in soil samples in Northeast Antarctic Peninsula, South Shetland Islands, South Orkney Islands and South Georgia. *Chamaepinnularia krasskei* was observed in freshwater at the mouth of a river (Lange-Bertalot & Genkal 1999) whereas *C. minutissima* is only a subaerial species (Bourrelly & Manguin 1952). At last, *C. rupicola* is known as typical of freshwater habitats and aerophytic (Hustedt 1937, Simonsen 1987, Wetzel *et al.* 2013). It was first observed in moorland (Hustedt 1937). For *M. turritus*, it was observed on sediments of a lake and in a cave (C.E. Wetzel, personal communication; Figs 46–64). The particular ecology of the new *Chamaepinnularia* species is highlighted by the presence of several taxa in the same samples such as *Crenotia thermalis*, *Fragilaria famelica*, *Navicula sanctamargaritae*, *N. veneta*, *Nitzschia vitrea*, *Pinnularia kuetzingii*, *Sellaphora labernardierei*, species found in other mineral springs in Europe (Krammer 2000, Lange-Bertalot 2001, Stavreva-Veselinovska & Todorovska 2010, Wojtal 2013, Beauger *et al.* 2015, 2020, Hindáková & Hindák 2015).

To have a more precise information on the ecology of the species, additional analysis was done on the Sail spring. The EC of the Sail spring ($> 5000 \mu\text{S cm}^{-1}$) is quite different from EC observed in river Allier and alluvial aquifer by Mohammed *et al.* (2014), respectively $183 \mu\text{S cm}^{-1}$ mean value and $1194 \mu\text{S cm}^{-1}$ mean value. This high value reveals mineralized water, within the range of mineral springs near the river Allier (Negrel *et al.* 1997a). Moreover, several high elements concentrations denote deep water-rock interactions e.g.: strontium, barium, lithium, boron, arsenic (Criaud 1983, Michard 1990, Gal & Gadalia 2010). This deep origin is crystalline basement (Stettler 1977, Fouillac & Michard 1979). At last, strontium isotopes are good tracers of water-rock interactions as $^{87}\text{Sr}/^{86}\text{Sr}$ reflects the signature of

minerals that constitute the drained aquifer lithologies (Petelet-Giraud & Négrel 2007, Gourcy & Brenot 2011). The strontium-isotope ratio measured in water sample of the Sail spring during the survey of November 2018 is 0.713354 (Table 2), very close to the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio measured in 1995: 0.713356 (Negrel *et al.* 1997b). These values are observed in waters draining granitic and gneissic rocks (Petelet-Giraud *et al.* 2018). These rocks are the crystalline basement of our study.

Moreover, examination of Cl versus Na concentrations (Fig 65) showed that the Sail spring display elemental compositions below the seawater dilution line (Berner & Berner 1996) suggesting Na enrichment which could be related to ion exchange with clays (Fig 65) (Brenot *et al.* 2008). The high chloride content (Figs 45, 65) is in agreement with mineral springs near the river Allier between Issoire and Clermont-Ferrand (Negrel *et al.* 1997a). Temporal fluctuations of the chemical composition of the Sail spring noticed during our study have been reported before (Negrel *et al.* 1997b) but not yet understood. One explanation could be a dilution of mineral water in the shallow alluvial aquifer. The recharge of this aquifer by surface waters contaminated by human activities could explain the rise of nitrate content in water.

In conclusion, this study of the diatom community of a salt and mineral spring with a deep origin of the water, revealed a small species with a previously unknown combination of the morphological characteristics (elevated sternum, developed virgae between striae, central area rectangular, forming a rather broad fascia) hence our description of *C. salina*. This species was observed in other mineral springs of the French Massif Central characterized by high conductivity and by high sodium and potassium contents.

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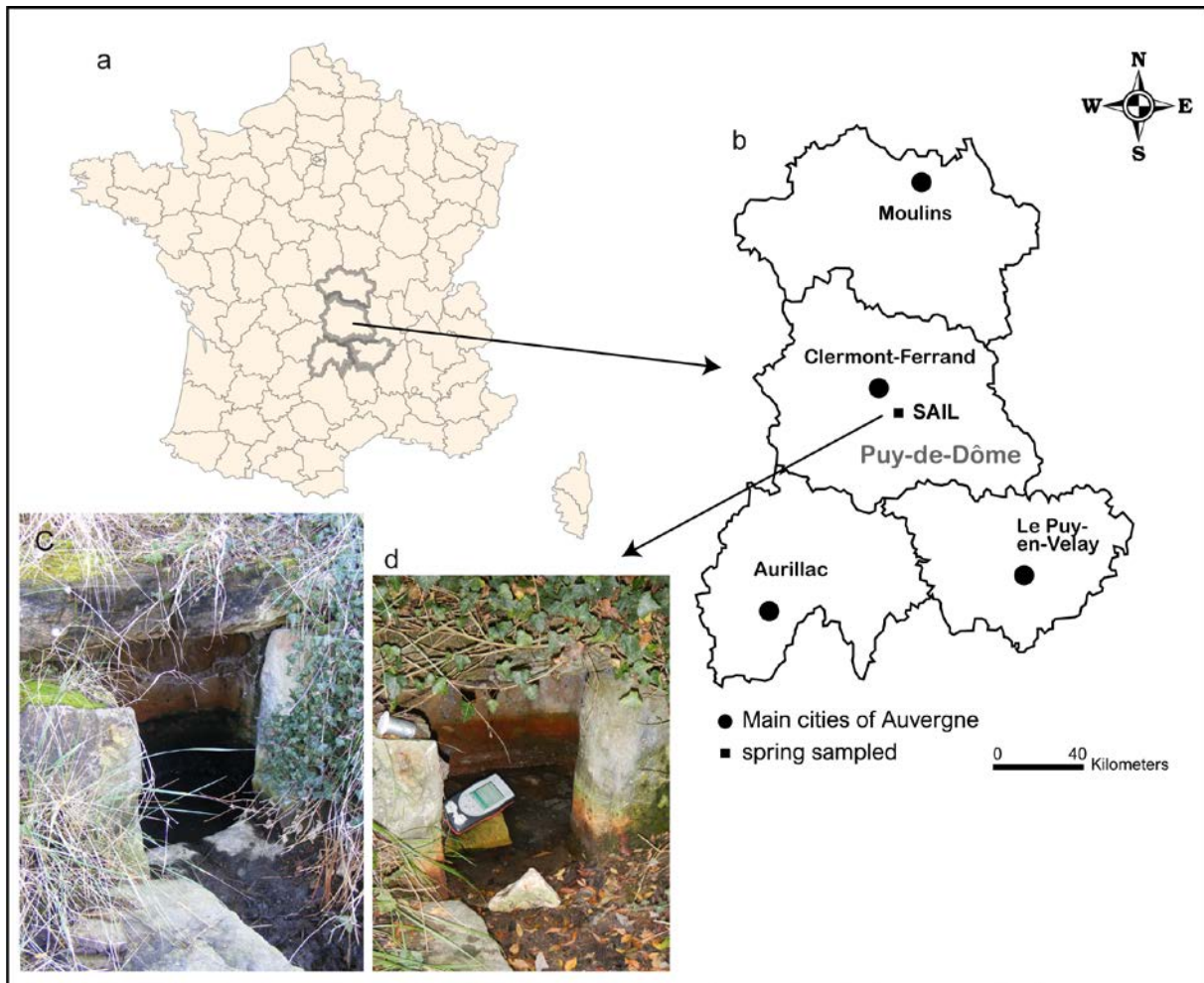
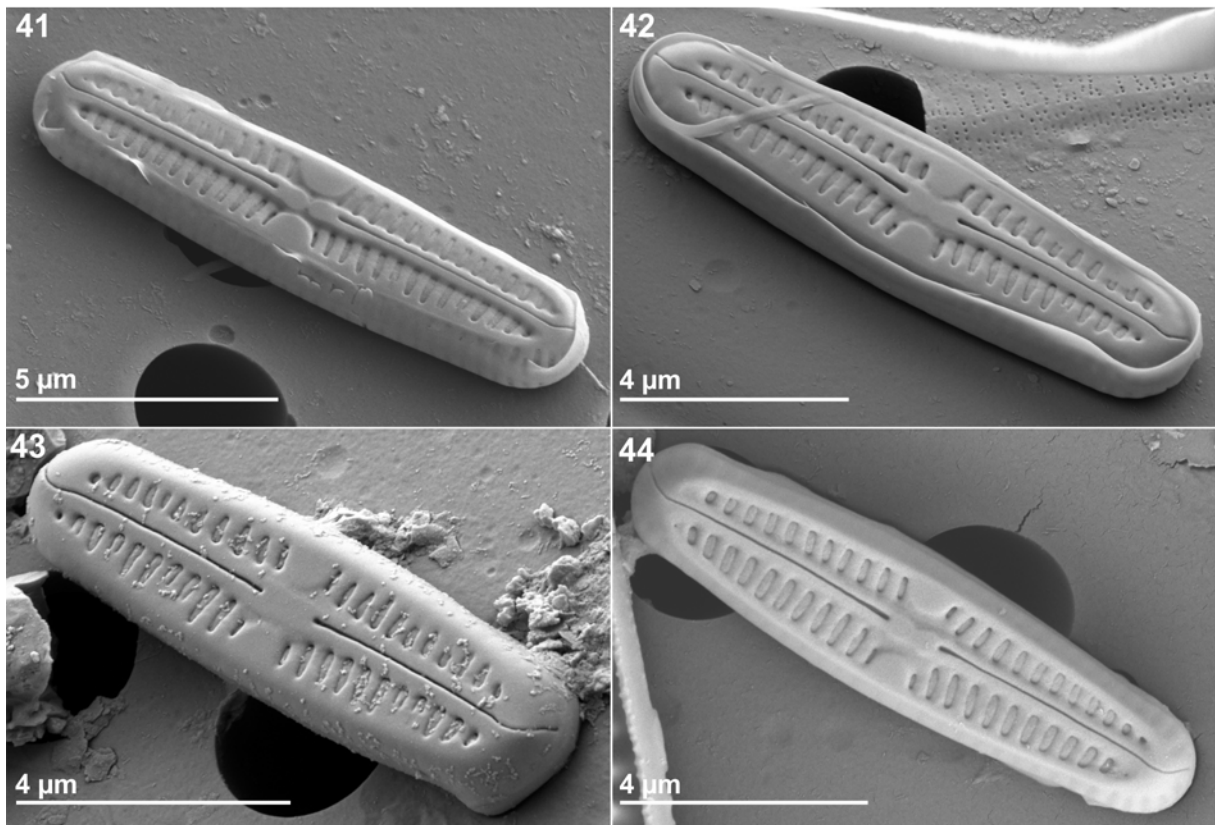


FIGURE 1. Map of the study spring. a: map of France; b: position of the Sail spring in the Puy-de-Dôme department (square); c and d: photographs of the spring taken in December 2014 and November 2018 respectively.

FIGURES 2–35. *Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector *sp. nov.*, LM. (Figs 2–28). Type population from Sail spring. SEM. (Figs 29–35) Type population from Sail spring. 29–35. External view of an entire valve showing the raphe structure and the striae. The striae are composed of simple, chamber-like areolae, with external openings covered by vela. The external proximal raphe endings are inconspicuous.

FIGURES 36–40. *Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector *sp. nov.*, Type population from Sail spring. SEM. 36. External detail of the central area and of the striae. Note the proximal raphe endings and the sternum. 37. External detail of the hooked distal raphe endings. 38, 40. Internal view of an entire valve showing the striae, the central nodule, the proximal and distal raphe endings. The proximal raphe endings are unilaterally hooked. The distal raphe fissures terminate internally with a small helictoglossa. 39. Internal detail showing the distal raphe endings. Internally, the areola openings are divided with internal plates of silica. Scale bar = 1 μm (Figs 36–37, 39), 5 μm (Figs 38, 40).



FIGURES 41–44. *Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector *sp. nov.* from 41. Zagat spring, 42: Saurier spring; 43: Camuse spring (CLF!, slide no. CLF103305) and 44. Felix spring. Scale bar = 5 µm (Fig. 41) and 4 µm (Figs 42–44).

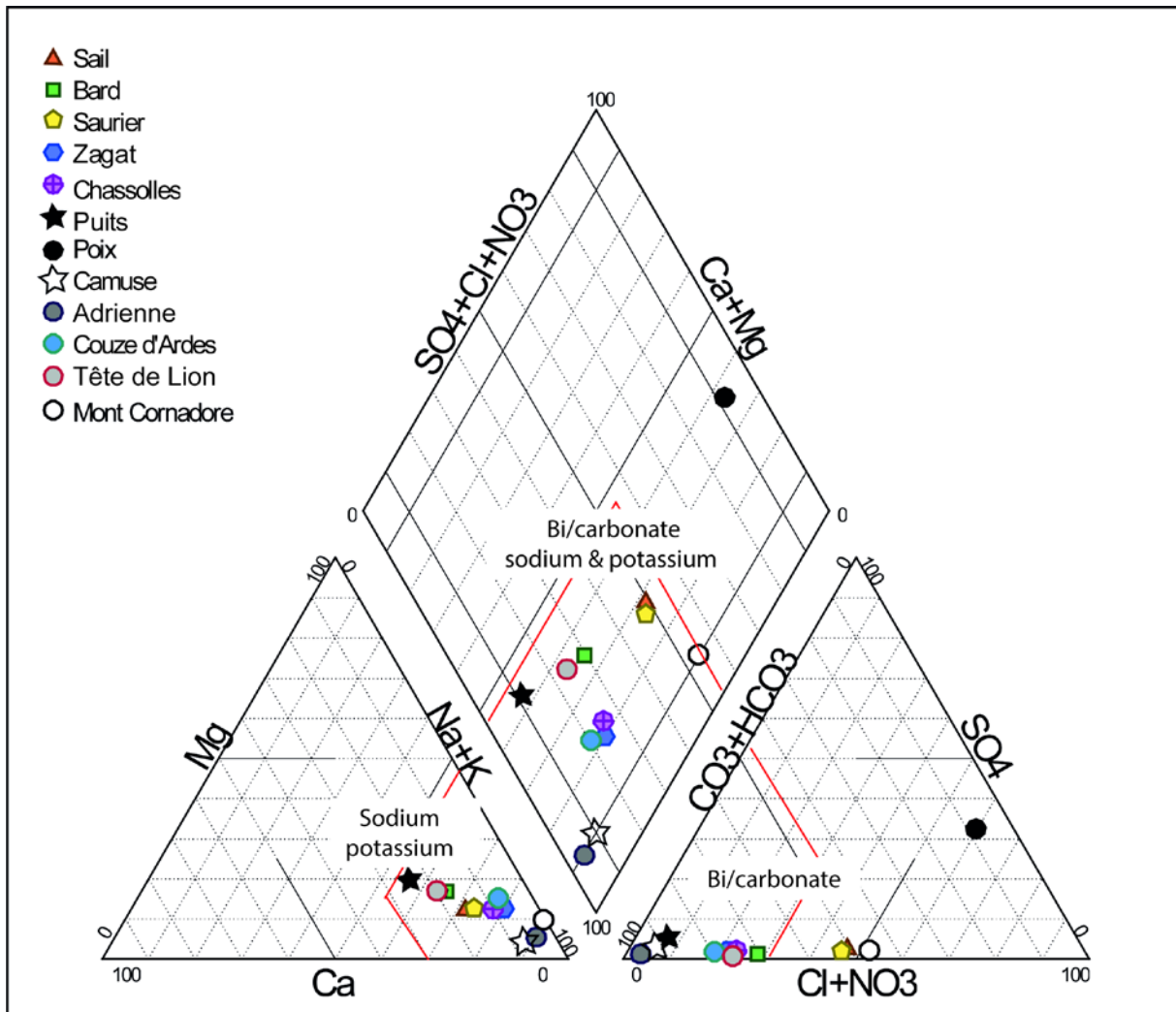


FIGURE 45. Piper diagram done on the 12 springs where *Chamaepinnularia salina* was observed.

FIGURES 46–64. *Microcostatus turritus* from Cave Bossea, Italy. LM. Figs 46–58. Population from Cave Bossea, Italy. SEM. Figs 59–64. Population from Cave Bossea, Italy. 59–62. External view of an entire valve showing the raphe structure and the microcostae. 63. Internal view of an entire valve showing the microcostae, the central nodule, the proximal and distal raphe endings. 64. Internal detail showing the striae. Scale bar = 10 μm (Figs 46–58), 4 μm (Fig. 63), 3 μm (Figs 59–62) and 500 nm (Fig. 64).

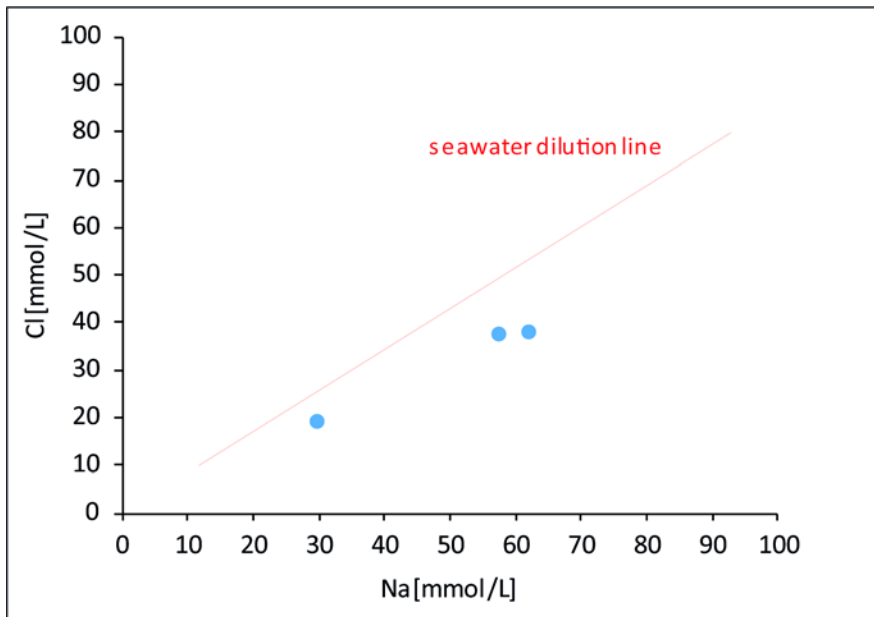


FIGURE 65. Cl versus Na concentrations for Sail spring samples.

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

Date	Sail spring			Camuse spring
	20/12/2014	23/11/2018	15/10/2019	26/09/2017
Conductivity ($\mu\text{S cm}^{-1}$)	7140	7670	5390	4290
pH (pH units)	6.8	6.8	7.0	6.5
Temperature ($^{\circ}\text{C}$)	12.9	10.3	16.6	15.8
Dissolved oxygen (%)	36.9	31.7	9.2	31.8
Li^+ (mg L^{-1})	7.1	7.8	3.5	4.5
Na^+ (mg L^{-1})	1323.3	1426.9	686.6	1006.8
NH_4^+ (mg L^{-1})	1.8	0.4	0.7	0.2
K^+ (mg L^{-1})	155.2	164.1	83.5	72.1
Mg^{2+} (mg L^{-1})	124.6	131.1	52.8	29.9
Ca^{2+} (mg L^{-1})	273.9	171.2	85.7	53.6
F^- (mg L^{-1})	0.6	0.6	0.5	2.4
Cl^- (mg L^{-1})	1326.4	1349.3	671.6	79.9
NO_3^- (mg L^{-1})	4.1	1.9	8.9	1.3
NO_2^- (mg L^{-1})	0.01	0.05	0.61	Bdl
Br^- (mg L^{-1})	3.1	3.1	1.1	Bdl
PO_4^{3-} (mg L^{-1})	0.1	0.3	0.5	0.1
HCO_3^- (mg L^{-1})	2480	2530	1150	2510
SO_4^{2-} (mg L^{-1})	94.6	101.2	51.8	51.5

TABLE 2. Additional analysis for Sail spring done in November 2018.

$\mu\text{g L}^{-1}$										mg L^{-1}	
Al	As	B	Ba	Be	Co	Mn	Ni	Pb	Sr	SiO_2	$^{87}\text{Sr}/^{86}\text{Sr}$
2.82	122	11634	142	5.5	1.42	139	2.07	<0.05	4517	94.4	0.713354

TABLE 3. Main characteristics of the new *Chamaepinnularia* and similar small species.

Species	Valve length (μm)	Valve width (μm)	Number of striae in $10 \mu\text{m}$	Sternum	Pole shape	Central area shape	Ecology
<i>Chamaepinnularia salina</i>	4–13	2–3	30–40	visible in LM	rounded	rectangular fascia	brackish water (saline lake)
<i>Chamaepinnularia gandrpii</i> (Genkal & Yarushina 2018)	9.0–21.3	1.5–4.7	21–26	not visible in LM	broadly rounded to capitate	elliptical	freshwater/oligotrophic
<i>Chamaepinnularia gerlackei</i> (Van de Vijver <i>et al.</i> 2010)	12–30	3.3–4.9	17.5–20	not visible in LM	broadly rounded	rhomboid to rectangular	freshwater/aerophytic
<i>Chamaepinnularia krasskei</i> (Lange-Bertalot & Genkal 1999)	15–27	3.3–4	21–23	not visible in LM	broadly rounded	rhombic lanceolate	freshwater
<i>Chamaepinnularia minutissima</i> (Bourrelly & Manguin 1952)	12	2.5	9	not visible in LM	substrate	elliptical	aerophytic

<i>Chamaepinnularia rupicola</i> (Hustedt 1937, Simonsen 1987)	12–14	2.5–3	28–30	not visible in LM	broadly rounded to capitated	rhomboid to rectangul ar	freshwater/aerophytic
<i>Microcostatus turritus</i> (Antoniades <i>et al.</i> 2009)	8–9	3	35	visible in LM	rounded	stauros fascia	freshwater/aerophytic

ANNEX

<i>Chamaepinnularia abdita</i> Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia aerophila</i> Van de Vijver & Beyens 2002
<i>Chamaepinnularia alexandrowiczii</i> Witkowski, Lange-Bertalot & Metzeltin 2000
<i>Chamaepinnularia aliena</i> (Krasske) Van de Vijver & Le Cohu 2002
<i>Chamaepinnularia amphiborealis</i> Lange-Bertalot & Werum 2004
<i>Chamaepinnularia antarctica</i> Van de Vijver, Kopalová, Zidarova & E.J.Cox 2013
<i>Chamaepinnularia australomediocris</i> (Lange-Bertalot & Rol.Schmidt) Van de Vijver 2002
<i>Chamaepinnularia begeri</i> (Krasske) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia begeri</i> f. <i>constricta</i> (Krasske) Lange-Bertalot 2018
<i>Chamaepinnularia brasiliiana</i> Metzeltin & Lange-Bertalot 1998
<i>Chamaepinnularia brasilianopsis</i> Metzeltin & Lange-Bertalot 1998
<i>Chamaepinnularia bremensis</i> (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia bremoides</i> R.J.Flower 2005
<i>Chamaepinnularia brevissima</i> (Hustedt) C.E.Wetzel & Ector 2013
<i>Chamaepinnularia calida</i> (Hendey) Lange-Bertalot 1999
<i>Chamaepinnularia circumborealis</i> Lange-Bertalot 1999
<i>Chamaepinnularia clamans</i> (Hustedt) Witkowski, Lange-Bertalot & Metzeltin 2000
<i>Chamaepinnularia cymatopleura</i> (West & G.S.West) Cavacini 2006
<i>Chamaepinnularia distantepunctata</i> (Simonsen) Witkowski, Lange-Bertalot & Metzeltin 2000
<i>Chamaepinnularia elliptica</i> Zidarova, Kopalová & Van de Vijver 2016
<i>Chamaepinnularia elongata</i> (Manguin) Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia evanida</i> (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia falkiae</i> Lange-Bertalot & Werum 2004
<i>Chamaepinnularia ferrarioana</i> Metzeltin & Lange-Bertalot 1998
<i>Chamaepinnularia furtiva</i> (Manguin) Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia gandrupii</i> (J.B.Petersen) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia gandrupii</i> var. <i>simplex</i> (Krasske) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia gerlachei</i> Van de Vijver & Sterken 2010
<i>Chamaepinnularia gibsonii</i> Van de Vijver 2012
<i>Chamaepinnularia gracilistriata</i> Van de Vijver & Beyens 2002
<i>Chamaepinnularia hassiaca</i> (Krasske) Cantonati & Lange-Bertalot 2009
<i>Chamaepinnularia hippodontiforma</i> Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia justa</i> (Hustedt) Witkowski, Lange-Bertalot & Metzeltin 2000
<i>Chamaepinnularia krasskei</i> Lange-Bertalot apud Lange-Bertalot & Genkal 1999
<i>Chamaepinnularia krookiformis</i> (Krammer) Lange-Bertalot & Krammer in Lange-Bertalot & Genkal 1999
<i>Chamaepinnularia krookii</i> (Grunow) Lange-Bertalot & Krammer 1999
<i>Chamaepinnularia margaritacea</i> (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia margaritiana</i> (Witkowski) Witkowski in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia mediocriformis</i> (M.Coste & Ricard) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia mediocris</i> (Krasske) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia mediocris</i> var. <i>atomus</i> (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia minutissima</i> (Manguin) Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia muscicola</i> (J.B.Petersen) Kulikovskiy, Lange-Bertalot & Witowski 2010
<i>Chamaepinnularia obsoleta</i> (Hustedt) C.E.Wetzel & Ector 2013
<i>Chamaepinnularia oculus</i> (Østrup) Lange-Bertalot 1999
<i>Chamaepinnularia parsura</i> (Hustedt) C.E.Wetzel & Ector 2013
<i>Chamaepinnularia perfidissima</i> Lange-Bertalot, C.E.Wetzel & Ector in Wetzel <i>et al.</i> 2013
<i>Chamaepinnularia plinskii</i> Żelazna-Wieczorek & Olszyński 2016
<i>Chamaepinnularia reinventa</i> Lange-Bertalot & Wydrzycka 2001
<i>Chamaepinnularia reinventa</i> ssp. <i>urtroquarea</i> Wydrzycka & Lange-Bertalot 2001
<i>Chamaepinnularia resalutata</i> Lange-Bertalot & Wydrzycka 2001
<i>Chamaepinnularia rexii</i> Veselá & J.R.Johansen 2009

<i>Chamaepinnularia rhombelliptica</i> Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia rupicola</i> (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia schaupiana</i> Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia soehrensioides</i> Kulikowsky, Lange-Bertalot, Witkowski & Dorofeyuk 2010
<i>Chamaepinnularia soehrensis</i> (Krasske) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia soehrensis</i> var. <i>capitata</i> (Krasske) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia soehrensis</i> var. <i>hassiaca</i> (Krasske) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia submuscicola</i> (Krasske) Lange-Bertalot & Metzeltin 1998
<i>Chamaepinnularia thermophila</i> (Manguin) C.E.Wetzel & Ector 2016
<i>Chamaepinnularia truncata</i> (D.König) Witkowski, Lange-Bertalot & Metzeltin 2000
<i>Chamaepinnularia ventosa</i> (Hustedt) C.E.Wetzel & Ector 2013
<i>Chamaepinnularia vyvermanii</i> Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia weikertii</i> (Manguin) Metzeltin & Lange-Bertalot 2007
<i>Chamaepinnularia wiktoriae</i> (Witkowski & Lange-Bertalot) Witkowski & Lange-Bertalot in Lange-Bertalot & Metzeltin 1996
<i>Chamaepinnularia witkowskii</i> (Lange-Bertalot & Metzeltin) Kulikovskiy & Lange-Bertalot 2010