

**DIATOM COMMUNITY COMPOSITION
WITHIN *OPHRYDIUM* COLONIES IN NORTHERN MICHIGAN
AND THE DESCRIPTION OF A NEW SPECIES OF
ENCYONOPSIS KRAMMER**

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ABSTRACT

Among the diverse group of organisms categorized as algae, there are some common and well understood symbiotic relationships, such as those between protozoa and *Zoochlorella*. However, there are some similar relationships that have received less attention, such as that between the colonial ciliate *Ophrydium* and diatoms. This study investigates this relationship, specifically comparing the diatom communities within *Ophrydium* colonies with those of the water in which they occur. *Ophrydium* and benthic algal samples were collected from three lakes in northern Michigan (Larks Lake, French Farm Lake, and Osmun Lake). Permanent diatom slides were prepared, and the diatom community composition of *Ophrydium* samples and samples from surrounding periphyton in each lake were analyzed and individual algae were counted and identified. A Jaccard Similarity Index suggests that some of the *Ophrydium* samples were more similar to other *Ophrydium* samples than they were to benthic samples from the same lake. When accounting for species relative abundance through the use of the Shannon-Wiener Index, the stark difference in diversity between benthic and *Ophrydium* samples was revealed, indicating much greater diversity in benthic algal samples. This difference in diversity was greatly influenced by one diatom species, which does not appear to have been described in existing literature which is described here as a new species, *Encyonopsis ophrydiensis* sp. nov., and which accounted for 75% to 93% of the diatom species composition in the *Ophrydium* samples. This species showed a preference for the habitat offered within *Ophrydium* colonies and was not identified in any benthic algal samples. Although it shares some similarities with other *Encyonopsis* species, its unique shape and affinity for *Ophrydium* colonies suggest that it is a newly discovered species. Further research is required to understand how the relationship between *Ophrydium* and *Encyonopsis ophrydiensis* sp. nov. evolved and how each organism is impacted by this symbiosis.

Keywords: *Ophrydium*, diatoms, *Encyonopsis*, new species

INTRODUCTION

Algae are a diverse group of organisms that find many unique ways to exploit their environment. One way in which they may achieve this is through symbiotic relationships. Symbiotic relationships of algae with lichens (e.g., Ahmadjian and

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Jacobs 1981) and corals (Muscantine and Porter 1977) are well-known, but other associations, with groups such as amoebae and ciliated protozoa, are less well known. One example of this kind of relationship is the green algae in the genus *Zoochlorella* K. Brandt living within cells of the ciliated genus *Ophrydium* Bory. *Ophrydium* is classified in the order Peritrichida, as they are single-celled protozoa containing endosymbiotic *zoochlorellae* (Woelfl et al. 2010). The most common endosymbiotic *Zoochlorella* species is *Zoochlorella parasitica* K.Brandt (Winkler and Corliss 1965). Within the cell of the protozoa live photosynthetic *Z. parasitica*, which provides the *Ophrydium* with carbohydrates (Sand-Jensen et al. 1997). These protozoa also filter feed on bacteria in order to obtain additional nutrients. The carbohydrates from *Z. parasitica* are used to build a thick mucilage around the protozoa that can combine with the mucilage of other *Ophrydium* cells, thereby forming a large colony. Each individual within a colony is in its own cylindrically shaped tube, or lorica, which prevents the individual from touching the mucilage matrix (Winkler and Corliss 1965). Individuals are attached to each other by scopula, or basal ciliature. The mucilage holding *Ophrydium* loricas together contains diatoms and soft algae, as well as heliozoans, bacteria, rotifers, nematodes, other ciliates, and crustaceans (Duval and Margulis 1995).

Ophrydium spend a majority of its life benthically in oligotrophic lakes and slow creek beds that lack excess chlorophyll a (Woelfl et al. 2010; Oberholster et al. 2010). Eventually they become planktonic once their colony produces enough gases from photosynthesizing *Zoochlorella* to promote buoyancy (Sand-Jensen et al. 1994). Normally, colonies remain benthic, often attached to rocks, plants, and, in northern Michigan, often on the macro alga *Chara*. Sometimes young colonies can be very dense and firm like a glass marble. Older colonies can take on a squashed doughnut shape, in which their width is larger than their height and the center caves in on one side (Oberholster et al. 2010; Winkler and Corliss 1965). This enables the colony to move up and down the water column. Becoming mobile, however, can be risky for the longevity of the colony as it may be destroyed by external disturbances that break the mucilage apart, thereby causing *Ophrydium* individuals to abandon the colony (Sand-Jensen et al. 1994). It is unclear what the fate of the *Zoochlorella* is in these situations.

Individual *Ophrydium* cells can eject themselves from the colony and become free living, using their buccula (or mouth) cilia and locomotor fringe to propel themselves through the water (Winkler and Corliss 1965). Seasonally, the majority of a colony may asexually produce telotrochs, or "larvae," that bud off from the adult cell and attach to a nearby substrate. This is one of the few times an *Ophrydium* cell becomes free floating and serves as a successful dispersive strategy for the colony (Winkler and Corliss 1965).

Previous research has been conducted on the endosymbiont *Zoochlorella* and their *Ophrydium* hosts, while research into the composition of algal communities, specifically diatoms, within the mucilage of their colonies, is less common. Geitler (1975) explored the algal species found within *Ophrydium versatile*. Czarnecki (1995) added to the existing body of research, contributing data for diatom composition within *Ophrydium* assemblages at Lake Itasca State Park. He concluded that within the *Ophrydium* assemblages of three nearby

lakes, three taxa (*Cymbella* C. Agardh, *Nitzschia* Hassall, *Brachysira* Kützing) constituted 70% of the identified diatom species. However, his data also suggest that there is no significant similarity in diatom community composition within *Ophrydium* between the three lakes studied. *Ophrydium* studies in Massachusetts (Duval and Margulis, 1995) found that diatom communities within *Ophrydium* collected from Hawley and Leverett bog wetlands, contained diatoms within the genera *Navicula* Bory, *Pinnularia* Ehrenberg, *Gyrosigma* Hassall, and *Cymbella*, while also containing coccoid cyanobacteria, and bacteria (Duval and Margulis 1995). *Navicula* dominated the community within the mucilage, while not being the dominant genus in surrounding waters (Duval and Margulis 1995). Reports of *Ophrydium* colonies have recently come from Russia (Bazhenova and Igoshkina 2016), South Africa (Oberholster et al. 2010) and Argentina (Peso et al. 2015).

While there has been some research to determine the common species of diatoms found in *Ophrydium* colonies, little has been done to compare the diatom communities within *Ophrydium* colonies to the surrounding benthic diatom communities. We investigated the diversity of diatom communities within *Ophrydium* colonies, comparing the diatom composition within *Ophrydium* colonies from three lakes in northern Michigan to the surrounding benthic diatom communities within the lake water from which they were sampled. Specifically, we wanted to determine whether diatom communities within *Ophrydium* colonies from different lakes are more similar to each other or are more similar to benthic diatom communities in the surrounding waters. Within the mucilage of *Ophrydium* colonies, we expected to find a different diatom assemblage than within the surrounding waters, and we expected the composition to be more similar between *Ophrydium* colonies than between each colony and the assemblage in the surrounding water.

METHODS AND MATERIALS

Collections

Samples of *Ophrydium* and surrounding benthic algae were collected at three sites in northern Michigan. Larks Lake (45°36'08.3"N, 84°55'42.7"W), located in Emmet County, is a shallow, spring-fed lake with a marl and sand bottom. This 600-acre inland lake has a maximum depth of 9 feet (Tip of the Mitt Watershed Council 2019). French Farm Lake (45°45'09.2"N, 84°45'57.9"W) (Institute for Fisheries Research 2019) in Emmet County is a shallow, 585-acre inland lake with a marl bottom rich in benthic cyanobacteria. French Farm Lake is partially surrounded by wetlands and contains a multitude of habitats including large patches of reeds and patches of lily pads along the shore. Osmun Lake (45°19'33.8"N, 84°23'24.4"W) is a 48-acre inland lake in Cheboygan County with a marl bottom. Most of the lake is less than 4 feet deep, but with a maximum depth of 10 ft (Cwalinski and Newman 2008). Sampling of all three lakes took place between July 11 and July 22, 2019. Each location was visited on a different day and both benthic algal samples and *Ophrydium* samples were collected on the same date from the same lake.

At all sites, benthic samples were collected from the surrounding area near the *Ophrydium* using a baster and placed in two 4-ounce Whirl-Paks, which were transported back to the lab. *Ophrydium* samples were collected by scooping between 10-20 individual *Ophrydium* colonies in two Whirl-Paks at each site. In Larks Lake, *Ophrydium* samples were collected above the sandy bottom near an aggregate of reeds. In French Farm Lake, *Ophrydium* samples were collected from the benthos, above the marl next to an aggregate of lily pads. *Ophrydium* colonies at this location were

exceptionally large, up to 30 cm wide. Finally, at Osmun Lake, *Ophrydium* colonies were collected above the marl bottom underneath lily pads; several of these colonies were planktonic.

In the lab, the two Whirl-Pak benthic samples from Larks Lake were combined into a 1-liter glass beaker up to 100 ml. This process was completed with the benthic samples from French Farm Lake and Osmun Lake, resulting in a total of 3 beakers, each filled with 100 ml of benthic sample from a particular sampling location. To remove organic matter from each sample, leaving a clean diatom media for ease of viewing and identification using a microscope, the following process was completed. Each beaker received 100 ml of 30% hydrogen peroxide followed by the addition of a small amount of potassium dichromate (Van Der Werff 1955). Following an exothermic reaction, each site sample was then transferred into its own 250 ml beaker and filled with deionized water. Over the course of a week, samples were decanted to about 50 ml and refilled with deionized water every 12 hours until colorless. The cleaned diatoms were transferred onto a cover slip and dried on a warming plate. After drying completely, the coverslips were permanently mounted onto glass slides using Naphrax mounting medium.

For the *Ophrydium* samples, we first extracted the *Ophrydium* colonies from their water environment inside of the Whirl-Paks and placed them into petri dishes. We then removed any *Chara* remnants and thoroughly rinsed the colonies with deionized water to remove any algae clinging to the exterior of the *Ophrydium* colonies. This cleaning was done for every site *Ophrydium* sample. *Ophrydium* samples were then separated into three separate 1-L beakers (one for each site). Each sample was then processed to become diatom slides through the same diatom media cleaning and mounting process as the benthic samples. All samples and slides (benthic and *Ophrydium* samples from each location) have been accessioned into the Diatom Collection of JPK at COLO.

Samples were analyzed with an Olympus BX-51 compound microscope with a 100× objective and DIC optics with a numerical aperture of 1.4. To determine the structure of the diatom communities present in each of the six samples, 600 valves were identified and enumerated. For SEM microscopy, cleaned material was air-dried onto 18 mm round cover glasses, which were then attached to aluminum stubs with double-sided tape. The stubs were sputter coated with 1 nm gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd., Watford, UK). Materials were examined using a JEOL JSM 7401 field emission SEM (JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 3 kV.

Comparison of Communities

To assess the similarity between samples, we calculated Jaccard Similarity Index values (Jaccard 1912). Additionally, a Shannon-Wiener Index of diversity (Spellerberg and Fedor 2003) was calculated to assess the diatom composition of the samples. Again, all species counted from each location and each sample type were included.

RESULTS

Overall, 85 taxa from 39 genera of diatoms were identified from the six samples from the three lakes (Table 1). The number of taxa in each of the 600 valve counts ranged from 13 (*Ophrydium* colonies from French Farm Lake) to 60 (benthic sample from French Farm Lake). The number of taxa from *Ophrydium* colonies averaged 14.6 taxa/sample, while the number of taxa from benthic samples averaged 43.3 taxa/sample. The *Ophrydium* samples were dominated by an *Encyonopsis* species comprising from 75% to just over 93% of the community from *Ophrydium* samples. This species of *Encyonopsis* was not encountered in any of the benthic samples surrounding the colonies. While this *Encyonopsis* species was unique to the *Ophrydium* diatom communities, it was the only species unique to those communities. The benthic communities had 39 taxa that were unique to these communities, that is, not found in the *Ophrydium* samples.

TABLE 1. List of diatom taxa collected from Larks Lake, Osmun Lake, and French Farm Lake and the number of each from a 600-valve collection from each of two substrate types in each lake.

| Taxa | Larks Lake | | Osmun Lake | | French Farm Lake | |
|-----------------------------------|------------------|---------|------------------|---------|------------------|---------|
| | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic |
| <i>Achnanthydium deflexum</i> | | | | | | 13 |
| <i>Achnanthydium macrocephala</i> | | | | | | 4 |
| <i>Achnanthydium minutissimum</i> | 4 | 74 | | 12 | | 55 |
| <i>Achnanthydium rosenstockii</i> | | 5 | | | | 6 |
| <i>Adlafia bryophila</i> | | | 4 | 16 | | 4 |
| <i>Amphipleura pellucida</i> | | | | | 2 | 2 |
| <i>Amphora ovalis</i> | | 2 | | | | 2 |
| <i>Amphora thumensis</i> | | | | | | 6 |
| <i>Aneumastus tuscula</i> | | | | | | 2 |
| <i>Brachysira vitrea</i> | 2 | 12 | 5 | 93 | 4 | 12 |
| <i>Caloneis silicula</i> | | | | | | 2 |
| <i>Cocconeis placentula</i> | | 5 | | | | |
| <i>Cyclotella comensis</i> | 2 | | | | | 8 |
| <i>Cyclotella distinguenda</i> | | | | | | 3 |
| <i>Cymbella affinis</i> | | 2 | | 6 | | 10 |
| <i>Cymbella hustedtii</i> | | | | 2 | | |
| <i>Cymbella leptoceros</i> | | | 48 | 93 | | |
| <i>Cymbopleura amphicephala</i> | | 2 | | | | |
| <i>Cymbopleura cuspidata</i> | | | | | | 4 |
| <i>Cymbopleura naviculiformis</i> | | | | | 2 | 2 |
| <i>Cymbopleura subaequalis</i> | | 2 | | | | |
| <i>Delicataptychus delicatula</i> | | 4 | 4 | 62 | | |
| <i>Encyonema minuta</i> | | 10 | 2 | 2 | | 2 |
| <i>Encyonema muelleri</i> | | | | | | |
| <i>f. ventricosa</i> | | 1 | | | | |
| <i>Encyonema norvegica</i> | | | | | | 12 |
| <i>Encyonema sp.</i> | | | | 4 | 2 | |
| <i>Encyonopsis cesatii</i> | | 24 | | 32 | 2 | |
| <i>Encyonopsis descripta</i> | | | | 4 | | 1 |
| <i>Encyonopsis falaisensis</i> | | 8 | | 2 | | |
| <i>Encyonopsis microcephala</i> | 6 | 82 | 4 | 38 | | 52 |
| <i>Encyonopsis ophrydiensis</i> | 545 | | 452 | | 559 | |
| <i>Encyonopsis subminuta</i> | | | 18 | 53 | | |
| <i>Eolimna minima</i> | | 2 | | | | 2 |
| <i>Epithemia adnata</i> | | 2 | | | | |
| <i>Epithemia argus</i> | | 2 | 6 | 4 | | 2 |
| <i>Epithemia zebra</i> | | | | | | 2 |
| <i>Eucocconeis flexella</i> | | 10 | | 12 | | 2 |
| <i>Eucocconeis laevis</i> | 2 | 8 | | | | |
| <i>Eunotia flexuosa</i> | | 4 | | | | |
| <i>Eunotia implicata</i> | 2 | 6 | 2 | 6 | | |
| <i>Eunotia sp. 1</i> | | | | | | 2 |
| <i>Fragilaria capucina</i> | | 4 | | | | |
| <i>Fragilaria sp.</i> | | 6 | 2 | 4 | 10 | |
| <i>Fragilaria tenera</i> | | | | | | 35 |
| <i>Fragilaria vaucheriae</i> | | | 2 | 4 | | |
| <i>Frustulia rhomboides</i> | | | | | | 1 |
| <i>Gomphonema affine</i> | | 2 | | | | 4 |
| <i>Gomphonema intricatum</i> | 11 | 93 | 2 | 2 | | 35 |
| <i>Gomphonema sp.</i> | | 2 | | | | |
| <i>Gomphonema truncatum</i> | | | | 2 | | |

(Continued on next page)

TABLE 1. (Continued).

| Taxa | Larks Lake | | Osmun Lake | | French Farm Lake | |
|--------------------------------|------------------|---------|------------------|---------|------------------|---------|
| | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic |
| Halamphora perpusilla | | 2 | | | | 8 |
| Halamphora veneta | | | | | | 2 |
| Karayevia clevei | | | | | | 1 |
| Lindavia radiosa | | | | | | 18 |
| Mastogloia lacustris | 4 | 2 | 1 | 5 | 1 | 1 |
| Mastogloia smithii | | 4 | | | | 7 |
| Navicula cryptocephala | | | | 4 | | |
| Navicula hasta | | | | | | 2 |
| Navicula oblonga | | 2 | | | | 5 |
| Navicula radiosa | 8 | 85 | 8 | 38 | 4 | 6 |
| Navicula schadei | | | | | | 8 |
| Navicula sp. 2 | | | | | | 2 |
| Navicula trivialis | 2 | 12 | | 10 | | 11 |
| Navicula veneta | 8 | 103 | 24 | 64 | 3 | 58 |
| Neidium ampliutum | | 4 | | 4 | | 4 |
| Nitzschia brunoii | | 2 | | | | |
| Nitzschia denticula | 2 | 4 | 16 | 15 | 8 | 58 |
| Nitzschia palea | | | | 3 | 2 | |
| Nitzschia sp. | 2 | | | | | 4 |
| Nitzschia tenuirostris | | | | | | 9 |
| Pinnularia microstaureon | | | | | | 4 |
| Pinnularia sp. 2 | | | | | | 2 |
| Placoneis clementis | | | | | 1 | |
| Planothifium lanceolatum | | | | | | 2 |
| Rhopalodia gibba | | 2 | | 2 | | |
| Sellaphora pupula | | | | | | 6 |
| Stauroneis anceps | | | | | | 4 |
| Stauroneis phoenicenteron | | | | | | 6 |
| Staurosira construens | | | | | | 75 |
| Staurosirella martyi | | | | | | 2 |
| Surirella sp. 1 | | | | | | 2 |
| Surirella sp. 2 | | | | | | 2 |
| Tabellaria fenestrata | | 3 | | | | |
| Tabellaria flocculosa | | | | | | 2 |
| Ulnaria ulna | | 1 | | | | 2 |
| Unidentified Naviculoid Diatom | | | | 2 | | |
| TOTALS | 600 | 600 | 600 | 600 | 600 | 600 |

The benthic samples were not only more species-rich than the *Ophrydium* samples, but they were also more diverse, as measured by Shannon-Wiener Index. The *Ophrydium* samples had a range of Shannon-Wiener diversity values from 0.401 to 1.095 (average 0.672), while the benthic samples had a range from 2.67 to 3.25 (average 2.86).

The Jaccard's Similarity Index values for the matrix of samples analyzed are given in Table 2. Interestingly, the *Ophrydium* communities were more similar to one another than they were to the benthic communities taken from the same lake, or other benthic communities taken from other lakes. This was the case for both the Lark's Lake *Ophrydium* sample and the French Farm *Ophrydium* sample. In

TABLE 2. Jaccard's similarity values for cross-sample comparisons between benthic and *Ophrydium* colonies in each of the three lakes.

| | Larks Lake | | Osmun Lake | | French Farm Lake | |
|------------------|------------------|---------|------------------|---------|------------------|---------|
| | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic | <i>Ophrydium</i> | Benthic |
| Larks Lake | | | | | | |
| <i>Ophrydium</i> | 1 | | | | | |
| Benthic | 0.262 | 1 | | | | |
| Osmun Lake | | | | | | |
| <i>Ophrydium</i> | 0.410 | 0.272 | 1 | | | |
| Benthic | 0.286 | 0.373 | 0.500 | 1 | | |
| French Farm Lake | | | | | | |
| <i>Ophrydium</i> | 0.285 | 0.156 | 0.304 | 0.257 | 1 | |
| Benthic | 0.159 | 0.282 | 0.136 | 0.200 | 0.104 | 1 |

the case of Osmun Lake, however, the *Ophrydium* sample was more similar to the benthic sample from that lake than the other two *Ophrydium* samples.

While the *Ophrydium* samples had lower species richness, diversity, and far fewer unique species as compared to the benthic samples, the dominant species in each of the three lakes in terms of relative abundance was a species of *Encyonopsis* that appears to be new to science. We describe this new species below:

***Encyonopsis ophrydiensis* Mark, McKim, Lowe & Kociolek, sp. nov.**

Figures 2–24.

Description: Valves narrowly linear-lanceolate, very weakly asymmetrical to the apical axis, dorsal and ventral margins convex, with apices protracted slightly, narrowly-rounded. Length 17–33 μm , breadth 3.5–5.0 μm . Axial area extremely narrow, no central area evident. Raphe filiform, arched to slightly undulate, with external proximal ends dilated slightly and deflected dorsally. Striae not punctate, radiate, strongly so at the apices, 32–36 / 10 μm in the center, 35–38 / 10 μm at the ends.

Holotype: Slide 216001, in the collection of JPK at the University of Colorado, Boulder (COLO).

Type Locality: Osmun Lake, Cheboygan County, Michigan.

Etymology: The specific epithet "*ophrydiensis*" refers to the habitat of the species on *Ophrydium* colonies.

In the SEM images, the valve exterior is dominated by a distinctly undulate raphe, whose proximal raphe ends are wider (Figures 17–20). The proximal raphe ends are deflected towards the dorsal margin. Distally, the raphe ends are hooked slightly and extend onto the valve mantle (Figure 20). Striae are radiate, strongly so near the apices. Areolae are narrow, linear to barely triangular (Figures 19, 20). Internally, the raphe is straight and continuous across the barely distinguished central nodule (Figures 21, 22). The raphe terminates at the apices as helictoglossae (Figures 21, 22, 24), which are located away from the terminus

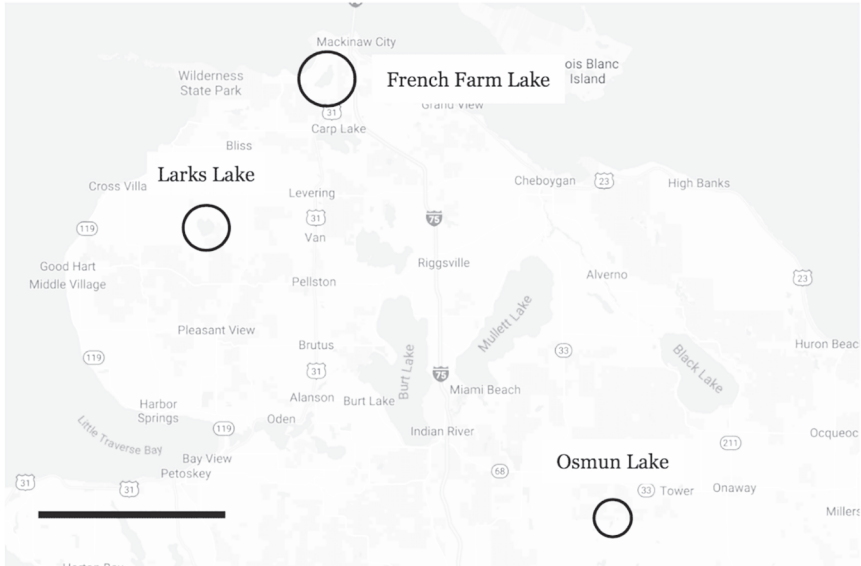
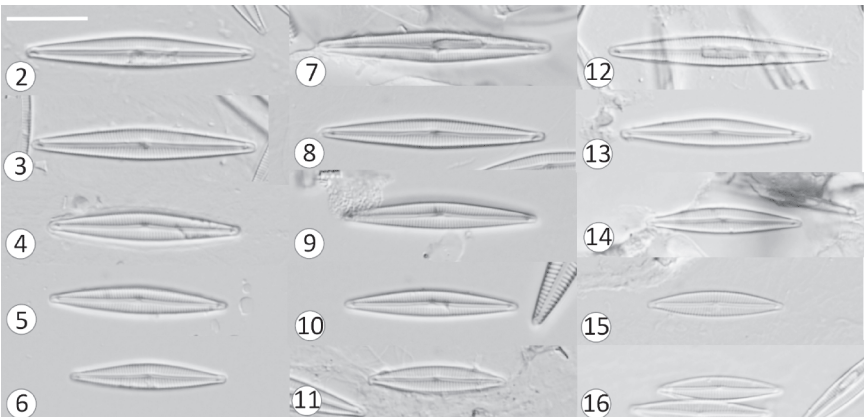


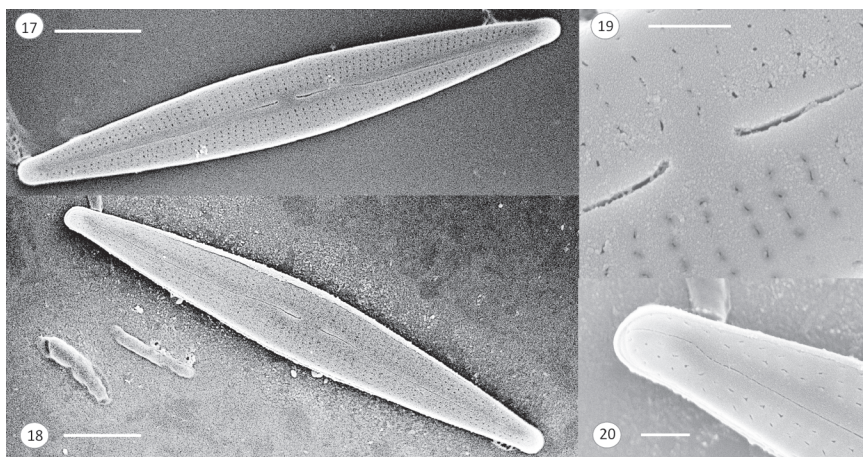
FIGURE 1. Map of the northern Lower Peninsula of Michigan showing the locations of the three study sites. Scale bar = 50 km.

of the valve. Areolae are round openings, located in narrow troughs that are constricted (Figures 21–24).

In his establishment and treatment of the genus *Encyonopsis*, Krammer (1997a) indicated that its diagnostic features include cells that are small, barely asymmetrical, and without apical pore fields and stigmata. Bahls (2015) established the genus *Kurtkrammeria* for those species previously included in



FIGURES 2–16. Light micrographs of *Encyonopsis ophrydiensis* sp. nov. Figures 2–6 are from French Farm Lake. Figures 7–11 are from Osmun Lake (holotype slide). Figures 12–16 are from Larks Lake. Scale bar = 10 μ m for all images.



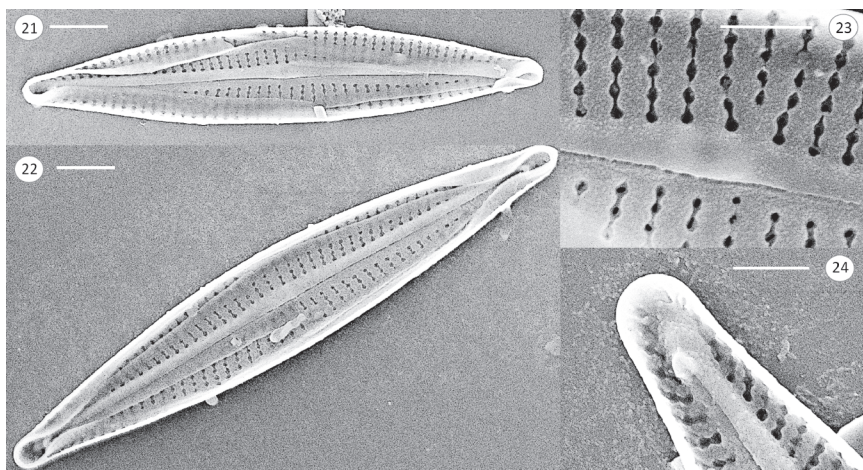
FIGURES 17–20. SEM images of *Encyonopsis ophrydiensis* sp. nov. External valve views. Figures 17 and 18: Whole valve views. Scale bars = 3 μ m. Figure 19: Central area, showing proximal raphe ends curved slightly towards the dorsal margin. Areolar openings are small and linear. Scale bar = 0.75 μ m. Figure 20: Apex of the valve showing distal raphe end deflected ventrally. Scale bar = 1 μ m. All specimens are from Osmun Lake.

Encyonopsis that differ on the basis of the direction of the striae at the apices, the structure of the areolae, and the presence of stigmata and apical pore fields. Lack of these characteristics in the new Michigan taxon places it in the genus *Encyonopsis*.

In his monograph of *Encyonopsis*, Krammer (1997b) considered species from all over the world. There are several taxa that resemble the new species from northern Michigan either in shape or size range. *Encyonopsis delicatissima* (Hustedt) Krammer, *Encyonopsis subruttneria* Krammer, and *Encyonopsis ruttneri* var. *obtusata* (Hustedt) Krammer are all similar in shape to *E. ophrydiensis*, but differ in their smaller length and breadth. All three of these taxa were described originally from Indonesia and, as Krammer suggests, the report by Foged (1959) of *E. delicatissima* from Afghanistan needs verification. The dimensions of those taxa described originally by Hustedt (1938) were confirmed by Simonsen (1987).

Encyonopsis lanceola (Grunow) Krammer has in general a shape similar to *E. ophrydiensis* and similar length and breadth. Grunow's original illustration (in Van Heurck 1880), confirmed in Krammer (1997b, p. 118, Plate 161, fig. 2; Plate 162, figs 1–7) suggests that this species is less asymmetrical to the apical axis than our new species. It had been reported previously from the United States by Patrick and Reimer (1966), but as a species of *Navicula*. This species also has protracted ends that are more broadly rounded than is evident in *E. ophrydiensis*.

Encyonopsis falaisensis (Grunow) Krammer has dimensions similar to those of *E. ophrydiensis*, but the original drawing by Grunow (in Van Heurck 1880) and subsequent light micrographs published by Krammer (1997b, Plate 162, figs 8–24) show that this species does not have protracted apices. Striae density is much coarser (18–20 / 10 μ m) (Krammer 1997b) than in *E. ophrydiensis*.



FIGURES 21–24. SEM images of *Encyonopsis ophrydiensis* sp. nov. Internal valve views. Figures 21 and 22: Whole valve. Very thin girdle bands have fallen in to the valve. Straight raphe is evident. Scale bars = 2 μ m. Figure 23: Central area, with a slight central nodule, is evident. The raphe is continuous. Areolar openings are round, contained in constricted troughs. Scale bar = 1 μ m. Figure 24: Apex of the valve, showing helictoglossa positioned away from end of valve. Striae are radiate. Scale bar = 1 μ m. Figures 21, 22, and 24 are from Larks Lake, and Figure 23 is from Osmun Lake.

Encyonopsis ophrydiensis is similar in size and shape to *E. neerlandensis* Van der Vijver (see Van der Vijver et al. 2012), a species found in acid waters of the Netherlands, but also reported from mountain lakes in the Rocky Mountain region (Bahls, 2013). The two species differ in that the species from Michigan may be slightly larger and has finer striae, and in that the species described from the Netherlands has distinctly subcapitate apices.

DISCUSSION

Observations of the diatoms occurring in mucilage of *Ophrydium versatile* show that while the communities in the mucilage of the ciliate are less species-rich and less diverse than those in the surrounding benthic environments, the colonies are quite similar to one another even across lakes separated by nearly 75 km. This suggests that not all species from the surrounding benthic communities are able to survive in the mucilage of *Ophrydium*.

Dute et al. (2000) suggested that the surrounding physical and chemical properties of the water in South Alabama bogs determined the diatom species found in *Ophrydium* colonies there. Since there was only one species unique to *Ophrydium* colonies in the three lakes in northern Michigan investigated here, our data support this general conclusion by Dute et al. (2000). However, the most common species in the mucilage of *Ophrydium* colonies of the three lakes, a new species of *Encyonopsis*, accounted for between 75% and 93% of the entire community present in the colonies and was not encountered in any of the benthic

samples studied herein. This suggests that this species, which forms such a high proportion of the mucilage communities, was able to thrive in these communities relative to the surrounding lake environment and may be an obligate endosymbiont.

The evolutionary relationship between *Ophrydium* and *Encyonopsis ophrydiensis* has not been studied and is especially intriguing in light of the overwhelming high relative abundance of this diatom in *Ophrydium* paired with its absence from the 600 valve counts in the nearby benthic samples. Additional sampling of the benthic waters surrounding the *Ophrydium* colonies may need to be conducted before confidently concluding that *E. ophrydiensis* is obligately found in *Ophrydium*. If this diatom species is found only in *Ophrydium* colonies and not the surrounding environment, it raises many questions concerning the mechanics of how the diatom gets passed on to daughter colonies and how each organism is affected by the relationship (e.g., benefits to *E. ophrydiensis* from living within the *Ophrydium* colony's mucilage).

As noted above, when planktonic *Ophrydium* colonies are disturbed, the *Ophrydium* cells split up and abandon their colony (Sand-Jensen et al. 1994). This raises the questions: (i) when the colonies break, does the *Encyonopsis* species get released into the benthic water or does it remain in the mucilage? (ii) will the *Encyonopsis* species abandon the mucilage if conditions are no longer favorable? and (iii) how does *E. ophrydiensis* remain in the colony if the probability of the colony splitting due to external factors is high? Additional research on the evolutionary relationship between *Ophrydium* and *Encyonopsis ophrydiensis* will contribute to our knowledge of co-evolution concepts as well as mutualistic relationships over time.

ACKNOWLEDGMENTS

We thank the University of Michigan Biological Station for providing the facilities to conduct our research as well as fostering an amazing scientific community that supported us through every step. We would also like to thank the Freshwater Algae Class of 2019 for all their support and identification help as well as Vera Ting for assisting in the collection of samples.

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