Analysis of the type material of *Gomphosphenia tackei* (Bacillariophyceae) and comparison to epizoic diatom populations on freshwater snails

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Background and aims – Hustedt (1942) originally described *Gomphosphenia tackei* from Germany under the name *Gomphonema tackei*. Because of the small cell size and the lack of scanning electron microscopy (SEM) images from the type material, it is often confused with other species from this genus, especially with *G. stoermeri*. The aim of this paper was to present detailed morphological characteristics of *G. tackei* based on the analysis of the type material and of several epizoic populations from Central Europe.

Material and methods – The material in this study was collected from the shells of the freshwater snails *Lymnaea stagnalis*, *Planorbarius corneus*, and *Planorbis planorbis*. Additionally, for an unambiguous species identification, the type material for *Gomphosphenia tackei* was analyzed using light and scanning electron microscopes.

Key results – The presence of *Gomphosphenia tackei* was confirmed in the studied material. The largest population (up to 19%) was recorded on the shell surfaces of living snails, whereas on empty shells, the diatom did not seem to be present or only in very low numbers. Valves are typically clavate with rounded apices. Valves are frequently observed in girdle view, often joint together in pairs. The valves in the studied populations had a valve length of 7–29 µm, a valve width of 3–4 µm, and a stria density of 25–29 striae in 10 µm. In the type population, valve length ranged from 7.5 to 27 µm with a valve width of 3.0–4.0 µm and a stria density of 23–29 striae per 10 µm. Striae were composed of 2–4 elongated to rounded areolae per stria. At the apices, the striae were composed of one single areola. The cells were attached to the substratum by their footpole.

Conclusion – Published illustrations of *Gomphosphenia tackei* do not always correctly represent this species. Individual cells are attached to the substratum by secreted mucilage, probably via their areolae or girdle band pores located on the footpole.

Keywords – Bacillariophyceae; Central Europe; diatoms; ecology; epizoic; gastropods; *Gomphosphenia*; *Gomphosphenia stoermeri*; Hustedt collection; mucilage secreting.
INTRODUCTION

The genus *Gomphosphenia* Lange-Bert. was separated from *Gomphonema* Ehrenb. (Ehrenberg 1832) based on several ultrastructural features, such as the raphe structure (straight raphe branches – internal T-shaped central raphe endings), stria and areola morphology, the absence of an apical porefield and the absence of isolated stigma (Lange-Bertalot 1995). Typus generis for the new genus became *G. lingulatiformis* (Lange-Bert. & E.Richardt) Lange-Bert.

*Gomphosphenia tackei* (Hust.) Lange-Bert. was originally described under the name *Gomphonema tackei* Hust. from boggy meadows in Germany (Hustedt 1942). The species is characterized by its small valve size and dense striation. Because of the small cell size and the lack of detailed scanning electron microscopy images (SEM) from the type material, the species is often confused with similar *Gomphosphenia* species such as primarily *G. stoermeri* Kociolek & E.W.Thomas. Moreover, the ecological preferences of *G. tackei* are also insufficiently known due to its rare occurrence and often incorrect identification.

In the present study, valves of *G. tackei* were found on the shells of freshwater snails. Previous literature on epizoic diatoms, especially from snails, is extremely limited and mainly deal with the relationships between diatoms and snails or other invertebrates, both freshwater and marine (Falniowski et al. 1988; Gillan & Cadée 2000; Cante et al. 2008; Radea et al. 2008; D’Alelio et al. 2011; Totti et al. 2011).

This study presents a morphological characterization of *Gomphosphenia tackei*, and for the first time, compares it with the type material of the species using light microscopy (LM) and scanning electron microscopy (SEM). It also attempts to improve our ecological knowledge of *G. tackei*, especially with regard to its substratum preferences.

MATERIAL AND METHODS

The material for this study was collected from the shells of several living freshwater snails: *Lymnaea stagnalis* (Linnaeus, 1758), *Planorbarius corneus* (Linnaeus, 1758), and *Planorbis planorbis* (Linnaeus, 1758), as well as from the empty shells of the same species (table 1). Sampling was performed in a small waterbody located in the territory of the city of Rzeszów, Poland (50°00′54.50″N, 21°59′47.89″E). Material was taken by scraping off the snail shells using a plastic brush. A total of 10 samples was collected, seven from living snails and three from the shells of dead specimens (table 1).

Samples were cleaned in 30% H$_2$O$_2$ with the addition of HCl. After complete dissolution of all organic matter, the samples were washed several times in a centrifuge at 2500 rpm, until all hydrogen peroxide had been removed. The resulting cleaned material was permanently mounted using the synthetic Pleurax resin (refractive index 1.75), identified and counted under a Carl Zeiss Axio Imager A2 light microscope (LM) at 1000× magnification equipped with differential interference contrast (DIC). Stubs were made for SEM analysis and observed using a Hitachi SU8010 scanning electron microscope.

In order to observe the diatom life form, diatom assemblages were directly examined with SEM on the *P. planorbis* shell surface. One snail, preserved in ethyl alcohol, was air-dried, mounted onto aluminium stubs, coated with 20 nm of gold using a turbo-pumped Quorum Q 150T ES coater, and subsequently observed using the Hitachi SU8010 SEM.

Additionally, the type material for *Gomphosphenia tackei* was analyzed using both LM and SEM observations. The original sample was retrieved from the Hustedt collection (BRM, Bremerhaven, Germany). Sample no. E1016 was collected on 28 Jul. 1937 from mosses near a small waterbody on the Warturmer Heerstrasse 5, Bremen, Germany. Samples and slides are conserved in the author’s diatom collection at the University of Rzeszów (Poland).

Diatoms were identified according to Krammer & Lange-Bertalot (1986) and Lange-Bertalot et al. (2017). Snails were identified using Piechocki & Wawrzyńiak-Wydrowska (2016). Diatom terminology follows Round et al. (1990). The species composition was determined by counting 300 valves on randomly selected fields under LM.

Conductivity, temperature, and pH of the sampling locality were measured in situ using a MARTINI PH65 and a MARTINI EC59. A detailed physico-chemical analysis of

| No. sample | Sampling date | Substratum                  |
|------------|---------------|------------------------------|
| E 1016     | 28 Jul. 1937  | *Lymnaea stagnalis* (Linnaeus, 1758); live |
| 2017/43    |               | *Planorbarius corneus* (Linnaeus, 1758); live |
| 2017/45    | 15 May 2017   | *Lymnaea stagnalis* (Linnaeus, 1758); live |
| 2017/46    |               | *Planorbarius corneus* (Linnaeus, 1758); empty shell |
| 2017/365   |               | *Planorbarius corneus* (Linnaeus, 1758); live |
| 2017/366   |               | *Lymnaea stagnalis* (Linnaeus, 1758); live |
| 2017/367   | 10 Sep. 2017  | *Lymnaea stagnalis* (Linnaeus, 1758); live |
| 2017/368   |               | *Lymnaea stagnalis* (Linnaeus, 1758); empty shell |
| 2017/369   |               | *Planorbarius corneus* (Linnaeus, 1758); live |
| 2017/370   |               | *Planorbarius corneus* (Linnaeus, 1758); empty shell |
Table 2 – Physico-chemical parameters of the sampling locality.

| Parameter            | Value       |
|----------------------|-------------|
| Temperature [°C]     | 18.5–24.5   |
| pH                   | 6.5–7.4     |
| Conductivity [µS×cm⁻¹] | 333–405   |
| Cl⁻ [mg×l⁻¹]        | 13.58–14.50 |
| NO₃ [mg×l⁻¹]        | < 0.01–0.32 |
| PO₄³⁻ [mg×l⁻¹]      | < 0.01     |
| SO₄²⁻ [mg×l⁻¹]      | 3.63–16.45  |
| Na⁺ [mg×l⁻¹]        | 13.94–15.38 |
| NH₄⁺ [mg×l⁻¹]       | < 0.01–0.171|
| K⁺ [mg×l⁻¹]         | 2.14–3.59   |
| Mg²⁺ [mg×l⁻¹]       | 11.44–26.32 |
| Ca²⁺ [mg×l⁻¹]       | 53.49–57.82 |

Additional observations in SEM (fig. 3)

*Gomphosphenia tackei* was observed mostly on the shells of living snails. Cells often formed colonies, consisting of less than ten individuals (fig. 3A–B). The cells were attached to the substratum by small mucilage excretions at the footpole apex of the valves (despite the lack of any typical secretion structures) (fig. 3C–F).

Species co-occurring in the examined samples

Both on living individuals and empty shells, the most abundant diatom species included *Cocconeis lineata* Ehrenb. (17.1%), *Gomphonema paludosum* E.Reichardt (31.0%), *Lemnicola hungarica* (Grunow) Round & Basson (8.1–10.9%), *Nitzschia amphibia* Grunow (8.2%), *Planolithidium frequentissimum* (Lange-Bert.) Lange-Bert. (6.8–21.8%), *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bert. (9.6–45.2%), and *Sellaphora nigri* (De Notaris) C.E.Wetzel & Ector (6.8–21.8%).

In the type material, the number of *Gomphosphenia tackei* valves reached ca 9% of the diatom assemblage. Most diatom taxa in the material occurred in low numbers, usually a few valves per species, and presented highly variable ecological preferences. On the one hand, taxa related to oligotrophy and low pH such as *Cavinula variostratiata* (Krasske) D.G.Mann & A.J.Stickle and various *Eunotia* species (*E. bidens* Ehrenb., *E. glacialisipiosa* Lange-Bert. & Cantonati, *E. sedina* Lange-Bert., Bąk & Witkowski, and *E. seminulum* Nörpel-Schempp & Lange-Bert.) occurred in the type material. But on the other hand, also taxa showing a wide ecological spectrum, tolerating eutrophic and highly conductive waters (such as *Epithemia adnata* (Kützing) Brēb., *E. turgida* (Ehrenb.) Kütz., *Navicula cineta* (Ehrenb.) Ralfs, *N. veneta* Kütz., *Nitzschia amphibia* Grunow, and *Rhopalodia gibba* (Ehrenb.) O.Müller) were recorded. Additionally, several brackish and marine taxa such as *Cosmioneis pusilla* (W.Smith) D.G.Mann & A.J.Stickle and *Tryblionella navicularis* (Brēb.) Ralfs were observed as well.

RESULTS

The results of the physico-chemical water analysis indicated a circumneutral pH and moderate electrolyte content. For each sampling period, the amount of nutrients was low (table 2).

The occurrence of *Gomphosphenia tackei* was confirmed in the examined material. The largest population (up to 19% of all counted diatoms) was recorded on the surface of the shells of living snails. On empty shells, the species was not observed or only recorded with very low frequencies (up to only a few valves per slide).

Detailed morphological description of *Gomphosphenia tackei* valves (figs 1–2)

Valves typically clavate having rounded apices. Valves frequently observed in LM lying in girdle view, often in pairs (fig. 1B, G–K). Valve dimensions of the studied population (n=100): length 7–29 µm, width 3–4 µm, stria density 25–29 striae in 10 µm. For the type population, the following dimensions were observed (n=20): length 7.5–27.0 µm, width 3.0–4.0 µm, stria density 23–29 striae in 10 µm. Central area rounded, bordered by a few shortened striae. Axial area narrow and linear. Striae slightly radiated to parallel at the valve apices, composed of 2–4 elongated to rounded areolae (fig. 2A–G, J–L, N–O). At the apices, striae composed of a single areola (fig. 2D, G, O). Raphe straight, filiform, with recognizable helictoglossae (fig. 1A, C–F). In SEM, external raphe endings small, tear-drop shaped (fig. 2D, R). Internal raphe endings T-shaped (fig. 2I, L). Terminal raphe endings terminating on large, elongated helictoglossae (fig. 2P–Q). In girdle view, frustules cuneate (fig. 1B, G–K). One row of areolae present surrounding the valve mantle (fig. 2D–E, P).

DISCUSSION

The valve dimensions of *G. tackei*, both from the studied samples and from the type material, coincided with those from the literature (Hustedt 1942; Krammer & Lange-Bertalot 1986; Bąk et al. 2012). A few valves were slightly smaller, but the striaion and valve shape were typical for the species.

Our SEM analysis of the type material clearly indicates that the illustrated valves in *Gomphosphenia tackei* are not conspecific with the type of *G. tuckei*, but resemble more the similar *Gomphosphenia stenneri* Kociolek & E.W.Thomas. The latter species was described from the Great Smoky Mountains National Park, USA (Thomas et al. 2009) and is also reported from Russia (Genkal & Yarushina 2016) and from several Swedish rivers (Van de Vijver et al. 2012). In contrast, other publications incorrectly cite the species (Lange-Bertalot & Krammer 1987: plate 40, figures 1–5; Rumrich et al. 2000: plate 132,
Figure 1 – LM images of *Gomphosphenia tacketi*. A–B. Specimens from the type material. C–K. Specimens from snail shells. A, C–E. Valve face views of entire valves. B, G–K. Valves in girdle view. F. Initial valve. Scale bar = 10 µm.
Figure 2 – SEM images of *Gomphosphenia tackei*. A–I. Valves from Hustedt type material. A–C. External view of the entire valve. D–E. External view of the valve headpole. F. External view of the central area. G. External view of the valve footpole. H. Internal view of the valve. I. Internal view of the central area. J–R. Valves from our study material. J–L, N. External view of the entire valve. M. Internal view of the valve. O. External view of the footpole and the central area. P. Internal view of the headpole. Q. Internal view of the footpole with helicoglossae. R. Internal view of the central area. Scale bars: A–C, H, J–N = 5 µm; D–G, I, O, Q = 2 µm; P, R = 1 µm.
In other studies dealing with Russian diatoms (Kulikovskiy et al. 2016: plate 124, figures 52–56; Potapova 2014: figure 344), and the valves identified as G. tacei most likely also represent Gomphosphenia stoermeri. The SEM analysis of the type material of G. tacei allows not only to eliminate future mistakes in the identification, it also offers the possibility to verify earlier studies. Both species are easily distinguished using SEM observations. In contrast to Gomphosphenia tacei, valves of G. stoermeri show small, blunt marginal spines. The striae of both species are also different as they are composed of 2–4 areolae in G. tacei, but of only one transapically elongated, slit-shaped areola in G. stoermeri (Thomas et al. 2009).

Following frequent mistakes in the correct identification of G. tacei, the real occurrence and ecology of this species are difficult to determine. The observations of the type material do not allow an unambiguous ecological characterization of the diatom community due to the considerable diversity of ecological preferences of diatoms in the sample, making it difficult to determine the ecological preferences of G. tacei. On the other hand, a study carried out in the reservoir from where the snail shells were sampled for our study did not report G. tacei (Kocielska-Streb et al. 2014). In more recent studies, G. tacei occurred mostly on the shells of living freshwater snails. On dead individuals, the species was much less frequent. Based on the ecological preferences of the studied snails, it is clear that they mostly prefer standing to slow-moving waters, with a eutrophic muddy substratum (Piechocki & Wawrzyniak-Wydrowska 2016), which corresponds to reservoir environment conditions. Gomphosphenia tacei is likely to be only associated with epibiont communities, but its rare occurrence makes it impossible to precisely determine the correct ecological preferences of this species.

The genus Gomphosphenia is characterized by a lack of apical porefields producing mucilage stalks (Round et al. 1990). Our observation of G. tacei shows that cells of this species are devoid of secretion structures, but are nevertheless able to attach to substrates. The cells are attached by secreting mucilage, probably originating from areolae or girdle band pores located on the footpole. In most cases, mucilage production occurs from pores at the apex of the cells (apical porefields) or through the raphe slit (Round et al. 1990; Aumeier & Menzel 2012). Detailed information about the secretion of mucilage without specialized structures is however lacking, although some genera are able to form mucilage tubes, as can be observed in some species belonging to the genera Amphipleura, Cymbella, Gyrosigma, Navicula, and Nitzschia (Round et al. 1990). For example, in Encyonema ventricosum, mucilage is secreted through the areolae but in same cells mucilage could also be formed through the raphe. In the latter species, mucilage plays an important role in the cell division process by fastening cells together (Bedoshvili et al. 2018). This suggests that more taxa can also produce pads or stalks even lacking apical porefields.

Future studies on the correct identification will be needed to increase our knowledge of the ecological preferences of this species and its ability to adhere its cells to substrates.

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Figure 3 – SEM images of Gomphosphenia tacei attached directly to snail shells. A–B. Specimens in colonies. C–D. Single specimens attached to snail shell. E–F. Footpole with mucilage excretions. Scale bars: A–D, F = 5 µm; E = 2 µm.
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