

Supplementary materials

Methods

Microscopy observations. Samples were prepared for imaging and visualizing on the Zeiss LSM 780 Confocal microscope using two methods. Briefly, high cell densities samples ($<10^5$ *Richelia* L⁻¹) were transferred to a glass slide by carefully dragging the filter with the cells side down into a 10 µL droplet of milliQ. Subsequently, a coverslip was added, and cells immediately viewed. A second approach was optimized where small pieces (5 mm diameter) of the filters were excised and directly mounted onto a 170 µm thick coverslip (#1.5) using ProLong® Diamond Antifade Mountant (Molecular Probes). The latter method was also used for the *H. hauckii*-*R. intracellularis* enrichment culture samples and the *R. clevis*-*R. intracellularis* and *C. compressus*-*C. rhizosoleniae* symbioses samples collected from the South China Sea. A 1:100 mix of 0.5 mg mL⁻¹ DAPI (4',6-diamidino-2-phenylindole) and anti-fading medium was applied for 15 min in order to visualize the host cell nucleus. Each cyanobacteria was measured for 1) length of the trichome, 2) the number and diameter of vegetative cells and 3) heterocyst diameter. Moreover, the cell diameter of the diatom hosts in girdle view was also measured, specifically in the region where the symbiont was housed.

PCR, cloning and sequencing. The PCR primer selection for the diatom hosts was based on previous studies showing high resolution in the class Bacillariophyceae using the 1D form of *rbcL* (Paul *et al.*1999, 2000; Mann *et al.*2001; Wawrik *et al.*2002; Tamura *et al.*2005; MacGillivray and Kaczmarek 2011; Li *et al.*2013) and the variable regions of the SSU (Sorhannus 2004; Theriot *et al.*2009, 2010; Zimmermann *et al.*2011; Luddington

26 *et al.*2012; Pernice *et al.*2013). All PCRs were performed with a MJ Mini Thermal
27 Cyclor (Bio-Rad; Hercules, California, USA) in 25 μ L reaction containing: 18.15 μ L
28 sterile distilled water, 2.5 μ L 10 X Taq buffer (Thermo Scientific), 0.75 μ L MgCl₂ (50
29 mM), 0.5 μ L dNTP mixture (10 mM), 0.5 μ L each primer (10 μ M), 1 U Taq DNA
30 polymerase (Thermo Scientific) and 2 μ L DNA template. The *rbcL* PCR thermocycler
31 was programmed with initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94
32 °C for 1 min, annealing at 52 °C for 1 min, 72 °C for 1.5 min with a final elongation at 72
33 °C for 15 min. The 18S rRNA was amplified with the following reaction conditions:
34 initial denaturation at 94 °C for 2 min, then 5 cycles of denaturation at 94 °C for 45 s,
35 annealing at 54 °C for 45 s and 72 °C for 1 min, followed by 35 cycles of 94 °C for 45 s,
36 annealing 52 °C for 45s and 72 °C for 1 min, and final elongation at 72 °C for 10 min.
37 *NifH* and 16S rRNA reactions were performed as previously described in Foster and Zehr
38 (2006). The PCR products were gel electrophoresed and appropriately sized products
39 were excised and purified using the QIAquick Gel Extraction Kit (Qiagen) and eluted in
40 20 μ L of EB Buffer (Qiagen). Three μ L of the purified PCR product was ligated with the
41 pGEM®-T Easy Vector System (Promega) following the manufacturer's protocol. Eight
42 to 24 white colonies were inoculated in 1mL aliquot of 2X Luria Bertani (LB) media
43 amended with ampicillin (100 μ g mL⁻¹) in sterile 96 well blocks and incubated overnight
44 at 37 °C with agitation. Eight to twelve clones were picked for each gene library and are
45 summarized in Supplementary Table S6. Plasmids were purified using the Montage
46 Plasmid Miniprep₉₆ Kit (Millipore) following the manufacturers full lysate protocol.
47 The purified plasmids (15 μ L) was sequenced using T7 primer (5 pmol μ L⁻¹) with an ABI
48 3730XL sequencer (Eurofins Genomics).

Phylogenetic Analyses. Compared to previous studies estimating the appearance of other symbiotic N₂-fixing cyanobacteria through molecular clock analyses (e.g. Nakayama *et al.* 2011; Cornejo-Castillo *et al.* 2016), we implemented the time calibrated reconstructions to reflect a biologically more realistic model. Specifically, an independent branch rate relaxed-clock model (IGR; Lepage *et al.*, 2007) was applied and combined with the fossilized birth-death process (FBD; (Zhang *et al.*, 2016)) to account for lineage specific rate variation and incomplete taxon sampling. The IGR model was compared with a strict molecular clock, and one other relaxed clock model (autocorrelated lognormal, TK02; Thorne and Kishino, 2002) implemented in the software (MrBayes v.3.2.7; Ronquist *et al.*, 2012). The alternative clock models were compared using Bayes factors (Kass and Raftery, 1995; Lepage *et al.*, 2007). Model likelihoods were not, however, estimated by the computationally demanding approach of thermodynamic integration (Lartillot and Philippe, 2006), but were based on the harmonic means of the likelihoods as reported by the MrBayes software. For both the Cyanobacteria and Diatom data sets, the IGR model appears to provide a larger Bayes factor (where a $2 \cdot \log BF > 10$ represents "very strong evidence of the first model over the second" (Kass and Raftery, 1995)) compared to both the strict, or TK02 clock models (Cyanobacteria: IGR vs. Strict: 196.72, IGR vs. TK02: 60.66; Diatoms: IGR vs. Strict: 582.48, IGR vs. TK02: 274.03), and was selected as the preferred clock model

For both data sets, we assumed that taxa were sampled to maximize diversity, and with a sample probability of 48/8,000 for Cyanobacteria, and 62/20,000 for Diatoms, based on an estimate of the number of extant species for those taxa (Guiry, 2012). For further

settings for the FBD, we assumed an exponential prior with mean 10 for the net speciation rate, a beta (1,1) distribution as prior for the relative extinction rate, and a fixed (0) value for the fossilization rate (no fossil samples included). For the IGR, we assumed an exponential distribution with mean 10.0 as a prior for the variance of the gamma distribution from which the branch lengths are drawn, and a log normal distribution with mean -7.0 and variance 1.0 as a prior for the base substitution rate for the tree. We applied multiple calibration points for the trees, with information about ages for specific taxa taken from the literature. See Supporting Information Table S5A for details about assumed ages and priors used. For each data set, respectively, the concatenated genes were assumed to have evolved on the same underlying topology, but allowed to have partition (gene) specific substitution models and model parameters. Partition-specific nucleotide substitution models were allowed to vary using a reversible-jump Markov chain Monte Carlo (rjMCMC; Ronquist *et al.*, 2012), and combined with an assumption about rate variation following a Gamma distribution (Yang, 1993). Four parallel runs of the rjMCMC were run, applying a stopping rule where an average standard deviation of split frequencies below 0.01 would indicate satisfying convergence (Lakner *et al.*, 2008). 25% of the samples were discarded for the final summary.

Ancestral Traits. In order to assign a state for each character (or trait) literature searches were performed (e.g. Round *et al.* 1990; Tomas 1997; Garrity *et al.* 2004; Mann 2010; Cox 2011). Additionally, the trait “life-history” in the Diatoms ancestral state reconstruction exclusively refers to symbiosis with cyanobacteria. In addition, the state “facultative” was assigned depending on observations found in literature, i.e. if a specific strain of

symbiont (or host) was found both as free-living and symbiotic, then it was characterized as “facultative”; conversely, if in literature there were only reports of that specific strain as symbiotic, then the state “obligate” was assigned.

Results.

Non-target sequences in clone libraries. All sequences retrieved from each clone library were characterized using BLASTn (NCBI). Specifically, the symbiont sequences were identified based on the similarity (>98%) with previously sequenced DDA symbionts (Supplementary Table S7A). Given the lack of sequences in the database for any of the diatom-hosts, these were characterized based on the similarity to the genus they belong to (Supplementary Table S7B). In two of the 18S rRNA libraries (*C. compressus* sample ID 10010, *H. hauckii* sample ID 404, 405) one clone out of eight (Supplementary Table S6) was 99% identical to a fungus belonging to the phylum Ascomycota. Fungi belonging to this phylum have been recently found in marine environments (Zhang *et al.* 2014; Raghukumar 2017). It is not uncommon to observe other algae caught in the siliceous projections (spines) typical of diatoms, especially the subclass Chaetocerotophycidae (Round *et al.* 1990), and since these samples were initially used in a WGA step, contaminating templates were likely also amplified. Since our objective was to sequence the eukaryotic diatom hosts, we did not report the fungal 18S rRNA sequences. The second phylotype in the 5239 *rbcL* library was a minor component (2 sequences out of 24 clones) and was 93% similar to eukaryotic clone from North Pacific, station ALOHA (Accession no. KC533903). We suspect that collection by

plankton net for this sample also contained non-symbiotic cells, which was reflected in the multiple phylotypes.

References for supporting information (includes Supplementary Tables S1-8)

Alonso C, Gómez-Pereira P, Ramette A, et al. Multilevel analysis of the bacterial diversity along the environmental gradient Río de la Plata–South Atlantic Ocean. *Aquat Microb Ecol* 2010;**61**(1):57–72.

Alonso S, Sáez L, Díaz P, and Morán XAG. The hidden seasonality of the rare biosphere in coastal marine bacterioplankton. *Environ Microbiol* 2015;**17**(10):3766–3780.

Balzano S, Lattaud J, Villanueva L, et al. A quest for the biological sources of long chain alkyl diols the ubiquitous in the marine realm western tropical North Atlantic Ocean. *Biogeosciences* 2018;**15**:5951–5968.

Beaudoin DJ, Carmichael CA, Nelson RK, et al. Impact of protists on a hydrocarbon-degrading bacterial community from deep-sea Gulf of Mexico sediments: A microcosm study. *Deep Sea Res Part II Top Stud Oceanogr* 2016;**129**:350–359.

Bekker A, Holland HD, Wang PL, et al. Dating the rise of atmospheric oxygen. *Nature* 2004;**427**:117–120.

Bik EM, Costello EK, Switzer AD, et al. Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nat commun* 2016;**7**:1–13.

Bombar D, Turk-Kubo KA, Robidart J, et al. Non-cyanobacterial *nifH* phylotypes in the North Pacific Subtropical Gyre detected by flow-cytometry cell sorting. *Environ Microbiol Rep* 2013;**5**(5):705–715.

- Bowler C, Allen AE, Badger JH, et al. The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature* 2008;**456**:239–244.
- Brocks JJ, Logan GA, Buick R, et al. Archean molecular fossils and the early rise of eukaryotes. *Science* 1999;**285**:1033–1036.
- Capone DG, Bronk DA, Mulholland MR, et al. (2008). *Nitrogen in the marine environment* 2nd edition. Amsterdam, The Netherlands: Elsevier, Academic Press, 1–1757.
- Carpenter EJ and Janson S. Intracellular cyanobacterial symbionts in the marine diatom *Climacodium frauenfeldianum* (Bacillariophyceae). *J Phycol* 2000;**36**:540–544.
- Choi CJ, Bachy C, Jaeger GS, et al. Newly discovered deep-branching marine plastid lineages are numerically rare but globally distributed. *Curr Biol* 2017;**27**(1):15–16.
- Cornejo-Castillo FM, Cabello AM, Salazar G, et al. Cyanobacterial symbionts diverged in the late Cretaceous towards lineage-specific nitrogen fixation factories in single-celled phytoplankton. *Nat Comms* 2016;**11071**:1–9.
- Countway PD, Gast RJ, Savai P, et al. Protistan diversity estimates based on 18S rDNA from seawater incubations in the Western North Atlantic. *J Eukaryot Microbiol* 2005;**52**(2):95–106.
- Cox EJ. Morphology, cell wall, cytology, ultrastructure and morphogenetic studies. In: Seckbach J, Kociolek J, and Kociolek P (eds). *The Diatom World*. Dortrecht: Springer, 2011, 21–45.

- De Corte D, Sintes E, Yokokawa T, et al. Comparison between MICRO–CARD–FISH and 16S rRNA gene clone libraries to assess the active versus total bacterial community in the coastal Arctic. *Environ Microbiol Rep* 2013;**5**(2):272–281.
- Domínguez-Escobar J, Beltrán Y, Bergman B, et al. Phylogenetic and molecular clock inferences of cyanobacterial strains within Rivulariaceae from distant environments. *FEMS Microbiol Lett* 2011;**316**:90–99.
- Edgcomb V, Orsi W, Bunge J, et al. Protistan microbial observatory in the Cariaco Basin, Caribbean. I. Pyrosequencing vs Sanger insights into species richness. *ISME J* 2011;**5**(8):1344–1356.
- Falcon LI, Carpenter EJ, Cipriano F, et al. N₂ fixation by unicellular bacterioplankton from the Atlantic and oceans: phylogeny and in situ rates. *Appl Environ Microbiol* 2004;**70**(2):765–770.
- Falcón LI, Magallón S and Castillo A. Dating the cyanobacterial ancestor of the chloroplast. *ISME J* 2010;**4**:777–783.
- Ferrario ME, Villafañe V, Helbling W et al. The occurrence of the symbiont *Richelia* in *Rhizosolenia* and *Hemiaulus* in the North Pacific. *Braz J Biol* 1995;**55**:439–443.
- Fong AA, Karl D, Lukas R, et al. Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean. *ISME J* 2008;**2**:663–676.
- Foster RA and Zehr JP. Characterization of diatom-cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences. *Environ Microbiol* 2006;**8**:1913–1925.

- Frias□Lopez J, Thompson A, Waldbauer J, et al. Use of stable isotope□labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. *Environ Microbiol* 2009;**11**(2):512–525.
- Garrity GM, Bell JA, and Lilburn TG. *Taxonomic outline of the prokaryotes. Bergey's manual of systematic bacteriology, 2nd ed.* New York: Springer-Verlag 2004, 1–399.
- George A. (2017). Characterizing Gross Lesions in Corals on Fringing Reefs of Taiwan and Hainan Island, China (Doctoral dissertation, University of South Florida).
- Gómez F, Furuya K, and Takeda S. Distribution of *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean. *J Plankton Res* 2005;**27**:323–330.
- Green-Garcia AM. *Characterization of the lucinid bivalve-bacteria symbiotic system: the significance of the geochemical habitat on bacterial symbiont diversity and phylogeny.* Master of Science (MS), Louisiana State University (USA), 2008.
- Guiry MD. How many species of algae are there? *J Phycol* 2012;**48**:1057–1063.
- Hasle GR. The fossil diatom *Thalassiosira oranica*, n. sp. *Micropaleontology* 1985;**31**:280–284.
- Hewson I, Moisander PH, Morrison AE, et al. Diazotrophic bacterioplankton in a coral reef lagoon: phylogeny, diel nitrogenase expression and response to phosphate enrichment. *ISME J* 2007;**1**(1):78–79.
- Hu S, Guo Z, Li T, et al. Detecting in situ copepod diet diversity using molecular technique:

233 development of a copepod/symbiotic ciliate-excluding eukaryote-inclusive PCR
 234 protocol. *PLoS ONE* 2014;**9**(7):1–10.
 235
 236 Janson S, Rai AN, and Bergman B. Intracellular cyanobiont *Richelia intracellularis*:
 237 ultrastructure and immuno-localisation of phycoerythrin, nitrogenase, rubisco, and
 238 glutamine synthetase. *Mar Biol* 1995;**124**:1–8.
 239
 240 Janson S, Wouters J, Bergman B, et al. Host specificity in the *Richelia*-diatom symbioses
 241 revealed by *hetR* gene sequence analyses. *Environ Microbiol* 1999;**1**:431–438.
 242
 243 Jensen S, Bourne DG, Hovland M, et al. High diversity of microplankton surrounds deep-
 244 water coral reef in the Norwegian Sea. *FEMS Microbiol Ecol* 2012;**82**(1):75–89.
 245
 246 Kaczmarek I, Beaton M, Benoit AC, et al. Molecular phylogeny of selected members of
 247 the order Thalassiosirales (Bacillariophyta) and evolution of the fucoxanthin. *J*
 248 *Phycol* 2006;**138**:121–138.
 249
 250 Kass RE and Raftery AE. Bayes factors. *J Am Stat Assoc* 1995;**90**(430):773–795.
 251
 252 Kim DY, Countway PD, Gast RJ, et al. Rapid shifts in the structure and composition of a
 253 protistan assemblage during bottle incubations affect estimates of total protistan
 254 species richness. *Microb Ecol* 2011;**62**(2):383–398.
 255
 256 Kok SP, Tsuchiya K, Komatsu K, et al. The protistan microplankton community along
 257 the Kuroshio Current revealed by 18S rRNA gene clone analysis: a case study of
 258 the differences in distribution interplay with ecological variability. *Plankton*
 259 *Benthos Res* 2014;**9**(2):71–82.
 260
 261 Kong L, Jing H, and Liu H. Phylogenetic diversity and spatio-temporal distribution of
 262 nitrogenase gene (*nifH*) in the northern South China Sea. *Aquat Microb Ecol*
 263 2011;**65**:15–27.

264

265 Kooistra WH, Gersonde RA, Medlin LK, et al. The origin and evolution of the diatoms:

266 their adaptation to a planktonic existence. In: Falkowski PG and Knoll AH (eds).

267 *Evolution of primary producers in the sea*. Burlington, MA: Academic, 2007, 207–

268 249.

269

270 Lakner C, Van der Mark P, Huelsenbeck J, et al. Efficiency of markov chain monte carlo

271 tree proposals in Bayesian phylogenetics. *Syst Biol* 2008;**57**:86–103.

272

273 Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (eds).

274 *Nucleic acid techniques in bacterial systematics*. New York: John Wiley and Sons,

275 1991, 115–175.

276

277 Lartillot N and Philippe H. Computing Bayes factors using thermodynamic integration.

278 *Syst Biol* 2006;**55**(2);195–207.

279

280 Lemmermann E. Die Algenflora der Sandwich-Inseln. Ergebnisse einer Reise nach dem

281 Pacific, H. Schauinsland 1896/97. *Enger's Bot Jb* 1905;**34**:607–663.

282

283 Lepage T, Bryant D, and Lartillot N. A General Comparison of Relaxed Molecular Clock

284 Models. *Mol Biol Evol* 2007;**24**:2669–2680.

285

286 Li B, Karl DM, Letelier RM, et al. Variability of chromophytic phytoplankton in the

287 North Pacific Subtropical Gyre. *Deep Sea Res. Part II Top Stud Oceanogr*

288 2013;**93**:84–95.

289

290 Li N, Yu SX, Wang YC, et al. Diversity of phototrophic phytoplankton in Northern South

291 China Sea indicated by *rbcL* analysis. *J Appl Phycol* 2016;**28**(2):773–781.

292

293 Lie AA, Liu Z, Hu SK, et al. Investigating Microbial Eukaryotic Diversity from a Global

294 Census: Insights from a Comparison of Pyrotag and Full-Length Sequences of

295 18S rRNA Genes. *Appl Environ Microbiol* 2014;**80**(14):4363–4373.

296

297 Lim HC, Lim PT, Teng ST, et al. Genetic structure of *Pseudo-nitzschia pungens*

298 (Bacillariophyceae) populations: implications of a global diversification of the

299 diatom. *Harmful Algae* 2014;**37**:142–152.

300

301 Loescher CR, Bourbonnais A, Dekaezemacker J, et al. N₂ fixation in eddies of the eastern

302 tropical South Pacific Ocean. *Biogeosci Discuss* 2015;**12**(1):18945–18972.

303

304 Luddington IA, Kaczmarska I, Lovejoy C. Distance and characterbased evaluation of the

305 V4 region of the 18S rRNA gene for the identification of diatoms

306 (Bacillariophyceae). *PLoS ONE* 2012;**7**:e45664.

307

308 Lyimo TJ. Distribution and abundance of the cyanobacterium *Richelia intracellularis* in

309 the coastal waters of Tanzania. *J Ecol Nat Environ* 2011;**3**:85–94.

310

311 MacGillivray ML, and Kaczmarska I. Survey of the efficacy of a short fragment of the

312 *rbcL* gene as a supplemental DNA barcode for diatoms. *J Eukaryot Microbiol*

313 2011;**58**:529–536.

314

315 Madhu NV, Paul M, Nanappan U, et al. Occurrence of cyanobacteria (*Richelia*

316 *intracellularis*) diatom (*Rhizosolenia hebetata*) consortium in the Palk Bay,

317 southeast coast of India. *Indian J Mar Sci* 2013;**42**:453–457.

318

319 Mann DG. Discovering diatom species: is a long history of disagreements about species-

320 level taxonomy now at an end? *Plant Ecol Evol* 2010;**143**:251–264.

321

322 Mann DG, Simpson GE, Sluiman HJ, et al. *rbcL* gene tree of diatoms: a second large

323 data-set for phylogenetic reconstruction. *Phycologia* 2001;**40**:1–2.

324

325 Marie D, Shi XL, Rigaut-Jalabert F, et al. Use of flow cytometric sorting to better assess

326 the diversity of small photosynthetic eukaryotes in the English Channel. *FEMS*

327 *Microbiol Ecol* 2010;**72**(2):165–178.

328

329 Massana R, Balague V, Guillou L, et al. Picoeukaryotic diversity in an oligotrophic

330 coastal site studied by molecular and culturing approaches. *FEMS Microbiol Ecol*

331 2004;**50**(3):231–243.

332

333 Mazard SL, Fuller NJ, Orcutt KM, et al. PCR analysis of the distribution of unicellular

334 cyanobacterial diazotrophs in the Arabian Sea. *Appl Environ Microbiol*

335 2004;**70**(12):7355–7364.

336

337 Miyake S, Ngugi DK, and Stingl U. Phylogenetic Diversity, Distribution, and

338 Cophylogeny of Giant Bacteria (*Epulopiscium*) with their Surgeonfish Hosts in the

339 Red Sea. *Front Microbiol* 2016;**7**(285):1–15.

340

341 Moisander PH, Morrison AE, Ward BB, et al. Spatial-temporal variability in diazotroph

342 assemblages in Chesapeake Bay using an oligonucleotide nifH microarray. *Environ*

343 *Microbiol* 2007;**9**(7):1823–1835.

344

345 Nakayama T, Ikegami Y, Nakayama T, et al. Spheroid bodies in rhopalodiacean diatoms

346 were derived from a single endosymbiotic cyanobacterium. *J Plant Res*

347 2011;**124**:93–97.

348

349 Olson JB, Steppe TF, Litaker RW, et al. N₂-fixing microbial consortia associated with the

350 ice cover of Lake Bonney, Antarctica. *Microb Ecol* 1998;**36**:231–238.

351

352 Ostensfeld CH and Schmidt J. Plankton fra det Rode Hav og Adenbugten (Plankton from

the Red Sea and the Gulf of Aden.) Videsnkabelige Meddelelser fra Dansk
Naturhistorisk Forening, Copenhagen, 1901, 141–182.

Padmakumar KB, Menon NR, and Sanjeevan VN. Occurrence of endosymbiont *Richelia*
intracellularis (Cyanophyta) within the diatom *Rhizosolenia hebetata* in Northern
Arabian Sea. *Int J Biodivers Conserv* 2010;**2**:70–74.

Paul JH, Alfreider A, and Wawrik B. Micro-and macrodiversity in *rbcL* sequences in
ambient phytoplankton populations from the southeastern Gulf of Mexico. *Mar Ecol*
Prog Ser 2000;9–18.

Paul JH, Pichard SL, Kang JB, et al. Evidence for a clade-specific temporal and spatial
separation in ribulose biphosphate carboxylase gene expression in phytoplankton
populations off Cape Hatteras and Bermuda. *Limnol Oceanogr* 1999;**44**:12–23.

Pernice MC, Logares R, Guillou L, et al. General patterns of diversity in major marine
microeukaryote lineages. *PLoS One* 2013;**8**:1–11.

Raghukumar S. The marine environment and the role of fungi. In: *Fungi in coastal and*
oceanic marine ecosystems, 2017. Cham: Springer, 17–38.

Ronquist F, Teslenko M, van der Mark P, et al. MrBayes 3.2: efficient Bayesian
phylogenetic inference and model choice across a large model space. *Syst Biol*
2012;**61**:539–542

Rothpletz A. Ueber die Flysch-Fucoiden und einige andere fossile Algen, sowie über
liasische, Diatomeen führende Hornschwämme. *Z Dtsch Geol Ges* 1896;**48**:854–
914.

- Round FE, Crawford RM, and Mann DG. *The Diatoms: Biology and morphology of the genera*. Cambridge (UK): Cambridge University Press, 1990.
- Shiozaki T, Chen YLL, Lin YH, et al. Seasonal variations of unicellular diazotroph groups A and B, and Trichodesmium in the northern South China Sea and neighboring upstream Kuroshio Current. *Cont Shelf Res* 2014;**80**:20–31.
- Sims PA, Mann DG, Medlin LK, et al. Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia* 2006;**45**:361–402.
- Sinha RK, Krishnan KP, Hatha AA, et al. Diversity of retrievable heterotrophic bacteria in Kongsfjorden, an Arctic fjord. *Braz J Microbiol* 2017;**48**:51–61
- Sintes E, Witte H, Stodderegger K, et al. Temporal dynamics in the free-living bacterial community composition in the coastal North Sea. *FEMS Microbiol Ecol* 2013;**83** (2):413–424.
- Sorhannus U. Diatom phylogenetics inferred based on direct optimization of nuclear-encoded SSU rRNA sequences. *Cladistics* 2004;**20**:487–497.
- Sorhannus U. A nuclear-encoded small-subunit ribosomal RNA timescale for diatom evolution. *Mar Micropaleontol* 2007;**65**:1–12.
- Speck MD and Donachie SP. Widespread *Oceanospirillaceae* bacteria in porites spp. *J Mar Bio* 2012;1–7.
- Strelnikova NI. Evolution of diatoms during the Cretaceous and Paleogene periods. In: Simola H (ed.) *Proceedings of the 10th International Diatom Symposium*. Koeltz Scientific Books, Koenigstein, 1990, 195–204.

410 Sunagawa S, DeSantis TZ, Piceno YM, et al. Bacterial diversity and White Plague
 411 Disease-associated community changes in the Caribbean coral *Montastraea*
 412 *faveolata*. *ISME J* 2009;**3**(5):512–521.
 413
 414 Sunagawa S, Woodley CM, and Medina M. Threatened corals provide underexplored
 415 microbial habitats. *PLoS ONE* 2010;**5**(3):1–7.
 416
 417 Sundström BG. Observations on the *Rhizosolenia clevei* Ostenfeld (Bacillariophyceae)
 418 and *Richelia intracellularis* Schmidt (Cyanophyceae). *Bot Mar* 1984;**27**:345–355.
 419
 420 Tamura M, Shimada S, and Horiguchi T. *Galeidinium rugatum* gen. et sp. nov.
 421 (Dinophyceae), a new coccoid dinoflagellate with a diatom endosymbiont. *J Phycol*
 422 2005;**41**:658–671.
 423
 424 Takishita K, Kakizoe N, Yoshida T, et al. Molecular evidence that phylogenetically
 425 diverged ciliates are active in microbial mats of deep-sea cold-seep sediment. *J*
 426 *Eukaryot Microbiol* 2010;**57**(1):76–86.
 427
 428 Takishita K, Yubuki N, Kakizoe N, et al. Diversity of microbial eukaryotes in sediment at
 429 a deep-sea methane cold seep: surveys of ribosomal DNA libraries from raw
 430 sediment samples and two enrichment cultures. *Extremophiles* 2007;**11**(4):563–
 431 576.
 432
 433 Teeling H, Fuchs BM, Becher D, et al. Substrate-controlled succession of marine
 434 bacterioplankton populations induced by a phytoplankton bloom. *Science*
 435 2012;**336**(6081):608–611.
 436
 437 Theriot EC, Ashworth M, Ruck E, et al. A preliminary multigene phylogeny of the
 438 diatoms (Bacillariophyta): challenges for future research. *Plant Ecol Evol*

439 2010;**143**(3):278–296
 440 Theriot EC, Cannone JJ, Gutell RR, et al. The limits of nuclear-encoded SSU rDNA for
 441 resolving the diatom phylogeny. *Eur J Phycol* 2009;**44**:277–290.
 442
 443 Thorne JL and Kishino H. Divergence time and evolutionary rate estimation with
 444 multilocus data. *Syst Biol* 2002;**51**(5):689–702.
 445
 446 Tomas CR. *Identifying marine phytoplankton*. Tomas CR and Hasle GR (eds). San
 447 Diego: Academic press., 1997.
 448
 449 Tomitani A, Knoll AH, Cavanaugh CM, et al. The evolutionary diversification of
 450 cyanobacteria : Molecular - phylogenetic and paleontological perspectives. *Proc*
 451 *Natl Acad Sci USA* 2006;**103**:5442–5447.
 452
 453 Turk KA, Rees AP, Zehr JP, et al. Nitrogen fixation and nitrogenase (*nifH*) expression in
 454 tropical waters of the eastern North Atlantic. *ISME J* 2011;**5**(7):1201–1212.
 455
 456 Villareal TA. Division cycles in the nitrogen-fixing *Rhizosolenia* (Bacillariophyceae)-
 457 *Richelia* (Nostocaceae) symbiosis. *Eur J Phycol* 1989;**24**:357–365.
 458
 459 Villareal TA. Marine nitrogen-fixing diatom-cyanobacteria symbioses. In: Carpenter EJ,
 460 Capone DG, Reuter JG (eds.). *Marine Pelagic Cyanobacteria: Trichodesmium* and
 461 other Diazotrophs. Dordrecht: Kluwer, 1992,163–75.
 462
 463 Walsh DA, Zaikova E, Howes CG, et al. Metagenome of a versatile chemolithoautotroph
 464 from expanding oceanic dead zones. *Science* 2009;**326**(5952):578–582.
 465
 466 Wawrik B, Paul JH, and Tabita TR. Real-time PCR quantification of *rbcL* (ribulose-1,5-
 467 biphosphate carboxylase/oxygenase) mRNA in diatoms and pelagophytes. *Appl*

Environ Microbiol 2002;**68**:3771–3779.

Wolf C, Frickenhaus S, Kiliyas E.S, et al. Protist community composition in the Pacific sector of the Southern Ocean during austral summer 2010. *Polar Biol* 2014;**37**(3):375–389.

Yang Z. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Mol Biol Evol* 1993;**10**:1396–1401.

Zeev EB, Yogev T, Man-Aharonovich D, et al. Seasonal dynamics of the endosymbiotic, nitrogen-fixing cyanobacterium *Richelia intracellularis* in the eastern Mediterranean Sea. *ISME J* 2008;**2**:911–923.

Zehr JP, Montoya JP, Jenkins BD, et al. Experiments linking nitrogenase gene expression to nitrogen fixation in the North Pacific subtropical gyre. *Limnol Oceanogr* 2007;**52**(1):169–183.

Zhang C, Stadler T, Klopstein S, et al. Total-evidence dating under the fossilized birth–death process. *Syst Appl Microbiol* 2016;**65**:228–249.

Zhang XY, Tang GL, Xu X-Y, et al. Insights into deep-sea sediment fungal communities from the East Indian Ocean using targeted environmental sequencing combined with traditional cultivation. *Plos One* 2014;**9**(10):1–11.

Zimmermann J, Jahn R, and Gemeinholzer B. Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. *Org Divers Evol* 2011;**11**(3):1–20.

Zöllner E, Hoppe HG, Sommer U, et al. Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). *Limnol Oceanogr* 2009;**54**(1):262–275.

499 Zuendorf,A., Bunge,J., Behnke,A., et al. Diversity estimates of microeukaryotes below
500 the chemocline of the anoxic Mariager Fjord, Denmark. *FEMS Microbiol Ecol*
501 2006;**58**(3): 476–491.
502