

Research



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Cooperative motility, force generation and mechanosensing in a foraging non-photosynthetic diatom

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Diatoms are ancestrally photosynthetic microalgae. However, some underwent a major evolutionary transition, losing photosynthesis to become obligate heterotrophs. The molecular and physiological basis for this transition is unclear. Here, we isolate and characterize new strains of non-photosynthetic diatoms from the coastal waters of Singapore. These diatoms occupy diverse ecological niches and display glucose-mediated catabolite repression, a classical feature of bacterial and fungal heterotrophs. Live-cell imaging reveals deposition of secreted extracellular polymeric substance (EPS). Diatoms moving on pre-existing EPS trails (runners) move faster than those laying new trails (blazers). This leads to cell-to-cell coupling where runners can push blazers to make them move faster. Calibrated micropipettes measure substantial single-cell pushing forces, which are consistent with high-order myosin motor cooperativity. Collisions that impede forward motion induce reversal, revealing navigation-related force sensing. Together, these data identify aspects of metabolism and motility that are likely to promote and underpin diatom heterotrophy.

1. Introduction

Eukaryotes fall into fundamentally distinct groups based on their means of energy acquisition. Photoautotrophs—land plants and algae—derive energy from sunlight. By contrast, heterotrophs, such as animals and fungi, obtain energy by feeding on primary producers or each other. Mixotrophs combine these two strategies.

Eukaryotic photoautotrophs evolved several times through endosymbiosis between a heterotrophic eukaryote and a photosynthetic microbe. Such an association between the ancestor of the Archaeplastida and a cyanobacterium led to the emergence of land plants, green and red algae, and glaucophytes. Afterwards, distinct algal lineages arose through secondary and tertiary endosymbiotic events, where the photosynthetic capacity was acquired from a eukaryotic red or green alga [1–4].

The stramenopiles (also known as heterokonts) obtained their plastid through a secondary or higher endosymbiotic event with a red alga [5]. This group includes oomycetes, multicellular brown algae and unicellular diatoms [6]. With an estimated 100 000 species [7], diatoms are one of the most abundant and diverse group of marine and freshwater microalgae [8–12]. Notably, they

employ biomineralization to construct silica-based [13,14] cell walls (frustules) that fit together like the two halves of a petri dish. Diatoms possess either radial (centric diatoms) or bilateral (pennate diatoms) symmetry. A group of pennate diatoms evolved a fine longitudinal slit through the frustule known as the raphe. These raphid pennate diatoms can move using a lineage-specific form of gliding motility [15,16] and have undergone substantial evolutionary radiation to comprise the most species-rich and diverse lineage of diatoms [9].

The raphe acts as a channel for the secretion of a complex mixture of proteins and glycoproteins [17–21] known collectively as extracellular polymeric substances (EPS). Motility can be blocked by an antibody to EPS [17] and by actomyosin inhibitors [22,23], suggesting that both systems play essential roles. Actin filaments occur in two prominent bundles that underlie the raphe just adjacent to the plasma membrane [24]. This arrangement supports a model of motility where myosin motors exert pushing forces on the extracellular EPS through a transmembrane protein [25]. However, this protein has yet to be identified, and since actin is likely to be required for EPS secretion [26,27], an alternative model where force is generated from EPS polymerization has not been excluded [15,16].

Within each photosynthetic lineage, loss of photosynthesis led to secondary heterotrophs, many of which are parasites that derive energy from their host [28]. Transitions to epizoid [29] and free-living [30,31] heterotrophy are also well documented. However, in most of these cases, the manner of energy acquisition remains unclear. In the diatom genus *Nitzschia*, loss of photosynthesis led to a group of free-living heterotrophs [32,33]. These apochlorotic diatoms have been isolated from the nutrient-rich waters of the intertidal zone where they occur as epiphytes on seaweeds, on decaying plant matter and in the surrounding waters [32,34–37]. As with many photoautotrophs that transition to heterotrophy [30], they have retained their plastid genomes and certain plastid-localized metabolic functions, but have lost key photosynthetic genes [38]. Early work showed that apochlorotic diatoms can grow on a variety of simple and complex carbohydrates including cellulose and the red algal cell wall polysaccharides agarose and carrageenan [34,35,39]. Recent genome sequence studies have identified lignin-degrading enzymes in *Nitzschia* Nitz4 [40] and the expansion of secreted proteins and functions related to organic carbon acquisition in *Nitzschia putrida* [41]. Thus, candidates for key heterotrophy-related functions are beginning to emerge.

Here, we isolate new strains of apochlorotic diatoms from Singapore's intertidal zone. Live-cell imaging documents EPS trail deposition and complex motility-related behaviours that include high force generation (approx. 800 pN), cooperative motility and collision-induced reversal. Variations in motility and metabolism suggest that apochlorotic diatoms are undergoing substantial ecophysiological radiation. We propose that these new isolates provide excellent models to study the evolutionary transition to free-living heterotrophy.

2. Results

2.1. Isolation and characterization of apochlorotic diatoms

Diatoms were cloned from organic materials collected from the intertidal zone on Sentosa Island, Singapore (see Material

and methods). Five clones were initially isolated from decaying plant matter, the brown alga *Sargassum* and the green alga *Bryopsis* (figure 1a). Subsequent work revealed the ability of these diatoms to metabolize the brown algal cell wall polysaccharide alginate (figure 2a). Thus, we isolated an additional seven clones from *Sargassum*. Phylogenetic analysis indicates that these 12 isolates fall into three distinct clades (figure 1a). Isolates were named *Nitzschia* singX–Y, where X designates the clade number and Y the isolate number. Isolates in clade 1 and 2 are sister taxa, with clade 2 having an affinity for *N. alba*, while clade 3 is distantly related to clades 1 and 2.

Isolated diatoms grown on agarose seawater [42] media form a radially expanding colony. Growth rates vary substantially both within and between clades. Rates of colony expansion for clade 1 and 2 diatoms vary between 100 and 300 nm s^{−1} (electronic supplementary material, figure S1a). By contrast, all clade 3 diatoms and the recently sequenced apochlorotic *N. putrida* [41] show very little colony expansion, with cells dividing to form aggregates at the site of inoculation (electronic supplementary material, figure S1b,c).

Clade 1 diatoms were isolated from green and brown algae, and decaying plant matter, suggesting that they occupy diverse ecological niches. They are also among the fastest-growing isolates. Thus, we chose *N. sing1*–1 as our model apochlorotic diatom (hereafter referred to as *N. sing1*). F-actin staining reveals characteristic bands underlying the raphe (figure 1b), and scanning electron microscopy (SEM) of frustules identifies eccentric raphes, hymenate pore occlusions and strongly hooked distal raphe ends (electronic supplementary material, figure S1d).

2.2. Growth on algal polysaccharides and catabolite repression

We next examined the growth of *N. sing1* on seawater media solidified with red algal cell wall polymers agarose and carrageenan. As previously observed with *N. alba* [39], both of these substrates could be used as the sole carbon source (electronic supplementary material, figure S2). In addition, *N. sing1* grows on the brown algal polysaccharide alginate. For each polysaccharide substrate, the rate of radial colony expansion generally has a concentration optimum and tends to decrease with increasing concentration (electronic supplementary material, figure S2). In the case of alginate, the medium underwent liquefaction and browning, indicative of polysaccharide hydrolysis (figure 2a). This is confirmed by a heat-sensitive alginate lyase enzyme activity detected in the media of *N. sing1* grown with alginate but not glucose (figure 2b). Representatives of each clade of Singaporean diatoms liquefy alginate, but *N. putrida* does not. This suggests that modes of heterotrophy vary substantially within the apochlorotic lineage. Neither agarose nor carrageenan undergo liquefaction. However, on these media, the diatoms tunnel to grow invasively (electronic supplementary material, figure S3), as has been documented for *N. albicostalis* [35] and *N. alba* [39].

We next examined cellular extracts of diatoms grown on seawater agar medium with and without 0.5% glucose. SDS-PAGE reveals two *N. sing1* proteins, p40 and p60, that are abundant on agarose media, but substantially diminished when glucose is present. This repression is observed for representatives of each *N. sing* clade, but not *N. putrida* (figure 2c). Alginate also promotes catabolite repression in *N. sing1*,

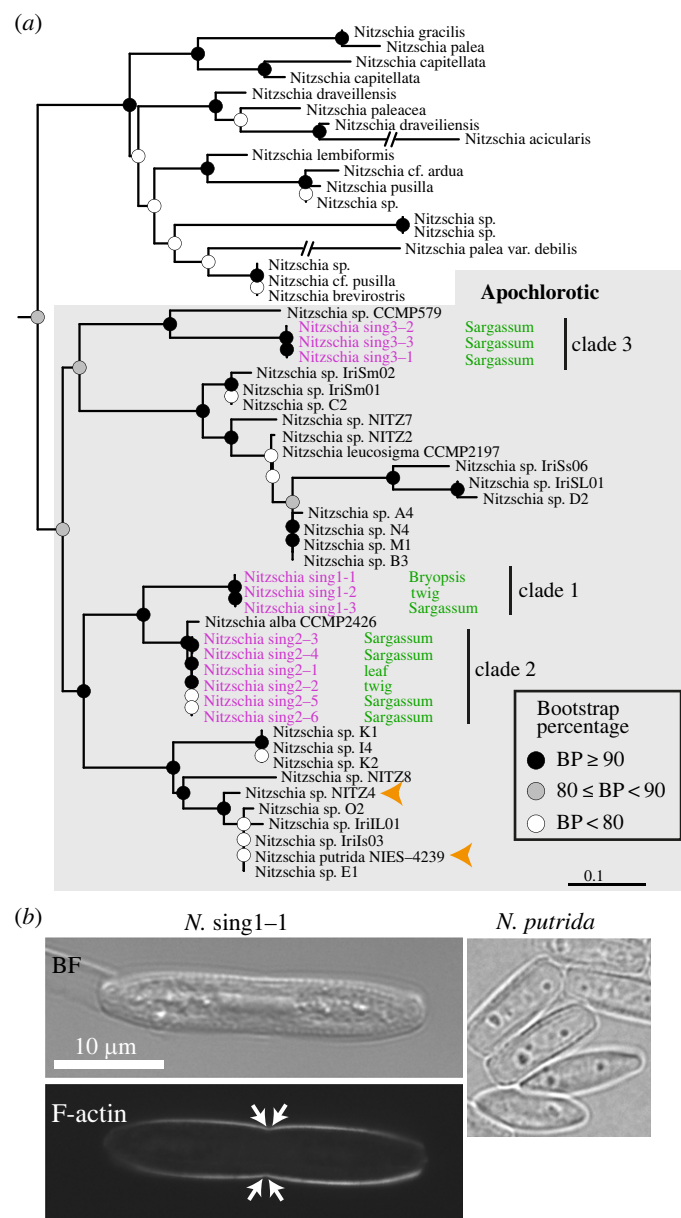


Figure 1. Characterization of Singaporean apochlorotic diatoms. (a) Maximum-likelihood phylogeny of apochlorotic *Nitzschia* (shaded box) and Bacillariales photosynthetic outgroup taxa. The Singaporean isolates are identified in magenta. The material from which they were isolated is indicated in green. Filled circles show simplified bootstrap support values. Scale bar = 0.1 nucleotide substitutions. Orange arrowheads identify apochlorotic species with sequenced genomes. (b) F-actin staining of *N. sing1-1*. The arrows point to the approximate position of proximal raphe ends. A bright-field (BF) image of *N. putrida* is shown for comparison. Scale bar = 10 μm .

indicating that it is also a preferred carbon source (electronic supplementary material, figure S4a). *N. sing1* accumulates large lipid droplets in the presence but not absence of glucose. By contrast, *N. putrida* lipid droplets have a similar appearance irrespective of glucose presence (figure 2d and electronic supplementary material, figure S4b). This provides further evidence for the metabolic responsiveness of *N. sing1* to a preferred carbon source.

2.3. Environmental control of EPS trails and motility

While measuring *N. sing1* growth, we found that the EPS can be seen as a refractive trail by bright-field microscopy (figure 3a). This is probably due to swelling of the EPS to form a refractive convex cross-sectional profile. EPS trails formed on 1% agarose have a uniform width and appearance. By contrast, on 2% agarose, where motility is substantially diminished, the trails take on a broken appearance and the EPS forms refractive spherical structures. To examine how the availability of seawater affects

motility, we overlaid the medium with seawater (figure 3b). In this condition, a dramatic increase in the speed of motility is observed as compared to plates without a seawater overlay (figure 3c,d). Here, trails are not seen because the EPS is not at the air interface. Together, these findings link nascent EPS swelling and material properties with the promotion of motility. Interestingly, diatoms are also observed gliding in a monolayer at the seawater–air interface, indicating that *N. sing1* motility is not strictly dependent on substratum attachment (figure 3c).

2.4. Cooperative motility, force generation and force sensing

Movies of EPS deposition allowed us to differentiate diatoms laying fresh EPS trails from those moving on pre-existing trails (figure 4a). We refer to these as trail blazers (blazers) and trail runners (runners), respectively. Blazers instantly accelerate upon joining a trail, while runners that leave a trail instantly decelerate (figure 4b–e). These observations

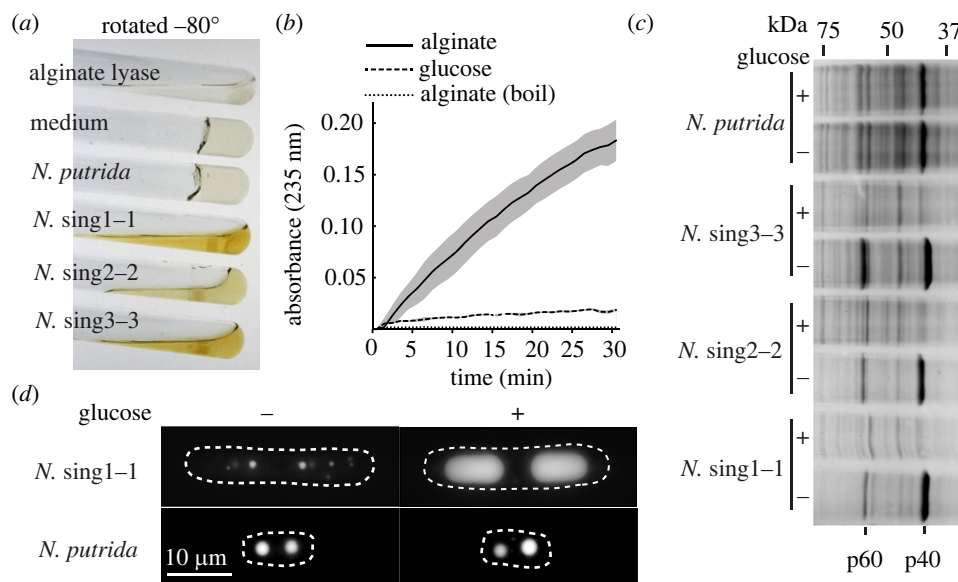


Figure 2. Diatom alginate utilization and catabolite repression. (a) The image shows cultures of diatoms on 1.5% seawater alginate medium. The tubes are rotated approximately -80° to show alginate gel liquefaction. Alginate lyase enzyme and medium alone serve as positive and negative controls, respectively. (b) The graph shows *N. sing1-1* alginate lyase enzyme activity in the indicated media after 4 days of growth. Note that boiling (boil) abolishes the enzyme activity. (c) SDS-PAGE of total cell extracts from diatoms grown on seawater agar medium in the absence (–) or the presence (+) of 0.5% glucose. Glucose-repressed proteins p40 and p60 are identified. (d) Lipid droplet accumulation of *N. sing1-1* and *N. putrida* on seawater agarose media in the presence (+) and absence (–) of glucose. The dotted line traces the cell periphery. Scale bar = 10 μm . Related to electronic supplementary material, figure S5.

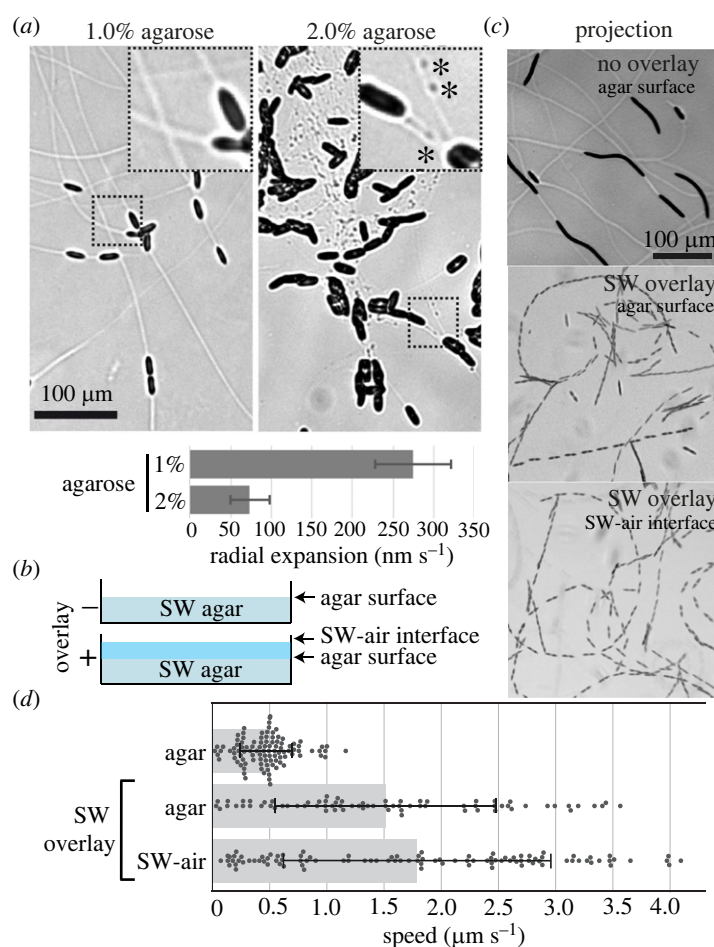


Figure 3. Environmental control of EPS and motility. (a) Appearance of EPS trails on 1 and 2% seawater agarose medium. The dashed box is magnified on the upper right. Scale bar = 100 μm . Asterisks identify refractive EPS puncta. The graph shows the speed of growth on each medium. (b) The diagram depicts a side view of assay plates in the presence (+) or the absence (–) of a seawater (SW) overlay. (c) The projections show diatom motility under the indicated conditions. Total time = 305 s. Scale bar = 100 μm . (d) The graph shows the speed of diatoms shown in (c). The bar shows the average with standard deviation indicated. Related to electronic supplementary material, movies S1, S2 and S3.

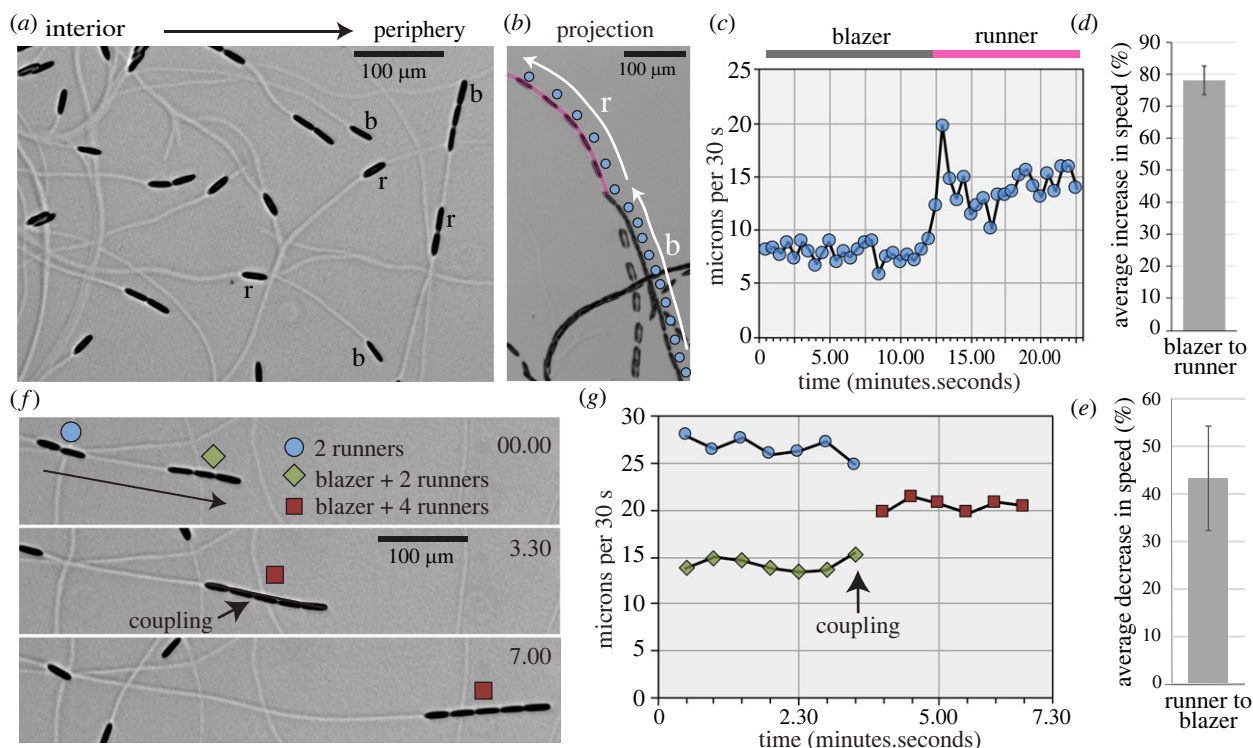


Figure 4. Live-cell imaging of gliding and EPS deposition by *N. sing1-1*. (a) The image shows a field of diatoms near the colony edge. The direction from the colony interior to colony periphery is indicated. Diatoms laying a new trail are blazers (b), while diatoms gliding on a pre-existing trail are runners (r). Note that these identities are interchanged as diatoms reverse or transition on or off trails. Scale bar = 100 μ m. Related to electronic supplementary material, movie S4. (b) A blazer (blue circle) instantaneously accelerates upon joining a pre-existing trail (magenta line). White arrows indicate the periods spent as blazer (b) and as runner (r). Scale bar = 100 μ m. The projection is derived from electronic supplementary material, movie S5. (c) The graph shows the speed of the diatom from (b). (d) The graph quantifies the average increase in speed when a blazer becomes a runner. Standard deviation is indicated ($n = 6$). (e) The graph quantifies the average decrease in speed when a runner becomes a blazer. Standard deviation is indicated ($n = 6$). (f) Runners can push blazers to make them move faster. A chain consisting of a blazer and two runners is caught by a pair of runners to make a chain of five. The groups are identified at the indicated frames by symbols given in the legend. The moment when cell-cell coupling occurs is indicated. Scale bar = 100 μ m. Related to electronic supplementary material, movie S6. (g) The graph shows the speed of the groups as defined in (f).

indicate that gliding motility is inherently more efficient when occurring on an EPS trail. Because of this relationship, runners tend to catch up to blazers and form chains of cells, particularly at the colony's expanding edge. When fast-moving runners catch up to blazers, the blazer can instantly accelerate (figure 4f,g). Thus, runners can exert pushing forces to make blazers move faster.

Mathematical modelling shows that the movement of diatoms is well approximated by Brownian motion over large space and time scales (electronic supplementary material). A quasi-steady-state analysis of the model provides a mathematical relationship between motion characteristics and establishes that colony diffusivity increases with diatom speed. This relationship is preserved in runner–blazer groups, which exhibit an increase in speed compared with lone blazers (figure 4f,g). Thus, modelling confirms the tendency for cooperative motility to increase colony diffusivity.

Broadside collisions between diatoms are readily observed and these frequently lead to reversal of the impacting diatom. In figure 5a, diatom 1 undergoes three successive impacts with a relatively stationary diatom 2. The initial collision is followed by a rapid reversal. However, the second and third collisions are characterized by longer periods spent pushing. This is coincident with increasing degrees of deflection of diatom 2 (figure 5a). Thus, the capacity for force generation appears to be dynamic. Not all collisions lead to reversal. In some cases, the impacting diatom slows dramatically upon collision and continues to move forward as it pushes the other diatom out

of its path (figure 5b,c). Together, these observations reveal the ability of moving *N. sing1* diatoms to impart force, which impacted diatoms resist through substratum adhesion.

We next employed a method that allows the measurement of forces exerted by single diatoms. Here, a force-calibrated glass micropipette is placed in the path of moving diatoms and the force is estimated through the degree of pipette deflection [43,44]. These data reveal forces between approximately 100 and approximately 800 pN (figure 6a,b; electronic supplementary material, figure S5). Lower force measurements are associated with glancing contact with the pipette. By contrast, high force measurements occur within the context of head-on contact and adhesion between the diatom and pipette. Adhesion is evidenced by diatom detachment from the agar surface at a load of approximately 740 pN (figure 6b, no. 3) and an apparent EPS tether that attained a length of 3–4 μ m before snapping at a load of approximately 800 pN (figure 6b, no. 2). Diatoms pass underneath the pipette while it undergoes deflection, suggesting that they are subject to downward pushing forces of approximately 84 pN while pushing/pulling on the pipette (based on an estimated diatom height of 4 μ m). Overall, these data show that *N. sing1* is capable of producing surprisingly high forces. For comparison, single intact muscle filaments produce forces of approximately 200–300 pN [45,46].

Collision-associated reversals suggest that *N. sing1* diatoms can sense force. However, reversals do not always follow collisions (figure 5c,d) and also occur in free-running diatoms.

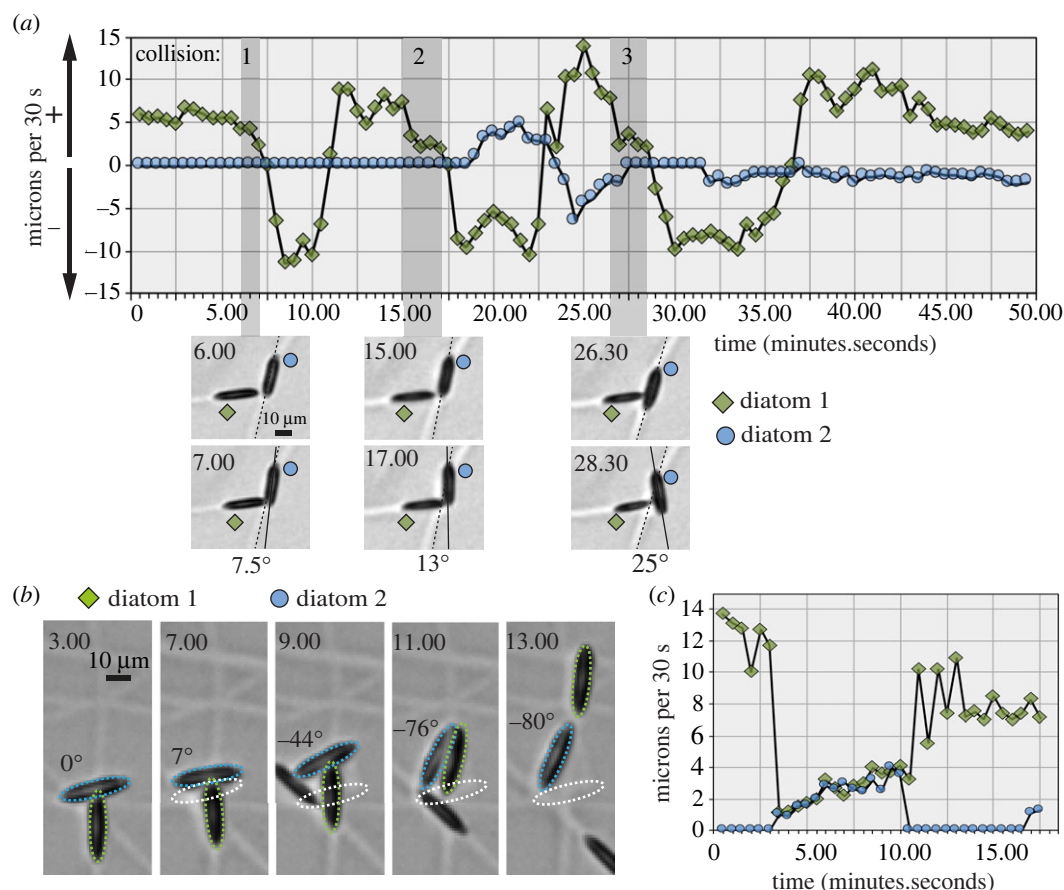


Figure 5. Pushing and collision-triggered reversals. (a) Diatom 1 collides with a stationary diatom 2 three consecutive times. Frame-to-frame speed is shown in the graph. The images at the bottom show the first (upper panel) and last (lower panel) frames of the collisions. Scale bar = 10 μm . Related to electronic supplementary material, movie S7. (b) Diatom 1 collides into a stationary diatom 2, which is pushed laterally and rotates until diatom 1 frees itself. The original position of diatom 2 is given by the dotted white outline and its degree of rotation is indicated numerically. Scale bar = 10 μm . Related to electronic supplementary material, movie S8. (c) The graph shows the speeds of diatom 1 and diatom 2 from (b).

To compare these two events, diatoms were grown on an agar surface close to a coverslip embedded perpendicular to the medium. Diatoms undergoing collisions with the coverslip were then compared to nearby diatoms that did not experience collision (figure 6c). In this experiment, 100% of colliding diatoms reverse within 400 s of being immobilized. By contrast, 13% of individuals whose motility is unimpeded reverse within the same time interval (figure 6d). In these experiments, colliding diatoms spend a longer period immobile prior to reversal when compared with free-running reversals. Free-running diatoms also slow prior to stopping and reversing, suggesting underlying distinctions between free-running and collision-induced reversals (electronic supplementary material, figure S6). Irrespective of this, these data show that collisions that impede forward motion significantly increase the probability of reversal. This type of force sensing is likely to increase colony diffusivity, especially where substrates possess complex morphologies.

3. Material and methods

3.1. Diatom isolation

Organic materials were collected at low tide from the intertidal zone on Sentosa island, Singapore (latitude 1.259895, longitude 103.810843; Singapore National Parks Board permit no. NP/RP20-016). Samples were inoculated at the

centre of synthetic seawater [42], 2% (w/v) agar plates supplemented with 100 $\mu\text{g ml}^{-1}$ ampicillin (Sigma-Aldrich, A9518) and 50 $\mu\text{g ml}^{-1}$ kanamycin (Sigma-Aldrich, K4000). After 2–3 days of incubation at 30°C, single diatoms gliding away from the source inoculum were excised with a scalpel and transferred to a fresh plate. To ensure that all isolates are single-cell clones, the isolation process was repeated. Diatom clones were cryopreserved according to the method of Stock *et al.* [47]. The *Sargassum* species present at the collection site were identified as a mixture of *S. polycystum*, *S. cf. granuliferum* and *S. ilicifolium* (electronic supplementary material, figure S8) through a maximum-likelihood (ML) analysis of ITS-2 alignment as previously described [48]. New sequences are available in GenBank under accessions OQ165106 to OQ165109. *N. putrida* (strain NIES-4239) was obtained from The Microbial Culture Collection at the National Institute for Environmental Studies 16-2, Onogawa, Tsukuba, Ibaraki 305-8506, Japan.

3.2. Diatom phylogenetic analysis

We sequenced one nuclear gene (28S d1–d2 rDNA) and two mitochondrial genes (*cob* and *nad1*) for each of the Singapore isolates following the PCR and sequencing protocols outlined in Onyshchenko *et al.* [33]. Raw Sanger sequences were edited and assembled with GENEIOUS v. 7.1.4 (Biomatters Ltd.). New sequences are available in GenBank under

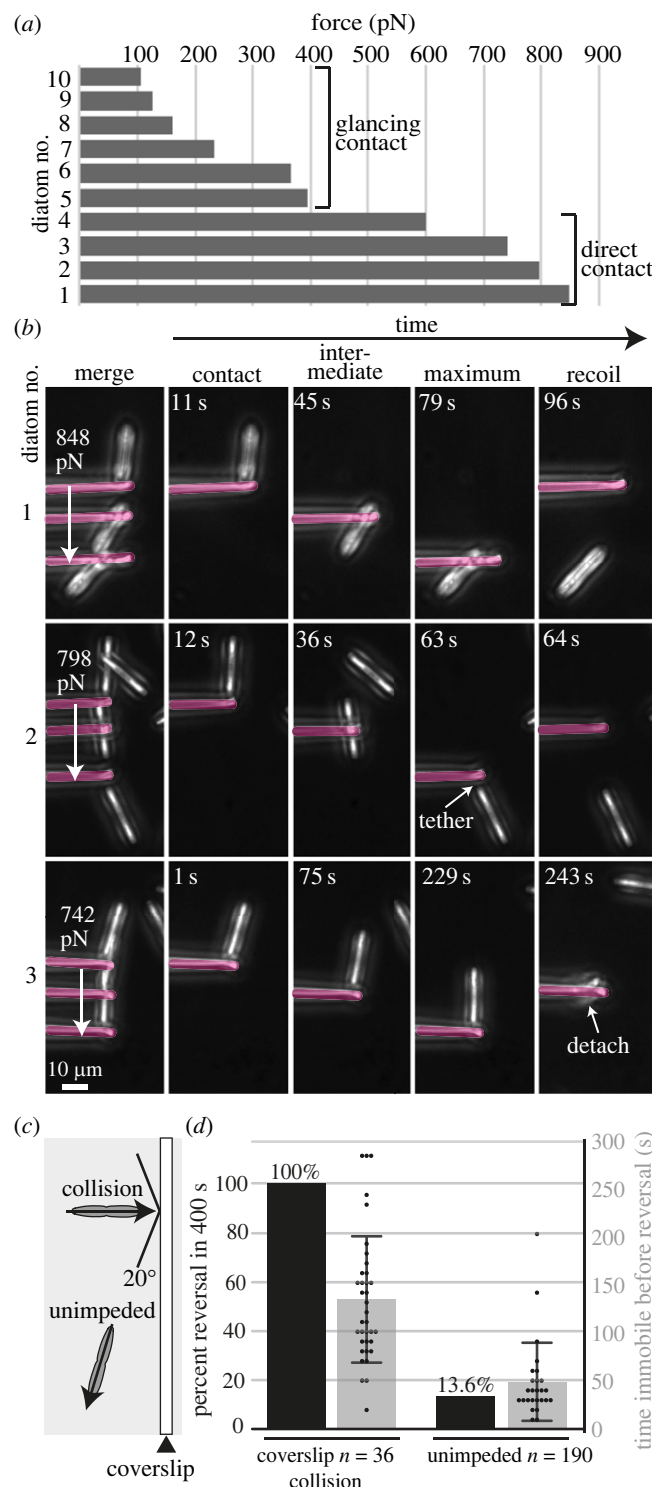


Figure 6. Forces exerted by *N. sing1* diatoms and mechanosensing. (a) The graph shows the maximum force produced by single diatoms pushing/pulling on the pipette. (b) Images taken from the indicated movies show first contact (contact), an intermediate time point (intermediate), the point of maximum pipette deflection (maximum) and pipette recoil (recoil). The arrows in the merge panel show maximum force values. The pipette is overlaid in opaque magenta. Note that for measurements 1 and 2 the diatom passes underneath the pipette. Scale bar = 10 μ m. Related to electronic supplementary material, movies S9, S10 and S11. (c) The schematic shows the experimental set-up for immobilization-triggered reversal. Only diatoms that hit the wall at an angle of more than 20° are included in the dataset. (d) The graph shows the percentage of cells reversing within 400 s (left y-axis). The scatterplot (right axis) shows the duration of time spent immobile prior to reversal. The light grey bar identifies the mean. Standard deviation is indicated.

accessions OQ319050–OQ319073 and OQ317929–OQ317940. The protein-coding *cob* and *nad1* genes were aligned by eye in MESQUITE v. 3.6 [49] using the predicted amino acid translations as a guide to preserve codon structure. Partial 28S rDNA genes were aligned using SSU-ALIGN ver. 0.1 [50] with a heterokont-specific covariance model [51]. The three alignments were concatenated with AMAS [52]. We used

IQ-TREE ver. 1.6.12 [53] for phylogenetic reconstruction. The concatenated alignment was partitioned by gene and codon position, and IQ-TREE's partitioning and model selection procedure (-m TESTMERGE) was used to identify the best fitting partition and nucleotide substitution model [54,55]. The model used edge-proportional branch lengths (IQ-TREE's '-spp' option) to account for differences in

evolutionary rates among partitions. Branch support was based on 1000 ultrafast bootstrap replicates [56].

3.3. Diatom growth, measurement, microscopy and movies

Diatoms were cultured on a synthetic seawater medium [42] with the following modification. NaCl was employed at a final concentration of 180 mM, and for growth in liquid cultures Na_2SiO_3 was employed at a final concentration of 840 μM . For solid media, salt solutions I and II were prepared as 4 $\times$ stock solutions, while polysaccharides were prepared at 2 $\times$ concentrations. Where necessary, polysaccharides were boiled and equilibrated to 60°C before mixing with salt solutions I and II to yield 1 $\times$ final concentrations. Polysaccharides were obtained from the following sources: Bacto agar (BD, 214010), agarose (Vivantis, PC0701), carrageenan (Sigma-Aldrich, C1013), low viscosity sodium alginate (Sigma-Aldrich, A1112) and medium viscosity sodium alginate (Sigma-Aldrich, A2033). For the alginate liquefaction assay (figure 2c), medium viscosity sodium alginate was employed.

To measure the speed of radial colony expansion (electronic supplementary material, figures S1a and S2), a starter culture was prepared on a seawater agar petri dish. From these confluent plates, a small block (approx. 0.5 $\times$ 0.5 cm) was excised with a scalpel and used to inoculate fresh plates from which measurements were derived. After overnight growth at 30°C, the edge of the radially expanding colony was marked. A subsequent measurement was made after 24 h. These marks were used to calculate speed, in nm s^{-1} . Each speed measurement was done in triplicate.

For movies, diatoms were grown on seawater agar medium in petri dishes. Movies were made using an Olympus BX51 upright microscope equipped with a 5 $\times$ objective using a Photometrics CoolSNAP HQ (Teledyne Photometrics) camera controlled by MetaVue software (Molecular Devices). Frames were acquired every 30 s at an exposure time of 20 ms with bright-field illumination. For movies shown in figure 3c, frames were acquired every 5 s. Graphs of diatom speed were made by manually measuring frame-to-frame diatom movement in ImageJ (<https://imagej.nih.gov/ij/index.html>). For the quantification shown in figure 4d,e, the average increase or decrease in speed was calculated from the average speed over six frames before and after joining the trail.

To examine total cell proteins (figure 2c and electronic supplementary material, figure S4a) by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), plates on which diatoms had been grown to confluence were flooded with 7.5 ml phosphate-buffered saline + (PBS+) (10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 240 mM NaCl), and cells were scraped off gently using a cell scraper (Corning Incorporated Costar, 3010). The cells were pelleted by centrifugation at 3.9 $\text{k} \times g$ for 10 min and washed once with 7 ml of PBS+ before being lysed by boiling in SDS–PAGE loading buffer. Proteins were resolved using 10% polyacrylamide gels.

For staining with phalloidin (ThermoFisher, A12379) (figure 1b), a block of seawater agar medium from a confluent plate was transferred to the centre of a coverslip. The coverslip was placed in a 60 mm petri dish, overlaid with 6 ml of liquid synthetic seawater medium (0.5% (w/v) glucose), and incubated at 30°C for 24 h. The agar block was subsequently removed, and the coverslip placed cells-up on a piece of

parafilm in a 90 mm petri dish. The cells were washed once with PBS+ and then fixed in PBS containing 4% paraformaldehyde (Electron Microscopy Sciences, 15713) for 30 min at room temperature. Samples were washed three times with PBS+ and then incubated with 0.17 μM phalloidin 488 (stock: 66 μM in dimethyl sulfoxide (DMSO)) in PBS+ for 1 h in the dark. Samples were washed with PBS+ (3 $\times$ fast wash + 3 $\times$ 10 min wash) at room temperature. After the last wash, 5 μl mounting medium (PBS, 20% glycerol, 2 $\mu\text{g ml}^{-1}$ DAPI, 1 $\times$ antifade), (100 $\times$ antifade stock: 20% (w/v) *n*-propyl gallate (Sigma-Aldrich, P3130) in DMSO) was added and the coverslip was placed on a microscope slide cells down and sealed with parafilm. For staining of lipid droplets with BODIPY, diatoms were grown on seawater agar media. After 2 days growth at 30°C, a block (approx. 1 cm $\times$ 1 cm) was excised with a scalpel and transferred cells-up onto a microscope slide. The diatoms were overlaid with 4 μl of a solution containing 30 $\mu\text{g ml}^{-1}$ BODIPY 505/515 (Invitrogen, D3921) in PBS+. After approximately 30 min a coverslip was added and the diatoms were imaged by fluorescence microscopy. The diatom frustules were prepared for SEM as previously described [57].

3.4. Force measurement

To measure the force produced by diatom movement (figure 6a,b), a small block, approximately 0.25 $\times$ 0.5 cm, was excised from a starter culture and used to inoculate 1.5% agarose seawater medium contained by a u-shaped thin-wall chamber made of polydimethylsiloxane (DOW, 4019862) on top of a coverslip [43]. After incubation at room temperature for 1 day, the chamber was flooded with seawater (Electrostatic attraction between the pipette and medium necessitated that these experiments be conducted with a seawater overlay as in figure 3b). The chamber was then placed on a microscope stage with a slide holder and a micropipette, prepared as described below, was inserted through the open side of the chamber and positioned in front of a diatom using an MP-285 motorized micromanipulator (Sutter Instruments). Movies were made using an Olympus IX81 equipped with a 40 $\times$ objective manipulated with MetaMorph software (Molecular Devices), with frames acquired every 0.5 s. Frame-to-frame micropipette and diatom movement were manually measured using ImageJ and used to produce force/velocity graphs (electronic supplementary material, figure S5).

Micropipette production: briefly, thin-wall glass capillaries (1 mm outer diameter, 0.78 mm inner diameter; World Precision Instruments, TW100F-6) were pulled using a P-97 micropipette puller (Sutter Instruments) and the tip was cut to the desired inner diameter of approximately 2 μm with an in-house developed heated platinum wire. The stiffness of the micropipette was calibrated using a standard micro glass rod ($k_s = 21.09 \pm 4.22 \text{ pN } \mu\text{m}$). The preparation of a standard micro glass rod and calibration of the working micropipette were conducted as previously described [43]. Before use, the tip of the micropipette was incubated overnight with 3% fetal bovine serum (Sigma-Aldrich, A7030).

3.5. Alginate lyase enzyme activity assay

Diatoms were grown in liquid medium for 3 days at 30°C, after which cells were removed using a cell strainer (SPL Life Sciences, 93040). The medium was then centrifuged at 3.9 $\text{k} \times g$ for 15 min and concentrated using a Pierce protein

concentrator with a 3 kilodalton cut-off (ThermoFisher, 88526). The concentrated medium was diluted 1 : 5 with synthetic seawater medium before use. For heat-inactivated controls, the diluted culture media was heated at 100°C for 5 min and then briefly centrifuged. To perform the enzyme assay, 5 µl of diluted sample was added to 45 µl sodium alginate buffer (10 mM Tris (pH 7.4), 200 mM NaCl, 200 mM KCl, 2 mM CaCl₂, 0.01% sodium azide and 0.1% low-viscosity sodium alginate (Sigma-Aldrich, A1112)) in a 384-well UV-STAR microplate (Greiner Bio-One International). Alginate lyase activity was determined by measuring the increase in absorbance at 235 nm at 1-min intervals using a Tecan Spark Multimode Microplate Reader (Tecan Inc.).

4. Discussion

The apochlorotic diatoms described here were isolated from a variety of organic materials (figure 1*a*) and can grow on a broad range of algal polysaccharides (figure 2*a*; electronic supplementary material, figure S2). Evidence for catabolite repression mediated by the presence of preferred carbon sources (figure 2*c*; electronic supplementary material, figure S4*a*) identifies an aspect of metabolism classically associated with heterotrophy in fungi [58] and bacteria [59]. Overall, these findings are consistent with a general role for apochlorotic diatoms in coastal marine nutrient cycling—one akin to the role of osmotrophic fungi [60] in terrestrial environments. Interestingly, unlike the diatoms identified here, *N. putrida* does not metabolize alginate nor show evidence of catabolite repression (figure 2*a,c*). A high degree of ecophysiological variation is further evidenced by poor motility of clade 3 isolates and *N. putrida* as compared to clade 1 and 2 isolates (electronic supplementary material, figure S1*a–c*). Together, these findings suggest that apochlorotic diatoms exploit distinct feeding strategies and are undergoing substantial evolutionary radiation.

A series of evolutionary innovations culminating in force generation from motility are likely to have predisposed the diatoms to a successful transition to heterotrophy. These include the advent of the silica-based cell wall, bilateral symmetry, the raphe and forceful motility. Certain marine bacteria are highly evolved for alginate metabolism [61,62], but are unlikely to generate forces necessary for tunnelling [63]. Thus, high forces from diatom gliding motility (figure 6*a,b*) are likely to underlie invasive growth (electronic supplementary material, figure S3) and allow access to nutrient pools unavailable to competing microorganisms. This is consistent with *N. alba* invasive growth on brown algal tissues [39] and tunnelling in both *N. alba* and *N. albicostalis*, which appears to be stimulated by the presence of bacteria [35,39]. In terrestrial environments, the fungi have a similar advantage where the force from pressurized hyphal networks underlies invasive growth [64]. Thus, distinct manners of force generation appear to provide an advantage to eukaryotic heterotrophs over their bacterial counterparts.

Diatom EPS trails have been visualized and characterized by electron microscopy [65], atomic force microscopy [66,67] and various staining techniques [15,17,19,68]. The direct observation of EPS deposition by living cells provides the opportunity to investigate the relationship between EPS trails and motility. On agarose concentrations that favour motility, uniform refractive EPS trails are presumably visible due to their convex cross-sectional profile. With increasing

agarose concentration, motility slows substantially and EPS trails lose their uniform cross-sectional profile to form aberrant refractive puncta (figure 3*a*). This suggests that freshly secreted EPS undergoes rapid swelling that is sensitive to timely hydration and/or the availability of critical seawater ionic constituents. A critical role for hydration is also consistent with dramatically enhanced motility when agar plates are overlaid with seawater (figure 3*b–d*). Faster motility on pre-existing trails and increased motility with a seawater overlay have also been observed in *Phaeodactylum tricornutum* [69]. Thus, these aspects of motility are likely to be general features of raphid pennate diatoms. The sea surface microlayer (SSM) is known to have a distinct physical, chemical and biological composition [70]. The finding that gliding occurs in a monolayer at the SSM (figure 3*c*) suggests that the EPS has an affinity for the seawater surface underside. This provides a physical basis for previous work showing that apochlorotic diatoms are enriched at the SSM [36].

The relationship between runners and blazers leads to cooperative motility (figure 4) and is likely to be related to the dual function of EPS in adhesion and motility. Runners that go off-trail instantly decelerate. This is consistent with more nascent EPS being consumed by the adhesive function. By contrast, blazers joining a trail instantly accelerate (figure 4*b–d*) because adhesive contacts are already present, and only EPS–EPS contacts are required. Interestingly, line scans of trails do not change dramatically between a fresh trail and one that has been passed over by runner diatoms (electronic supplementary material, figure S7). Thus, runners may be secreting substantially less EPS than blazers. This could also factor into their tendency to move at higher speeds.

Our force measurements are consistent with force generation through myosin motors; however, they do not exclude a role for EPS polymerization. Single myosin molecules exert forces of 3–4 pN [71], while isolated muscle filaments can generate maximum forces of approximately 200–300 pN [45,46]. Thus, *N. sing1* peak forces of 700–800 pN (figure 6*a, b*) are consistent with the cooperative action of multiple myosin motors arrayed along the length of raphe-associated actin filaments. Force measurements were made with single diatoms and are likely to be higher in runner/blazer chains. Thus, cooperative motility is expected to increase both colony diffusivity (electronic supplementary material) and the ability to tunnel effectively.

A related set of equations describes colony diffusivity of *N. sing1* (electronic supplementary material) and the mixotrophic diatom *Navicula* [72]. Thus, periodic reversal combined with random turning is likely to be a general strategy used by raphid pennate diatoms to avoid immobilization after encountering an obstacle. By sensing immobilization (figure 6*c*), *N. sing1* is able to reduce the period of immobility, while presumably maintaining an independent frequency of free-running reversal. Distinct mechanisms could underlie *N. sing1* force sensing. For example, mechanosensitive ion channels are established force sensors that could trigger a signalling cascade leading to reversal. In another model, strain on the force-generation machinery could trigger reversal through a feedback mechanism. More work will be required to determine the mechanism of force sensing.

The transition from photoautotrophy to obligate heterotrophy is likely to have been accompanied by a variety of physiological adaptations. However, because many pennate diatoms are highly evolved mixotrophs [73–76], it remains

unclear whether alginate utilization, cooperative motility and force sensing are unique to apochlorotic diatoms or pre-date their emergence. Identifying the genetic basis for diatom obligate heterotrophy will require an integrated approach that combines comparative genomics with molecular, biochemical, cellular and physiological studies.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The data are provided in electronic supplementary material [77].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. P.Z.: data curation, investigation, methodology, writing—review and editing; K.K.: formal analysis, investigation, methodology, writing—review and editing; C.Q.: data curation, formal analysis, investigation; Z.H.L.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; Y.A.: investigation, writing—original draft, writing—review and editing; Z.T.Y.: formal analysis, investigation, methodology; J.N.: funding acquisition, investigation, methodology, supervision, writing—review and editing; A.J.A.: formal analysis, investigation, methodology, writing—review and editing; Y.J.: funding acquisition, investigation,

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References

- Gould SB, Waller RF, McFadden GI. 2008 Plastid evolution. *Annu. Rev. Plant Biol.* **59**, 491–517. (doi:10.1146/annurev.arplant.59.032607.092915)
- Keeling PJ. 2013 The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu. Rev. Plant Biol.* **64**, 583–607. (doi:10.1146/annurev-arplant-050312-120144)
- Sibbald SJ, Archibald JM. 2020 Genomic insights into plastid evolution. *Genome Biol. Evol.* **12**, 978–990. (doi:10.1093/gbe/evaa096)
- Reyes-Prieto A, Weber APM, Bhattacharya D. 2007 The origin and establishment of the plastid in algae and plants. *Annu. Rev. Genet.* **41**, 147–168. (doi:10.1146/annurev.genet.41.110306.130134)
- Dorrell RG, Bowler C. 2017 Secondary plastids of stramenopiles. In *Advances in botanical research* (ed. Y Hirakawa), pp. 57–103. New York, NY: Academic Press.
- Derelle R, López-García P, Timpano H, Moreira D. 2016 A phylogenomic framework to study the diversity and evolution of stramenopiles (= heterokonts). *Mol. Biol. Evol.* **33**, 2890–2898. (doi:10.1093/molbev/msw168)
- Mann DG, Vanormelingen P. 2013 An inordinate fondness? The number, distributions, and origins of diatom species. *J. Eukaryot. Microbiol.* **60**, 414–420. (doi:10.1111/jeu.12047)
- Malviya S *et al.* 2016 Insights into global diatom distribution and diversity in the world's ocean. *Proc. Natl Acad. Sci. USA* **113**, E1516–E1525. (doi:10.1073/pnas.1509523113)
- Nakov T, Beaulieu JM, Alverson AJ. 2018 Accelerated diversification is related to life history and locomotion in a hyperdiverse lineage of microbial eukaryotes (Diatoms, Bacillariophyta). *New Phytologist* **219**, 462–473. (doi:10.1111/nph.15137)
- Armbrust EV. 2009 The life of diatoms in the world's oceans. *Nature* **459**, 185–192. (doi:10.1038/nature08057)
- Bowler C, Vardi A, Allen AE. 2010 Oceanographic and biogeochemical insights from diatom genomes. *Ann. Rev. Mar. Sci.* **2**, 333–365. (doi:10.1146/annurev-marine-120308-081051)
- Sims PA, Mann DG, Medlin LK. 2006 Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia* **45**, 361–402. (doi:10.2216/05-22.1)
- Kröger N, Poulsen N. 2008 Diatoms—from cell wall biogenesis to nanotechnology. *Annu. Rev. Genet.* **42**, 83–107. (doi:10.1146/annurev.genet.41.110306.130109)
- Hildebrand M, Lerch SJL, Shrestha RP. 2018 Understanding diatom cell wall silicification—moving forward. *Front. Mar. Sci.* **5**, 1–19. (doi:10.3389/fmars.2018.00125)
- Poulsen N, Davutoglu MG, Suchanova JZ. 2022 Diatom adhesion and motility. In *The molecular life of diatoms* (eds A Falcione, T Mock), pp. 367–396. Berlin, Germany: Springer.
- Bedoshvili YD, Likhoshvay YV. 2021 Cellular mechanisms of raphid diatom gliding. In *Diatom gliding motility* (eds SA Cohn, KM Manoylov, R Gordon), pp. 65–73. New York, NY: Wiley.
- Lind JL, Heimann K, Miller EA, Van Vliet C, Hoogenraad NJ, Wetherbee R. 1997 Substratum adhesion and gliding in a diatom are mediated by extracellular proteoglycans. *Planta* **203**, 213–221. (doi:10.1007/s004250050184)
- Poulsen N, Kröger N, Harrington MJ, Brunner E, Paasch S, Buhmann MT. 2014 Isolation and biochemical characterization of underwater adhesives from diatoms. *Biofouling* **30**, 513–523. (doi:10.1080/08927014.2014.895895)
- Lachnit M, Buhmann MT, Klemm J, Kröger N, Poulsen N. 2019 Identification of proteins in the adhesive trails of the diatom *Amphora coffeaeformis*. *Phil. Trans. R. Soc. B* **374**, 20190196. (doi:10.1098/rstb.2019.0196)
- Poulsen N, Hennig H, Geyer VF, Diez S, Wetherbee R, Fitz-Gibbon S, Pellegrini M, Kröger N. 2023 On the role of cell surface associated, mucin-like glycoproteins in the pennate diatom *Craspedostauros australis* (Bacillariophyceae). *J. Phycol.* **59**, 54–69. (doi:10.1111/jpy.13287)
- Zackova Suchanova J *et al.* In press. Diatom adhesive trail proteins acquired by horizontal gene transfer from bacteria serve as primers for marine biofilm formation. *New Phytologist*. (doi:10.1111/nph.19145)
- Poulsen NC, Spector I, Spurck TP, Schultz TF, Wetherbee R. 1999 Diatom gliding is the result of an actin-myosin motility system. *Cell Motil. Cytoskeleton* **44**, 23–33. (doi:10.1002/(SICI)1097-0169(199909)44:1<23::AID-CM2>3.0.CO;2-D)
- Yamaoka N, Suetomo Y, Yoshihisa T, Sonobe S. 2016 Motion analysis and ultrastructural study of a colonial diatom, *Bacillaria paxillifer*. *Microscopy* **65**, 211–221. (doi:10.1093/jmicro/dfv375)
- Edgar LA, Pickett Heaps JD. 1983 The mechanism of diatom locomotion. I. An ultrastructural study of the motility apparatus. *Proc. R. Soc. Lond. B* **218**, 331–343. (doi:10.1098/rspb.1983.0042)
- Wetherbee R, Lind JL, Burke J, Quatrano RS. 1998 Minireview—the first kiss: establishment and control of initial adhesion by raphid diatoms. *J. Phycol.* **34**, 9–15. (doi:10.1046/j.1529-8817.1998.340009.x)
- Pollard TD, Goldman RD. 2018 Overview of the cytoskeleton from an evolutionary perspective. *Cold*

- Spring Harb. Perspect. Biol. **10**, a030288. (doi:10.1101/cshperspect.a030288)
27. Titus MA. 2018 Myosin-driven intracellular transport. *Cold Spring Harb. Perspect. Biol.* **10**, a021972. (doi:10.1101/cshperspect.a021972)
 28. Barkman TJ, McNeal JR, Lim SH, Coat G, Croom HB, Young ND, Depamphilis CW. 2007 Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evol. Biol.* **7**, 1–15. (doi:10.1186/1471-2148-7-248)
 29. Frankovich TA, Ashworth MP, Sullivan MJ, Theriot EC, Stacy NI. 2018 Epizoic and apochlorotic tursiocola species (Bacillariophyta) from the skin of Florida manatees (*Trichechus manatus latirostris*). *Protist* **169**, 539–568. (doi:10.1016/j.protis.2018.04.002)
 30. Dorrell RG *et al.* 2019 Principles of plastid reductive evolution illuminated by nonphotosynthetic chrysophytes. *Proc. Natl Acad. Sci. USA* **116**, 6914–6923. (doi:10.1073/pnas.1819976116)
 31. Kayama M *et al.* 2020 A non-photosynthetic green alga illuminates the reductive evolution of plastid electron transport systems. *BMC Biol.* **18**, 1–15. (doi:10.1186/s12915-020-00853-w)
 32. Kamikawa R *et al.* 2015 Multiple losses of photosynthesis in *Nitzschia* (Bacillariophyceae). *Phycol. Res.* **63**, 19–28. (doi:10.1111/pre.12072)
 33. Onyschenko A, Ruck EC, Nakov T, Alverson AJ. 2019 A single loss of photosynthesis in the diatom order Bacillariales (Bacillariophyta). *Am. J. Bot.* **106**, 560–572. (doi:10.1002/ajb.2.1267)
 34. Lewin, J, Lewin, RA. Culture and nutrition of some apochlorotic diatoms of the genus *Nitzschia*. *J. Gen. Microbiol.* **46**, 361–367 (1967). (doi:10.1099/00221287-46-3-361)
 35. Rogerson A, Hannah FJ, Wilson PC. 1993 *Nitzschia albicostalis*: an apochlorotic diatom worthy of ecological consideration. *Cah. Biol. Mar.* **34**, 513–522.
 36. Blackburn MV, Hannah F, Rogerson A. 2009 First account of apochlorotic diatoms from mangrove waters in Florida. *J. Eukaryot. Microbiol.* **56**, 194–200. (doi:10.1111/j.1550-7408.2008.00388.x)
 37. Li CW, Volcani BE. 1987 Four new apochlorotic diatoms. *Brit. Phycol. J.* **22**, 375–382. (doi:10.1080/00071618700650441)
 38. Kamikawa R, Azuma T, Ishii KI, Matsuno Y, Miyashita H. 2018 Diversity of organellar genomes in non-photosynthetic diatoms. *Protist* **169**, 351–361. (doi:10.1016/j.protis.2018.04.009)
 39. Armstrong E, Rogerson A, Leftley JW. 2000 Utilisation of seaweed carbon by three surface-associated heterotrophic protists, *Stereomyxa ramosa*, *Nitzschia alba* and *Labyrinthula* sp. *Aquat. Microb. Ecol.* **21**, 49–57. (doi:10.3354/ame021049)
 40. Onyschenko A, Roberts WR, Ruck EC, Lewis JA, Alverson AJ. 2021 The genome of a nonphotosynthetic diatom provides insights into the metabolic shift to heterotrophy and constraints on the loss of photosynthesis. *New Phytol.* **232**, 1750–1764. (doi:10.1111/nph.17673)
 41. Kamikawa R *et al.* 2022 Genome evolution of a nonparasitic secondary heterotroph, the diatom *Nitzschia putrida*. *Sci. Adv.* **8**, eabi5075. (doi:10.1126/sciadv.abi5075)
 42. Berges JA, Franklin DJ, Harrison PJ. 2001 Evolution of an artificial seawater medium: Improvements in enriched seawater, artificial water over the last two decades. *J. Phycol.* **37**, 1138–1145. (doi:10.1046/j.1529-8817.2001.01052.x)
 43. Huang W, Fu C, Yan J. 2023 Single-cell quantification of the mechanical stability of cell–cell adherens junction using glass micropipettes. In *Mechanobiology: methods and protocols* (ed. R Zaidel-Bar), pp. 267–280. US: Springer.
 44. Biswas R *et al.* 2021 Mechanical instability of adherens junctions overrides intrinsic quiescence of hair follicle stem cells. *Dev. Cell* **56**, 761–780. (doi:10.1016/j.devcel.2021.02.020)
 45. Cheng YS, de Souza Leite F, Rassier DE. 2020 The load dependence and the force-velocity relation in intact myosin filaments from skeletal and smooth muscles. *Am. J. Physiol. Cell Physiol.* **318**, C103–C110. (doi:10.1152/ajpcell.00339.2019)
 46. Liu X, Pollack GH. 2004 Stepwise sliding of single actin and myosin filaments. *Biophys. J.* **86**, 353–358. (doi:10.1016/S0006-3495(04)74111-9)
 47. Stock W, Pinseel E, De Decker S, Sefton J, Blommaert L, Chepurnova O, Sabbe K, Vyverman W. 2018 Expanding the toolbox for cryopreservation of marine and freshwater diatoms. *Sci. Rep.* **8**, 4279. (doi:10.1038/s41598-018-22460-0)
 48. Yip ZT, Quek RZ, Low JK, Wilson B, Bauman AG, Chou LM, Todd PA, Huang D. 2018 Diversity and phylogeny of sargassum (Fucales, Phaeophyceae) in Singapore. *Phytotaxa* **369**, 200–210. (doi:10.11646/phytotaxa.369.3.3)
 49. Mesquite Project Team. 2014 Mesquite: a modular system for evolutionary analysis. See <https://www.mesquiteproject.org/>.
 50. Nawrocki EP, Kolbe DL, Eddy SR. 2009 Erratum: Infernal 1.0: Inference of RNA alignments (Bioinformatics). *Bioinformatics* **25**, 1335–1337. (doi:10.1093/bioinformatics/btp157)
 51. Nakov T, Ruck EC, Galachyants Y, Spaulding SA, Theriot EC. 2014 Molecular phylogeny of the Cymbellales (Bacillariophyceae, Heterokontophyta) with a comparison of models for accommodating rate variation across sites. *Phycologia* **53**, 359–373. (doi:10.2216/14-002.1)
 52. Borowiec ML. 2016 AMAS: A fast tool for alignment manipulation and computing of summary statistics. *PeerJ* **4**, e1660. (doi:10.7717/peerj.1660)
 53. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015 IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**, 268–274. (doi:10.1093/molbev/msu300)
 54. Chernomor O, von Haeseler A, Minh BQ. 2016 Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* **65**, 997–1008. (doi:10.1093/sysbio/syw037)
 55. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS. 2017 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589. (doi:10.1038/nmeth.4285)
 56. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018 UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **35**, 518–522. (doi:10.1093/molbev/msx281)
 57. Harding WR, Taylor JC. 2007 A methods manual for the collection, preparation and analysis of diatom samples diatoms of Indian subcontinent view project new and relevant taxa: ecological and taxonomic characterization (NATEC) view project. See <https://www.researchgate.net/publication/264195296>
 58. Adnan M, Zheng W, Islam W, Arif M, Abubakar YS, Wang Z, Lu G. 2017 Carbon catabolite repression in filamentous fungi. *Int. J. Mol. Sci.* **19**, 48. (doi:10.3390/ijms19010048)
 59. Deutscher J. 2008 The mechanisms of carbon catabolite repression in bacteria. *Curr. Opin. Microbiol.* **11**, 87–93. (doi:10.1016/j.mib.2008.02.007)
 60. Richards TA, Talbot NJ. 2018 Osmotrophy. *Curr. Biol.* **28**, R1179–R1180.
 61. Zhang L, Li X, Zhang X, Li Y, Wang L. 2021 Bacterial alginate metabolism: an important pathway for bioconversion of brown algae. *Biotechnol. Biofuels* **14**, 1–18. (doi:10.1186/s13068-020-01854-1)
 62. Hehemann JH *et al.* 2016 Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes. *Nat. Commun.* **7**, 12860. (doi:10.1038/ncomms12860)
 63. Sabass B, Koch MD, Liu G, Stone HA, Shaevitz JW. 2017 Force generation by groups of migrating bacteria. *Proc. Natl Acad. Sci. USA* **114**, 7266–7271. (doi:10.1073/pnas.1621469114)
 64. Lew RR. 2011 How does a hypha grow? The biophysics of pressurized growth in fungi. *Nat. Rev. Microbiol.* **9**, 509–518. (doi:10.1038/nrmicro2591)
 65. Edgar LA. 1983 Mucilage secretions of moving diatoms. *Protoplasma* **118**, 44–48. (doi:10.1007/BF01284745)
 66. Chen L, Weng D, Du C, Wang J, Cao S. 2019 Contribution of frustules and mucilage trails to the mobility of diatom *Navicula* sp. *Sci. Rep.* **9**, 1–12. (doi:10.1038/s41598-018-37186-2)
 67. Higgins MJ, Crawford SA, Mulvaney P, Wetherbee R. 2002 Characterization of the adhesive mucilages secreted by live diatom cells using atomic force microscopy. *Protist* **153**, 25–38. (doi:10.1078/1434-4610-00080)
 68. Molino PJ, Wetherbee R. 2008 The biology of biofouling diatoms and their role in the development of microbial slimes. *Biofouling* **24**, 365–379. (doi:10.1080/08927010802254583)
 69. Iwasa K, Shimizu A. 1972 Motility of the diatom, *Phaeodactylum tricornutum*. *Exp. Cell Res.* **74**, 552–558. (doi:10.1016/0014-4827(72)90416-8)
 70. Cunliffe M *et al.* 2013 Sea surface microlayers: A unified physicochemical and biological perspective

- of the air–ocean interface. *Prog. Oceanogr.* **109**, 104–116. (doi:10.1016/j.pocean.2012.08.004)
71. Finer JT, Simmons RM, Spudich JA. 1994 Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113–119. (doi:10.1038/368113a0)
 72. Hu WS, Huang M, Zhang HP, Zhang F, Vyverman W, Liu QX. 2020 Movement behavioral plasticity of benthic diatoms driven by optimal foraging. *bioRxiv*. (doi:10.1101/682153)
 73. Villanova V, Spetea C. 2021 Mixotrophy in diatoms: molecular mechanism and industrial potential. *Physiol. Plant* **173**, 603–611. (doi:10.1111/ppl.13471)
 74. Lewin JC, Lewin RA. 1960 Auxotrophy and heterotrophy in marine littoral diatoms. *Can. J. Microbiol.* **6**, 127–134. (doi:10.1139/m60-015)
 75. Meyer N, Rydzyk A, Pohnert G. 2022 Pronounced uptake and metabolism of organic substrates by diatoms revealed by pulse-labeling metabolomics. *Front. Mar. Sci.* **9**, 821167. (doi:10.3389/fmars.2022.821167)
 76. Tuchman NC, Schollett MA, Rier ST, Geddes P. 2006 Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions. *Hydrobiologia* **561**, 167–177. (doi:10.1007/s10750-005-1612-4)
 77. Zheng P *et al.* 2023 Cooperative motility, force generation and mechanosensing in a foraging non-photosynthetic diatom. Figshare. (doi:10.6084/m9.figshare.c.6836749)