

Novel pennate diatom symbionts support high N₂ fixation rates

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Abstract

Diazotrophy is the most important nitrogen source in the oligotrophic surface ocean, but the organisms involved and their contributions are incompletely understood due to limited observations. Only diazotrophic organisms possess the *nifH* gene to reduce dinitrogen to ammonium, but their distribution and activity can only be quantified through sampling and experiments during research cruises. Some recent studies document small diatoms with symbionts able to fix nitrogen, a new source of biologically available nitrogen in addition to the well-known cyanobacterial species such as *Trichodesmium* or symbionts of haptophytes (UCYN-A) and diatoms (Diatom–Diazotroph Associations, or DDAs). Here, we document a very active symbiosis between small pennate diatoms such as *Mastogloia* and *Haslea* with rhizobial and cyanobacterial symbionts in waters of the Western tropical North Atlantic influenced by the Amazon River plume. We used NanoSIMS analysis of ¹⁵N₂ tracer experiments to quantify high rates of nitrogen fixation in generally abundant, symbiont-bearing pennate diatoms. This newly described symbiosis may contribute a previously unquantified flux of biologically available nitrogen to oceanic systems. Pennate diatoms and their symbionts may close a key gap in our understanding of the supply of nutrients to the ocean and provide a previously unknown biological sink for carbon dioxide.

Keywords: nitrogen fixation; symbiosis; diatoms; tropical North Atlantic Ocean; NanoSIMS; cell-specific N₂ fixation

Dinitrogen (N₂) fixation plays a key role in supplying reactive nitrogen to pelagic ecosystems, and filamentous cyanobacteria were long thought to be the only marine diazotrophs. In recent years, the application of fluorescence analysis, molecular and bioinformatic approaches, and single-cell mass spectrometry has generated increasing evidence for a significant contribution of new nitrogen by other groups including non-cyanobacterial diazotrophs [1] and newly discovered endosymbionts of haptophytes [2] and diatoms [3, 4]. Interestingly, these latter diazotrophs could represent an early stage of organogenesis [5].

Among the enigmatic marine diazotrophs are spheroid bodies containing N₂ fixation genes [6] in rhopalodiate diatoms such as *Rhopalodia gibba* [7]. Similarly, Nakayama [8] found non-photosynthetic intracellular cyanobacteria in the rhopalodiate *Epithemia turgida* [9], and N₂ fixation has been documented in other rhopalodiate diatoms [4]. More recently, Tschitschko et al. [3] have described a rhizobial symbiont, *Candidatus Tectiglobus diatomicula*, of biraphid diatoms belonging to the genus *Haslea*. The rhizobial symbiont appears to be broadly distributed in the world ocean [3], but its host specificity and activity remain poorly

characterized. Taken together, these different lines of evidence suggest an overlooked contribution of symbiosis between diatoms and multiple diazotroph lineages to nitrogen supply in the ocean.

Here, we report unexpectedly high ¹⁵N enrichment within pennate diatoms tentatively identified as *Mastogloia* spp. and *Haslea* spp. from ¹⁵N₂—tracer experiments carried out in the northern Amazon River Plume (Figs 1 and 2, Supplementary Fig. S1, Supplementary Tables S1–S3). We could not visualize the rhizobial symbionts reported from *Haslea* [3] and *Epithemia* [4], but many of the diatoms we observed contained symbionts with phycoerythrin fluorescence flanking the nucleus axially, in contrast to the lateral arrangement of the previously described rhizobial symbionts (Fig. 2, Supplementary Fig. S3). Our NanoSIMS analyses of ¹⁵N in individual diatoms provide definitive evidence of direct assimilation of ¹⁵N₂ by cells containing these endosymbionts (Fig. 1).

Although pennate diatoms are known to acquire reactive nitrogen from sympatric, free-living diazotrophs [4], this pathway cannot generate ¹⁵N enrichments greater than those

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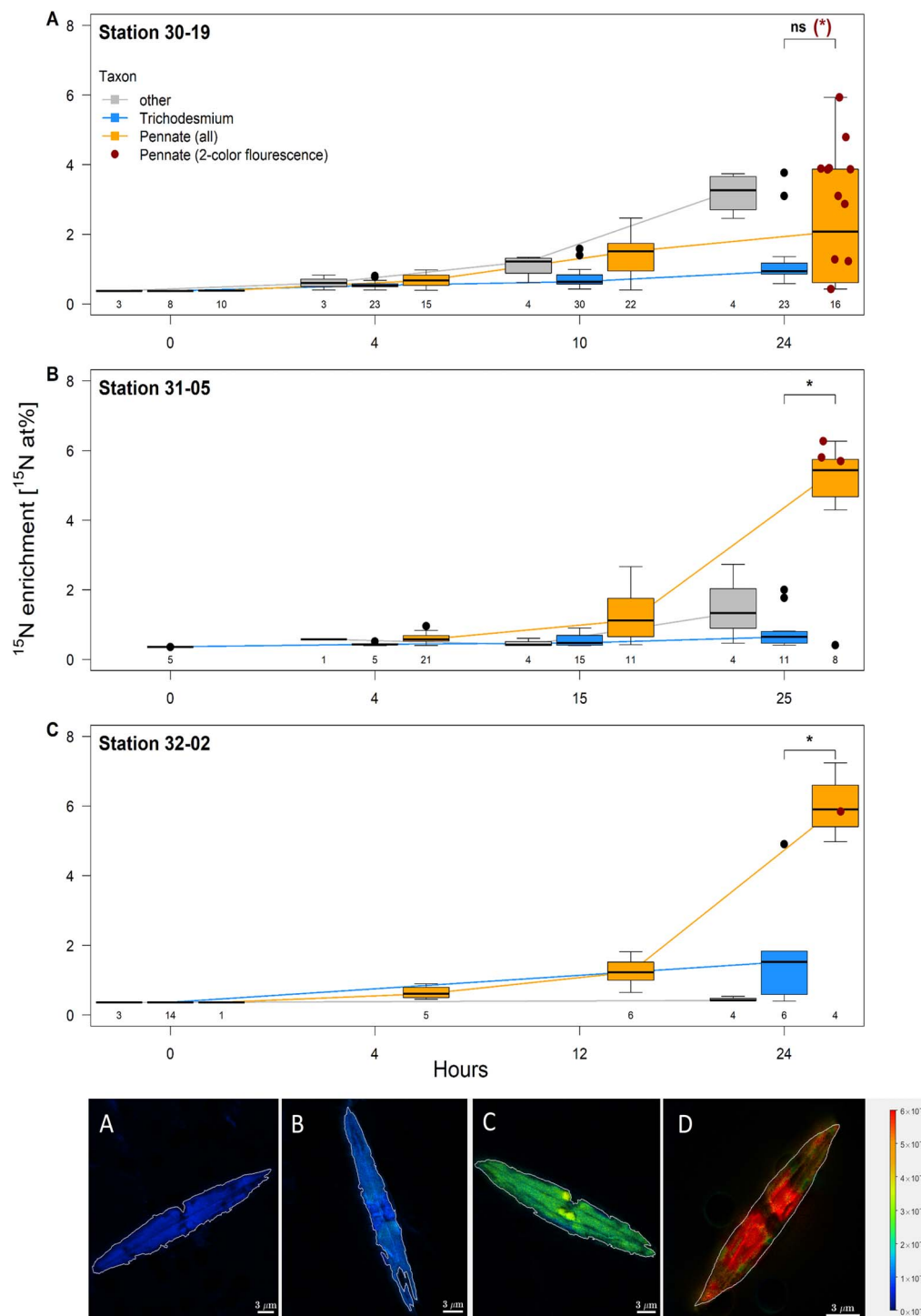


Figure 1. ^{15}N enrichment in at% in cells of *Trichodesmium*, pennate diatoms, and other diazotrophs such as *Rhizosolenia-Richelia*, *Chaetoceros* over the incubation time of up to 25 h from three stations (A, station 30-19; B, station 31-05; and C, station 32-02) in the Amazon river plume. Boxplots display the median and interquartile range with whiskers extending to the minimum and maximum, excluding outliers. Outliers are shown as dots and defined as values more than 1.5 times the interquartile range removed from either the lower or upper quartile. Numbers below the boxplot indicate the number of cells measured. Significant differences in ^{15}N enrichment after 24–25 h between *Trichodesmium* and all analyzed pennate diatoms are indicated by asterisks, ns means not significant. Depth was not considered as a factor since it did not affect ^{15}N enrichment (Wilcoxon rank sum test, $P > .05$). At the end of the incubation, the ^{15}N enrichment of pennate diatoms with two color fluorescence is shown as red points and the significance of the difference with resp. to *Trichodesmium* enrichment is indicated for station 30-19 in parentheses. Typical examples of the enrichment of pennates are shown in the panels (D–G) below for the different time points 0 (D), 4 h (E), 10–14 h (F), and 24–25 h (G). The scale bar illustrates enrichments with ^{15}N from zero to 6×10^{-2} ‰ (cold to warm colors from left to right).

in the free-living diazotrophs. In all, we identified 108 pennate diatoms that became enriched in ^{15}N , of which 56 contained two-color fluorescent inclusions indicative of cyanobacteria (Supplementary Table S4). At the end of our incubations, many

pennate diatoms had significantly higher ^{15}N enrichments than *Trichodesmium* and other Diatom-Diazotroph Associations previously known to supply reactive nitrogen to the Amazon River Plume [9–11] (Fig. 1, Supplementary Tables S4–S6).

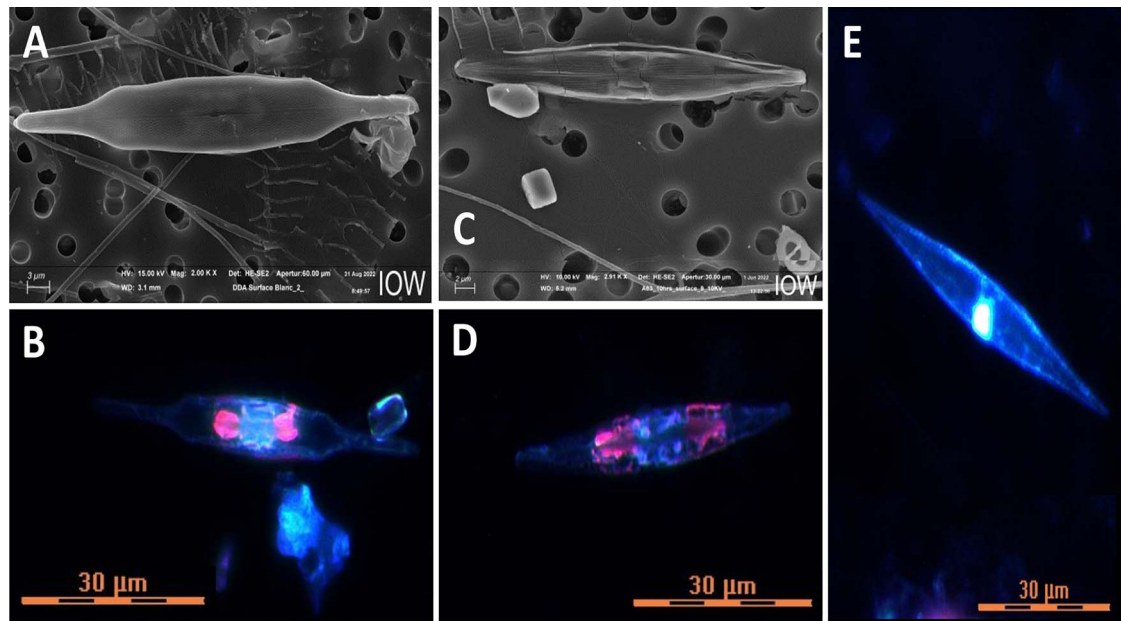


Figure 2. Examples of pennate diatoms showing putative *Mastogloia* sp. (A, B) and *Haslea* sp. (C, D) cells with two-color fluorescence. Panels (A and C) show general morphology, while panels (B and D) show fluorescence under trichromatic excitation (see [Supplementary Fig. S3](#) for details). In contrast, panel (E) shows a diatom lacking putative symbionts and exhibiting monochromatic fluorescence.

The high ^{15}N enrichment of the pennate diatoms with fluorescent symbionts implies that they were not acquiring ^{15}N secondarily from other diazotrophs via a recycling pathway. As a quantitative test, we estimated that the maximum possible enrichment of ^{15}N in pennates via release of labeled $^{15}\text{N}\text{-NH}_4^+$ by other active diazotrophs in the surrounding water is ~ 1.15 at% ^{15}N , a third of the median enrichment of 3.89 at% ($n = 28$) we found in pennates after 24 h of incubation (SI-Methods). The ^{15}N enrichment of pennate diatoms with symbionts increased through our 24 h incubations to as high as 7.24 at% ^{15}N ([Fig. 1](#), [Supplementary Table S4](#)), while the significantly lower ^{15}N enrichments of pennates without symbionts are consistent with acquisition of ^{15}N via a recycling pathway (Dunn's test, $z = 4.12$, $P < .001$).

Because these findings were serendipitous, we lack molecular analyses allowing us to unambiguously resolve the identity of these fluorescent endosymbionts. However, we collected samples for metatranscriptomics and interrogated them for community-level signals of *nifH* gene transcription as well as diatom taxonomy to explore the potential identity of both symbionts and hosts. For taxonomic assessment, Kaiju, a protein-level classification tool [12], could classify between 13.6% and 45.7% of metatranscriptomic reads ([Supplementary Table S3](#)) revealing relatively similar diatom communities across the sampled region with site-by-site variability ([Supplementary Fig. S4](#)).

Among the diatoms, transcripts attributed to *Mastogloia* spp. occurred at all stations with the highest relative abundance at station 29-04 ([Supplementary Fig. S4B](#)), while transcripts attributed to *Haslea* spp. were highest at station 31-05 ([Supplementary Fig. S4B](#)), suggesting broad presence but relatively low contribution of these pennates to the diatom community. Pennate diatoms of these genera benefit from elevated silicate concentrations e.g. in the river plumes like that of the Amazon ([Supplementary Table S1](#)) [11, 13] and may be observed elsewhere.

Although we could not identify the symbionts of our diatoms unambiguously, an evaluation of putative *nifH* gene expression within the assemblage using a combination of blast searches versus curated and public databases revealed the presence of the rhizobial symbiont, *Candidatus Tectiglobus diatomicola*, in our

samples despite its absence in our microscopic observations ([Supplementary Fig. S5](#)). Overall, the highest *nifH* transcript proportion was observed at Station 28-01 for transcripts attributed to *Trichodesmium* (3.84 TPM), while transcripts belonging to the recently identified rhizobial symbiont (*Candidatus Tectiglobus diatomicola*, [3]) showed the highest expression at Station 29-04 (1.87 TPM; [Supplementary Fig. S5](#)). Our samples also contained transcripts attributed to *Richelia*, *Crocospaera*, UCYN-A, and an unclassified cyanobacterium ([Supplementary Fig. S5](#)). Although we cannot yet identify the inclusions associated with high rates of assimilation of ^{15}N by pennate diatoms, they have morphological and fluorescence characteristics of cyanobacteria. Our methods did not allow us to visualize the previously described rhizobial symbionts of *Haslea*, but our molecular data show them to be present ([Fig. S5](#)). Our observations thus provide strong evidence that pennate diatoms may form symbiotic associations with both rhizobia and currently unidentified cyanobacteria.

In summary, multiple broadly distributed diatom genera may be associated with rhizobial and/or cyanobacterial symbionts, but the small size of the pennate diatom host and the challenge of characterizing Diatom–Rhizobial Associations (DRAs) and some Diatom–Cyanobacterial Associations (DCAs) by light or fluorescence microscopy has led to them being largely overlooked. Pennate diatoms are abundant and broadly distributed but whether they play an important role in supplying new nitrogen to tropical waters of the North Atlantic cannot yet be assessed. Further field studies are needed to explore the possibility that DRAs and DCAs with pennate diatom hosts are active in other ocean basins, and to quantify their overall contribution to the oceanic nitrogen budget.

Author contributions

Bhavya S. Panthalil and Maren Voss (Conceptualization and experiment design), Bhavya S. Panthalil (Experiments for cell-specific analysis), Bhavya S. Panthalil and Angela Vogts (Fluorescence, SEM analysis, and NanoSIMS analysis), Maren Voss, Joseph P. Montoya, Ajit Subramaniam, Mar Benavides, Bhavya S. Panthalil, and

Angela Vogts (Data evaluation, discussion, and figures), Matthew J. Harke (Genomic sample analyses and data evaluation), Christiane Hassenrück (Data evaluation, statistics, and figures), Ajit Subramaniam (Sampling, data interpretation, writing), Joseph P. Montoya (Sampling, data evaluation), and All (Writing of manuscript)

Supplementary material

Supplementary material is available at ISME Communications online.

Conflicts of interest

The authors declare no competing interests.

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Data availability

Fastq files have been deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) <https://www.ncbi.nlm.nih.gov/sra> under accession number PRJNA1226575.

METEOR cruise M174 [Dataset]. Hydrographic properties of water masses in the Amazonas River plume obtained in April/May 2021 by CTD measurements during RV METEOR cruise M174 PANGAEA, <https://doi.org/10.1594/PANGAEA.942346>. A, NanoSIMS data published as supplementary tables with this article.

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