

New data on the ultrastructure of stomatocysts, plate and spines scales of *Chrysosphaerella coronacircumspina* (Chrysophyceae, Chromulinales)

Anna Yu. Bessudova

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, 3 Ulan-Batorskaya, Irkutsk, 664033, Russia

Alena D. Firsova

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, 3 Ulan-Batorskaya, Irkutsk, 664033, Russia

Yelena V. Likhoshway

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, 3 Ulan-Batorskaya, Irkutsk, 664033, Russia

An updated and refined description of the ultrastructure of siliceous elements – plate and spine scales, as well as the morphotype of the stomatocyst *Chrysosphaerella coronacircumspina* is provided using scanning and transmission electron microscopy. A wide range of sizes of plate scales of *C. coronacircumspina* allows us to distinguish two types of them – large and small elliptical. Also, single small subcircular plate scales were discovered for the first time. The spine scales of *C. coronacircumspina* can grow up to 22.8 μm in length. The collar of the found morphotypes of stomatocysts has a smaller diameter (2.6 μm) than previously described. In the studied reservoirs, *C. coronacircumspina* cells were found mainly in the summer and autumn season, and stomatocysts only in autumn.

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Corresponding author: Alena D. Firsova (adfir71@yandex.ru)

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Keywords

Chromulinales, *Chrysosphaerella*, ultrastructure, stomatocyst

Introduction

The genus *Chrysosphaerella* Lauterborn (1896: 16) belongs to the Class Chrysophyceae, Order Chromulinales, Family Chrysosphaerellaceae and, according to electron microscopy data, contains 12 species (Kapustin and Kulikovskiy 2022). These free-living microeukaryotes have two flagella of

unequal length and can form cell colonies. Their cells are covered with siliceous shield elements with a characteristic ultrastructure of plate and spine scales for each species. At the end of the growth season or under unfavorable conditions the cells form silica stomatocysts. Morphotypes of stomatocysts of different species may differ in shape, size, surface ornamentation, as well as features of the structure of the collar and pores. The genus *Chrysosphaerella* is known for a limited number of stomatocyst morphotypes (Duff et al. 1995). Currently, stomatocysts are known for three species of the genus *Chrysosphaerella* – *C. brevispina* Harris and Bradley, *C. longispina* Lauterborn (Voloshko 2016) and *C. coronacircumspina* Wujek et Kristiansen (Firsova et al. 2017). Since after the destruction of cells, their siliceous elements and individual stomatocysts remain in the samples, it is important to know about the limits of their morphological variability. Most species of the genus *Chrysosphaerella* are common inhabitants of northern reservoirs, but have different environmental requirements (Siver 1993; Voloshko 2016), so their exact species identification is important. The aim of this study was to extend the description of stomatocyst, plate and spine scales of *Chrysosphaerella coronacircumspina* based on the analysis of samples from a lake located in the basin of the Lower Yenisei, Southern Baikal and the Irkutsk Reservoir.

Materials and methods

The material was samples collected in September 2009 in Lake Sopochnaya Karga, the Yenisei River basin (Bessudova et al. 2018), as well as in June, August and October 2023 in Southern Baikal and the Irkutsk Reservoir. To detect *Chrysosphaerella* cells, 7–20 mL of a barometric sample was passed through a filter with a diameter of 13 mm and pores of 0.8 μm (Whatman Part of GE HealthCare, Chicago, IL, USA). Then the filter with the test material was dried at room temperature, coated with gold in a vacuum evaporator SDC 004 (SD 004 Balzers, Liechtenstein) and examined using a scanning electron microscope SEM QUANTA 200 (FEI Company; Hillsboro, OR, USA). The standard method of sample preparation for the detection of plate and spine scales using transmission electron microscopy (TEM) was given earlier (Bessudova et al. 2021).

Results

The first description of *C. coronacircumspina* (Wujek et al. 1977: 191–193) provides data on elliptical plate scales, 2–3 \times 0.7–1.7 μm . According to L. Voloshko (2016), the plate scales of *C. coronacircumspina* are larger, oval, 2–3.5 \times 1.5–2.5 μm . Since the size limits of plate scales vary widely, we propose to distinguish two types of plate scales, small and larger. We found both small and larger elliptical plate scales on the studied cells of *C. coronacircumspina*. Also, we found single, small subcircular plate scales, which were not previously reported and which we describe below. The size of the spines in the first description varies widely from 7–20 μm (Wujek et al. 1977). We found even longer spine scales. The following are extended descriptions of the ultrastructure of stomatocysts, plate and spine scales of the studied species.

Chrysosphaerella coronacircumspina (Fig. 1)

Description: The cells are usually single. There are two types of elliptical scales, small, 0.9–1.6 \times 0.7–1.2 μm and larger, 2–3.5 \times 1.4–2.5 μm (Fig. 1G, H, I). The subcircular scales, 1–1.3 μm in diameter, are rarely found (Fig. 1F, G). The plate scales have a raised, unpatterned rim along the edge, measuring 0.3–0.5 μm in width. On the inner surface of the rim, a series of short radial ribs, 0.09–0.13 μm in diameter, converge towards the center, enclosing a central unpatterned area of oval shape (Fig. 1E, F). The spine scales, measuring 7–22.8 μm in length (Fig. 1A, B, C), taper towards a forked tip and consist of a shaft and a primary base plate in the form of a funnel-shaped base, 3–6 μm in diameter to which a secondary base plate, attached forms a "crown" around the base of the spine (Fig. 1D, G, I). A large circular hole, 0.4–0.8 μm in diameter, is present at the base of the spine (Fig. 1D, I). The shaft's thickness from the base to the tip has a ratio of approximately 3:1.

Ecology: The cells of the species were found in the studied reservoirs in the summer and autumn season at a water temperature of 4.5–18.3 °C, pH = 7.3–8.7 (Bessudova et al. 2018; Bessudova et al. 2024). The maximum growth in Southern Baikal and the Irkutsk Reservoir was noted at a water temperature of 5.1–15.6 °C, pH = 7.6–8.7.

Stomatocyst 156, Zeeb and Smol 1993 (Fig. 2)

Biological affinity: *Chrysophaerella coronacircumspina* (Firsova et al. 2017)

Negative number: 19112 cysta_012

Location: Lake Baikal

SEM description: The stomatocyst is spherical, 8.7–10.7 µm in diameter or slightly oblate, 9.3–10.7 × 8.7–10.5 µm. The pore is regular, 0.7–1 µm in diameter (according to Duff et al. 1995 – 0.5–0.8 µm and up to 1 µm according to Shadrina and Safronova 2020) surrounded by a planar annulus (Firsova et al. 2017; Bazhenova 2021; according to Duff et al. 1995 – annulus is swollen), 0.8–1 µm width and a short, conical collar with a rounded outer margin, 2.6–3.9 µm in diameter (according to Duff et al. 1995 – 3.0–4.1 µm in diameter) and 0.3–0.7 µm height (according to Duff et al. 1995 – 0.2–0.7 µm height). The surface of the stomatocyst is smooth, without ornamentation. The ratio of the pore diameter to the diameter of the stomatocyst is 0.08–0.09.

References: The morphotype of the stomatocyst have previously been found in reservoirs in the USA (Sandgren and Carney 1983 as cyst 10; Zeeb and Smol 1993; Duff and Smol 1994; Zeeb 1994; Duff et al. 1995) and Russia (Firsova et al. 2017; Shadrina and Safronova 2020; Bazhenova 2021).

Ecology: Previously, morphotype of the stomatocyst were found in the reservoirs of Peterhof parks at a water temperature of 16.5–21.5 °C, pH = 7.2–8.0, conductivity of 461–532 µS cm⁻¹ (Shadrina and Safronova 2020) and Lake Baikal in September at a water temperature of 5.2–14 °C, pH about 8 (Bessudova et al. 2017; Firsova et al. 2017). In this study, stomatocysts were found in Southern Baikal in October at a water temperature of 8.8–9.6 °C, pH = 8.2–8.5.

Discussion

C. coronacircumspina belongs to the section *Brevispinae* Kapustin due to the presence of plate scales with a thickened oval ring in the central part on the exterior surface and ornaments with a scalloped oval shaped pattern on the undersurface, as well as spine scales consist of two base plates and a spine with a large hole at its base (Kapustin and Kulikovskiy 2022). *C. coronacircumspina* cells differ from other species in the section *Brevispinae* of spine scales morphology, their secondary base plate, attached to the primary base plate, forms a "crown" around the base of the spine. An analysis of the previously presented descriptions of *C. coronacircumspina* and the present ultrastructure study showed that *C. coronacircumspina* cells cover two types of plate scales, small and larger elliptical, subcircular are rarely found. The first description of the plate scales of *C. coronacircumspina* contained data on one type of plate scales corresponding to small and larger (Wujek et al. 1977). The studies of another author included only data on large plate scales, the size of which exceeded the first description (Voloshko 2016). In the first description, the length of the spine scales of *C. coronacircumspina* is 7–20 µm (Wujek et al. 1977), whereas in the other, only 10–16 µm (Voloshko 2016). In the lake located in the basin of the Lower Yenisei, Sopochnaya Karga, we previously found *C. coronacircumspina* cells covered with spine scales up to 22.8 µm (Fig. 1A) (Bessudova et al. 2018, Fig. 2B, C). This exceeds the maximum size for the previously described spine scales of the species. Spine scales with a maximum length of 16.6 µm were found in Southern Baikal. The difference in the size of plate and spine scales may be the result of intraspecific variability and continuous morphological changes in the ultrastructure of the siliceous elements of the cell.

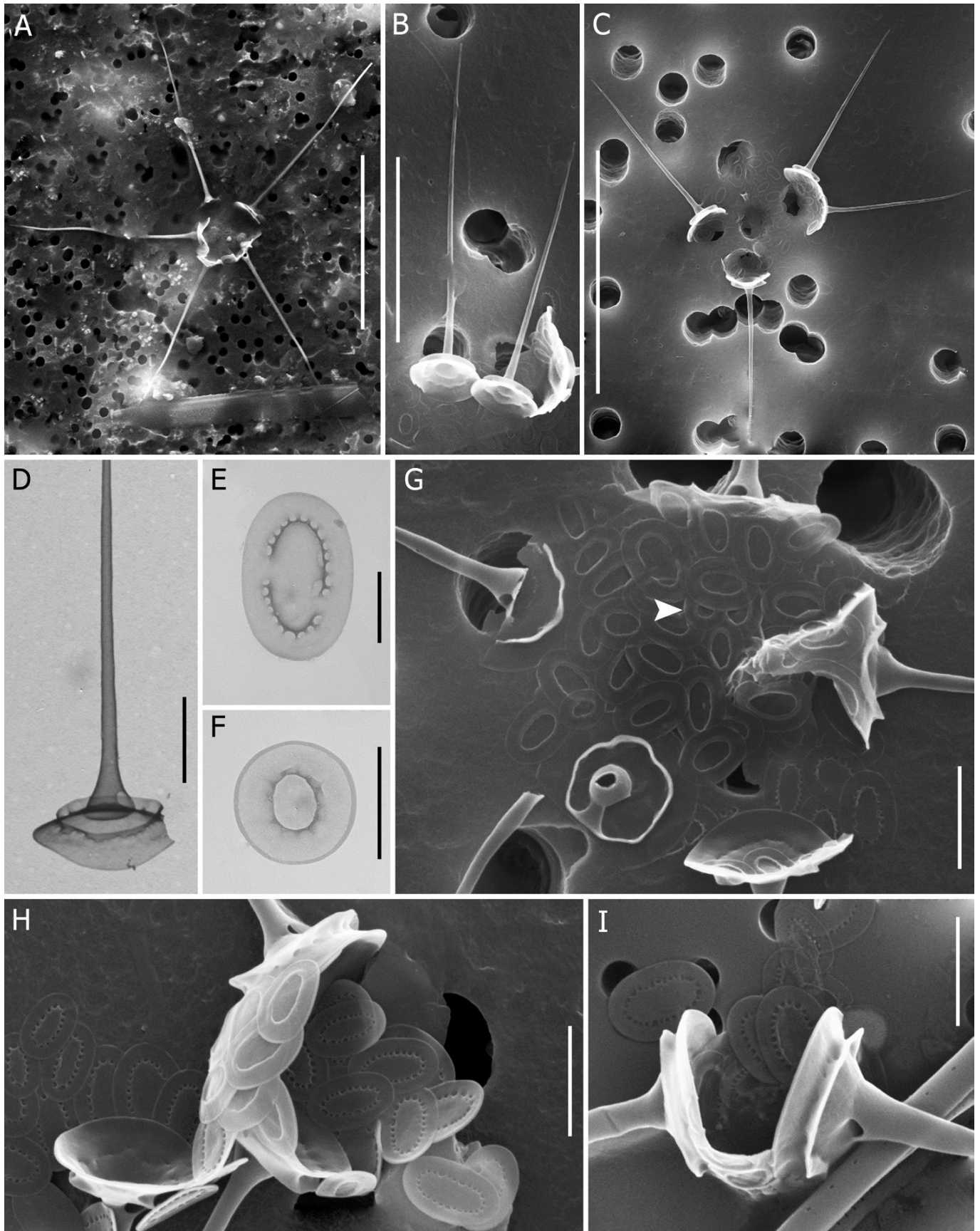


Figure 1. Micrographs of the *Chryso-sphaerella coronacircumspina*. SEM (A-C, G-I), TEM (D-F). **A**, a decayed cell with long spine scales (more than 20 μm), Sopochnaya Karga Lake, the Yenisei River basin; **B**, a long spine scales (more than 16 μm), Southern Baikal; **C**, a decayed cell, Irkutsk Reservoir; **D**, an individual spine scale, Southern Baikal; **E**, an individual elliptical

plate scale, Southern Baikal; **F**, an individual subcircular plate scale, Southern Baikal; **G**, the base plates of the spines and plate scales, small and larger elliptical, as well as the arrow shows a subcircular scale, Southern Baikal; **H, I**, the base plates of the spines and elliptical plate scales, Southern Baikal. Scale bars: **E, F** – 1 μm ; **D, G-I** – 2 μm ; **B** – 10 μm ; **A, C** – 20 μm

Previously, the morphology of stomatocysts of representatives of the genus *Chrysosphaerella* as a separate taxonomic criterion was not given sufficient attention. The known morphotypes of the stomatocysts *C. brevispina* and *C. longispina* (Stomatocysta 42, Duff and Smol 1989; Stomatocysta 49, Duff and Smol 1991; Stomatocysta 120, Duff and Smol 1992) have a simple structure: medium or largesized stomatocysts, spherical or slightly oblate, without a collar, with a concave or regular pore, sometimes surrounded by swollen pseudoannulus that appears planar in apical view, smooth or microtextured surface without ornamentation (Duff et al. 1995). The morphotypes belonging to *C. coronacircumspina* – Stomatocysta 156, Zeeb and Smol, is distinguished by a pore are surrounded by a short, conical wide collar with a rounded outer margin. According to Shadrina and Safronova (2020), the stomatocysts morphotypes found in the reservoirs of the Peterhof parks, the pore diameter, as in our "Baikal" morphotypes, reaches 1 μm . All the morphotypes that we found in Lake Baikal earlier (Firsova et al. 2017), and now had a flat annulus, unlike the described Zeeb and Smol (Duff et al. 1995).

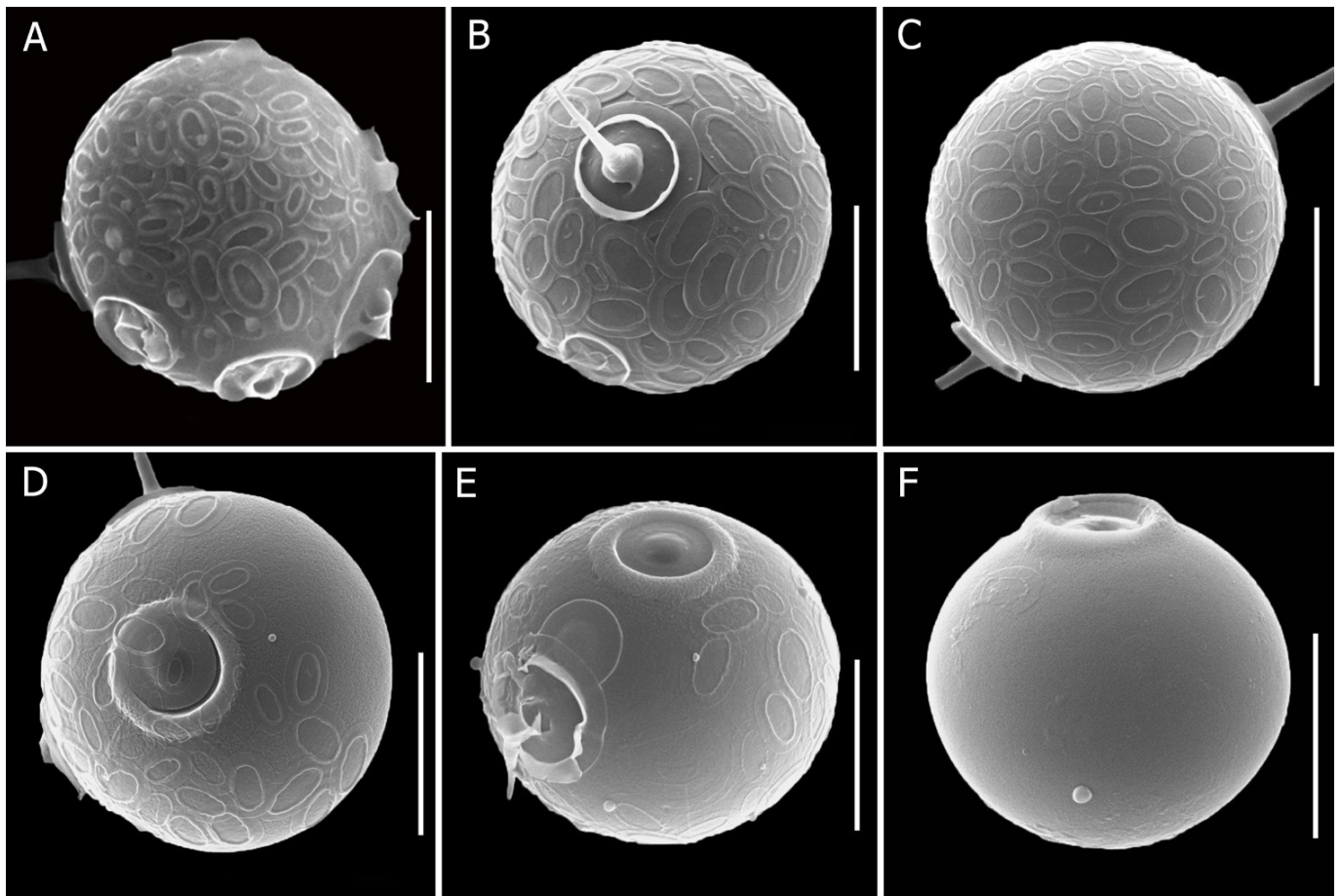


Figure 2. Micrographs of *C. coronacircumspina*. SEM. **A**, a cell covered plate scales and the base plates of the spines, Sopochnaya Karga Lake, the Yenisei River basin; **B, C**, cells covered plate scales and the base plates of the spines, Southern Baikal; **D-F**, stomatocysts covered with individual plate scales and the base plates of the spines, Southern Baikal. Scale bars 5 μm

Populations of silica-scaled chrysophytes may be potential markers of climate change (Wolfe and Perren 2001; Rühland et al. 2023). Each species shows growth during a certain season. However, the growth season may shift depending on the latitude of the reservoir location or the water temperature (Siver 1993). Using the example of a natural model with a temperature gradient of water – Southern Baikal – Irkutsk Reservoir, the growth of *C. coronacircumspina* cells was revealed

mainly in August-October, at temperature from 5.8 to 18.3 °C (Bessudova et al. 2024). However, in warmer waters there was a shift in phenology, *C. coronacircumspina* began to grow earlier - in June, where the water temperature already reached 9.9–11.5°C (Bessudova et al. 2024). The *C. coronacircumspina* cells we found in the Lower Yenisei basin grew at a temperature of 4.3 °C (Bessudova et al. 2018). Also, *C. coronacircumspina* cells were found in reservoirs of the Southern Urals at temperatures from 2.4 °C (Snitko et al. 2018) and in a reservoir in Portugal at temperatures up to 24.7 °C (Santos et al. 1996). The maximum growth in Southern Baikal and the Irkutsk Reservoir was noted at a water temperature of 5.1–15.6 °C.

Thus, the present study expands the range of the collar diameter of the *C. coronacircumspina* stomatocyst from 3.0–4.1 µm (according to Duff et al. 1995) to a minimum of 2.6 µm. Previously undescribed subcircular plate-scales were found, and the range of spine-scales was expanded from 7–20 µm (Wujek et al. 1977) to 22.8 µm. The temperature limits of *C. coronacircumspina* growth and cyst formation in the studied reservoirs have been revealed, which can later be applied in monitoring reservoirs and used as modern environmental markers. Data on the morphotype of stomatocyst can be used as paleoindicators when they are detected in microfossils.

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