Taxonomic revision of *Chamaepinnularia krookiformis* Lange–Bertalot et Krammer with a description of *Chamaepinnularia plinskii* sp. nov.

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**Abstract:** The benthic diatoms collected during our recent research in athalassic habitats exemplify a high morphological variability within *Chamaepinnularia krookiformis* Lange–Bertalot et Krammer. The species has already been known from freshwater ecosystems as well as from brackish waters in different regions of Europe. This variability has been also confirmed by the analysis of the literature. However, this is the first time that the morphological variability of the species has been addressed. In order to revise *C. krookiformis* and describe a new species, which has been distinguished on the basis of our material, we compared it with the type material of *Pinnularia krookiformis* Krammer 1992 from the periodic saline pool in Nordrhein–Westfalen (Germany). This study deals with a morphological analysis based on a large populations of *C. krookiformis* from Pelczyska (Central Poland). Selected morphological features were analysed, i.e.: valve length and width, number of striae in 10 µm, apices width, constrictions width, apices/constrictions width ratio, central–area length, and valve/central area length ratio. The results of light (LM) and scanning (SEM) microscopy, supported by multidimensional scaling (MDS), allowed for the distinction of three different morphotypes. Two of them match Krammer concept of *Pinnularia krookiformis* from 1992, while the third, occurring in our samples in three sampling sites, is clearly different. Thus, we described a new species *Chamaepinnularia plinskii* sp. nov., by the following major features: the valves are linear, symmetric, with rounded, capitate apices, the axial area is narrow, expanding to a wide central area forming a fascia, distal raphe endings are hooked to the same side of the valve and striae slightly radiate in the middle and in the rounded apices, becoming parallel in constrictions, 20–26 in 10 µm. This species occurs in inland waters with a wide range of chloride concentration, from 1000 to 3500 mg.l⁻¹.

**Key words:** brackish inland waters, *Pinnularia*, *Chamaepinnularia*, type material, new species

**INTRODUCTION**

The *Chamaepinnularia* Lange–Bertalot et Krammer genus was described in 1996, based on generitype *Chamaepinnularia vyvermanni* Lange–Bertalot et Krammer from the periodic saline pool in Nordrhein–Westfalen (Germany). According to the first description, representatives of the genus are characterized by small cells with their dimension not exceeding 25 µm in length and 4 µm in width (Lange–Bertalot & Metzeltin 1996); a slightly larger dimension range is characteristic for *Chamaepinnularia gerlachii* Van de Vijver et Sterken from Antarctica (Van de Vijver et al. 2010).

Diatom frustules of this genus are linear through linear–elliptic to linear–lanceolate shapes with rounded apices. The symmetry and arrangement of the raphe system do not deviate significantly from those observed in representatives of *Navicula Bory de Saint–Vincent* and *Pinnularia Ehrenberg*. Distal raphe endings are hooked towards the same direction. Proximal raphe endings are deflected. Distal raphe arms terminate on a helictoglossa on the valve internal side, while the proximal raphe endings are hooked. Externally, distinguishable single–row striae are formed out of one large areola create foramina, occluded by vela. Internally, striae are open with silica bridges (Lange–Bertalot & Metzeltin 1996; Wetzel et al. 2013).

The genus include species, which were earlier classified as *Navicula* or *Pinnularia* (Lange–Bertalot & Metzeltin 1996), e.g. those within the *Navicula* genus – *Chamaepinnularia begeri* (Krasske) Lange–Bertalot, *C. mediocris* (Krasske) Lange–Bertalot, *C. soehrensis* (Krasske) Lange–Bertalot et Krammer and those within the *Pinnularia* genus – *Chamaepin-
nularia krookii (Krammer) Lange–Bertalot et Karmmer, C. krookiformis (Krammer) Lange–Bertalot et Karmmer. Recently, Chamaepinnularia parsura (Hustedt) C.E. Wetzel et Ector, C. obsoleta (Hustedt) C.E. Wetzel et Ector, C. brevissima (Hustedt) C.E. Wetzel et Ector, C. peridisissma (Lange–Bertalot) C.E. Wetzel et Ector, and C. ventosi (Hustedt) C.E. Wetzel et Ector (Wetzel et al. 2013) were transferred to Chamaepinnularia.

Chamaepinnularia species occur in various fresh–water habitats and in waters with a varying range of salinity. Several species are typical of aerial habitats. Species occurring in antarctic regions are also known (Van de Vugt et al. 2010; Sterken et al. 2015). Some species were observed in the Sphagnum peat bogs (Kulikovskiy et al. 2010). In Europe, Chamaepinnularia schuuppiana Lange–Bertalot et Metzeltin was noted to occur in oligo–dystrophic waters in Finland but also in mire and fen pools (Cantonati & Lange–Bertalot 2009). It was also observed that several other species occurred in Europe both in low pH lakes (Kapetanovic et al. 2011) and in mountain fens with a high concentration of minerals (Frankova et al. 2009).

Chamaepinnularia krookiformis was noted to occur in the area of Poland both in seawaters and saline inland waters in the coastal zone (Witkowski 1994; Witkowska et al. 2000; Bak et al. 2006), in freshwater ecosystem such as upland rivers (Wojtal 2009; Peszek et al. 2015), quaternary springs located near Lodz (Zelazna–Wieczorek 2011), and in athalassic habitats in Central Poland (Zelazna–Wieczorek et al. 2015).

For many years, specimens of Chamaepinnularia krookiformis and C. krookii have been classified into different genera and species (Fig. 1). Navicula krookii was first described in 1882 by Grunow. The description contained information about a similarity of the species with Pinnularia globiceps Gregory (1856). The valve length was within 19–28 µm, its width was between 5.5 and 7 µm. The number of striae was between 15 and 17 in 10 µm (Grunow 1882). Several years later, Cleve (1891) transferred the species to the genus Pinnularia, at the same time introducing a correction in the species name giving it the name of Pinnularia krookii Cleve. The species’ name was given to honour Swedish botanist Krook (Cleve 1891). Several years later, Cleve (1895) considered P. krookii to be a variety of P. globiceps: P. globiceps var. krookii Cleve. The change resulted from the similarity of P. krookii to P. globiceps; the most significant difference was that of the cell size (Cleve 1895). Navicula ignobilis Krasske was described in 1938. However, specimens shown in the drawing were very similar to the specimens drawing of Navicula krookii Grunow published in 1882 (Grunow 1882; Krasske 1938). Krammer and Lange–Bertalot (1986), who referred to some earlier works by Grunow (1882), Cleve (1895) and Krasske (1938) marked Pinnularia “krocki” and Pinnularia ignobilis. Under the name of Pinnularia ignobilis, pictures showing in fact Pinnularia “krocki” (Krammer, Lange–Bertalot 1986, p. 416–417, tafel 187, figs 6–7, 9”; tafel 206, figs 12–19) were published. While photomicrographs of Pinnularia “krocki” Krammer, Lange–Bertalot (1986, p. 416, tafel 187, fig. 9; tafel 206, figs 8–9) showed the species that had not been previously described. Krammer (1992) described a new species of Pinnularia krookiformis making a reference to the specimens illustrated in the publication of Krammer & Lange–Bertalot (1986, p. 416, tafel 187, fig. 9; tafel 206, figs 8–9). Several years later, P. krookii and P. krookiformis were transferred to the Chamaepinnularia genus by Lange–Bertalot et Krammer in Lange–Bertalot & Genkal (1999) (see Fig. 1).

In this paper, we present results of the analysis of populations of Chamaepinnularia krookiformis from the Pelczyska (Central Poland) and the type material Pinnularia krookiformis from Nordrhein–Westfalen (Germany) described by Krammer (1992) with light (LM) and scanning electron microscope (SEM) techniques using multivariate analysis of multidimensional scaling (MDS) to explore the morphological variability of the different populations up to now identified as C. krookiformis. The analysis enabled us to describe one new species Chamaepinnularia plinski sp. nov., which is detailed below.

**Material and Methods**

The study area is located in Pelczyska (51°58’34.5”N, 19°14’20.4”E) (Central Poland), a village next to the city of Łęczyca in the Łódz Province. The study area included three sampling sites, i.e. outflow, ditch and pond, with a varying range of salinity. Chemical and physical parameters, also the structure of diatom assemblages, clearly differentiate the ditch environment from the two other ecosystems of an outflow and pond (Zelazna–Wieczorek et al. 2015). All the data is compiled in Table 1.

**The following samples have been examined.** 30 samples altogether were collected from all three habitats in 1964 (X, XI and XII); 1992 (IV); 1994 (VI, IX); 2013 (VII, XII); 2014 (II, III). For the diatomological analysis, 15 samples collected by Plinski in 1964/1965 (1969), 3 samples collected by Zelazna–Wieczorek (2002) in 1992/1994, and 12 samples collected recently, in 2013/2014, were used. The morphological analysis was based on samples in which Chamaepinnularia krookiformis occurred with relative abundance greater than 5%. The recent benthic samples were collected and purified according to Zelazna–Wieczorek (2011). The analysis of selected morphological features of Chamaepinnularia krookiformis was made by examining 4 permanent slides with a light microscope (Nikon YS 100 and Nikon Eclipse E400) with 1000× magnification (plan oil–immersion objective 100×/1.25). A total of 170 specimens were analysed in 4 permanent slides. The following features were taken into account: 1) valve width and 2) length, 3) number of striae in 10 µm, 4) width of capitate apices, 5) width of constrictions, 6) ratio of apices width and constrictions width, 7) length of central area and 8) ratio of valve length and central
area length (Fig. 2A) in the MDS analysis (Multi-Dimensional Scaling – MDS) based on the Bray–Curtis similarity (Żelazna–Wieczorek 2011) was used. The PRIMER 6.1.10 software was used for calculations.

The new permanent slides from unmouted type material of Pinnularia krookiformis Krämer 1992 from saline puddles in Nordrhein–Westfalen were prepared (Salzgebiet südlich von Salzkotten, Nordrhein–Westfalen Germany, leg. Wygasch, 17.04.1982 – KR604B Friedrich–Hustedt–Zentrum für Diatomeenforschung Institute for Polar and Marine Research, Bremerhaven). The unmouted sample was washed, first in 95% ethyl alcohol and then several times in deionized water. The new permanent slides were mounted with Naphrax® according to Żelazna–Wieczorek (2011). 45 specimens from the type material morphological features were analysed.

All materials were analysed using the Phenom Pro X (with 8 nm gold layer, at 10 kV) scanning microscope at the Laboratory of Microscopy Imaging & Specialist Biological Techniques at Faculty of Biology & Environmental Protection. SEM photomicrographs were taken at a resolution of 1024x1080 pixels.

RESULTS AND DISCUSSION

The Bray–Curtis similarity index was used to determine the similarity between Chamaeapinnularia krookiformis specimens. The MDS procedure distinguished three groups of specimens determined as: Morphotype 1 (N=66 specimens), Morphotype 2 (N=66 specimens) and Morphotype 3 (N=38 specimens) (Fig. 2B.). The variation range of morphological features of specimens and species variability by Lange–Bertalot & Metzeltin (1999) are listed in Table 2.

Chamaeapinnularia plinskii Żelazna–Wieczorek et Olszyński sp. nov. (Figs. 3–19)

Morphotype 1 (Fig. 2B)


Description: LM: Linear, symmetric valves with rounded capitate apices. Valve dimension: length 18–24 µm, width 4–5 µm, width of apices: 3–4 µm. The narrowest points of the valves are close to the capitate apices, width of constrictions 2.5–3.5 µm. The ratio of the width of apices to the width of constrictions is 0.9–1.4. The axial area is narrow, length 6–11 µm, width 3–5 µm, and expands to a wide central area, forming a fascia. The ratio of the valve length to the length of the central area is 1.9–3.4. Raphe is straight and filiform. Distal raphe endings are hooked towards the same direction. Proximal raphe endings are straight, expanded and pore–like. Striae are slightly–radiate in the middle and in the rounded apices to become parallel in the constrictions: 20–26 in 10 µm.

SEM: Externally, striae are composed of a chamber–like areola and covered by vela. Striae on the valve face are separated from the mantle striae by a hyaline area (Fig. 50). Distal raphe endings are hooked, curving down to the mantle (Fig. 51). Internally, striae on the valve face are interrupted and divided by a silica bridge to form two parts, the first one is small and rounded, and located on the valve face–mantle junction, while the second one is elongated, and located on the valve face (Fig. 52). Proximal raphe endings are hooked and deflected to the same direction, distal raphe endings terminate in a helictoglossa. The thick sternaum expands proximally to a wide, rhomboidal fascia, distally to polar bars (Fig. 52).

Holotype: slide: D.PEDB040713 No. 00065 Algae Collection Department of Algology and Mycology, University of Łódź. Holotype illustrated in Fig. 7 (designated here).

Type locality: POLAND. Łódź Province, Pelczyska village N 51°58'34.5", E 19°14'20.4", brackish inland waters.

Etymology: This species is named in honour of Professor Marcin Pliński, a Polish phycologist, who began exploring diatoms in Pelczyska village in 1964.

Differential diagnosis: Chamaeapinnularia plinskii possesses the characteristic feature of the genus, i.e. a chamber–like areola covered by vela divided into two parts by a silica bridge on the internal valve side (Lange–Bertalot & Metzeltin 1996). Unlike C. krookiformis, C. plinskii has wide fascia reaching the valve edge, a greater number of striae in 10 µm, wider constrictions and a lower ratio of the valve length to the width of the central area as shown in Table 2. C. plinskii may be also confused with Pinnularia bertrandii Krämer. However, P. bertrandii has a smaller number of striae in 10 µm, these are arranged more radially and they are shorter at the axial area apices. Frustules in P. bertrandii are larger and proximal endings of the raphe are slightly curved towards the same direction (Krämer 2000). Specimens similar to C. plinski were not found in the type material of Pinnularia krookiformis.

Ecology and Distribution: A description of Chamaeapinnularia plinskii was made based on specimens mainly from a small ditch through which water flows from the backfilled outflow. The water is characterized by slightly alkaline to alkaline conditions and high conductivity values up to 5170 µS.cm⁻¹. The concentration of chlorides varied from 1000 to 3500 mg.l⁻¹. As far as the type locality is concerned, this species was primarily found in benthic samples taken from the bottom of the ditch. C. plinskii was already reported in the freshwater springs of the Łódź Hills (Żelazna–Wieczorek 2011, p. 103, plate 103, fig. 34) wherein the concentration of chlorides was between 12.09
Table 1. Physical and chemical parameters of water at the studied sites analysed according to Želazna–Wieczorek et al. 2015 (modified).

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<td>7.20</td>
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<td>n/d</td>
<td>n/d</td>
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<td>1.4–1.9</td>
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<td>7.22</td>
<td>7.20</td>
<td>7.22</td>
<td>n/d</td>
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<td>n/d</td>
<td>n/d</td>
<td>4450.00</td>
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<tr>
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<td>7.00</td>
<td>7.20</td>
<td>7.22</td>
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<td>n/d</td>
<td>n/d</td>
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<td>3116.19</td>
<td>9172.03</td>
<td>3468.23</td>
<td>925.21</td>
<td>1585.00</td>
<td>1006.00</td>
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<td>2209.00</td>
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<td>2930.00</td>
<td>2930.00</td>
<td>2.0–3.5</td>
<td>1.9–3.5</td>
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Table 2. Range of selected morphological features of *Chamaepinnularia krookiformis* sensu lato in original and type material of *Pinnularia krookiformis* Krämer 1992.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Material from Pełczyska (Poland)</th>
<th>Chamaepinnularia plünski sp. nov. = Morphotype 1</th>
<th>Chamaepinnularia krookiformis Morphotype 2</th>
<th>Chamaepinnularia krookiformis Morphotype 3</th>
<th>Type material of <em>Pinnularia krookiformis</em> Krämer 1992</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>Width</td>
<td>Striae in 10 µm</td>
<td>Width of apices (µm)</td>
<td>Width of constrictions (µm)</td>
<td>Ratio of width of apices/width of constriction</td>
</tr>
<tr>
<td>(µm)</td>
<td>(µm)</td>
<td>4–5</td>
<td>3.0–4.0</td>
<td>2.5–3.5</td>
<td>0.9–1.4</td>
</tr>
<tr>
<td>18–24</td>
<td>18–24</td>
<td>17–22</td>
<td>16–20</td>
<td>2.5–3.0</td>
<td>1.3–2.0</td>
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<tr>
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<td>19–21</td>
<td>16–20</td>
<td>2.5–3.0</td>
<td>1.3–2.0</td>
<td>1.0–1.3</td>
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<tr>
<td>14–17</td>
<td>14–17</td>
<td>14–17</td>
<td>3.0–3.5</td>
<td>2.0–2.5</td>
<td>1.0–1.3</td>
</tr>
<tr>
<td>16–18</td>
<td>16–18</td>
<td>16–18</td>
<td>2.0–2.5</td>
<td>1.3–2.0</td>
<td>1.3–2.0</td>
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</table>

*Żelazna–Wieczorek & Olszyński: Taxonomic revision of Chamaepinnularia krookiformis*
Fig. 1. Timeline of interpretations of the *Chamaepinnularia krookiformis* species.
Fig. 2. The MDS procedure distinguished three groups of specimens based on eight features: (A) morphological features: (1) valve width and length, (3) number of striae in 10 µm; (4) width of capitate apices, (5) width of constrictions, (6) ratio of apices width to constrictions width, (7) length of central area, and (8) ratio of the valve length to the central area length; (B) MDS analysis revealed three morphotypes of Chamaepinnularia krookiformis.

to 19.49 mg.l\(^{-1}\). This species was noted in the costal shallows of Puck Bay (Witkowski 1994, p. 176–177, plate XXXVIII, fig. 16–17) where the concentration of chloride ions was higher. Żelazna–Wieczorek (2011) identified several specimens of this species in samples from springs; however, in samples from the Puck Bay the presence of this species was noted from rare to frequent (Witkowski 1994). In the present research, the species percentage exceeded 10% in samples from the ditch. Since Chamaepinnularia plinskii occurs largely in waters with an increased concentration of chloride ions (over 1000 mg.l\(^{-1}\) Cl\(^{-}\)), according to the halobion system of Van der Werr and Huls 1957–1974 (Denys et al. 1983; Żelazna–Wieczorek et al. 2015) Chamaepinnularia plinskii can be classified as a brackish species (the 4\(^{th}\) category of salinity).

Chamaepinnularia krookiformis (Krammer) Lange–Bertalot et Krammer 1999 (tafel 45, fig. 6–10)

Description species according to Krammer 1992

Valve length 14–40 µm; valve width 5–11 µm; number of striae 17–21/10 µm; ratio of the length to the width 2.8–4. The valve is elliptic in shape, convex, ends of the valve are rounded with constrictions. The axial area is narrow; the central area is wide and of rhomboid–elliptic shape. The raphe is straight and filiform.

Based on the MDS analysis two morphological forms defined as: Morphotype 2 and Morphotype 3, Chamaepinnularia krookiformis have been distinguished (Fig. 2B). Morphotype 2 (Fig. 20–31) and Morphotype 3 (Fig. 32–49).

Description: LM: the main differentiating feature of Morphotype 2 from Morphotype 3 is the central area length and width. In Morphotype 2, the central area dimensions are 6–10 µm in length and 2–3.5 µm in width. In specimens belonging to Morphotype 3, the central area dimensions are 11–14 µm in length and 2.5–5.0 µm in width; the ratio of the valve length to the central area length is 1.4–1.9. Morphotype 3 has more capitate apices than Morphotype 2 (Table 2).

SEM: Externally, striae are composed of simple, chamber–like areole, with external openings covered by vela. The valve striae are separated from the mantle striae by a hyaline area along the entire valve circumference. The raphe is straight, its proximal endings have a tear drop shape. Distal raphe endings are hooked in the case of Morphotype 2 (Figs 53–55), while they are strongly hooked in the case of Morphotype 3 (Figs. 56–58). Internal view: striae make foramina, they are arranged in the same way as those on the ex-
ternal side. Striae on the valve face are interrupted and separated by a silica bridge to form two parts, the first one is small and rounded and is located on the valve face–mantle junction, the second one is elongated, and located on the valve face. Raphe is straight, distal endings form a helictoglossa, the proximal ones are hooked in the same direction.

Specimens from the type material of *Pinnularia krookiformis* correspond to both morphotypes in the recent material: Morphotype 2 (Figs 62–69) and Morphotype 3 (Figs 70–77). The analysis of morphological features of Morphotypes 2 and 3 in SEM (Figs 59–61) allows a high similarity between the type and recent material to be confirmed. However, the valve dimensions in the
Figs 50–52. SEM images of *Chamaepinnularia plinskii* sp. nov. from Pelczyska (Central Poland): (50) External view, (arrow A) striae composed of chamber-like areola and covered by vela, (arrow B) striae on the valve face separate from the mantle striae by a hyaline area; (51) external view, (arrow) distal raphe endings hooked, curving down to the mantle; (52) internal view, (arrow A) silica bridge, divided areola, (arrow B) thick sternum forming a fascia. Scale bars 5 µm.

Figs 53–55. SEM images of *Chamaepinnularia krookiformis* – Morphotype 2 from Pelczyska (Central Poland): (53–54) internal view, chamber-like areola, tear-drop-shaped proximal raphe endings, distal raphe endings strongly hooked; (55) internal view, distal raphe endings form helictoglossa, the proximal ones are hooked and turned towards the same valve edge. Scale bars 5 µm.

Figs 56–58. SEM images of *Chamaepinnularia krookiformis* – Morphotype 3 from Pelczyska (Central Poland): (56–57) internal view, chamber-like areola, tear-drop-shaped proximal raphe endings, distal raphe endings strongly hooked; (58) internal view, distal raphe endings form helictoglossa, the proximal ones are hooked and turned towards the same side of the valve. Scale bars 5 µm.

Figs 59–61. SEM photomicrographs of the type material of *Pinnularia krookiformis* Krammer from Germany [= *Chamaepinnularia krookiformis* (Krammer) Lange-Bertalot et Krammer 1999]: (59) valve external view, (60) valve internal view; (61) valve internal view with hooked distal raphe endings. Scale bars 5 µm.
recent and type material are narrower (length 14–22 µm and width 4–5 µm) than those given in the description by Krammer (1992) for *Pinnularia krookiformis* (length 14–40 µm, width 5–11 µm).

**Summary**

In the athalassic habitats studied, a wide morphological variability of forms earlier classified as *Chamaepinnularia krookiformis* was observed. The analysis allowed three morphotypes to be distinguished. Morphotype 1 was distinguished as a new species, *Chamaepinnularia plinskii*, based on the following most important features: the presence of a wide fascia reaching the valve margin, number of striae in 10 µm, wider apical conceptacle, and classification as a separate species.

*Chamaepinnularia plinskii* and *C. krookiformis*, occurred in the same habitats in Poland. The species were noted in various types of ecosystems as *Chamaepinnularia krookiformis*; however, the analysis of micrographs in publications enables their verification and classification as *Chamaepinnularia krookiformis* and *C. plinskii*. Żelazna–Wieczorek (2011) observed both species in the springs of the Łódź Hills, i.e.: *Chamaepinnularia plinskii* as *C. cf. krookiformis* (plate 103, fig. 34) and *Chamaepinnularia krookiformis* – Morphotype 3 (plate 103, figs 35–36). Wottal (2009) noted *Chamaepinnularia krookiformis* in the Kobylanka River; however, the photomicrographs documentation does not provide sufficient information for the correct classification of Morphotype 2 and 3 (p. 164, plate 26, fig. 5; plate 82, fig. 3). In Puck Bay, Witkowski (1994) could also observe the occurrence of *Chamaepinnularia krookiformis* Morphotype 2 (plate XXXVIII, figs 14–15) and *Chamaepinnularia plinskii* (Plate XXXVIII, Figs 16–17).

*Chamaepinnularia plinskii* is a species occurring in various types of aquatic ecosystems which dry up periodically; however, conditions favorable for its development are alkaline water with high chlorides concentrations.

**References**


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