The riddle of the 'green streaks'.

In search of the first microorganism which Antoni van Leeuwenhoek described.

by Wim van Egmond, the Netherlands in collaboration with Frans Kouwets

During the summer of 1674 Antoni van Leeuwenhoek (1632-1723), a draper from Delft, filled a small bottle with water from a lake called the Berkelse Meer. He examined the contents with his microscope the next day. His description of the find and what he observed using his tiny but superb microscope can be regarded as the beginning of microbiology. He wrote about this discovery in Dutch. His letter, dated September 7th, was sent to the Royal Society in London, translated into English and an extract published in Philosophical Transactions. Although the language in which he wrote is a bit old-fashioned it is still a treat to read. Here is the English translation. (The original letter was one long paragraph, I split it into 3 parts for easier reading.)

"About two hours distant from this Town there lies an inland lake, called the Berkelse Mere, whose bottom in many places is very marshy, or boggy. Its water is in winter very clear, but at the beginning or in the middle of summer it becomes whitish, and there are then little green clouds floating through it; which, according to the saying of the country folk dwelling thereabout, is caused by the dew, which happens to fall at that time, and which they call honey-dew. This water is abounding in fish, which is very good and savoury.

Passing just lately over this lake, at a time when the wind blew pretty hard, and seeing the water as above described, I took up a little of it in a glass phial; and examining this water next day, I found floating therein divers earthy particles, and some green streaks, spirally wound serpent-wise, and orderly arranged, after the manner of the copper or tin worms, which distillers use to cool their liquors as they distil over. The whole circumference of each of these streaks was about the thickness of a hair of one's head. Other particles had but the beginning of the foresaid streak; but all consisted of very small green globules joined together: and there were very many small green globules as well.

Among these there were, besides, very many little animalcules, whereof some were roundish, while others, a bit bigger, consisted of an oval. On these last I saw two little legs near the head, and two little fins at the hindmost end of the body. Others were somewhat longer than an oval, and these were very slow a-moving, and few in number. These animalcules had divers colours, some being whitish and transparent; others with green and very glittering little scales; others again were green in the middle, and before and behind white; others yet were ashen grey. And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that 'twas wonderful to see: and I judge that some of these little creatures were above a thousand times smaller than the smallest ones I have ever yet seen, upon the rind of cheese, in wheaten flour, mould, and the like."



The Berkelse Meres were situated south east of Delft, where Van Leeuwenhoek lived.

There has always been a lot of speculation about the true nature of the organisms he described in this letter. Van Leeuwenhoek was a layman and it was long before Linnaeus provided us with a systematic way of describing living organisms. Almost everything Van Leeuwenhoek observed through his simple single lens microscope had never been seen before. Van Leeuwenhoek did not give the organisms names, he simply described their appearance, as detailed as he could.

He was one of the best observers of his time. In any case literally because he was the only one who could make a microscope with a magnification of up to several hundred times and with a very high resolving power.

One of the most influential scientists who studied the work of Van Leeuwenhoek was Clifford Dobell (1886 -1949). He was a protozoologist who studied intestinal amoebae but also published on algae. His classic biography about Van Leeuwenhoek from 1932 *Antony van Leeuwenhoek and his little Animals* from which the above translation was sourced was very popular and most of the names put to the descriptions of Van Leeuwenhoek can be credited to Dobell. He thought the spirally arranged green 'streaks' Van Leeuwenhoek described were *Spirogyra*, a filamentous alga that has coiled chloroplasts. Ever since, this is regarded as the organism Van Leeuwenhoek described.



Spirogyra photographed in darkfield illumination. Image width: approximately 0,9 mm.

Dobell comments (Footnote 2, p. 110): "The common green alga *Spirogyra*: the earliest recorded observations on this organism. The size of the filament negatives the suggestion that L. could have been referring to *Arthrospira* or *Spirulina*, (these are filamentous cyanobacteria)."

He furthermore gives suggestions for the other organisms Van Leeuwenhoek mentioned: ciliates, rotifers and *Euglena*. We can be pretty sure he observed at least one of these groups in the sample, but the description of these organisms is not as detailed as the first one. The present article is about that first observation, the most important one, the first observation of a living aquatic microorganism. Dobell dismisses *Arthrospira* and *Spirulina* because of their size. I agree. But could there be other candidates?

In 2009 our Dutch desmid club (<u>www.desmids.nl</u>) organised an excursion to the Czech Republic to collect some samples of this group of beautifully shaped green algae. During a field trip I was talking with Frans Kouwets, phycologist at the Ministry of Infrastructure and Environment in Lelystad. Somewhere during our conversation I mentioned *Spirogyra* as the organism found by Antoni van Leeuwenhoek. 'Aha', Frans said, 'that is something that I seriously doubt. I don't think it was *Spirogyra* and perhaps it is interesting to publish something about this.'

It is now 2016 and Frans and I have discussed this subject over the years. I have studied Van Leeuwenhoek for my work for a museum about microbes, continuously finding new clues. An extra reason why I am so interested is the fact that I grew up near the location and live near the former Berkelse Meer. Frans currently is very busy with his monograph of the desmid genus *Cosmarium* and encouraged me to write the article. But Frans is reading over



Spirogyra bloom, forming floating algal beds, photographed in June.

my shoulder so don't forget that he started all this.

Let's start to sum up the reasons why we think *Spirogyra* is an unlikely candidate. And perhaps it is a good moment to refer to the motto of the Royal Society; *Nullius in verba*, which can be translated as 'take nobody's word for it'.

Spirogyra is common in shallow ponds and ditches. A large lake as the Berkelse Meer was (1.5 kilometer wide) is not the usual habitat. Although it inhabits lake edges it is not planktonic.

Spirogyra does form floating masses but in early summer, and then their color is not whitish green but yellow or brown green.

Spirogyra blooms consist of very long filaments or strands, not microscopic streaks.

Being such a keen observer, Van Leeuwenhoek was always very accurate in his observations. He would certainly have described *Spirogyra* as long strands. If you find *Spirogyra* you can take it out of the water with your hands as scum, and it feels slippery.

Van Leeuwenhoek used capillary tubes at the time for studying liquids e.g. in his earlier letter dated <u>July 6th 1674</u>. If he did it would be impossible to put *Spirogyra* in such a tube.

There is enough reason for doubting it was *Spirogyra*. It is interesting to see that for such a long time everybody copied Dobell's suggestion, although it is understandable. What else could it have been? Before we search for other candidates, let's look again at the description.

"I found floating therein divers earthy particles, and some green streaks, spirally wound serpent-wise, and orderly arranged, after the manner of the copper or tin worms, which distillers use to cool their

liquors as they distil over. The whole circumference of each of these streaks was about the thickness of a hair of one's head. Other particles had but the beginning of the foresaid streak; but all consisted of very small green globules joined together: and there were very many small green globules as well."

and the original letter in Dutch:

"bevonde ick daer in te drijven, verscheijde aertsche deeltgens, ende eenige groene ranckjens, in geschickte ordre slanghs gewijse omgekrult, op gelijcke manier, als de copere off tinne slangen sijn, die de distelatuers gebruijcken, omme haer over gehaelde wateren te verkoelen, ende de gantsche circumferentie, van jder van dese ranckjens, hadt ontrent de dickte van een haer van ons hooft; andere deeltgens hadden maer een begin, van het boven verhaelde ranckje, alle bestaende uijt seer kleijne groene same gevoeghde clootgens, als mede seer veel kleijne groene clootgens,"

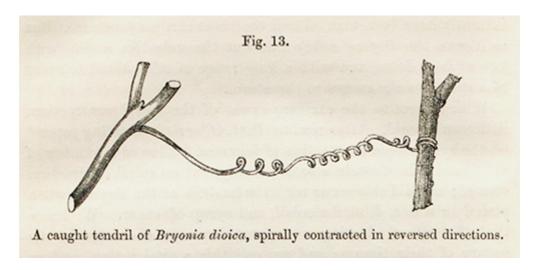


Illustration from Darwin's book on the Movements and Habits of Climbing Plants - 1854

Van Leeuwenhoek only wrote in Dutch. He did not know any other language and his Dutch is translated by others. That is why I think we should look at the original Dutch text and try to read it as carefully as possible. The actual Dutch word Van Leeuwenhoek used was 'ranckje' (plural ranckjens). While writing this article I noticed that the English translation of the word 'ranckjes' (both in the *Collected Letter's* and Dobell's), into 'streaks' is not accurate. The word streak is a bit misleading because it suggests an elongated shape. In my opinion a correct translation would be small tendril. And I am sure he used the Dutch equivalent because a tendril is often coiled. The Dutch text is even clearer on the coils than the translation.

It can be translated various ways. Here's what I would make of it: Several earthy particles and some green tendrils that are bent snake-wise to orderly arranged curls, like the copper or tin tubes distillers use to cool their transferred water.



A distiller's copper worm.

Next: why would Van Leeuwenhoek mention 'the whole circumference' for the thickness of *Spirogyra*? If you look at *Spirogyra* it is not necessary to add 'the whole circumference'. It should be enough to describe it as 'long strands the thickness of a human hair'. He only had a reason to mention 'the whole circumference' when it is not obvious whether that thickness refers to the coil or what the coil is made of. Could it be that he wrote 'the whole circumference' because the organism he described actually was a coil and not a tube with coils inside such as *Spirogyra*?

Finally, the last part of the sentence was translated as:

"Other particles had but the beginning of the foresaid streak; but all consisted of very small green globules joined together: and there were very many small green globules as well."

Apart from the streaks the translation is good; clootgens are little balls. Other particles had only a beginning of the small tendrils mentioned above, all made out of very small green little balls joined together, as well as many small green balls. [This last double remark is a bit odd but I think he means there were also separate little balls]

(Note: *Spirogyra* can be found as very small cells but the word particle would not be a good description. It is also questionable if these small cells of *Spirogyra* can be found floating.)

Van Leeuwenhoek literally describes his find as regularly ordered coils made out of little balls (clootgens). Resembling a vine tendril and a distiller's copper worm (shown above). Many of his descriptions are cryptic and hard to associate with certain organisms. That is true for the other organisms he describes in the latter part of the letter. But this section, the first time aquatic microbes are ever described, is clear and accurate. We also have the details of

the time and setting. In his boat on the Berkelse Meer, a nutrient rich lake (plenty of fish) Antoni van Leeuwenhoek describes a late summer cyanobacterial bloom and the first aquatic microbe captured in words could very well be the one shown here:



Cyanobacteria bloom of Dolichospermum, photographed in darkfield illumination. Image width: approximately 0,9 mm.

The image shows what Frans and I suggest to be the most likely candidate. It's a filament-forming cyanobacterium. They are made out of little balls, the individual cells, that form very regularly coiled strands. I found these in late summer in a large lake as a whitish green layer against the surface. The location was a quite similar type of lake as the Berkelse Meer, the Kralingse plas near Rotterdam, 12 kilometers from where the Berkelse Meer once was. During August there was a bloom of cyanobacteria. So the time and location also fits the description. I have collected them the same way Van Leeuwenhoek did. They float under the surface as a cloudy layer and when you put a bottle in the water you can easily fill it.

Cyanobacteria, or blue green algae, are bacteria that perform photosynthesis. Although they have a bad reputation they are important oxygen producers. The cyanobacterium that we think Van Leeuwenhoek described is in the genus *Dolichospermum*. Recently the planktic forms of *Anabaena* were separated from this genus and transferred to the genus *Dolichospermum*.

The coils of this species of *Dolichospermum* have a diameter that range between 70 and 90

microns. Van Leeuwenhoek is talking about the whole circumference (not the little cells) measuring about the same as the thickness of a hair from our head. According to the DNBL *Collected Letters* website the size of such a hair is between 60 and 80 microns.

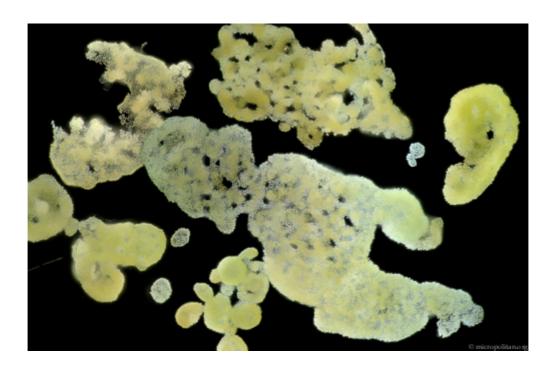


Cyanobacteria bloom, photographed in August.

Dobell ruled out the helical cyanobacteria *Arthrospira* and *Spirulina* and I think he was right. Their filaments are more slender but more importantly, they are not made out of little balls.

Frans and I can't think of any other suitable candidate. If we compare all the arguments in favor of *Dolichospermum* and all the arguments against *Spirogyra* there is only one conclusion. It is much more likely that Van Leeuwenhoek observed cyanobacteria like *Dolichospermum*. And that would mean it is not only the first account of living aquatic microorganisms, but also the first observation of bacteria, two years before Van Leeuwenhoek described them in his studies of 'pepper water' in the letter dated October 9th 1676.

I think it is important to state that we can only speculate here. We can't be 100% sure. But in the case of the greenish helix of little balls we can safely replace *Spirogyra* by *Dolichospermum*, the cyanobacterium formerly known as *Anabaena*, as the number one candidate of the first described aquatic microorganism.



Microcystis

Unless...

What about the 'earthy particles'? If we assume that the whitish water with the green clouds is a cyanobacterial bloom, then the earthy particles could represent cyanobacteria as well. Such a bloom often consists of several genera of cyanobacteria. The earthy particles could be *Microcystis*. Their colonies have shapes that can be described as earthy particles.

And then that would be the first aquatic microorganism ever described.

But I think here a Nullius in verba would do.

Footnotes

There are many different species of *Dolichospermum* with various dimensions that form coiled filaments so it could well be that Van Leeuwenhoek saw a different species. But there are not that many species with a regular coil of the diameter Van Leeuwenhoek mentions. If we follow the description of a regular coil with the diameter between 60 and 80 micron we can narrow it down to *D. circinale*, *D. spiroides* or *D. crassum*. The one I photographed may be *D. crassum* but not without doubt because these species vary in appearance and are hard to distinguish from one another.

A paper by Jiří Komárek and Eliška Zapomělová published in *Fottea* (Journal of the Czech Phycological Society), 2007, vol. 7, issue 1, 1-31, presents a detailed and richly illustrated review of these coiled cyanobacteria. <u>Planktic morphospecies of the cyanobacterial genus</u>

Anabaena = subg. Dolichospermum - 1. part: coiled types.

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The distiller's copper worm image is web sourced but exact credit uncertain, but will be pleased to add due credit if contacted.

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