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ALGOLOGICAL NOTE 5: A DIATOM OF THE GENUS *CYLINDROTHECA* WITH FLEXIBLE ENDS IN URUGUAY

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Abstract

A benthic diatom of the genus *Cylindrotheca* with flexible cell ends was found in a rockpool on a brackish water beach near the South Atlantic in Uruguay. Its unusual movements were filmed. As diatoms are usually rigid due to their silicified frustules, the discovery of this species with flexible cell ends and unusual movements caught our attention. We studied its morphology using light microscopy to identify it.

Keywords: diatoms, sea algae, epipsammon, rockpool, movement, flexibility, fragility, Atlantic, Uruguay, *Cylindrotheca*, video, microscopy.

Résumé

Une diatomée benthique du genre *Cylindrotheca* dont les terminaisons cellulaires sont souples a été trouvée dans un bassin intertidal sur une plage d'eau saumâtre près de l'Atlantique Sud en Uruguay. Ses mouvements, de caractère inhabituel, ont été filmés. Comme les diatomées sont d'habitude rigides grâce à leur frustule silicifié, la découverte de cette espèce aux terminaisons flexibles et aux mouvements particuliers nous a interpellés. Dans un but d'identification, nous avons étudié sa morphologie par microscopie photonique.

Mots-clés : diatomées, algue marine, épipsammon, mare intertidale, mouvements, flexibilité, fragilité, Atlantique, Uruguay, *Cylindrotheca*, microscopie, vidéo.

Zusammenfassung

Eine benthische Kieselalge der Gattung *Cylindrotheca* mit weichen Zellenden wurde in einem Gezeitentümpel an einem Brackwasserstrand in der Nähe des Südatlantiks in Uruguay gefunden. Ihre Bewegungen, die von ungewöhnlichem Charakter sind, wurden gefilmt. Da Kieselalgen aufgrund ihrer Silikatfrusteln normalerweise starr sind, war die Entdeckung dieser Art mit flexiblen Endungen und besonderen Bewegungen für uns sehr interessant. Um sie zu identifizieren, untersuchten wir ihre Morphologie mittels Lichtmikroskopie.

Stichwörter : Diatomeen, Meeresalgen, Epipsammon, Gezeitentümpel, Bewegungen, Flexibilität, Zerbrechlichkeit, Atlantik, Uruguay, *Cylindrotheca*, Mikroskopie, Video.

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DEFINITIONS

Epipsammic: species that live on the surface of sand grains.

Fibula: internal silica bar used as a bridge to support the raphe canal.

Frustule: silicified cell wall of diatoms, box-shaped formed by two valves. The frustule is only partially visible in living diatoms. To reveal this, the diatom cytoplasm must be destroyed by oxidation.

SEM: scanning electron microscope.

Naphrax®: resin with a high refractive index (index of 1.73) used to embed diatoms for examination by light microscopy.

Raphe: opening or slit in the silicified cell wall of raphid diatoms. The raphe system comprises the slit and mucilaginous material secreted by the cell. In diatoms with two raphe systems (one per valve), these structures are used for locomotion.

Stria: row of punctuation marks. In Latin, it is a groove or stripe. In centric diatoms, striae are generally oriented along the rays of a valve. In pennate diatoms, the striae are generally oriented transapically on the valve. The characteristics of the striae are oriented more or less transapically for the taxonomy and phylogeny of diatoms.

INTRODUCTION

This article concerns a diatom discovered on the sandy sediment of a residual intertidal pool (rockpool) on an Atlantic beach in Uruguay on several occasions (11 years ago, 3 years ago and in 2024). In this work, we take a closer look at this alga using a sample collected in 2024. The flexibility of the cell endings of this organism and its particular movements, filmed by the first author, attracted our attention. Such flexibility in a diatom has rarely been reported in the literature. Here, we describe and explain the movements and morphology of this alga.

We identified it at the generic level but do not have enough information to determine whether it is a new species.

MATERIALS AND METHODS

An epipsammic sample was collected from the coast of the seaside resort of Cuchilla Alta in the Department of Canelones, Uruguay, South America (fig. 1), on 25 January 2024. Summer in Uruguay runs from December to March. During this period, the water temperature is usually around 15°C (59°F). This brackish coastal area is part of the Rio de la Plata estuary, which opens into the Atlantic Ocean. The salinity of the water in this estuary varies according to the proportion of fresh water from the river and salt water from the ocean (tides) that feed it. At the time of sampling, salinity levels were high.

The sample (approximately 700 ml) containing water, sand and the green alga *Ulva intestinalis* was collected from an intertidal residual rockpool (fig. 2), placed in a jar (fig. 3) and stored at room temperature. Filtered fresh water was added periodically to compensate for evaporation.

For over one month after 29 January 2024, the presence of living diatoms was observed in samples periodically withdrawn from the jar. These observations were made using a microscope equipped with interference contrast (Olympus BX41, 40X and 100X objectives). Video recordings were made using a Panasonic Lumix GH5S camera.

To observe diatom frustules at high magnification, they were embedded in Naphrax®. As the first author was unable to carry out the *ad hoc* preparation procedure, a few drops of the sample dried on a piece of smooth cardboard were sent to the last author. A small amount of this deposit was taken up with a damp brush and then placed in a drop of water on a cover slip. After drying, we noticed that the high salinity of the sample, revealed by the presence of numerous cubic salt crystals, prevented microscopic observation. Therefore, we desalinated the

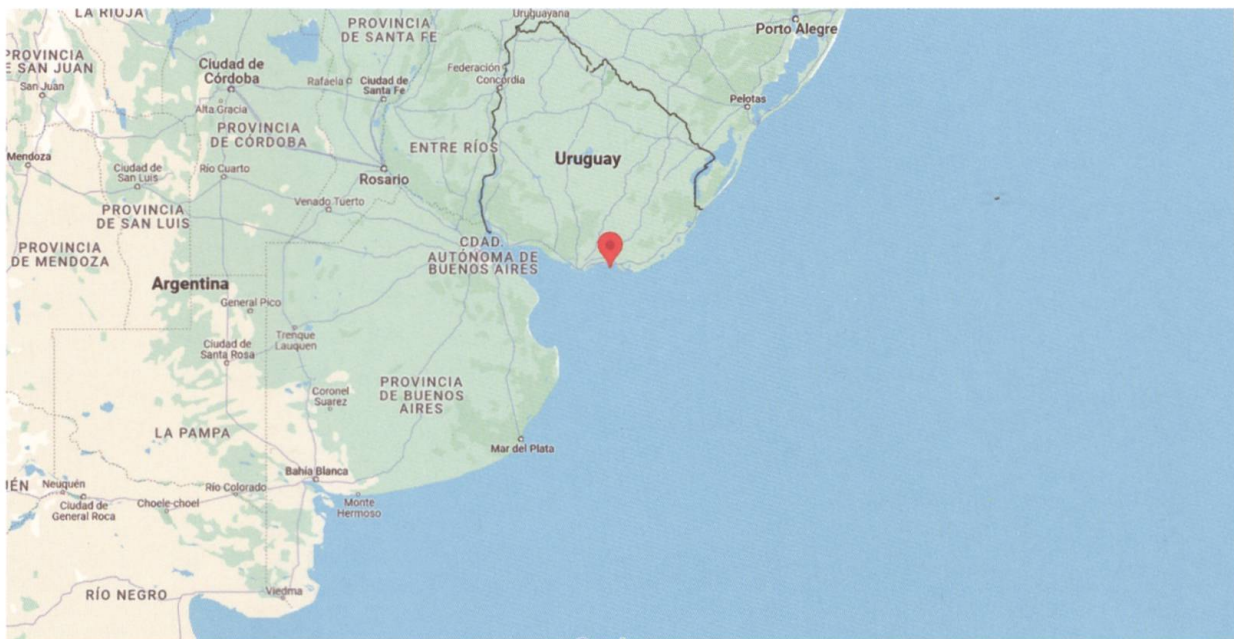


Figure 1: Geographical location of the sample. Coordinates: -34.7974 -55.4972



Figure 2: Sandy rockpool in which the sample was taken.



Figure 3: Jar containing the original sample.

deposit by adding and then removing deionised water three times. After drying, the preparation was mounted directly with Naphrax®, without oxidation or flame treatment. A second preparation was also mounted in Naphrax® after being flamed to oxidise the cytoplasm. The preparations were observed at high magnification using interference contrast with a Leica DMLB microscope and photographs were taken with a Moticam 10 camera. An attempt to examine the specimens using SEM did not yield any results.

OBSERVATION OF LIVE DIATOMS

In the original sample, the diatom of interest was the most abundant. It was fusiform, and its movements were filmed. Several recorded sequences were edited into a video, and the video was posted on YouTube with explanations (GOMEZ REVELLO & JENNI, ref. a).

Our alga showed the usual type of movement seen in diatoms with raphe: a back and forth movement along the longitudinal axis (apical sliding). The endings were well

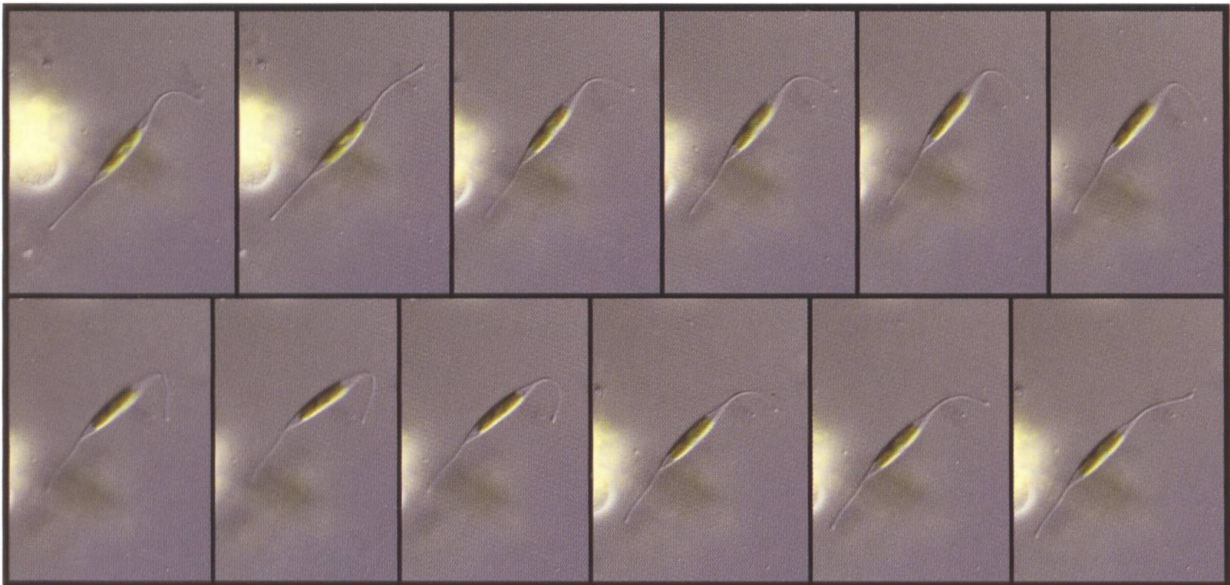


Figure 4: Frames from a video illustrating the formation of the hooked curvature of a flexible diatom cell.

aligned along the axis of the cell and did not deform during movement.

At certain moments, a progressive bending of one of the ends (fig. 4) occurred, as the cell advanced in the direction of this end. The resulting bend was more or less accentuated and more or less regular. The cell then moved backwards, unfolding the end, which returned it to its straight shape. This process was repeated several times, as if the tip of the terminal were preventing the cell from advancing by bending.

In some cases, the bending was very strong, to the point of twisting an end. An example of this movement was observed in the video mentioned above and illustrated in figure 5, with a segment (apical part) of the end «stuck» to debris. The cell moved forward and an S-shaped torsion of the end formed until the extremity of the cell body passed over this end. The glued end of the cell suddenly detached under the effect of the tension caused by the forward movement of the cell. This succession of movements is more obvious in the video. No helical sliding or tilting movements were observed on the flanks.

OBSERVATION OF NAPHRAX® PREPARATIONS

In the Naphrax® preparations without oxidation (fig. 6), only a few highly deformed fragments of the flexible diatom remained. Some remains corresponded to whole frustules but whose width was not preserved. Other remains were fragmented. Portions of the raphe were observed on several fragments, but it was impossible to count the fibulae or observe whether they were spaced out along the centre of the valves. This examination, from which we had hoped to describe the exact morphology of our diatom, was a failure. Only the length of the cells could be measured, and these data completed the series of measurements we had made using frames from our videos.

In addition, several other diatoms were observed in this preparation (fig. 7). These, unlike the flexible diatom, had rigid cell walls that withstood the treatments required to make them visible. Their frustules were very well preserved, making them easy to identify. Some taxa were totally marine, others

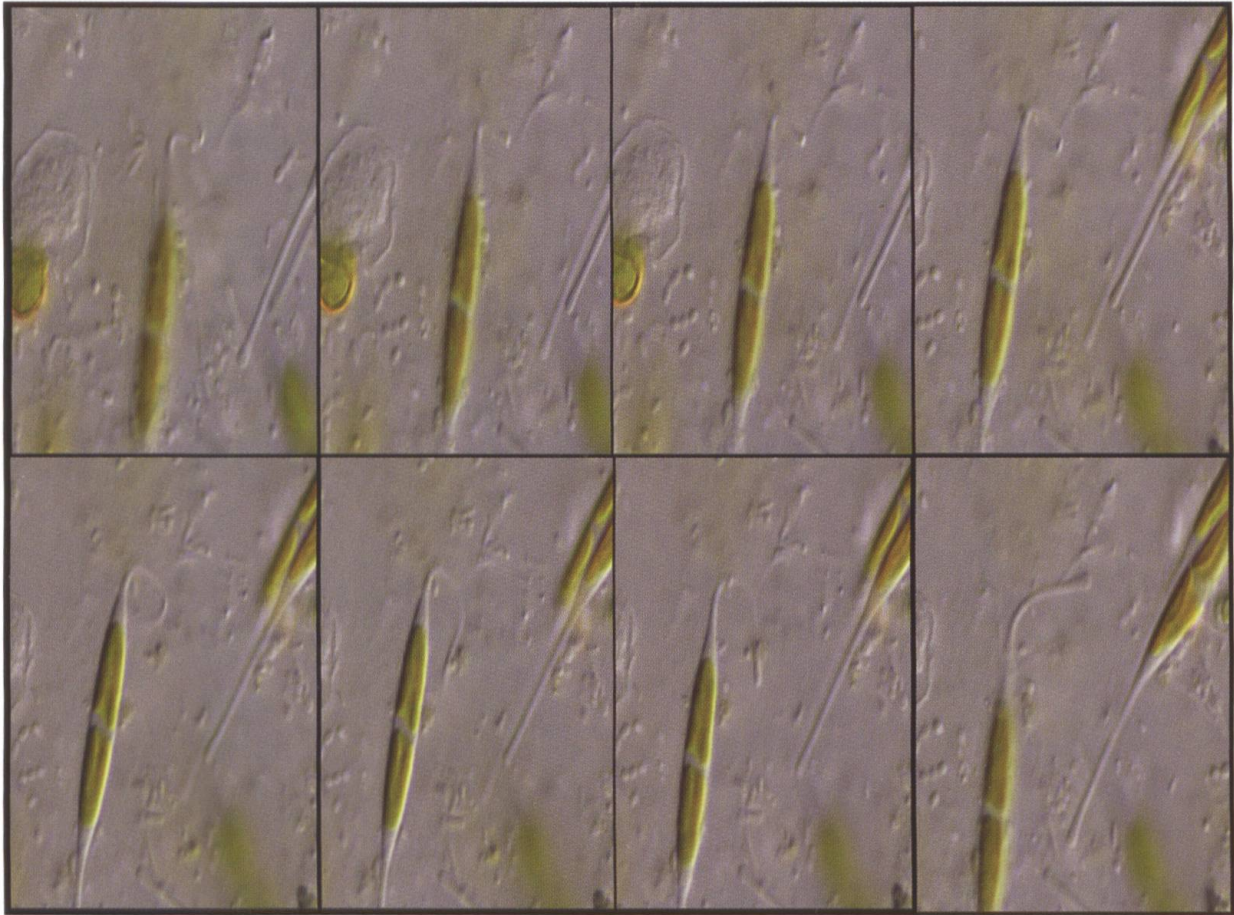


Figure 5: Frames from a video illustrating the formation of an S-twist followed by a loop by the end of the cell.

were from fresh or brackish water, and some were typical of freshwater. This confirmed that water salinity varies at this point in the estuary. The small pennate species were typical of the sandy substrate and were therefore part of the epipsammic community. The circular species (*Thalassiosira pseudonana*), which is of planktonic origin, was brought by drift with the tides.

However, in the preparation oxidised by flame heating, no trace of the flexible diatom was found. The frustules of this species were completely destroyed by this process. However, the other diatoms (fig. 7) were very well preserved despite this somewhat aggressive but commonly used treatment. If it had

been possible to prepare the fresh sample *ad hoc*, the results might have been better. However, the advantage of the preparation that we performed revealed the extreme fragility of this diatom.

IDENTIFICATION ATTEMPT

Our species had the following morphological characteristics:

- Cells abruptly stretched into longitudinal ends (fig. 8). Sometimes, the ends formed a slight curve in the same direction (fig. 9). They were imperceptibly twisted (figs. 9 and 10).

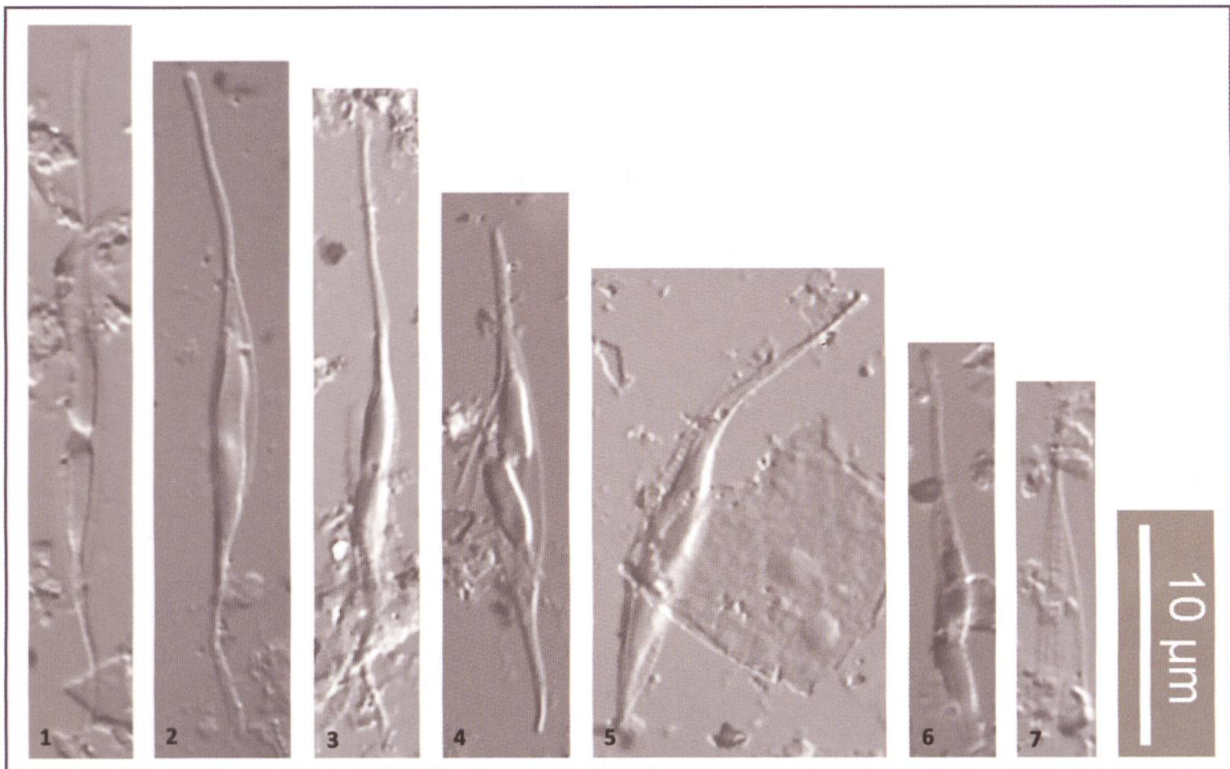


Figure 6: Remains of the flexible diatom in the Naphrax® mounted preparation. 1–3: frustules, whole but very deformed; 4: frustule, whole but laterally split; 5–6: frustules, fragmented with one end missing; 7: half a frustule on which part of the raphe fibulae can be seen. In most fragments, remnants of chloroplasts are still recognisable.

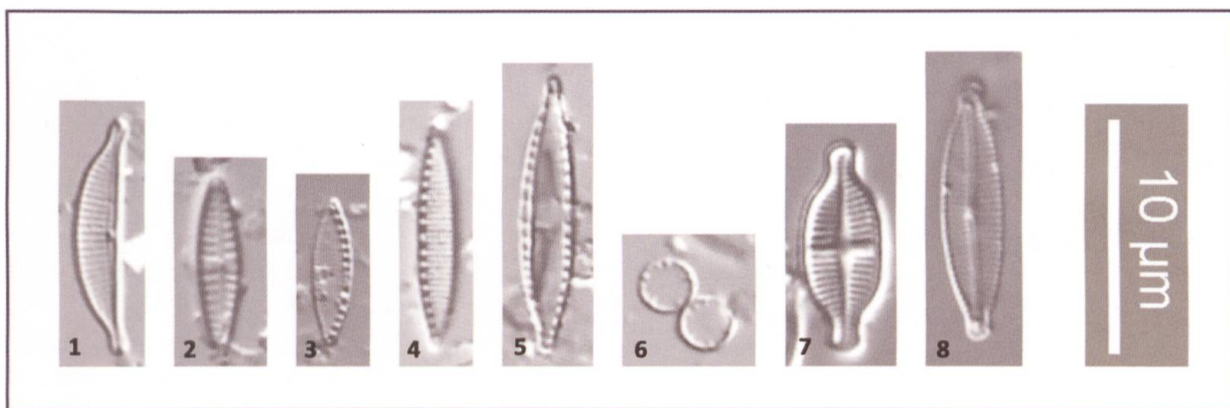


Figure 7: Frustules of species that retained their shape despite the various treatments mentioned in the text. Marine and brackish water species: 1 *Halamphora nawukiana*; 2 *Navicula perminuta*; 3 *Nitzschia liebethruthii*; 4 *Nitzschia frustulum*; 5 *Nitzschia tubicola*; 6 *Thalassiosira pseudonana*. Freshwater species: 7 *Gogorevia exilis*; 8 *Encyonopsis subminuta*.

- Very fragile frustules that were partially destroyed during drying and desalting of the samples (fig. 6).
- Lateral raphe with 16–18 fibulae/10 mm (counted on 2 frustules), arranged in a regular manner, with no central gap, indicating the absence of a central nodule.
- The cell was 51–52 μm long and 3–3.6 μm wide. The striae were invisible under photon microscopy, as they were more numerous than 40/10 μm .
- The cells contained 2 chloroplasts in a gutter (fig. 8).

With its fusiform cells abruptly stretched into long thin ends, this species, according to its shape, was in the following series of freshwater or marine *Nitzschia* and *Cylindrotheca*: *Nitzschia acicularis*, *N. draveillensis*, *N. reversa*, *N. ventricosa*, *Cylindrotheca closterium* (= *N. closterium*), *N. longissima* forma *parva*, or *Nitzschia rectilonga* (VAN HEURCK, 1881; KRAMMER & LANGE-BERTALOT, 1988; PAULMIER, 1997; WITKOWSKI *et al.*, 2000). Some of these species were classified in the former genus *Nitzschiella* (PERAGALLO & PERAGALLO, 1897-1908).

We cite this series, bearing in mind that it may be questionable whether we are basing ourselves on European or North American taxa (taxonomic force-fitting) when describing organisms from the southern hemisphere (HEGER *et al.*, 2009; VAN DE VIJVER & KOPALOVA, 2014).

Among these species, those with a twisted raphe were reclassified in the genus *Cylindrotheca* by REIMANN & LEWIN (1964). Some, such as *C. gracilis*, have two twisted raphes along their entire length. Others have twisted raphes only along the thin ends, which makes this character hardly noticeable. This was the case for our species, which therefore belongs to this genus now accepted by the scientific community (MEDLIN & MANN, 2007), despite the reservations of KRAMMER & LANGE-BERTALOT (1988) and JAHN & KUSBER

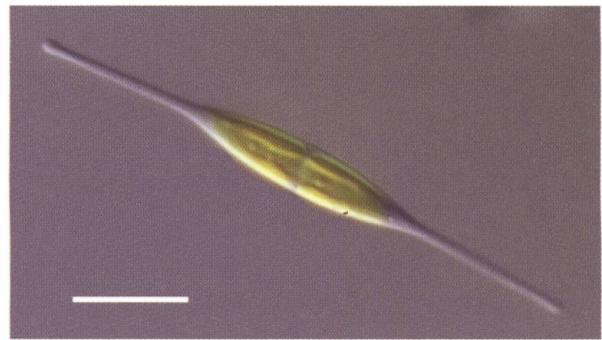


Figure 8: A living cell with two longitudinal ends and two guttered chloroplasts. Scale bar: 10 μm .

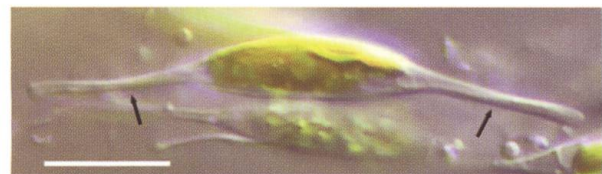


Figure 9: A living cell showing the twisting of the two ends at the points indicated by arrows. Scale bar: 10 μm .

(2005). Our diatom was similar to *C. closterium* in its general shape, its dimensions and its lateral raphe, which was barely twisted at the ends (figs. 9 and 10).

However, this well-known and frequently found species has central nodules on its two valves, structures whose presence can be deduced under light microscopy by the fact that the two central fibulae are separated from each other (REIMANN & LEWIN, 1964; MIŠIĆ RADIĆ *et al.*, 2023). However, in our species, the central fibulae were not spaced apart (fig. 10). Given the taxonomic importance of this criterion since the revisions of the genus *Nitzschia* (LANGE-BERTALOT, 1976, 1980; LANGE-BERTALOT & SIMONSEN, 1978), our diatom does not belong to any of the species in the series selected by REIMANN & LEWIN (1964).

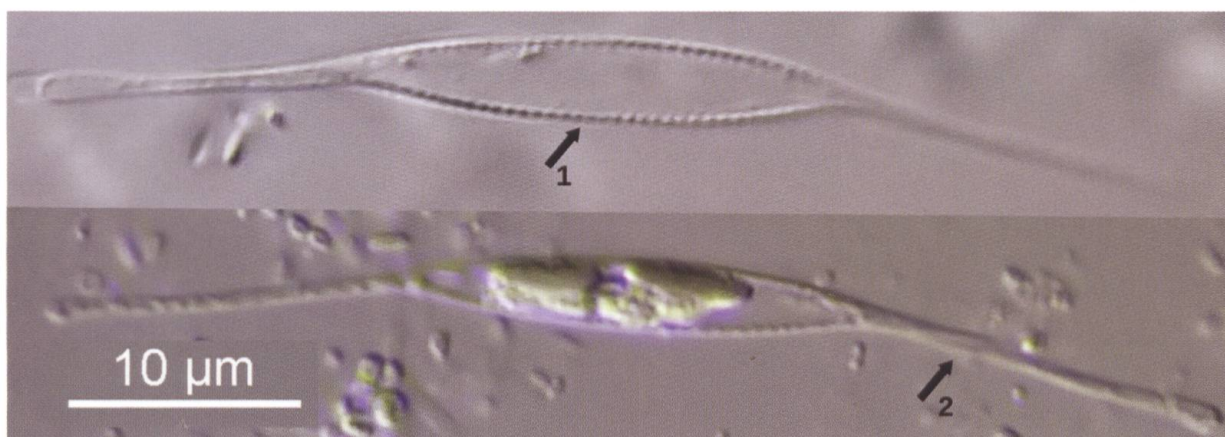


Figure 10: Frustules of dead diatoms (the wall is visible because the cytoplasm is degraded). Arrow 1 designates the regularity of the fibulae in the middle of the frustule; which indicates that the cell does not have a central nodule (area of interruption of the raphe in species that have two fibulae separated at this point; as in *Nitzschia tubicola* [fig. 7]). Arrow 2 indicates the torsion of an end.

It may be a new species. If this is the case, it would have to be described in due form, which unfortunately is presently impossible for us because of the rarity of the original material and the fragility of the frustules. We therefore propose that it be provisionally named *Cylindrotheca sp.*

In freshwater phytoplankton, there is a species, *Nitzschia acicularis* (KRAMMER & LANGE-BERTALOT, 1988), whose dimensions are identical to those of our diatom. Its frustule, which also lacks a central nodule, has proportionally shorter ends than the cell body. In addition, as far as we know, this species has no twisted ends. These differences preclude the attribution of our organism to this taxon.

DISCUSSION

This study highlights two aspects of the biology of our diatom: the flexibility of the ends, linked to the unusual movements that we as well as a few authors before us, have observed and documented, and the fragility of the wall. Although such fragility has been reported in several species, few authors mention «behaviour» similar to that observed

in *Cylindrotheca sp.* Consequently, we feel that these characteristics should be treated separately.

Flexibility and movements

Considering the movements based on our filmed and video-edited observations, a distinction should be made between an active element, the usual back-and-forth movement of diatoms, and a passive one, the flexing of the ends. We found no mention in the literature of such a distinction between active and passive movements.

Many hypotheses have been put forward to explain the various movements of diatoms. However, currently, there is no certainty as to the mechanisms behind these movements (EDGAR, 1979; EDGAR & PICKETT-HEAPS, 1983; BERTRAND, 2008; BEDOSHVILI & LIKHOSHWAY, 2021; WANG *et al.*, 2021).

When they were still classifying long-ended *Nitzschia* in the genus *Nitzschiella* described by Rabenhorst, Hippolyte and Maurice Peragallo wrote the following on page 292 of one of the first editions of their

flora (PERAGALLO & PERAGALLO, 1897-1908)
- translated from French:

«**Genus 18. - Nitzchiella Rab.**

The Nitzschiella, as delimited by Grunow, comprise two groups of rather different forms that deserve to be separated.

The Nitzschiella strictly defined have long filiform extensions which are very flexible and mobile. In the very rapid movements of these forms, these long ends bend in the direction of the movement like rudders: it really seems that these movements are voluntary and that the Diatom uses these appendages to direct its movement, which is much less automatic than that of the naviculae. Strictly speaking, therefore, it cannot be said that these forms are or are not sigmoid, as the extensions can be either in line with each other or curved in the same direction or in the opposite direction.»

This description more or less matches our observations. However, concerning our diatom, our interpretation differs on several points, in particular on the attribution of a «voluntary» (= active) movement of the ends. In fact, on page VIII of the supplementary notes to the definitive edition of their flora in 1908, one of the Peragallo brothers returned to this observation and cast doubt on it:

«**F. - Nitzchiella.** - *I must admit that, in what I said about the movements of the Nitzchiella, I was deceived first of all by appearances. In this movement, it is not the end of the Nitzchiella that changes direction, it is the cell that turns on itself as it moves; the end then acts as a rudder and directs the direction of the movement.*»

Did his observation of a helical movement in *Nitzschiella*, a movement well known in *Cylindrotheca gracilis* (DIATOMS OF NORTH AMERICA, ref. b) and in other species of this genus, sow doubt in the mind of this author? Moreover, such flexibility of the ends probably caused him to be astonished, just as we are.

The study by C. G. Ehrenberg in 1839 on the vivacity of movements in *Ceratoneis closterium* (= *Cylindrotheca closterium*), reproduced by JAHN & KUBSER (2005), did not specify that the endings of this species could bend. More recently, APOYA *et al.* (2006) described in detail, as shown in their figure 1J, the various types of movement of *Cylindrotheca closterium*, including the bending of the ends. This movement occurs particularly during apical sliding, when one of the ends has been retained by debris. This observation is largely consistent with our own if we exclude a much weaker curvature of the ends of *C. closterium*. This is also illustrated by a short film of *Cylindrotheca closterium*, strain NIES-1045, from the NIES Microbial Collection (NIES-1045 ref. c).

This important feature argues, in addition to the morphological characteristics mentioned above, in favour of the fact that our diatom does not belong to the species *Cylindrotheca closterium*.

In a sequence from a «mainstream» film (PICKETT-HEAPS & PICKETT-HEAPS, 2022), the movements of a diatom were observed to be very similar to those of our algae. The extract from the commentary on this sequence explains:

«*In summary, gliding movement is generated by mucilage secretion coupled with cytoplasmic transport. This motile system can generate significant force. When this Nitzschia gets stuck, the cell shows remarkable persistence in trying to move. The force it generates loads up the wall like a spring. This cell persisted in its behavior over 20 minutes. On some occasions, the force generated by the cell can permanently damage the wall.*»

Both in this sequence and in our films, it is the body of the cell that is actively moving. Following the immobilisation of an end, the algae is prevented from moving forward. It then moves backwards, more or less abruptly,

before trying to move forward again. Pickett's film even shows the fracture of a terminal under the pressure of the cell's movement, confirming that the movement of the terminal is not active. In comparison with our observations, the ends of this *Nitzschia* appear to be more rigid than those of our diatom and consequently do not reach such strong curvatures as those of our algae. This distinction also applies to *Cylindrotheca closterium*.

In 2018, A. N. KHANAYCHENKO (2018) showed that the tips of the ends of *Cylindrotheca closterium* secrete adhesive substances (mucopolysaccharides) enabling them to attach themselves in large numbers to copepod crustaceans of the species *Oithona davisae* (which are invasive in the Black Sea), immobilising and eliminating them. Based on this observation, it is unclear whether, in addition to bending when they encounter an obstacle (debris for example) as observed by APOYA *et al.* (2006), the ends of our algae also secrete adhesive substances. We sometimes found that the ends bent and slowed down the cell's advance in the absence of visible debris. At present, this question remains unanswered.

Fragility

Diatom cells have a rigid silicified wall. The broken frustules are very sharp. This is why some diatomites are used as insecticides, destroying the digestive system of insects that ingest them. The results of microscopic preparations with Naphrax® confirmed the fragility of the *Cylindrotheca sp.* frustules, of which only fragments were found after treatment. The frustules of the other diatoms from the same sample were complete and perfectly recognisable.

Thus, the failure of the SEM observation attempt was probably due to the total

destruction of the frustules during the preparation of the specimen. This fragility has been noted by several authors in *Cylindrotheca* species and in the fusiform species of the genus *Nitzschia* (REIMANN & LEWIN, 1964; KRAMMER & LANGE-BERTALOT, 1988; WITKOWSKI *et al.*, 2000), such as *N. acicularis* and *N. draveillensis*. It is thought to be linked to the low silicification of their frustules. It is also unclear whether the flexibility of the ends of our species is due to the lack of silica in their walls.

There is also the question of the influence of factors present in the environment at the time of sampling, such as nutrients, pollutants and salinity. For example, NOVOSEL *et al.* (2022) showed that *Cylindrotheca closterium* was tolerant to a decrease in salinity and that its rigidity and hydrophobicity changed according to the saline concentration in its environment.

CONCLUSION

Compared with previous work, our main contribution was to have documented the plasticity of the ends of our diatom *Cylindrotheca sp.* and the resulting movements, as shown in an easily accessible video. In addition, attempts to observe preparations using Naphrax (and SEM) have demonstrated the great fragility of the frustule of this diatom.

ACKNOWLEDGEMENTS

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WEB SITES

- GOMEZ REVELLO, C. & JENNI, B (ref. a)
https://www.youtube.com/playlist?list=PLyn9F8HbHjLIDdqp_NQKtVVtDPWQQ_VWT
- DIATOMS OF NORTH AMERICA (ref. b)
<https://diatoms.org/genera/cylindrotheca/guide>
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