Assessment of algal diversity in the Styx River catchment

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Abstract

Surveys of algae were carried out at 12 sites in the Styx River, Smacks Creek and Kaputone Stream over 3 days in November 2009 to document the diversity of algae at key points in the Styx catchment. Different species of algae were identified using traditional morphological identification as well as molecular techniques. Fifty different species were identified over all sites, plus 13 more species from environmentally cloned sequences. A number of novel algal lineages were found in the Styx catchment. The species data collected in this study provide a baseline for future monitoring of algal communities. One hypothesis to explain the distribution of algae in the Styx is that algal communities are likely to be more resilient to sedimentation in sites with slower flows, and that the presence of macrophytes may increase this resilience. Also, the site at greatest risk for incursion of *Didymosphenia geminata*, according to accepted preferences for this species, currently harbours the greatest diversity of other species. These observations have potential implications for management.

Introduction

The Styx River

The Styx River is a small (54.8 km^2) spring-fed catchment situated to the north of Christchurch City (Taylor et al. 2000). The Styx catchment, which includes three major tributaries (Kaputone Creek, Kainga Stream and Smacks Creek), is an important natural asset and drains suburban, horticultural, agricultural and industrial land. The river is 23.8 km in length, originates in the Harewood area and runs north-eastwards through reserves, rural pastures, horticultural areas and residential developments before reaching the sea via the Waimakariri River at Brooklands Lagoon (Hills 2002).

In recent years, due to changing land-uses and population growth through the northward expansion of Christchurch City, parts of the catchment that have not previously been urbanised have undergone extensive modification. This has put increasing pressure on the catchment and resulted in damage to the river environment in the form of a reduction of stream 'health' caused by loss of riparian vegetation, increased siltation, excessive macrophyte growths and other factors (Taylor et al. 2000).

Past and current monitoring programmes in the Styx River catchment have looked at water quality, water status, aquatic plants, streambed substrate, water velocity and depth, riparian vegetation, invertebrates, lizards and macrophytes (http://www.thestyx.org.nz/new-zealand/monitoring/).

What are algae?

The organisms referred to as 'algae' are a loose collection of groups with similar ecological characteristics; however, algae as a whole cannot be defined by shared features, due to the extremely disparate evolutionary origins of the various groups (e.g. Graham & Wilcox 2000). The term is a legacy from early microscopic

observations that did not reveal the phylogenetic diversity of the species concerned, but is still generally retained for pragmatic reasons. In simple terms algae may be regarded as aquatic microbes that are capable of oxygenic photosynthesis – although even this definition does not encapsulate all the organisms regarded as algae, including those presented here. In this report, the groups included are the Chlorophyta (green algae not of the land plant lineage), Streptophyta (which includes land plants, but the early divergent members are green algae), Bacillariophyceae (the diatoms), Xanthophyceae (yellow-green algae), and cyanobacteria (blue-green algae, which are actually bacteria and not close relatives of other algal groups, but are capable of photosynthesis and are often found in similar habitats to other algae).

Distribution patterns of algae

The distributions of most algal species, especially in fresh water, are poorly understood globally, let alone in New Zealand where relatively little research has been carried out. Nonetheless some patterns have become evident, especially in the diatom flora. Diatoms have received particular taxonomic attention because their ornamented frustules offer many more characters to assist classification compared with most groups of 'soft algae'. It seems that Australasia may be a 'centre of endemism' for certain species (e.g. Sabbe et al. 2001), which tend to be found in undisturbed habitats (Kilroy et al. 2007). These taxa are usually accompanied by others that are assumed to be cosmopolitan, and the proportion of endemism in the freshwater algal flora is assumed to be low (an estimate of 2.3% diatom endemism was provided by the Species2000 project). However, the true distributions of most taxa are simply unknown, due to the difficulties of applying rigorous morphology-based taxonomy to species with a paucity of characters to assist their classification. Molecular data, which have revolutionised algal systematics (e.g. Brodie & Lewis 2007), can help greatly in this regard. Very few New Zealand freshwater algae have been the subject of molecular investigation, and many of these have been small alpine species that might be expected to be widely dispersed (Novis et al. 2008). The use of molecular data in this project is its first application to surveying the algae of a lowland stream environment in New Zealand. It thus provides a first glimpse of distribution patterns in a number of algal groups, especially in a global context, and establishes a baseline for future investigations in the Styx and other catchments that will ultimately lead to much more detailed knowledge of freshwater algal biogeography.

Algal survey

To date there has been no systematic study focused on the diversity of algal species in the Styx River or its tributaries. Previous studies of aquatic macrophytes began in 1953 and have included large algae such as *Nitella* and *Cladophora* (Robb 1989). Consequently there is some information about the distribution of these species, e.g. *Nitella hookeri* and filamentous green algae as a group are especially common (Robb 1989), but there is no information about the other algal species present, or distribution of these species, in the river catchment. There is also no recognition of the diversity of filamentous green algae, which may represent many species.

Objectives

This study aimed to document the diversity of algae at key points in the Styx catchment. Techniques used included traditional morphological identification (including ultrastructural) as well as molecular techniques using DNA from both cultured strains and environmental samples.

Methods

Site selection

Twelve sites were selected within the Styx River catchment (Fig. 1). Ten of these sites are regularly monitored as part of the Styx Living Laboratory Trust's monitoring regime. The other two sites, Spencerville Rd Bridge and Teapes Rd Bridge, were included in this study as they were easy to access and believed to be good habitats for algae.

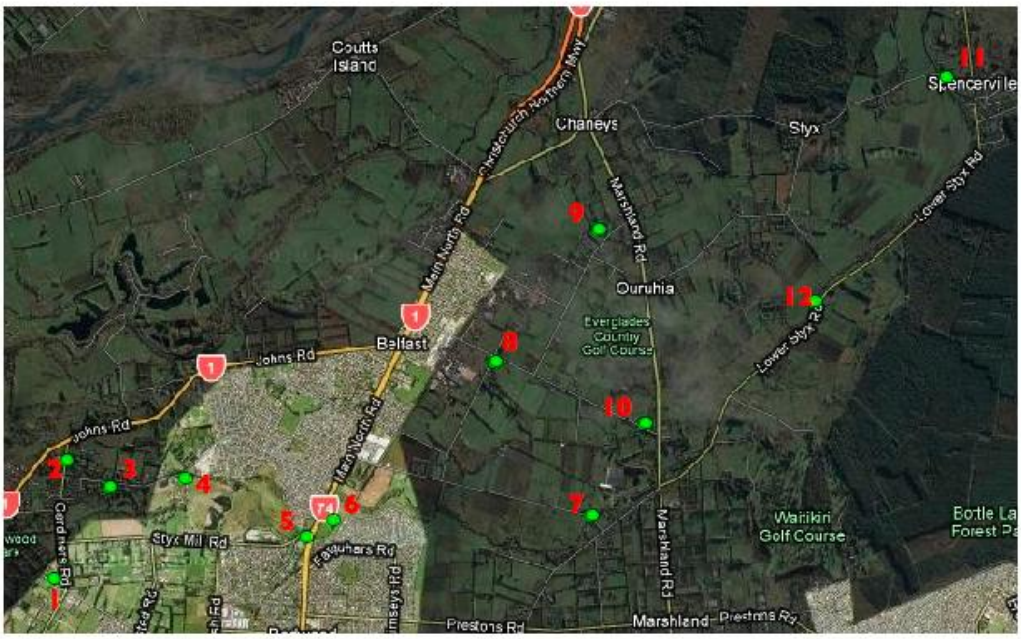


Figure 1. Map of Styx River catchment.

1) Styx River headwaters
2) Smacks Creek at Gardiners Rd
3) Smacks Creek at Willowbank Wildlife Reserve
4) Styx River at Styx Mill Conservation Reserve
5) Styx River at Main North Rd
6) Styx River at Redwood Springs
7) Styx River at Radcliffe Rd
8) Kaputone Stream at Belfast Rd
9) Kaputone Stream at Ouruhia Domain
10) Kaputone Stream at Belfast Rd Bridge
11) Styx River at Spencerville Rd Bridge
12) Styx River at Teapes Rd Bridge

Sample collection

Each site was visited on a single occasion: sites 1–4 on 15 November; sites 5–8 on 16 November, and sites 9–12 on 24 November 2009. Sites were examined in detail for algal growths, and representative samples were collected for subsequent analysis, and returned to the laboratory the same day. Samples included periphyton (filaments and coatings attached to submerged objects), phytoplankton (pigmented water samples), and specimens of Charales (large streptophyte green algae that resemble pond weeds). A total of 53 samples were collected over all the sites (see Appendix 1).

Three replicates were collected of each sample and samples were stored in sterile screw-cap test tubes. The pH and conductivity were also measured at each of the 12 sites.

Photomicroscopy

One replicate of each sample (A) was frozen and stored in the freezer for later DNA extraction. The second replicate (B) was preserved in 70% ethanol, and samples containing charophytes were dried in the Allan Herbarium at Landcare Research. The third replicate (C) was used for direct microscopic examination of field material. Numerous photos of each sample were taken using a photomicroscope (Leica DMLB microscope with bright field and Nomarski Differential Interference Contrast (DIC) optics and a Leica DC500 digital camera system). Algae present on each slide were identified (usually to genus level) if possible, for later comparison with cultured material.

Inoculation of cultures

Samples other than those containing charophytes were streaked onto agarised BG-11 medium in 5.5-cm Petri dishes, sealed using Parafilm tape, and incubated under natural light on the laboratory bench. Agar was washed five times overnight in distilled water to remove toxins from the first autoclaving step. An additional crude culture was made with samples containing Oedogoniales or Zygnematales to stimulate the sexual cycle and aid identification.

Cultures from each sample were examined under the stereo- and compound microscopes and each different colony type was inoculated onto a new plate. Plates were left to grow for a minimum of 3 weeks and then examined and subcultured again. This was done a third time, if necessary, to obtain as many unialgal cultures as possible. Each clean sample was examined under the microscope and photos were taken. One sample of each species was then inoculated into an agar slant for long-term storage and maintenance and the remaining sample was used for DNA extraction.

DNA extraction of cultures and environmental samples

In the case of environmental samples, material in the sample tube was used directly for extraction. Cultured material was removed from each plate by scraping a coverslip across the surface of the agar to collect the algae.

The following method was used to extract DNA from environmental and cultured samples:

1. Mortar, pestles and 1 ml of extraction buffer were heated at 65ºC on a heatblock.
2. The material was put into a mortar with warmed extraction buffer, and ground to a pulp.
3. The sample was incubated on a heatblock at 65ºC for 30 min.
4. The sample was then spun in a centrifuge for 5 min at 13200 rpm.
5. After spinning, the supernatant was removed from each sample and added to 1.5-ml tubes containing 750 µl of chloroform. Samples were spun again for 5 min.
6. In the fume hood, the supernatant was carefully removed and added to 1.5-ml tubes containing 750 µl phenol/chloroform. The samples were spun again for 5 min.
7. The supernatant was removed again and added to 1.5-µl tubes containing 750 µl of chloroform. The samples were spun for 1 min.
8. The supernatant was removed from each sample (~400 µl) and transferred to clean 1.5-ml tubes.

Samples from step 7 were subjected to the protocol of the i-genomic Plant DNA extraction Mini Kit, page 45: IV–VII. www.intronbio.com.

Polymerase chain reaction (PCR)

Three PCR reactions were run on the DNA from each environmental sample. Intron i-Taq DNA polymerase was used to amplify the DNA in each reaction.

PCR recipe

	× 1 (µl)
H_2O	11.5
10× buffer	2.0
dNTP (2.5 mM)	2.5
5% DMSO	0.8
Taq	0.2
P1	1.0
P2	1.0
DNA	1.0

PCR 1 (Chlorophyte rbc*L)*

Primers used: Nr4F–PR3R (see Appendix 2)
A master mix was made with the above ingredients, but excluding the DNA, then 19 µl was pipetted into 0.2-µl tubes for each sample. The DNA from each sample was then added to the appropriately labelled tube.

DNA was then amplified using the following program:

1. 4 min @ 94ºC
2. (30 s @ 94ºC, 45 s @ 56ºC, 45 s @ 72ºC) × 34 cycles

PCR 2 (Streptophyte rbc*L)*

Primers used: PNK1F–PNK2R (see Appendix 2)
Same as above, except the following program was used:

1. 4 min @ 94ºC
2. (30 s @ 94ºC, 45 s @ 45ºC, 45 s @ 72ºC) × 34 cycles

PCR 3 (Heterokont rbc*L)*

Primers used: HRF2–HRR4 (see Appendix 2)
Same as above, except the following program was used:

1. 3 min @ 94ºC
2. (1 min @ 94ºC, 1 min @ 50ºC, 2 min @ 72ºC) × 30 cycles

For primer sequences, see Appendix 2.

Cultured and charalean environmental samples

For cultured samples, two pairs of primers were used in each PCR reaction to create longer DNA strands for sequencing.

Primers used (see Appendix 2):
Heterokont cultures: HRF2–HRR4, HRF5–HRR2
Chlorophyte cultures: Nr1F–Nr6R, Nr4F–PR3R
Streptophyte cultures: PNK1F–PNK2R, PNK4F–Kkdown
Charalean environmental samples (1, 3, 9, 17, 27, 36): PNK1F– PNK2R

Cloning

Cloning of PCR products generated from environmental samples was used to separate the different sequence types generated from a community of species.

TA Cloning Procedure

1. PCR products were generated as described above.
2. Contaminating small fragments were removed by gel-cutting the product of interest, using the PerfectPrep Gel Cleanup kit (Eppendorf, Hamburg, Germany)
3. The gel-cut product was concentrated from 30 µL into 10 µL using the DNA clean and concentrator kit (Zymo, Orange, CA, USA)
4. The concentrated product was ligated to the PCR2.1 vector (Invitrogen, San Diego, CA, USA) by incubating the following mixture overnight at 22ºC: 1 µL vector, 1 µL DNA Ligase (Fermentas, Ontario, Canada), 1 µL 10X ligase buffer, 7 µL PCR product. A subsequent 10-min 65ºC incubation was used to destroy the enzyme.
5. Salts were removed from the ligation mix by repeating the clean and concentrate step.
6. *E.coli* JM107 cells in a 50-µL suspension were transformed with 1–2 µL of cleaned vector by electroporation.
7. Clones were incubated for 1 h in SOC medium to allow expression of the ampicillin-resistance genes, and then 75 µL of each sample was plated onto LB medium containing ampicillin and incubated overnight.

8. Clones containing inserts were identified by blue/white screening, and clones containing inserts of the correct size were chosen using colony PCR with M13 primers and standard PCR conditions.
9. Clones containing inserts of the correct size were sequenced.

Sequencing

Shrimp alkaline phosphatase (0.6 µl) and exonuclease I (0.3 µl) were added to 6 µL of each product and these were heated in the cycler for 30 min at 37ºC and 15 min at 80ºC.
A mixture of the following was then made and subjected to a standard sequencing program in the thermocycler:

Big Dye Terminator 3.1	1 µl
Sequencing buffer	1.5 µl
H_2O	6.34 µl
Primer	0.16 µl
Template	1 µl

The products of this reaction was precipitated in 600-µl tubes and sent to the Allan Wilson Centre at Massey University, Palmerston North, for capillary separation and sequencing.

Analysis of molecular data

Each DNA sequence was edited to correct ambiguous bases using Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). Pairs of sequences from cultured samples were assembled into contigs and then edited.

Blast searches of GenBank via the NCBI (National Center for Biotechnology Information) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were done for each sequence. Any sequences that were not of algal origin were discarded and the remaining sequences were added to the appropriate dataset (Charales, Diatoms, Spirogyra, Green Algae, Xanthophyceans) for alignment.

Each dataset was aligned using the program ClustalX 2.09 (Larkin et al. 2007). Alignments were checked by eye. A neighbour-joining analysis of phylogeny was run for each dataset in MEGA4 (Tamura et al. 2007), with 1000 bootstrap replicates, pairwise deletion, and a maximum composite likelihood nucleotide substitution model including transitions and transversions. This was judged appropriate for the aims of this study – to identify close relatives of our sequences in large, unwieldy datasets – rather than a more sophisticated analysis in which deeper phylogenetic relationships would have been the focus.

Ordination analyses of presence/absence data

In order to visualise the distributions of the species found and look for patterns in the data, the presence or absence of each algal species (including the cloned sequences) at each site was compiled into a matrix and analysed by two methods in the ordination program PC-Ord 5 (McCune & Mefford 2006). Detrended Correspondence Analysis (DCA) was used first on the complete dataset of species occurrences. Rare species were downweighted and axes rescaled. A subset of the complete dataset was then

used for Canonical Correspondence Analysis (CCA), including only those sites for which water quality data were available online (http://www.thestyx.org.nz/new-zealand/WaterQuality_Monitoring/). The water quality variables included in the CCA were clarity, flow, electrical conductivity, and three substrate size classes. The pH variable was omitted because there was almost no variation between sites, and the substrate size classes were combined into three: small substrate (sand/silt up to gravel), stones (small and large), and macrophytes, in order to reduce the number of variables to satisfy the constraints of the method. Row and column scores were standardised by centring and normalising, scores were scaled by optimising sites, and the sites scores for graphing were calculated as linear combinations of the environmental parameters.

Diatom cleaning and mounting

The diatoms in each sample were cleaned and mounted using the method of Biggs & Kilroy (2000, pp. 106–107).

Samples with diatoms (after cleaning): Nos 2, 6, 7, 8, 12, 13, 14, 15, 19, 20, 22, 23, 28, 29, 31, 34, 37, 39, 40, 41, 43, 44, 47, 50, 51

Samples without diatoms (after cleaning): Nos 4, 10, 18, 46

Results

Taxonomy

Class Coscinodiscophyceae
Subclass Coscinodiscophycidae
Order Melosirales
Family Melosiraceae

***Melosira varians* C. Agardh** Plate 1A–E
Reference: Biggs & Kilroy 2000, p. 153

Distribution: Found in Styx River at headwaters, Styx Mill Conservation Reserve, Main North Rd, Radcliffe Rd, and Spencerville Rd and Teapes Rd bridges; Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve; Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Cylindrical filaments approximately as long as they are wide, 10–40 µm in diameter. No ornamentation on cell walls in either valve or girdle view. Several to many lobed, brown-coloured chloroplasts line the cell walls.

Remarks: Cultured from sample 40. Molecular data from culture indicate the closest relative to this sample is *Melosira nummuloides* (Fig. 2). The *M. varians* sequence found in GenBank was omitted from the analysis because it did not appear to align. The branch leading from this sample to *M. nummuloides* is quite long indicating differences from our sample. Reported to be widespread in New Zealand in slow- to medium- flowing open lowland streams (Biggs & Kilroy 2000).

Class Mediophyceae
Order Thalassiosirales
Family Thallassiosiraceae

***Thalassiosira* cf. *weissflogii* (Grunow) G. Fryxell & Hasle** Plate 1F
Reference: Krammer & Lange-Bertalot 1991, vol. 3

Distribution: Found in Kaputone Stream at Ouruhia Domain (Fig.1).

Features: Cylindrical valve, 19 µm in diameter. Rectangular girdle view with rounded corners. Blunt processes around the valve margin.

Remarks: Not cultured. No molecular data. Only one specimen seen.

Class Bacillariophyceae
Order Eunotiales
Family Eunotiaceae

Cf. *Actinella indistincta* Vyverman & Bergey Plate 1G–J
Reference: Sabbe et al. 2001, figs 31–44

Distribution: Found in Styx River at Styx Mill Conservation Reserve and Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Cells slightly clavate in girdle view, 15–36.4 µm long. Valves slightly semi-arcuate and heteropolar, with ventral margin more or less concave and dorsal margin convex, 3.4–6.7 µm wide at midpoint. Striae difficult to resolve with the light microscope, parallel in the centre to slightly radiate at the poles. Sternum narrow and indistinct. Raphe short, largely situated on the valve mantle; distal ends bent onto the valve face.

Remarks: Not cultured. No molecular data. This species may be very difficult to separate from *Eunotia lunaris* as shown in Foged (1979). Sabbe et al. (2001) state that the difference between the two genera is that *Actinella* is asymmetrical about the median transapical plane, yet Foged's illustrations of *E. lunaris* include asymmetrical examples. It is possible that the two species are synonymous. Previously reported in one western (Oberon Tarn) and one corridor lake (Twisted Lake) in Tasmania, and in an unnamed tarn on Stewart Island (Sabbe et al. 2001).

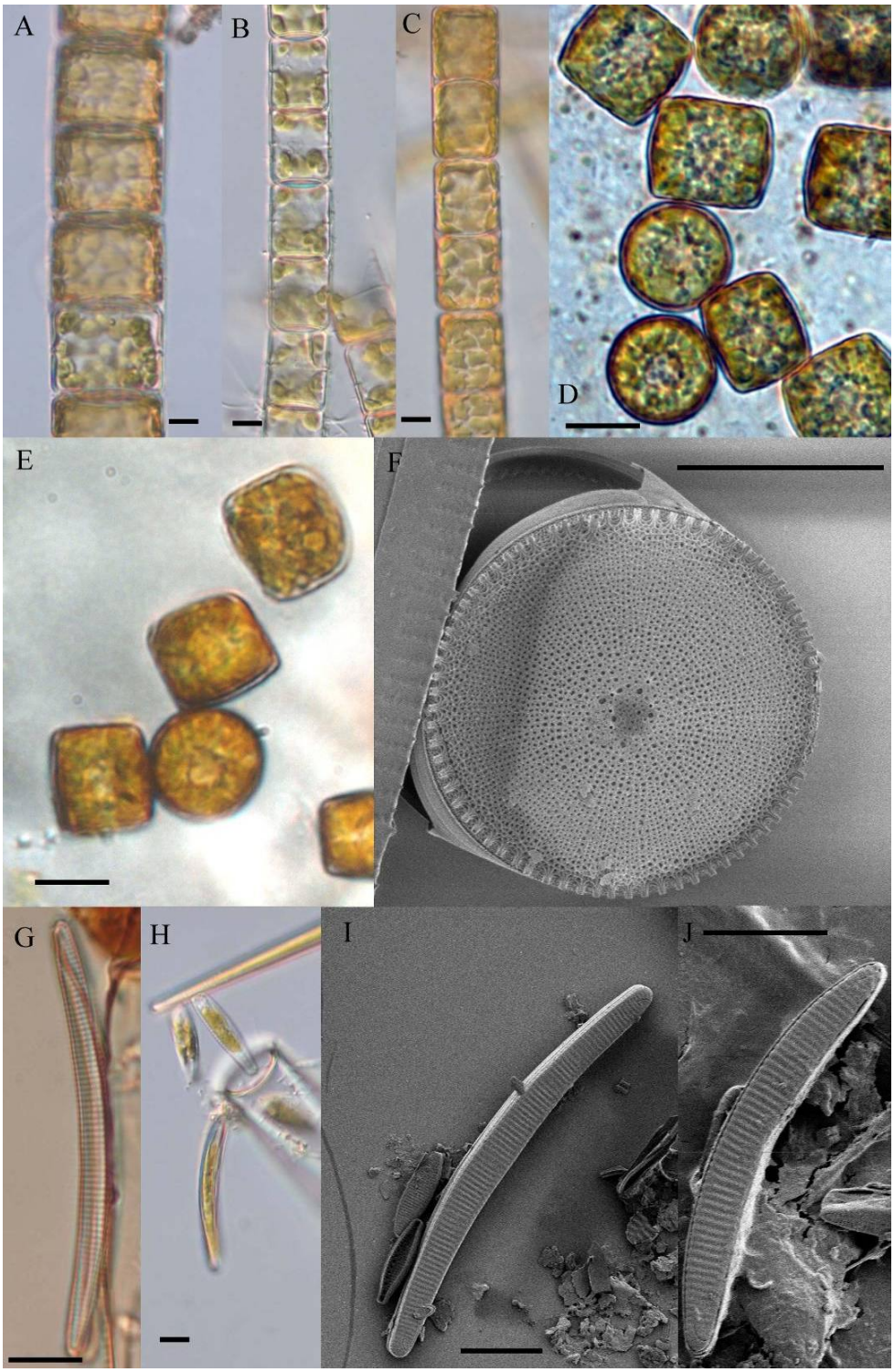


Plate 1

A–E, *Melosira varians*: **A–C,** field material (samples 47(13), 14B(2), 8B(3)); **D–E,** cultured material (cultures 50/3 and 46/3).

F, *Thalassiosira* cf. *weissflogii*, field material, SEM.

G–J, Cf. *Actinella indistinct*: **G,** cleaned field material (sample 15(5)); **H,** living material (sample 15B(12)), **I–J,** SEM (samples 15-1, 19-3).

All scales = 10 µm.

***Actinella* cf. *brasiliensis* Grunow** Plate 2A
Reference: Krammer & Lange-Bertalot 1991, vol. 3, fig. 160: 2–3

Distribution: Rare. Found in Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Valves clavate, 112 µm long, 7 µm wide, with one particularly swollen headpole. Clavate in girdle view. Biraphid, raphes located at margins. Raphe endings not visible in these specimens. Rimoportula opening on swollen headpole.

Remarks: Not cultured. No molecular data. Only one specimen seen. Identification is uncertain because raphe endings are hidden and there is a lack of material.

Subclass Eunotionphycidae
Order Eunotiales
Family Eunotiaceae

***Eunotia* cf. *epithemia* G.L. Rabenhorst** Plate 2B
Reference: Biggs & Kilroy 2000, p. 150

Distribution: Found in Styx River at Styx Mill Conservation Reserve (Fig. 1).

Features: Valve crescent-shaped, 22 µm long, 5 µm wide at centre, with rounded ends. Rectangular in girdle view. Rudimentary raphe system on both valves, raphes located at margins. Parallel striae.

Remarks: Not cultured. No molecular data.

Family Pinnulariaceae

***Pinnularia gibba* Ehrenberg** Plate 2C–E
Reference: Krammer & Lange-Bertalot 1991 vol.1, fig. 189: 1–9

Distribution: Found in Styx River at headwaters, Radcliffe Rd, and Redwood Springs; Smacks Creek at Gardiners Rd; Kaputone Stream at Belfast Rd Bridge (Fig. 1).

Features: Valves 80–120µm long, 15 µm wide with slightly capitate ends. Wide axial and central areas. Biraphid, straight raphe. Short striae, convergent at apex and radiate at centre, sometimes with a gap at the centre of the valve.

Remarks: Cultured from sample 22. Molecular data from culture suggest closest relative is *Gomphonema affine* (Fig. 2). Reported as widespread and not usually common in stream periphyton (Biggs & Kilroy 2000).

***Pinnularia interrupta* W. Smith** No images available
Reference: Krammer & Lange-Bertalot 1991 vol. 1, fig. 190: 1–11

Distribution: Found at Styx headwaters and Styx at Styx Mill Conservation Reserve (Fig. 1).

Features: Valve symmetrical with undulating margins, 40 µm long, 9.5 µm wide with rounded ends. Biraphid, straight raphes located in middle of valve. Striae convergent at apices and radiate towards central area; transverse fascia.

Remarks: Not cultured. No molecular data. Reported to be widespread (Biggs & Kilroy 2000).

Order Bacillariales
Family Bacillariaceae

***Nitzschia acicularis* (Kützing) W. Smith** Plate 2F–G
Reference: Krammer & Lange-Bertalot 1991 vol. 2, fig. 85: 1–3

Distribution: Found in Styx River at Main North Rd, Radcliffe Rd, near Redwood Springs and Spencerville Rd bridge; Smacks Creek at Gardiners Rd; Kaputone Stream at Ouruhia Domain and Belfast Rd bridge (Fig. 1).

Features: Valve symmetrical, 20–60 µm long, 3.5 µm wide, with fine drawn-out ends. Two parietal chloroplasts.

Remarks: Culture from sample 37. No molecular data. Reported as widespread but usually not very abundant (Biggs & Kilroy 2000).

***Hantzschia amphioxys* Grunow** Plate 2H–I
Reference: Krammer & Lange-Bertalot 1991 vol. 2, fig. 88: 1–7

Distribution: Found in Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Valve slightly curved, 26–64 µm long, 3–9 µm wide, with protracted axial areas and slight narrowing at centre. Raphe located on margins on inside of curve. Parallel striae.

Remarks: Not cultured. No molecular data.

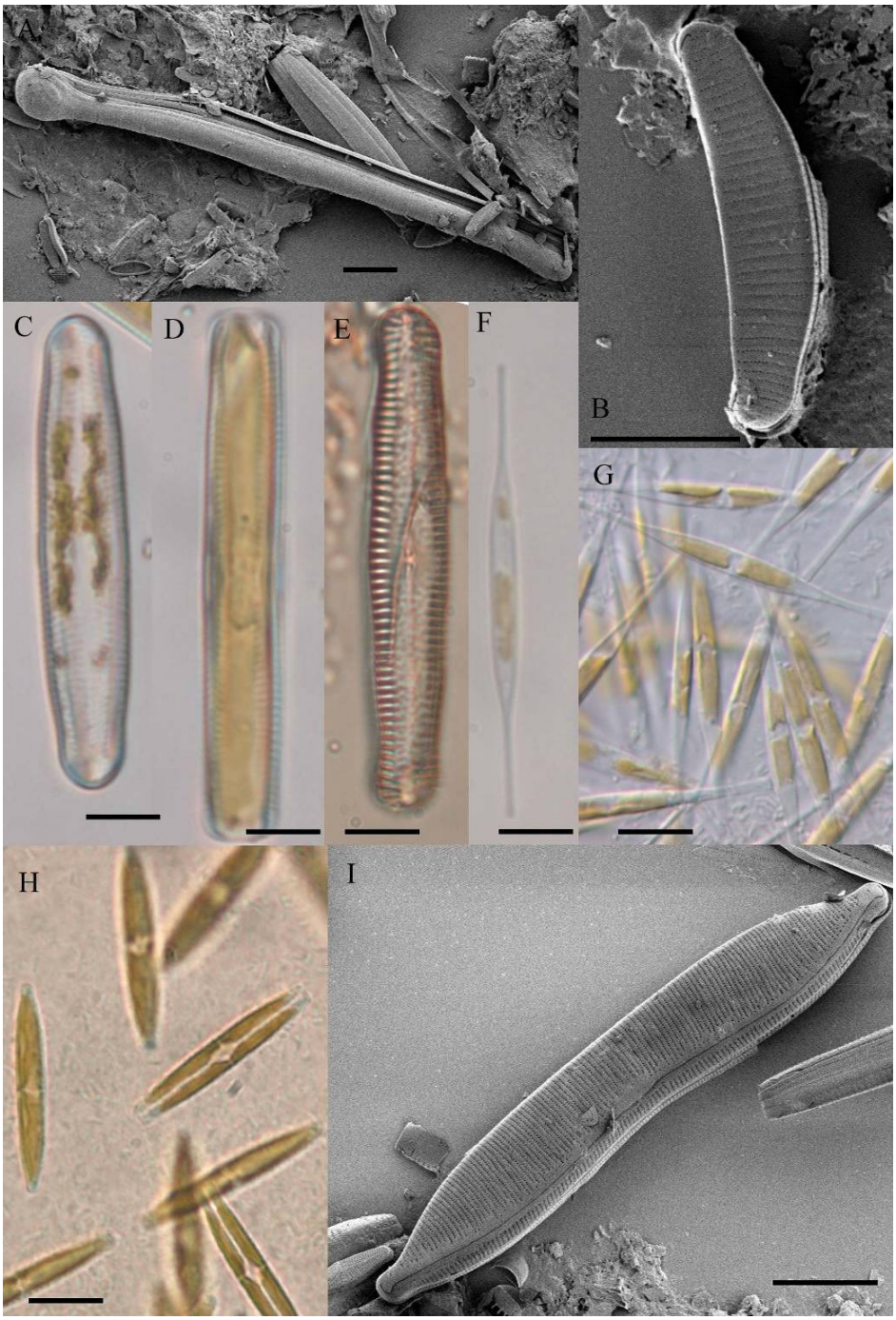


Plate 2
A, *Acintella* cf. *brasilensis*, SEM.
B, *Eunotia* cf. *epithemia*, SEM.
C–E, *Pinnularia gibba*: **C,** living material valve view (sample 8B(18)); **D,** living material girdle view (sample 8B(9)); **E,** cleaned field material (sample 34(1)).
F–G, *Nitzschia acicularis*: **F,** living field material (sample 12B(9)); **G,** cultured material (culture 37/3).
H–I, *Hantzschia amphioxys*: **H,** cultured material (culture 6-4-1); **I,** SEM (sample 15-8).
All scales = 10 µm.

Order Fragilariales
Family Fragilariaceae

***Fragilaria* cf. *famelica* (Kützing) Lange-Bertalot** Plate 3A–C
Reference: Krammer & Lange-Bertalot 1991, vol. 3, fig. 111: 4–12, 16, 17

Distribution: Found in Styx River at Styx Mill Conservation Reserve, Main North Rd and Radcliffe Rd (Fig. 1).

Features: Oval in valve view, 11–13 µm long, 4–6 µm wide. Sometimes swollen at the centre of the valve. Narrow rectangular in girdle view. Araphid. Striae discontinuous across the valve faces to form a central sternum. A fascia at the middle of the valve, extending halfway across.

Remarks: Not cultured. No molecular data. Common in lakes and slow-moving streams (Biggs & Kilroy 2000).

***Synedra ulna* Ehrenberg** Plate 3D–F
Reference: Biggs & Kilroy 2000, p. 150

Distribution: Found at all sites, except Styx River at North Rd Redwood Springs (Fig. 1).

Features: Valve symmetrical, up to 200 µm long, with parallel margins with protracted apices. Araphid, pseudoraphe runs the length of the cell. Parallel striae with a clear central area.

Remarks: Cultured from sample 29. No molecular data. Widespread and extremely common (Biggs & Kilroy 2000).

***Staurosira* cf. *construens* Ehrenberg** Plate 3G–I
Reference: Krammer & Lange-Bertalot 1991, vol. 3, fig. 132: 1–34

Distribution: Found in Styx River at Styx Mill Conservation Reserve, Smacks Creek at Gardiners Rd, and Kaputone Stream at the Belfast Rd Bridge (Fig. 1).

Features: Valve cruciform, 13–36 µm long, 10–13.3 µm wide. Rectangular in girdle view. Araphid, pseudoraphe that widens in centre of cell. Prominent parallel striae.

Remarks: Not cultured. No molecular data. Reported as widespread and common in clean, spring-fed streams (Biggs & Kilroy 2000).

***Meridion circulare* (Greville) C. Agardh** Plate 3J–L
Reference: Biggs & Kilroy 2000, p. 174

Distribution: Found at the Styx headwaters Fig. 1).

Features: Valve view clavate, 22–37.5 µm long, 6–9 µm wide. Wedge-shaped in girdle view. Costae cross the valve face and these are visible in girdle view as small knobs down each long edge. Several small chloroplasts in cell.

Remarks: Not cultured. No molecular data. Reported to prefer clean, cool streams and is not very common (Biggs & Kilroy 2000).

Family Gomphonemataceae

***Gomphonema acuminatum* Ehrenberg** Plate 3M–P
Reference: Krammer & Lange-Bertalot 1997, vol. 1, fig. 160: 1–12

Found in Styx River at Styx Mill Conservation Reserve, Spencerville Rd and Teapes Rd; Smacks Creek on Gardiners Rd and Willowbank Wildlife Reserve; Kaputone Stream at the Belfast Rd Bridge (Fig. 1).

Features: Valve similar shape to *G. truncatum* but with extra projection at headpole, 30–100 µm long, 11–13 µm wide. Girdle view wedge-shaped. Biraphid, raphes straight. Striae slightly radiate towards the footpole and at the swelling below the headpole.

Remarks: Not cultured. No molecular data. Reported as widespread (Biggs & Kilroy 2000).

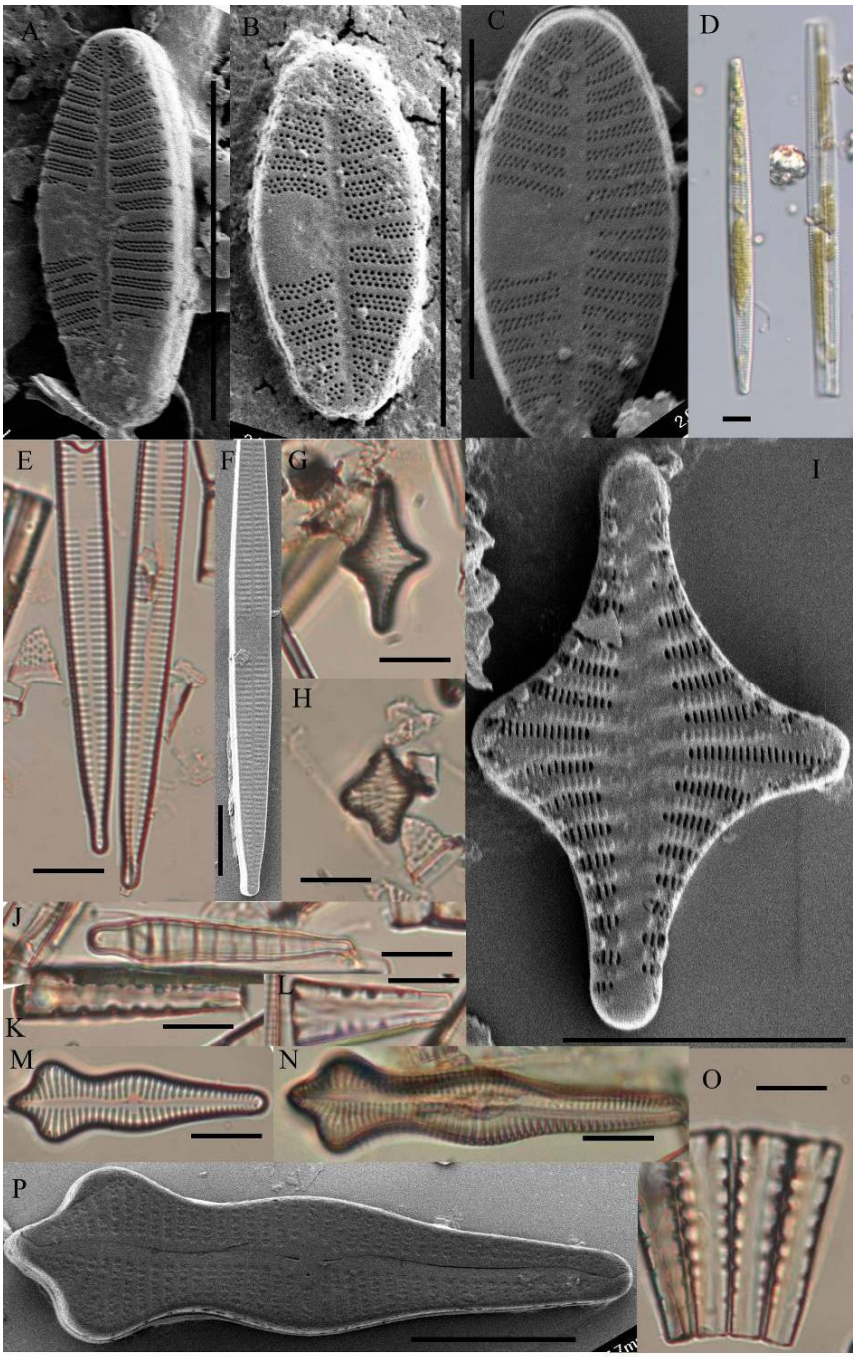


Plate 3
A–C, *Fragillaria* cf. *famelica*, SEM (samples 19-1, 22-3, 31-2).
D–F, *Synedra ulna*: **D,** living field material (sample 4B(1)); **E,** cleaned field material (sample 29C); **F,** SEM (sample 8-2).
G–I, *Staurosira* cf. *construens*: **G, H,** cleaned field material (samples 7(3), 8(14)); **I,** SEM (sample 19-8).
J–L, *Meridion circulare*, cleaned field material (sample 7(1)): **J,** valve view; **K,** half girdle view; **L,** girdle view.
M–P, *Gomphonema accuminatum*: **M–O,** cleaned field material (samples 8(15), 12(7)), valve and girdle views; **P,** SEM (sample 7-10).
All scales = 10 µm.

***Gomphonema minutum* C. Agardh** Plate 4A
Reference: Kociolek & Kingston 1999, fig. 91

Distribution: Found in Styx River at Main North Rd and Smacks Creek at Willowbank Wildlife Reserve (Fig 1).

Features: Longitudinally asymmetrical in valve view, 20 µm long, 5.5 µm wide. Wedge shape in girdle view. Biraphid with a straight raphe. Widely spaced radiate striae.

Remarks: Not cultured. No molecular data. Reported as widespread and can be very common (Biggs & Kilroy 2000).

***Gomphonema* cf. *parvulum* (Kützing) H.F. Van Heurck** Plate 4B–I
Reference: Krammer & Lange-Bertalot 1991 vol. 1, fig 154: 1–25

Distribution: Found at Styx River at North Rd and Spencerville Rd Bridge; Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve; Kaputone Creek at Ouruhia Domain (Fig. 1).

Features: Heteropolar leaf-shaped valve, 10–25 µm long, 5–10 µm wide, with protracted axial areas and slightly elongated at one end. Girdle view rectangular with thickened areas at each end indicating raphe endings. Biraphid, raphes slightly hooked at poles. Striae densely spaced and parallel. Space at one side of central area and stigma opposite the space.

Remarks: Cultured from samples 22, 39 and 46. No molecular data. There are many gomphonemoid diatoms present in environmental samples that are similar to this species but it is difficult to accurately distinguish between different species without first culturing and sequencing each morphotype.

***Gomphonema truncatum* Ehrenberg** Plate 4J
Reference: Krammer & Lange-Bertalot 1991, vol. 1, fig. 159: 11–18

Distribution: Found in Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Asymmetric valve view, 'Jelly-baby' shaped, 42 µm long, 12 µm wide with a rounded axial area. Biraphid, raphe straight. Striae radiate at the centre with a space at centre on both valves and a single stigma.

Remarks: Not cultured. No molecular data. Reported as widespread (Biggs & Kilroy 2000).

Subclass Bacillariophycidae
Order Cymbellales
Family Cymbellaceae

***Encyonema* cf. *sinicum* Krammer** Plate 4K
Reference: Krammer 1997, fig. 72: 1–9

Distribution: Found in Styx River at Styx Mill Conservation Reserve and Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Asymmetric lunate valve, 45 µm long, 12.6 µm wide, with rounded dorsal side. Biraphid with upward-pointing central raphe endings and downward-pointing terminal endings. Striae slightly radiate and widely spaced. Chloroplast in valve view is H-shaped.

Remarks: Not cultured. Molecular data from environmental clones of sample 22 show one group of clones is close to *Encyonema* cf. *sinicum*, and another group is close to *Cymbella affinis* and *C. lanceolata* (Fig. 2). Identification is uncertain due to the degree of difference between our sample and strains of this species in GenBank. However, morphology of specimen from sample 15 is similar to that of *Encyonema sinicum* from Krammer (1997).

***Cymbella aspera* Cleve** Plate 4L–N
Reference: Krammer & Lange-Bertalot 1991 vol. 1, fig. 131: 1–3

Distribution: Found in Styx River at North Rd and Redwood Springs;Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Valves asymmetric with wide axial and central areas, 70–150 µm long, 20–45 µm wide. Biraphid, raphes curved and located in centre of valve. Striae are large and slightly radiate. Stigmata absent.

Remarks: Not cultured. No molecular data. Reported as widespread and often abundant in low-conductivity streams (Biggs & Kilroy 2000).

Family Rhoicospheniaceae

***Rhoicosphenia* cf. *abbreviata* Lange-Bertalot** No images available
Reference: Krammer & Lange-Bertalot 1991 vol. 1, fig. 91: 1–28

Distribution: Found in Styx River at Main North Rd and at Teapes Rd Bridge (Fig. 1).

Features: Valve clavate, 20–60 µm long, up to 8 µm wide. Girdle view bent and wider at one end. Biraphid, straight raphes. Concave valve face has a more highly developed raphe than the convex face. Striae parallel.

Remarks: Not cultured. No molecular data. Very common and widespread (Biggs & Kilroy 2000).

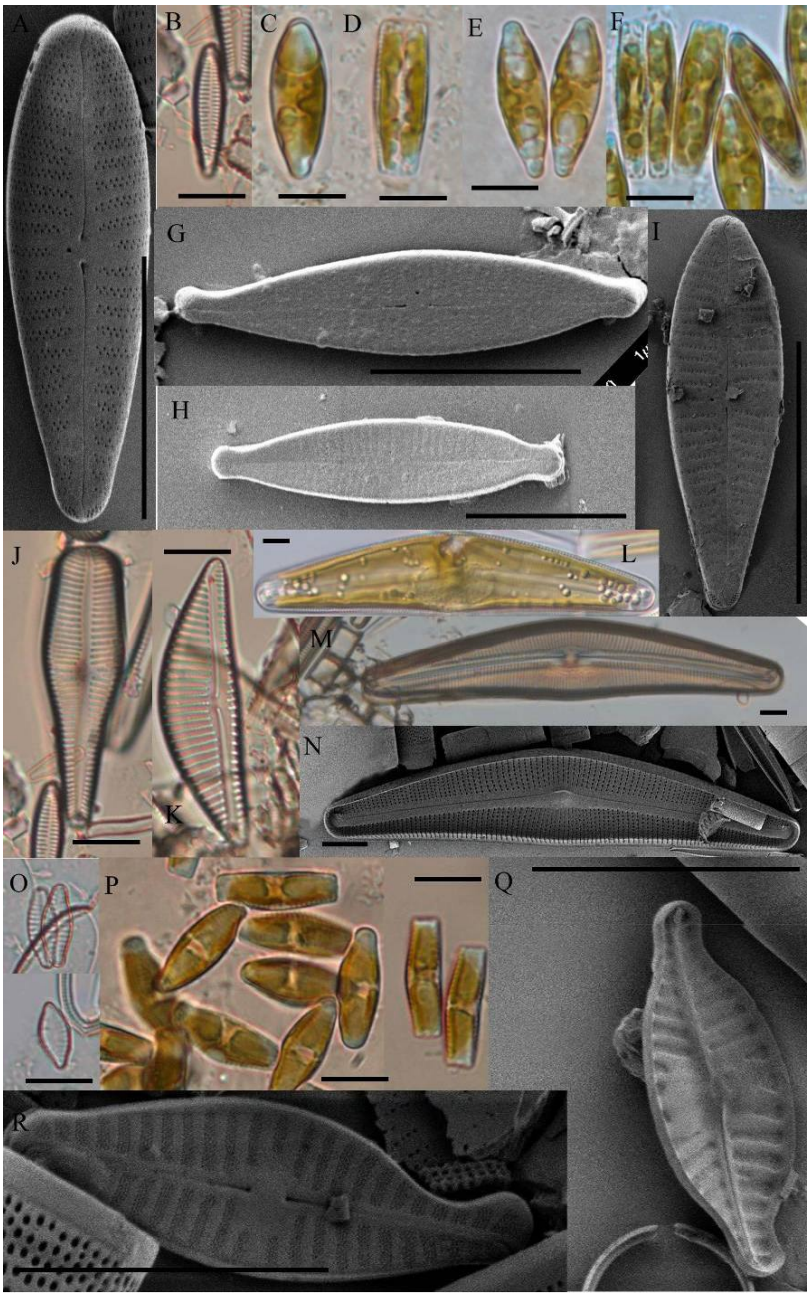


Plate 4

A, *Gomphonema minutum* SEM (sample 23(2)).

B–I, *Gomphonema* cf. *parvulum*: **B,** cleaned field material (sample 15-3); **C–F,** cultured material in valve and girdle views (cultures 22/1, 39/2, 46/1); **G–I,** SEM (samples 8-3, 8(1), 15-3).

J, *Gomphonema truncatum*, cleaned field material (sample 15-3).

K, *Encyonema* cf. *sinicum*, cleaned field material (sample 15-1).

L–N, *Cymbella aspera*: **L,** living field material (sample 8B(15)); **M,** cleaned field material (sample 8(16)); **N,** SEM of valve interior (sample 7-5).

O–R, *Achnanthes* cf. *lanceolata*: **O,** cleaned field material (sample 20(1)); **P–Q,** cultured material (culture 18/1/1), valve and girdle views; **Q–R,** SEM of interior and exterior of valve including bent headpole (samples 7-9 and 7-8).

All scales = 10 µm.

Order Achnanthales
Family Achnanthaceae

Achnanthes* cf. *lanceolata Plate 4O–R
Reference: Lange-Bertalot & Krammer 1989 p. 83, fig. 84:1–16

Distribution: Found in Styx River at Styx Mill Conservation Reserve and Smacks Creek at Gardiners Rd.

Features: Gomphonemoid in valve view, 12–17 µm long, 5–6 µm wide with protracted axial areas and one bent pole. Ventral valve is flat whereas dorsal valve is swollen and raised. Raphe straight with slightly hooked ends. Striae slightly radiate.

Remarks: Cultured from sample 18. Molecular data from culture suggest nearest relative is *Achnanthidium minutissimum* (Fig. 2). Identification uncertain due to differences between the valve shape and published descriptions of *A. minutissimum*.

Family Cocconeidaceae

***Cocconeis placentula* Ehrenberg** Plate 5A–C
Reference: Biggs & Kilroy 2000, p. 162

Distribution: Found in Styx River at Main North Rd, Radcliffe Rd, Spencerville Rd Bridge and Styx Mill Conservation Reserve; Smacks Creek at Gardiners Rd; Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Valve oval, 10–90 µm long, 8–40 µm wide. Monoraphid, distinctive ridge around the perimeter of the raphid valve. The ornamentation of the rapheless valve varies in different varieties. Single chloroplast and the punctae are often clearly visible.

Remarks: Not cultured. No molecular data. Reported as common and widespread (Biggs & Kilroy 2000).

Order Tabellariales
Family Tabellariaceae

Tabellaria flocculosa Kützing Plate 5D
Reference: Biggs & Kilroy 2000, p. 154

Distribution: Found in Smacks Creek at Willowbank Wildlife Reserve (Fig. 1)

Features: Valve narrow with swollen axial and central areas, 21–22 µm long, 11 µm wide. Girdle view oblong to square with prominent septa running towards centre of cell. Araphid with parallel striae. Several small chloroplasts.

Remarks: Not cultured. No molecular data. Widespread but not common (Biggs & Kilroy 2000).

Order Naviculales
Family Stauroneidaceae

Craticula molestiformis (Hustedt) Lange-Bertalot Plate 5E
Reference: Sabbe et al. 2003, p. 242, figs 64, 65, 88, 89

Distribution: Found in Kaputone Stream at Belfast Rd Bridge (Fig. 1).

Features: Valve elliptical-lanceolate, 35.6 µm long, 6.9 µm wide, with rostrate ends. Axial area thin; slightly widening towards central portion of valve.

Remarks: Cultured from sample 44. Molecular data from sample 44 support this identification (Fig. 2).

Order unassigned
Family unassigned

Mayamaea atomus (Kützing) Lange-Bertalot Plate 5F
Reference: Krammer & Lange-Bertalot 1986, p. 216, figs 10, 74

Distribution: Found in Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Valve linear-elliptical to elliptical, 10 µm long, 3.8 µm wide, with rounded ends. Axial area distinct; slight widening towards the central portion of the valve. Girdle view rectangular.

Remarks: Cultured from samples 10, 14 and 18. Molecular data from samples 10 and 14 support identification of this species as *Mayamaea atomus* (Fig. 2).

Order Naviculales
Family Naviculaceae

***Navicula* spp.** Plate 5G–N
Reference: Krammer & Lange-Bertalot 1991 vol. 1

Distribution: Found at all sites (Fig. 1).

Features: Valve view linear-lanceolate, 27.5–90 µm long, 7.5–19 µm wide with slightly protracted axial areas. Biraphid.

Remarks: Cultured from samples 32, 39, 44, and 47. Due to the abundance of naviculoid diatoms in the samples and the similarity between many of the species, it is difficult to identify many of these diatoms to species level due to a lack of molecular data.

Navicula veneta Kützing No images available
Reference: Krammer & Lange-Bertalot 1991 vol. 1

Distribution: Found in Kaputone Stream at Belfast Rd Bridge (Fig. 1).

Features: Valve view linear-lanceolate, 23 µm long, 6 µm wide with slightly protracted axial areas. Girdle view rectangular with slightly tapered ends. Biraphid.

Remarks: Molecular data from culture 34-3 supports identification of this isolate as *Navicula veneta* (Fig. 2).

Family Stauroneidaceae

Stauroneis phoenicenteron (Nitzsch) Ehrenberg Plate 5O–P
Reference: Krammer & Lange-Bertalot 1991 vol. 1, fig. 84: 1–3

Distribution: Found in Styx River at North Rd and Styx Mill Conservation Reserve; Smacks Creek at Willowbank Wildlife Reserve; Kaputone Stream at the Belfast Rd Bridge (Fig. 1).

Features: Valve elongate with slightly protracted axial areas, 45–223 µm long, 23–42 µm wide. Biraphid, raphes straight, thickened band of silica across valve in central area. Striae fine and radiate throughout valve. Two chloroplasts lie against girdle and extend under valve face.

Remarks: Not cultured. No molecular data.

Order Rhopalodiales
Family Rhopalodiaceae

Rhopalodia gibba (Ehrenberg) G.F.O Müller Plate 5R–S
Reference: Krammer & Lange-Bertalot 1991 vol. 2, fig. 111: 6

Distribution: Found in Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Cells rectangular, 50–123 µm long, 5–11.7 µm wide with slightly swollen central area. Raphes lie close to valve edges. Striae not visible.

Remarks: Cultured from sample 40-2. No molecular data.
Remarks: Not cultured. No molecular data.

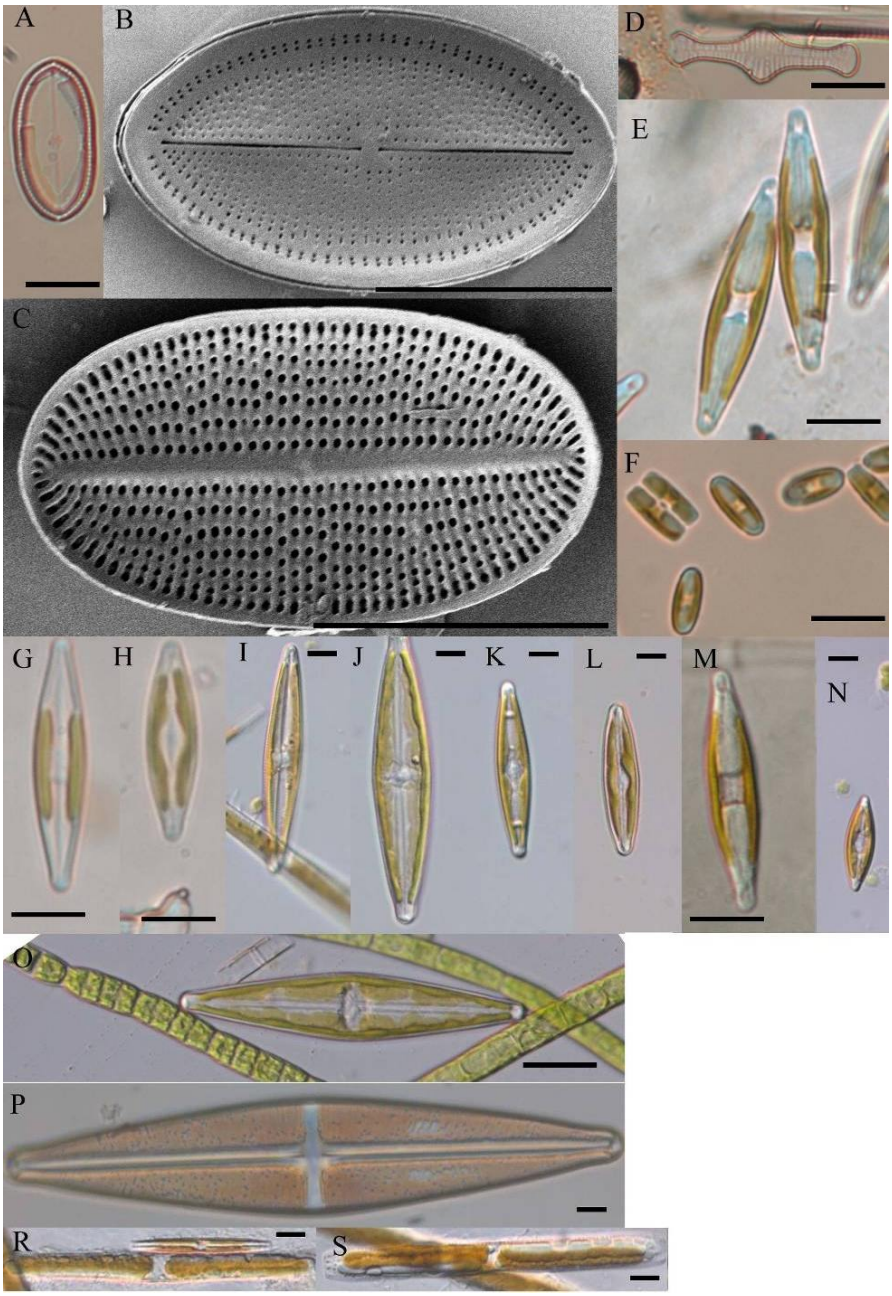


Plate 5

A–C, *Cocconeis placentula*: **A,** cleaned field material (sample 8(1)); **B-C,** SEM of exterior and interior of valve (samples 31-4, 41-3).
D, *Tabellaria flocculosa*, cleaned field material (sample 15(2)).
E, *Craticula molestiformis*, cultured material (culture 44/2).
F, *Mayamaea atomus*, cultured material (culture 14/1/1).
G–N, *Navicula* spp., living material (samples 7B(1), 10B(4), 7B(11), 15B(9), 19B(9), 22A(9), culture 32/1, sample 28A(3)).
O–P, *Stauroneis* cf. *phoenicenteron*: **O,** living field material (sample 18B(14)); **P,** cleaned field material (sample 14(2)).
Q, deleted photo.
R–S, *Rhopalodia gibba*, cultured material (culture 40/2).
All scales = 10 µm.

Class Chlorophyceae
Order Microsporales
Family Microsporaceae

Microspora amoena **(**Kützing) Rabenhorst Plate 6A–B
Reference: Novis 2004, fig. 3: A, B

Distribution: Found in Styx River at Radcliffe Rd and North Rd, and Kaputone Stream at the Belfast Rd Bridge (Fig. !).

Features: Unbranched filaments; cells are approximately square in longitudinal optical longitudinal section. Chloroplast is reticulate and parietal. H-shaped joins in the cell wall between cells. Cells 20–25 µm long, 13–18 µm wide.

Remarks: Not cultured. No molecular data.

Microspora **cf.** ***stagnorum*** (Kützing) Lagerheim Plate 6C
Reference: Novis 2004, fig. 3H–K, 4J, K

Distribution: Found in The Styx River at Styx Mill Conservation Reserve (Fig. 1).

Features: Filaments 8.3 µm wide. Cells cylindrical, 27 µm long. Cell wall <1 µm thick. Reddish pigment in walls between cells.

Cf. ***Gloeocystis papuana*** (Watanabe) Ettl & Gärtner Plate 6D
Reference: Novis 2001, p. 203, fig. 6.21e, q–ac

Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).

Features: Small colonies of green, spherical cells, each 10 µm in diameter, enclosed in a mucilage envelope. Single chloroplast can cover the entire cell wall. 1–2 pyrenoids visible.

Remarks: Not cultured. No molecular data. Distribution uncertain, but the genus has been found in abundance in a stream on the Coromandel Peninsula (Biggs & Kilroy 2000).

Order Sphaeropleales
Family Scenedesmaceae

Desmodesmus aldavei Hegewald Plate 6E
Reference: Hegewald & Silva 1988, p. 66, fig. 91

Distribution: Found in Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Cells oblong, 12 µm long, 7.5 µm wide. Forms linear coenobial colonies of eight cells.

Remarks: Cultured from sample 16. Molecular data from culture 16-1 and clone 23/1 show the closest relative as *Scenedesmus quadricauda* (Fig. 3).

Scenedesmus* cf. *acutus Meyen Plate 6F
Reference: Hegewald & Silva 1988, p. 60, fig. 79

Distribution: Found in the Styx River at Main North Rd and Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Cells oblong, 7.5 µm long, 2.5 µm wide. Forms colonies that look like stacks of four cells. Chloroplast covers most of the cell wall. Each cell has one pyrenoid.

Remarks: Cultured from sample 7. No molecular data.

Order Volvocales
Family Chlamydomonadaceae

Chlamydomonas* cf. *noctigama Ehrenberg Plate 6G–H
Reference: Ettl 1983, p. 322, fig. 370

Distribution: Found in Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Ovoid cells, 15–19 µm long, 12.5–18 µm wide. Contractile vacuoles, papilla, cup-shaped chloroplast with lobes/incisions, one to several pyrenoids in each chloroplast, presence of red eyespot.

Remarks: Cultured from sample 13-5. Molecular data from cultures suggests the closest relative to this isolate is *Chlamydomonas acidophila* (Fig. 3).

Chlamydomonas* cf. *quiescens Plate 6I
Reference: Ettl 1983, p. 328, fig. 381

Distribution: Found in the Styx River at Main North Rd (Fig. 1).

Features: Cells oval in longitudinal optical section, 9–11 µm long, 6–8 µm wide. No visible flagella. Contractile vacuoles.

Remarks: Cultured from sample 23-1. Molecular data from this culture indicates that the closest relative is *Radiofilum transversale* (Fig. 3). Despite the lack of flagella, this isolate was identified as *Chlamydomonas* due to the presence of contractile vacuoles.

Chlamydomonas* cf. *macrostellata Plate 6J
Reference: Ettl 1983, p. 391, fig. 512

Distribution: Found in the Styx River at Spencerville Rd Bridge (Fig. 1).

Features: Cells oval in longitudinal optical section, 9–13 µm long, 5–9 µm wide. No visible flagella. Contractile vacuoles. Presence of red eyespot.

Remarks: Cultured from sample 50-4. Molecular data from this culture; no close relatives are indicated by the molecular data (Fig. 3). Despite the lack of flagella, this isolate was identified as *Chlamydomonas* due to the presence of contractile vacuoles.

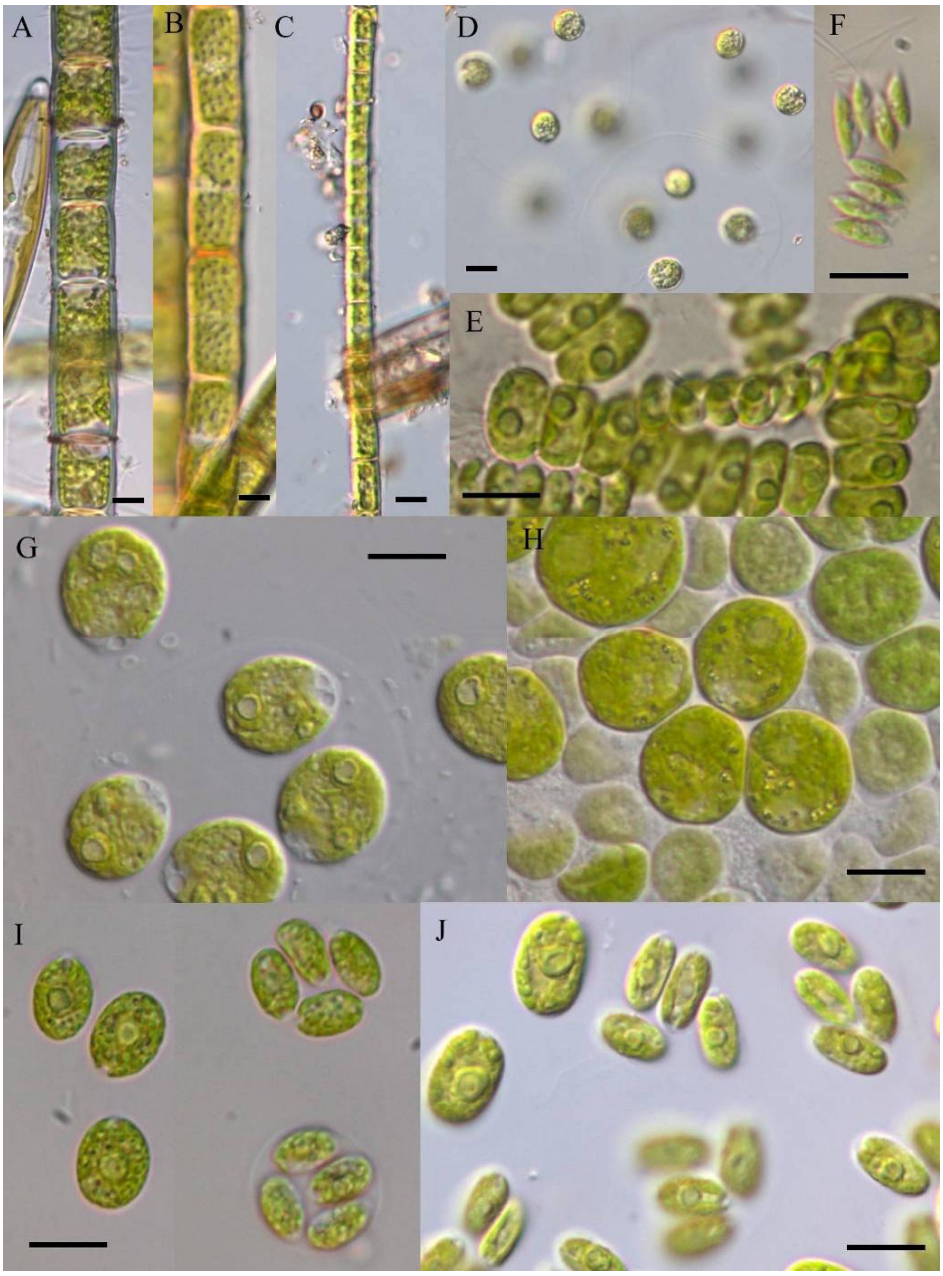


Plate 6

A–B, *Microspora amoena*, living field material: **A,** optical section of filament (sample 43(4)); **B,** showing reticulate chloroplast surface (sample 29A(3)).
C, *Microspora* cf. *stagnorum*, living field material (sample 19B(11)).
D, cf. *Gloeocystis papuana*, living field material (sample 12B(3)).
E, *Desmodesmus aldavei*, cultured material (culture 16/1).
F, *Scenedesmus* cf. *acutus*, cultured material (culture 7/1/1).
G–H, *Chlamydomonas* cf. *noctigama*, cultured material (culture 13/5): **G,** mature cells with papillae; **H,** old rounded cell in clump.
I, *Chlamydomonas* cf. *quiescens*, cultured material (culture 23/1), mature and dividing cells.
J, *Chlamydomonas* cf. *macrostellata*, cultured material (culture 50/4), mature and dividing cells.
All scales = 10 µm.

Order Oedogoniales
Family Oedogoniaceae

Oedogonium* cf. *cyathigerum Wittrock Plate 7A–G
Reference: Novis 2003, figs 7A–F, 8A–C

Distribution: Found in Styx River at Styx Mill Conservation Reserve and Smacks Creek at Willowbank Wildlife Reserve (Fig. 1).

Features: Dioecious. Vegetative cells cylindrical, 16–23 µm wide, 41–53 µm long, smooth-walled. Oogonia single, spherical to slightly ellipsoid, 16–25 µm wide, 18–23 µm long, pore opening in median position.

Remarks: Not cultured. No molecular data. Identification of this species is difficult due to a lack of fertile male material. The presence of an opening in the oogonium wall at the median position in Plate 7E supports identification of this species as *O. cyathigerum*.

Order Chaetophorales
Family Chaetophoraceae

Stigeoclonium* cf. *lubricum Vischer Plate 7H–I
Reference: Sarma 1986, fig. 280

Distribution: Found in the Styx River at Styx Mill Conservation Reserve and Radcliffe Rd and in Smacks Creek at Gardiners Rd (Fig. 1)

Features: Branched with small squarish to elongated cells, sometimes doliiform. Cells 10–16.7 µm long, 6.7–11 µm wide. Parietal chloroplast.

Remarks: Cultured from samples 10 and 18. Molecular data from cultures suggest closest relative is *S. helveticum* (Fig. 3). In New Zealand, this species has been found on the Coromandel Peninsula and in Lake Rotoiti, Lake Taupo, and Waitakere Stream (Sarma 1986).

Class Ulvophyceae
Order Cladophorales
Family Cladophoraceae

Cladophora glomerata Pilger Plate 7J
Reference: Biggs & Kilroy 2000, p. 144

Distribution: Found in the Styx River at Radcliffe Rd and Kaputone Stream at Spencerville Rd Bridge.

Features: Large branched filaments with very long cells, 93–183 µm long, 27–37 µm wide. Branches originate from cell cross walls. Branches taper towards rounded ends.

Cell has lace-like chloroplast lining the inside of the cell walls and numerous pyrenoids.

Remarks: Not cultured. No molecular data. Reported as widespread and found in high-conductivity streams and rivers (Biggs & Kilroy 2000).

Class Zygnematophyceae
Order Zygnematales
Family Desmidiaceae

Cosmarium* cf. *subcucumis Plate 7K–L
Reference: Croasdale & Flint 1986, fig 31: 6, 7

Distribution: Found in Smacks Creek at Gardiners Rd and at Willowbank Wildlife Reserve (Fig. 1).

Features: Cells oval in longitudinal optical section, 29 µm long, 20 µm wide, 11–17 µm thick, divided into two parts by an isthmus 1.2 µm wide. Smooth cell wall.

Remarks: Not cultured. No molecular data. Species reported as widespread (Croasdale & Flint 1986).

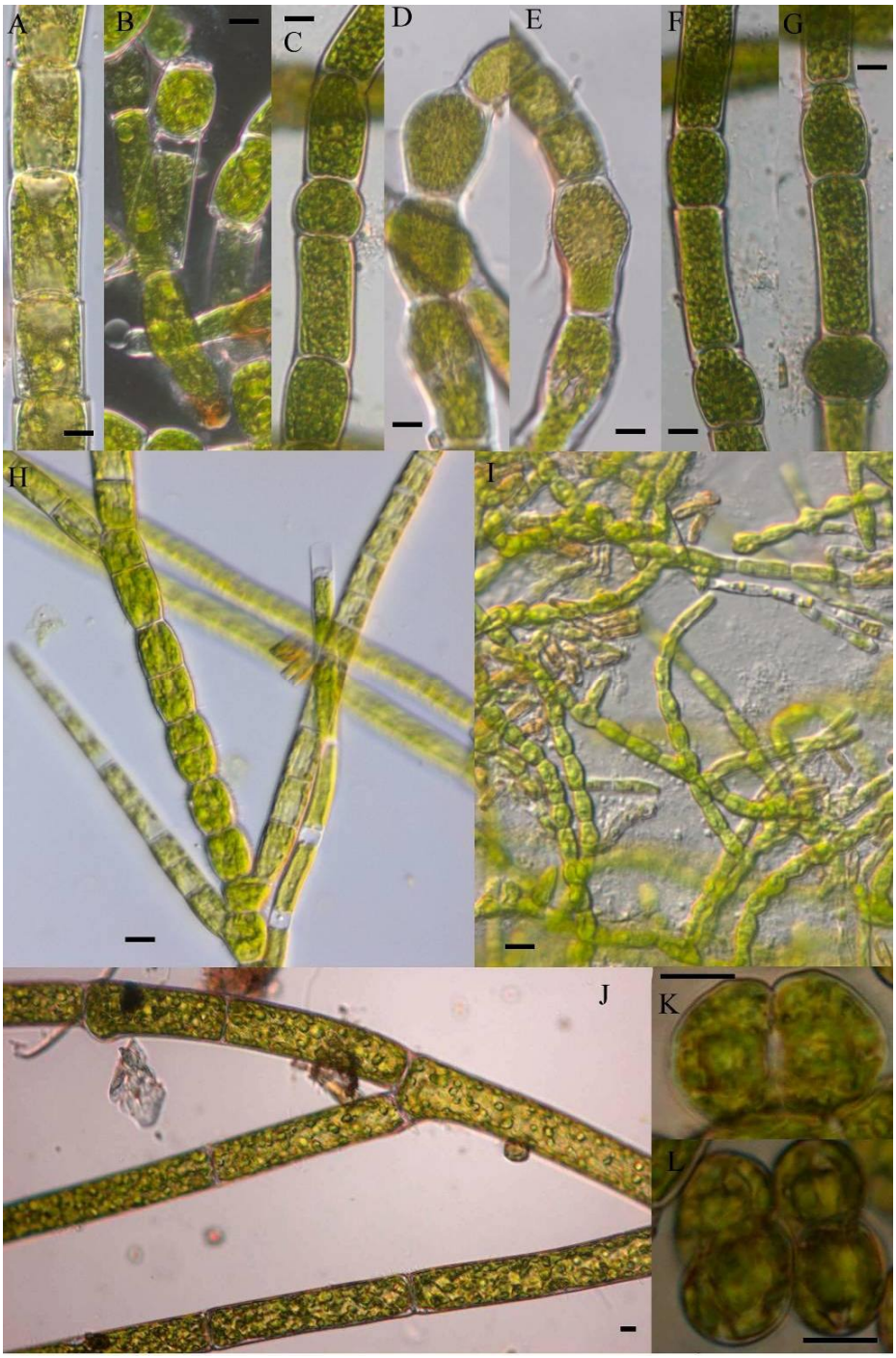


Plate 7

A–G, *Oedogonium* cf. *cyathigerum*: **A,** vegetative filament (sample 14-2); **B,** filaments with holdfasts (sample 14-2); **C–G,** oogonia at various stages of maturation (crude culture 20).

H–I, *Stigeoclonium* cf. *lubricum*: **H,** living field material (sample 18B); **I,** cultured material (culture 10-2-2).

J, *Cladophora glomerata*, living field material (sample 47A).

K–L, *Cosmarium* cf. *subcucumis*, living field material (sample 14-2): **K,** dorsal view showing isthmus and semicells; **L,** side view.

All scales = 10 µm.

Family Closteriaceae

Closterium ehrenbergii Meneghini ex Ralfs Plate 8A–B
Reference: Croasdale & Flint 1986, fig. 6: 11, 12

Distribution: Found in Styx River at Radcliffe Rd and Spencerville Rd Bridge, and Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Cells strongly curved, 465–475 µm long, 85–95 µm wide, c.100º of arc. Ventral margin concave with tumid middle portion, apices rounded, chloroplast with many scattered pyrenoids.

Remarks: Not cultured. No molecular data. Species reported as widespread (Croasdale & Flint 1986).

Family Zygnemataceae

Spirogyra* cf. *decimina (Müller) Kützing Plate 8C–I
Reference: Kadlubowska 1984, p.300, fig. 458

Distribution: Found in the Styx River at Spencerville Rd Bridge; Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve; Kaputone Stream at Belfast Rd, Ouruhia Domain and the Belfast Rd Bridge (Fig. 1)

Features: Smooth-sided filaments. Cells 41.5–55 µm long, 45–80 µm wide. One or two ribbon-like chloroplasts arranged in spiral. Pyrenoids spaced along the chloroplasts. Flat walls between cells.

Remarks: Cultured from sample 49. Molecular data from Clone 32/4 suggests that the closest relative is *Spirogyra gracilis* (Fig. 4). The variation in cell widths and number of chloroplasts of the different filaments may be the result of a polyploid series of the same species, where the diameter of the filament and number of chloroplasts increases with the copy number of chromosomes (although these were not measured in this study). These increases can be seen in Plate 8I which is the widest filament and has many chloroplasts. This is quite different to *S. maxima,* which is huge but does not have so many chloroplasts. Reported to be found in Europe, Asia, North and South America and Africa (Kadlubowska 1984), and in Australia (Day et al. 1995, 2000).

Spirogyra maxima (Hassall) Wittrock Plate 8J
Reference: Kadlubowska 1984, p. 431, fig. 674

Distribution: Found in the Styx River at Spencerville Rd Bridge and Kaputone Stream at Belfast Rd Bridge (Fig. 1).

Features: Smooth-sided filaments. Cells square in optical section, 110 µm long, 120 µm wide. Similar to *S. decimina*, but with fewer chloroplasts for a given size.

Remarks: Not cultured. No molecular data. Found throughout Europe, Argentina, North Africa and Asia (Kadlubowska 1984).

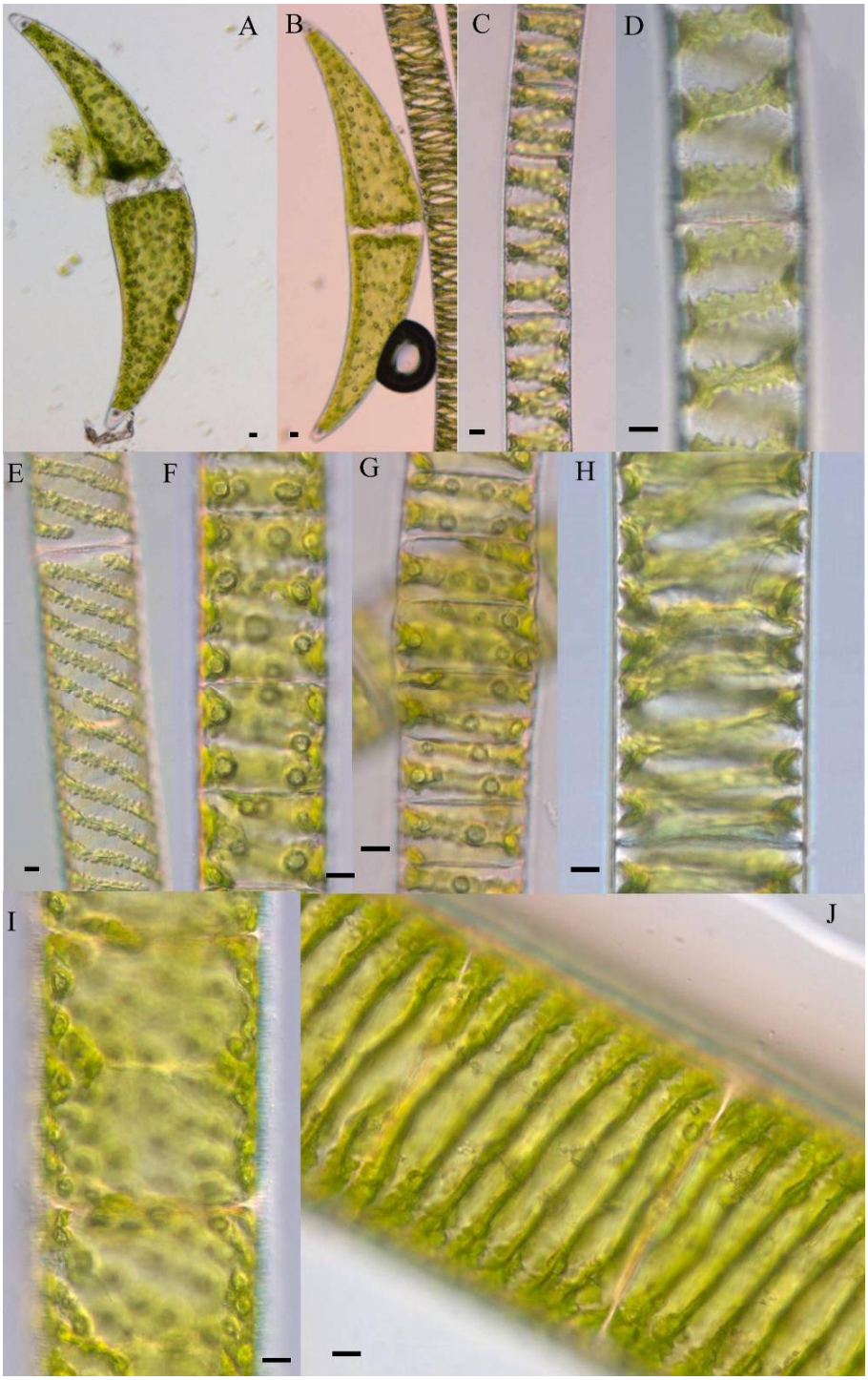


Plate 8
A–B, *Closterium ehrenbergii*, living field material (samples 28A, 40(2)).
C–I, *Spirogyra* cf. *decimina*, living field material: **D–G,** specimens with cells of varying length, but all containing a single chloroplast (samples 50(5), 44(7), 32A(1), 13B(3); **H, I,** filaments with cells containing more than one chloroplast and with increased width, representing possible polyploidy series (samples 40(4), 32A(2)).
J, *Spirogyra maxima*, living field material (sample 33A(2)).
All scales = 10 µm.

Class Charophyceae
Order Charales
Family Characeae

Nitella* cf. *hookeri Braun Plate 9A–C
Reference: Wood & Mason 1977, p. 148, figs 38, 39, 40, 41, 42

Distribution: Found in Smacks Creek at Gardiners Rd and Willowbank Wildlife Reserve (Fig. 1).

Features: Plants 8–15 cm high, light to dark green. Branching branchlets, 3–8 branchlets in a whorl, to approximately 7 cm long. Commonly entwined with macrophytes or grass at stream banks, and consequently difficult to obtain the whole specimens.

Remarks: Not cultured. Molecular data from clones and environmental samples suggest closest relative is *N. pulchella* (Fig. 5). A lack of fertile material made accurate species identification according to morphology impossible.

Class Xanthophyceae
Order Tribonematales
Family Tribonemataceae

Tribonema* cf. *regulare Pascher Plate 9D–E
Reference: Ettl 1978, p. 443

Distribution: Found in the Styx River at Styx Mill Conservation Reserve and Radcliffe Rd, and Kaputone Stream at Ouruhia Domain and the Belfast Rd Bridge (Fig. 1).

Features: Cell longer than wide, 13–22 µm long, 6.7–8.3 µm wide. Several disc-shaped pale green chloroplasts spaced through each cell.

Remarks: Not cultured. Molecular data from clones of samples 6, 7 and 12 suggest closest relatives are *Tribonema regulare* and *T. utriculosum* (Fig. 6).

Order Vaucheriales
Family Vaucheriaceae

***Vaucheria* sp.** Plate 9F
Reference: Biggs & Kilroy 2000, p. 147

Distribution: Found in the Styx River at Main North Rd, Smacks Creek at Gardiners Rd, and Kaputone Stream at Belfast Rd (Fig. 1).

Features: Large branched filaments with rounded tips and no cell cross walls. Multiple small chloroplasts line the cell wall. The filaments are up to 150 µm in diameter and decrease in size with branching. Siphonous.

Remarks: Not cultured. No molecular data. Reported as widespread and found in a wide range of conditions (Biggs & Kilroy 2000). Identification to species level is not possible due to absence of fertile material.

Class Cyanophyceae
Subclass Synechococcophycideae
Order Pseudanabaenales
Family Pseudanabaenaceae

Cf. *Leptolyngbya* Anagnostidis & Komárek Plate 9G
Reference: Biggs & Kilroy 2000, p. 195

Distribution: Found in Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Filaments 3 µm wide, cells longer than they are wide. Mucilage sheath, pale blue-green colour. Heterocysts absent.

Remarks: Not cultured. No molecular data. Reported to be found in clean-water upland streams and rivers, and widespread in New Zealand (Biggs & Kilroy 2000).

Subclass Oscillatoriophycideae
Order Oscillatoriales
Family Phormidiaceae

***Phormidium* sp. 1** Plate 9H–I
Reference: Biggs & Kilroy 2000, p. 198

Distribution: Found in the Styx River at Main North Rd, Redwood Springs and Spencerville Rd Bridge (Fig. 1).

Features: Filamentous; filaments unbranched. Cylindrical cells ~10 µm long, 10 µm wide. Mucilage sheath present, pale orange colour.

Remarks: Not cultured. No molecular data. Distribution widespread and often very abundant in high-conductivity streams and rivers (Biggs & Kilroy 2000).

***Phormidium* sp. 2** Plate 9K
Reference: Biggs & Kilroy 2000, p. 198

Distribution: Found in Kaputone Stream at Belfast Rd (Fig. 1).

Features: Filamentous; filaments unbranched. Cylindrical cells,10 µm long, 10 µm wide. Constriction at cross walls, < 1 µm deep.

Remarks: Not cultured. No molecular data. This species is distinguishable from *Phormidium* sp. 1. by constricted cross walls and different sheath colour.

Family Oscillatoriaceae

Cf. *Oscillatoria* Vaucher ex Gomont Plate 9J
Reference: Biggs & Kilroy 2000, p. 197

Distribution: Found in Kaputone Stream at Ouruhia Domain (Fig. 1).

Features: Cells discoid, ~2 µm long, ~5 µm wide. Trichomes are cylindrical and straight. Mucilage sheath, pale green colour.

Remarks: Not cultured. No molecular data. Reported as widespread in New Zealand and found in a range of conditions (Biggs & Kilroy 2000).

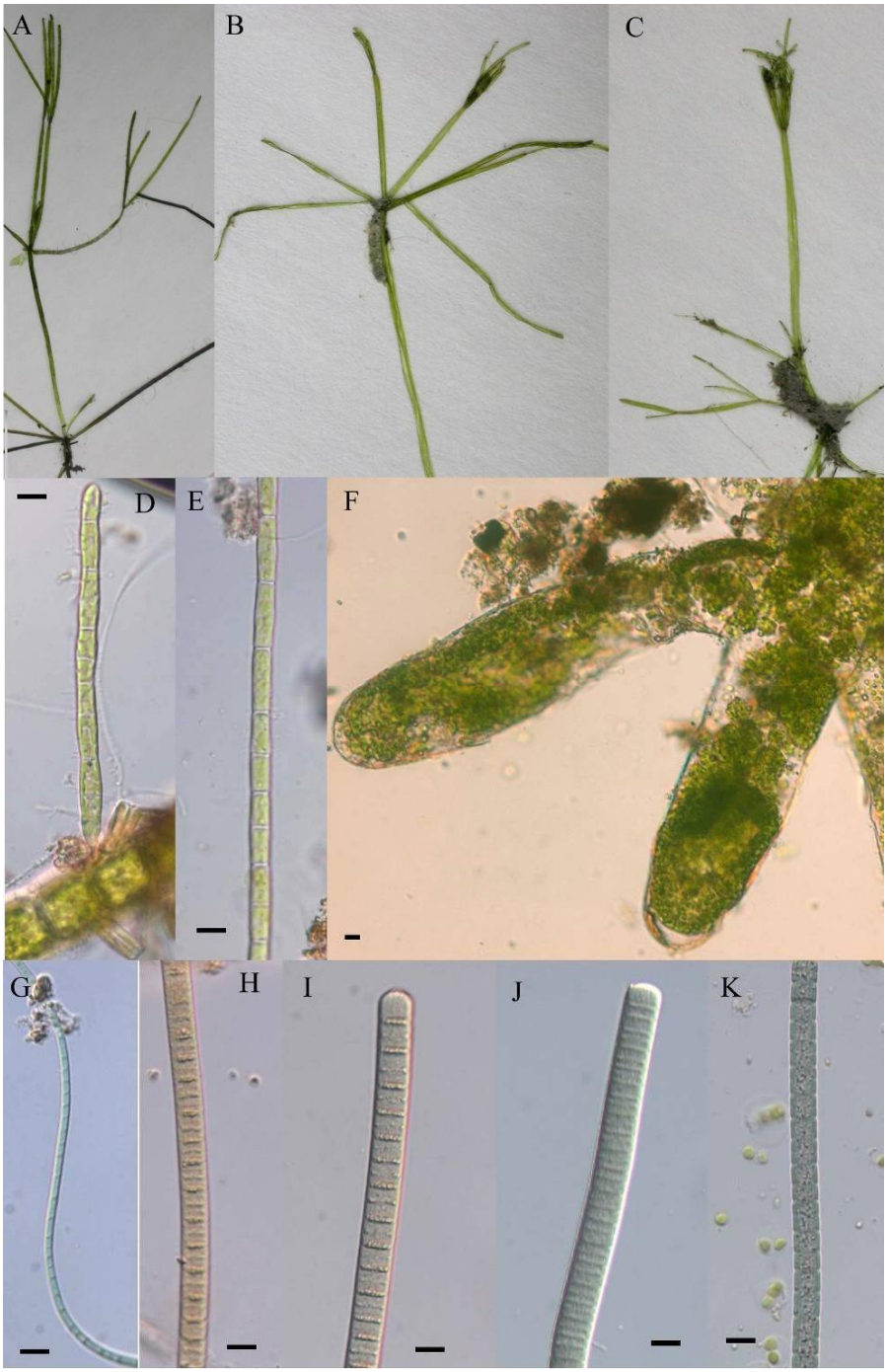


Plate 9
A–C, *Nitella* cf. *hookeri*, dried herbarium specimens (samples 17A, 9A, 9A).
D-E, *Tribonema* cf. *regulare*, living field material (samples 43(8), 43(2)).
F, *Vaucheria* sp., living field material (sample 23A)
G, cf. *Leptolyngbya*, living field material (sample 41(6)).
H-I, *Phormidium* sp. 1, living field material (samples 23A(1), 46(4)).
J, cf. *Oscillatoria*, living field material (sample 39(10)).
K, *Phormidium* sp. 2, living field material (sample 34A(8)).
All scales = 10 µm.

Sequences of environmental clones

The only data we have for the following cloned species are DNA sequences.

Diatom sequences

Clones 22-2/22-3/46-1/46-4
Reference: AM710493.1
Distribution: Found in Styx River at Main North Rd and Spencerville Rd Bridge (Fig. 1).
Remarks: Nearest relatives are *Cymbella lanceolata* and *C. affinis* (Fig. 2). The short branch and high bootstrap value of 91 between these clones and the nearest relatives suggest a close relationship, and their position in a clade of *Cymbella* species strongly suggests several species of this genus.

Chlorophyte sequences

Clones 6-1/6-2/6-3/6-5
Reference: AB127986
Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).
Remarks: There are no close relatives of these clones in the database (Fig. 3).

Clone 6-4
Reference: AB044171
Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).
Remarks: There are no close relatives of this clone in the database (Fig. 3).

Clone 25-1
Reference: EF113446.1
Distribution: Found in Styx River at Main North Rd (Fig. 1).
Remarks: There are no close relatives to this clone in the database (Fig. 3).

Clones 25-3/25-4
Reference: EF113468.1
Distribution: Found in Styx River at Main North Rd (Fig. 1).
Remarks: There are no close relatives to these clones in the database (Fig. 3).

Clone 28-3
Reference: NC_008372
Distribution: Found in Styx River at Radcliffe Rd (Fig. 1).
Remarks: Nearest relative is *Stigeoclonium helveticum* (Fig. 3). This clone is likely to be a species of *Stigeoclonium*.

Clone 31-3
Reference: EF113472.1
Distribution: Found in Styx River at Radcliffe Rd (Fig. 1).
Remarks: There are no close relatives of this clone in the database (Fig. 3).

Clone 34-1
Reference: AM260445.1
Distribution: Found in Kaputone Stream at Belfast Rd (Fig. 1).

Remarks: Nearest relative is *Diplosphaera mucosa* (Fig. 3). Very short branch and bootstrap value of 100 suggest this clone matches this species.

Xanthophyte sequences

Clones 7-5/12-2
Reference: U89900.1
Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).
Remarks: These sequences formed a well-supported clade and perhaps represent the same organism. The clade is closest to the *Botrydium* clade in the phylogeny (Fig. 6), but the two are not united by a robust branch.

Clone 12-5
Reference: AF064744.1
Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).
Remarks: Nearest relative is *Mischococcus sphaerocephalus* (Fig. 6). A short branch length and a bootstrap value of 73 suggest that this clone is close to this species.

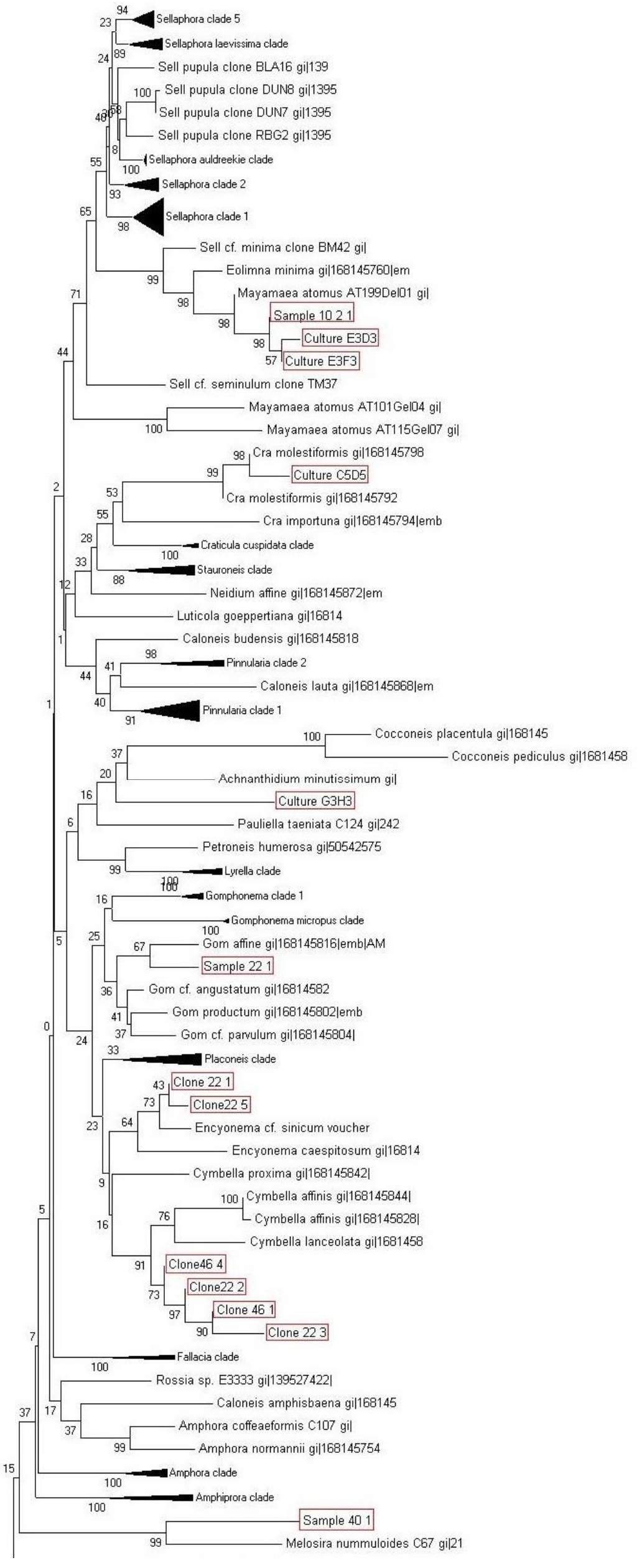
Charales sequences

Clones 9-1/9-2/9-4
Reference: AB076057.1
Distribution: Found in Smacks Creek at Gardiners Rd (Fig. 1).
Remarks: Nearest relative is *Nitella pulchella* (Fig. 5). These clones matched *Nitella* sequences from material collected in samples 9 and 17. They are united with *Nitella pulchella* by a poorly resolved branch.

Clones 28-1/28-4/28-5
Reference: AY823704.1
Distribution: Found in Styx River at Radcliffe Rd (Fig. 1).
Remarks: These clones are placed robustly within a clade comprising species of *Nitella* and *Tolypella* (Fig. 5). However, their position within this clade was unresolved. Nearest relative is *Tolypella porteri* strain X-907. Very long branch and bootstrap value of 45 indicate a large distance between the clone and nearest relative.

Clones 31-1/31-4
Reference: AB076063.1
Distribution: Found in Styx River at Radcliffe Rd (Fig. 1).
Remarks: This organism could be included in the species *Nitella gracilens*, but if so it is an early-divergent member of the clade (Fig. 5).

Phylogenies



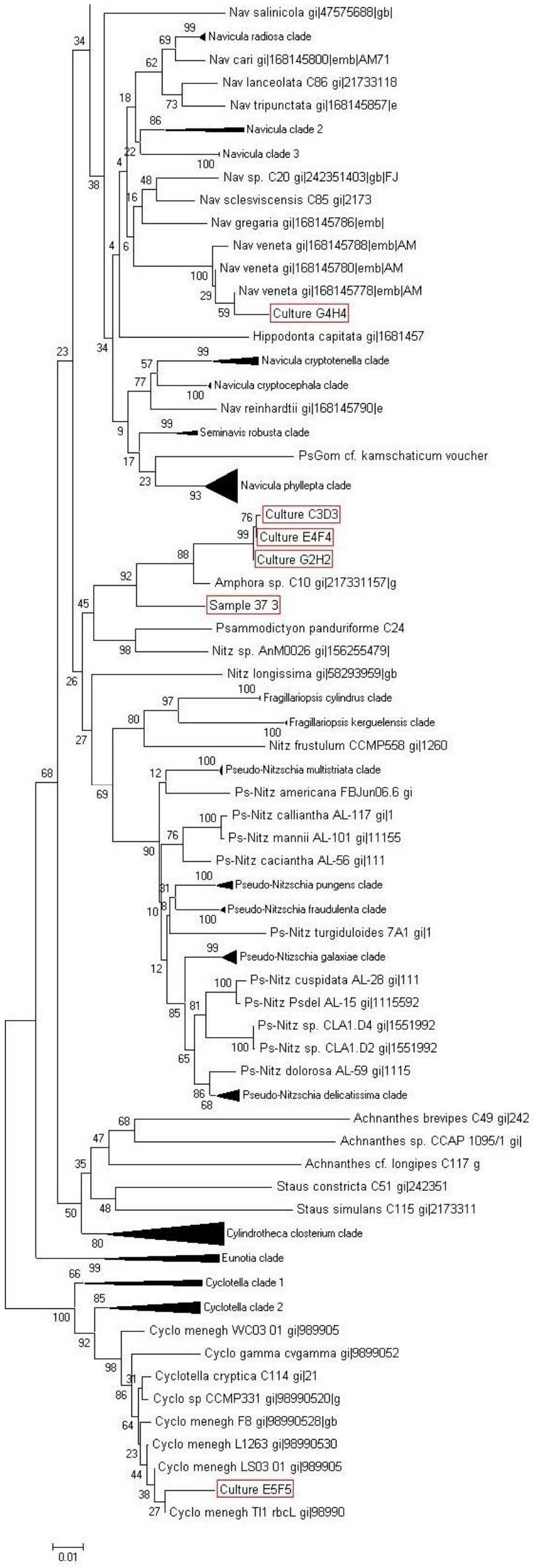


Figure 2. Phylogeny of diatom species. Sequences obtained in this study are surrounded by a red border.

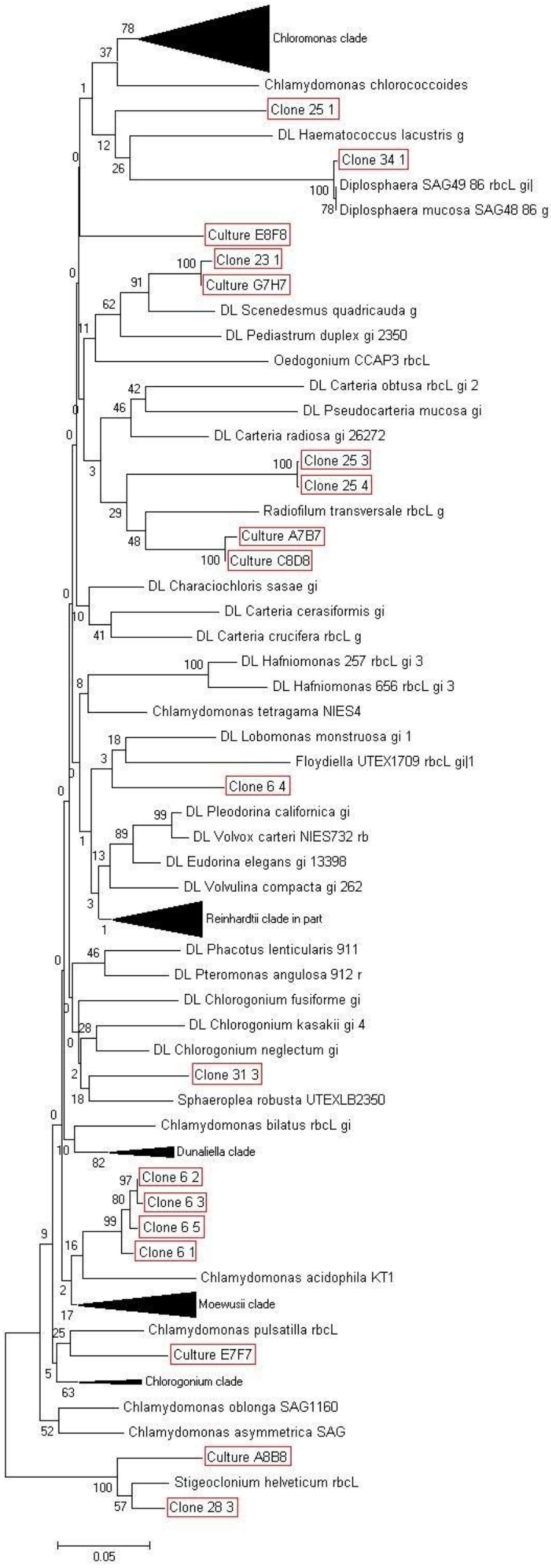


Figure 3. Phylogeny of chlorophyte species. Sequences obtained in this study are surrounded by a red border.

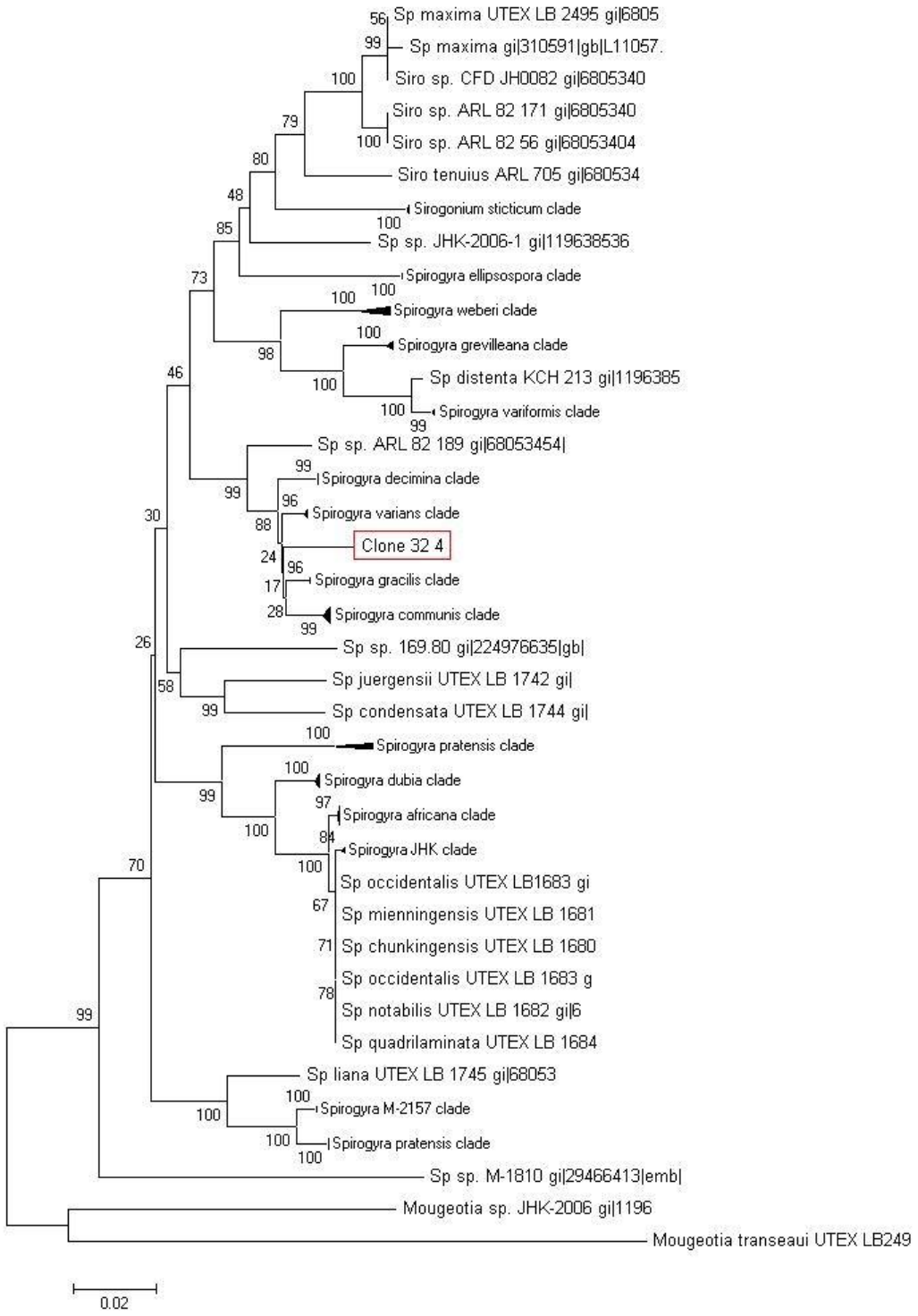
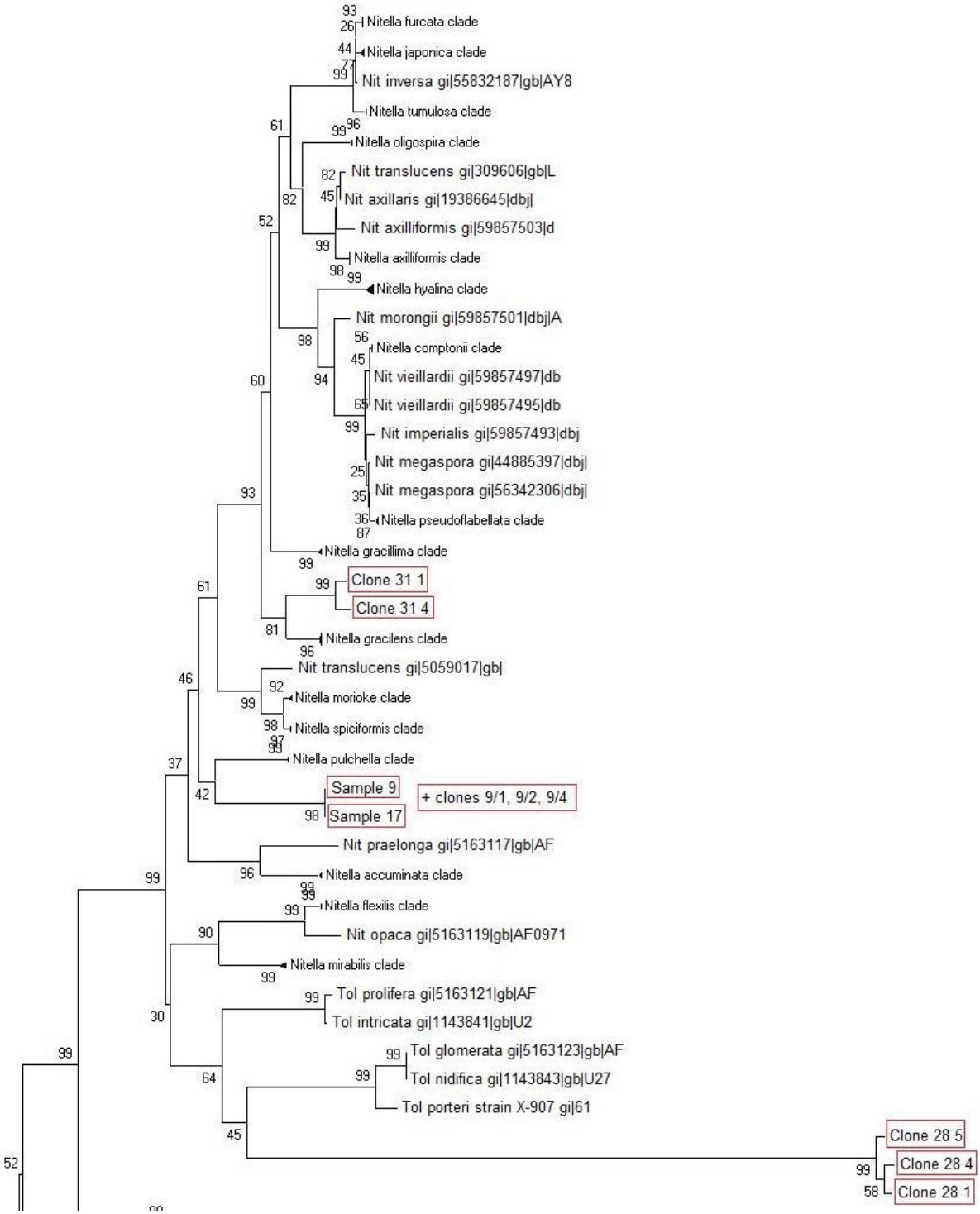


Figure 4. Phylogeny of *Spirogyra* species. Sequences obtained in this study are surrounded by a red border.


Nitella furcata clade
Nitella japonica clade
Nit inversa gi|55832187|gb|AY8
Nitella tumulosa clade
Nitella oligospira clade
Nit translucens gi|309606|gb|L
Nit axillaris gi|19386645|dbj|
Nit axilliformis gi|59857503|d
Nitella axilliformis clade
Nitella hyalina clade
Nit morongii gi|59857501|dbj|A
Nitella comptonii clade
Nit vieillardii gi|59857497|db
Nit vieillardii gi|59857495|db
Nit imperialis gi|59857493|dbj
Nit megaspora gi|44885397|dbj|
Nit megaspora gi|56342306|dbj|
Nitella pseudoflabellata clade
Nitella gracillima clade
Clone 31 1
Clone 31 4
Nitella gracilens clade
Nit translucens gi|5059017|gb|
Nitella morioke clade
Nitella spiciformis clade
Nitella pulchella clade
Sample 9
Sample 17
+ clones 9/1, 9/2, 9/4
Nit praelonga gi|5163117|gb|AF
Nitella accuminata clade
Nitella flexilis clade
Nit opaca gi|5163119|gb|AF0971
Nitella mirabilis clade
Tol prolifera gi|5163121|gb|AF
Tol intricata gi|1143841|gb|U2
Tol glomerata gi|5163123|gb|AF
Tol nidifica gi|1143843|gb|U27
Tol porteri strain X-907 gi|61
Clone 28 5
Clone 28 4
Clone 28 1

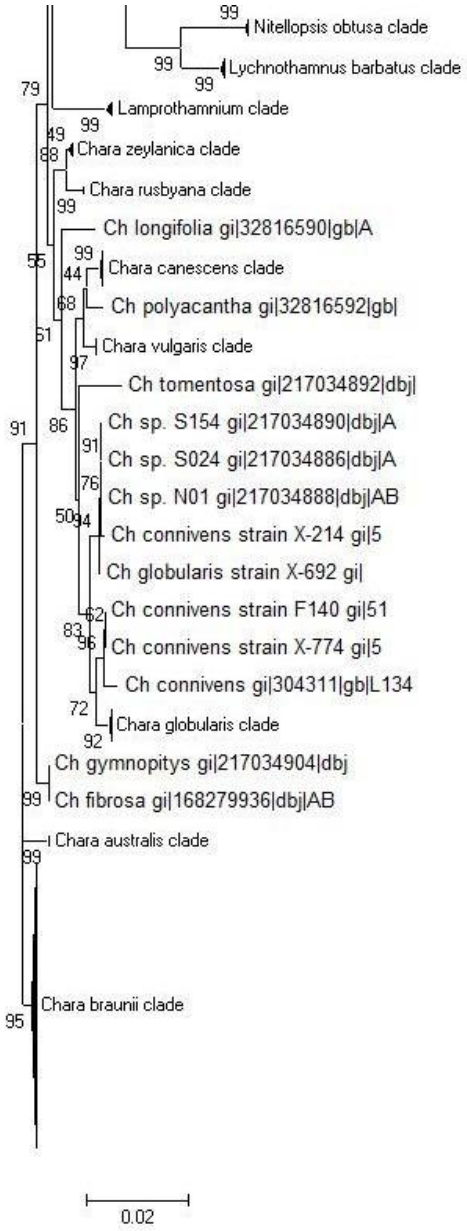


Figure 5. Phylogeny of Charales species. Sequences obtained in this study are surrounded by a red border.

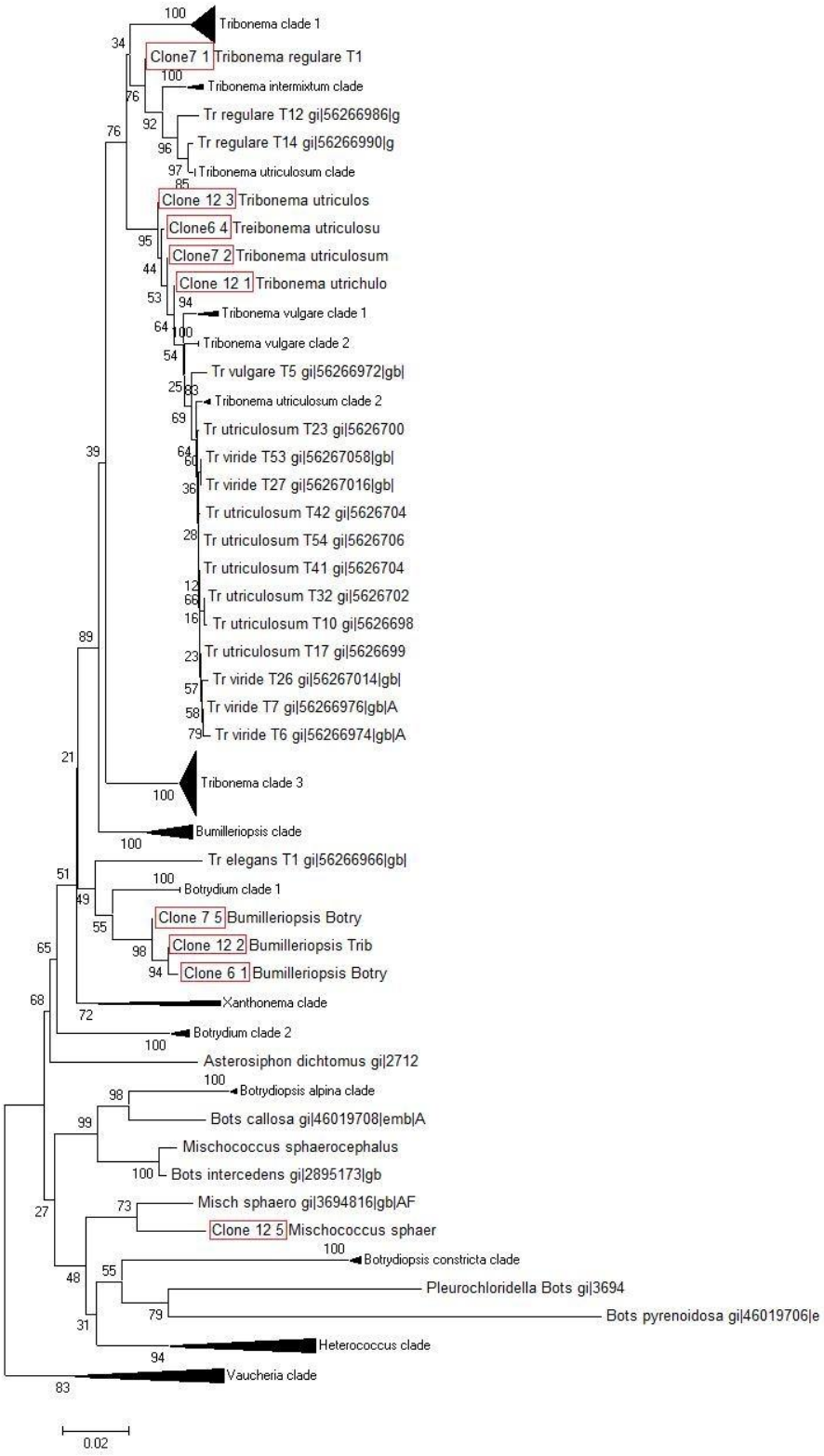


Figure 6. Phylogeny of xanthophyte species. Sequences obtained in this study are surrounded by a red border.

Ordinations

CCA: The variance explained by the first axis was 25%, by the second 18%, and by the third (not shown) 18%. A joint plot arranging taxa and sites in environmental space is shown in Fig. 7.

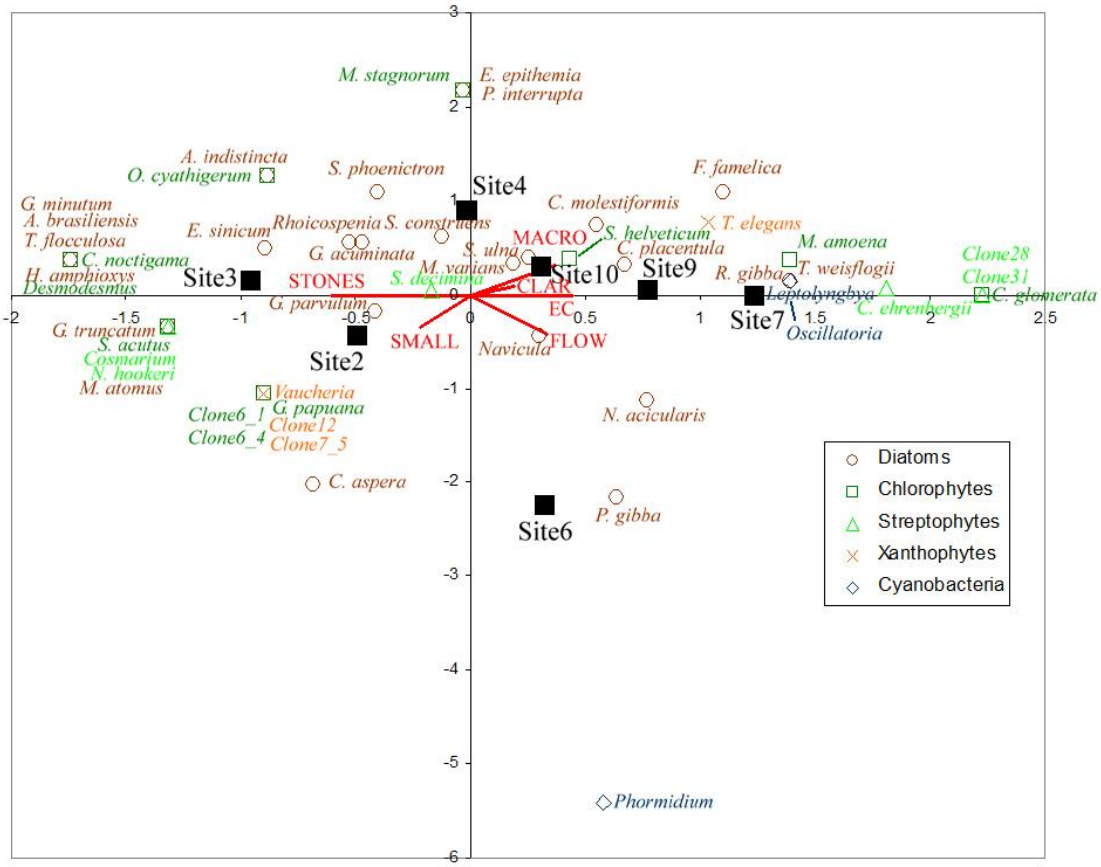


Figure 7. Canonical Correspondence Analysis (CCA) plot of algal species.

A DCA scatterplot arranging sites and species in species space is shown in Fig. 8.

Overview of diversity at each site

A total of 51 different species of algae were described throughout all sites in this study. In addition, another 13 possible species were found from clones of environmental samples. Altogether these species were a mixture of diatoms (31), chlorophytes (21), xanthophytes (4), charophytes (4), and cyanobacteria (4). The diversity of algal species varied greatly between the different sites (Fig. 9). Some sites provided a good habitat for algae, such as Smacks Creek at Gardiners Rd in which 26 species were identified. Other sites had a lower diversity of species, such as the Styx headwaters, where only six species were identified. The same is true for the sites in Kaputone Stream where only 20 different species were found overall, compared with 39 in the Styx River and 34 in Smacks Creek.

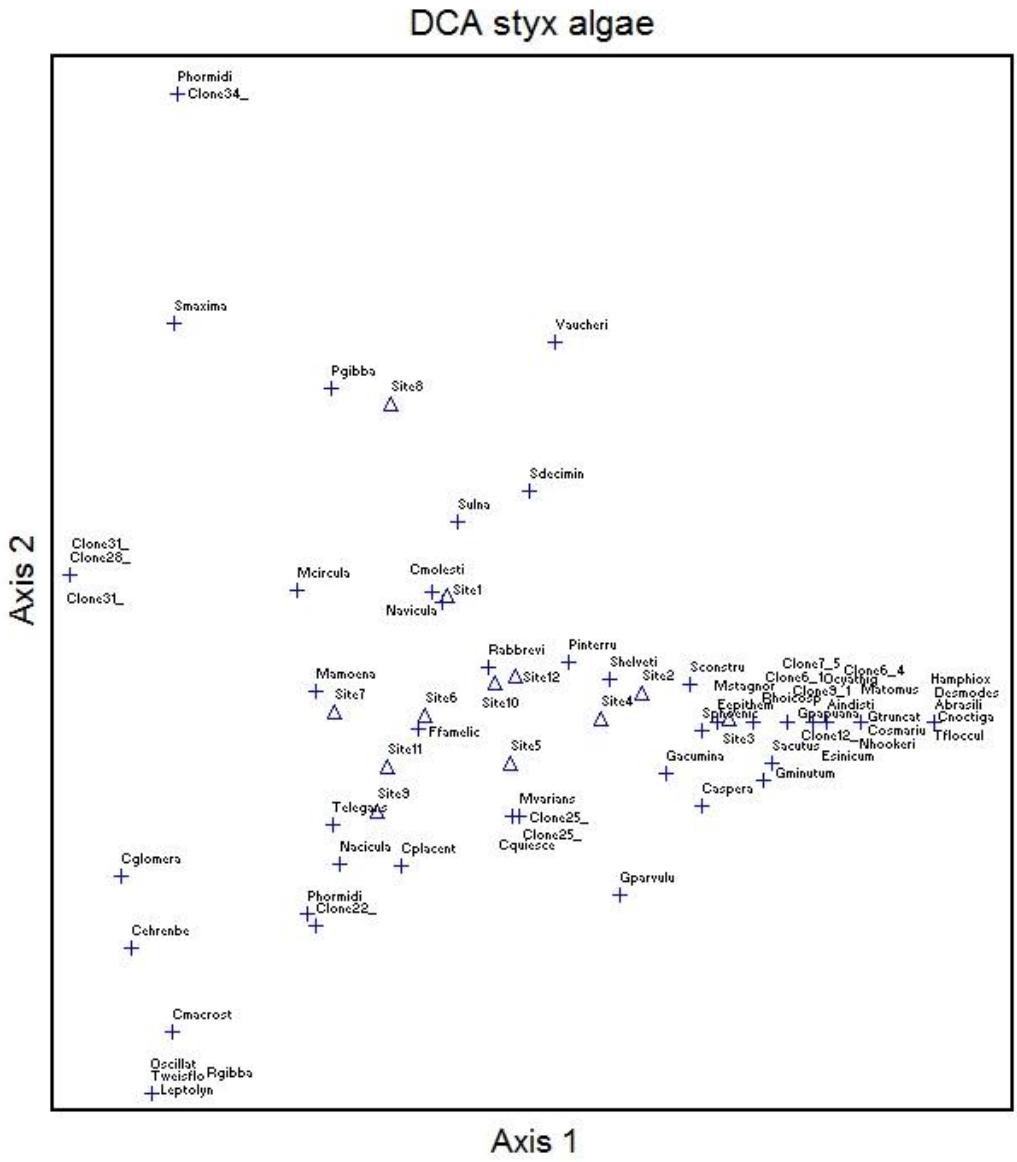


Figure 8. Detrended Correspondence Analysis (DCA) plot of algal species.

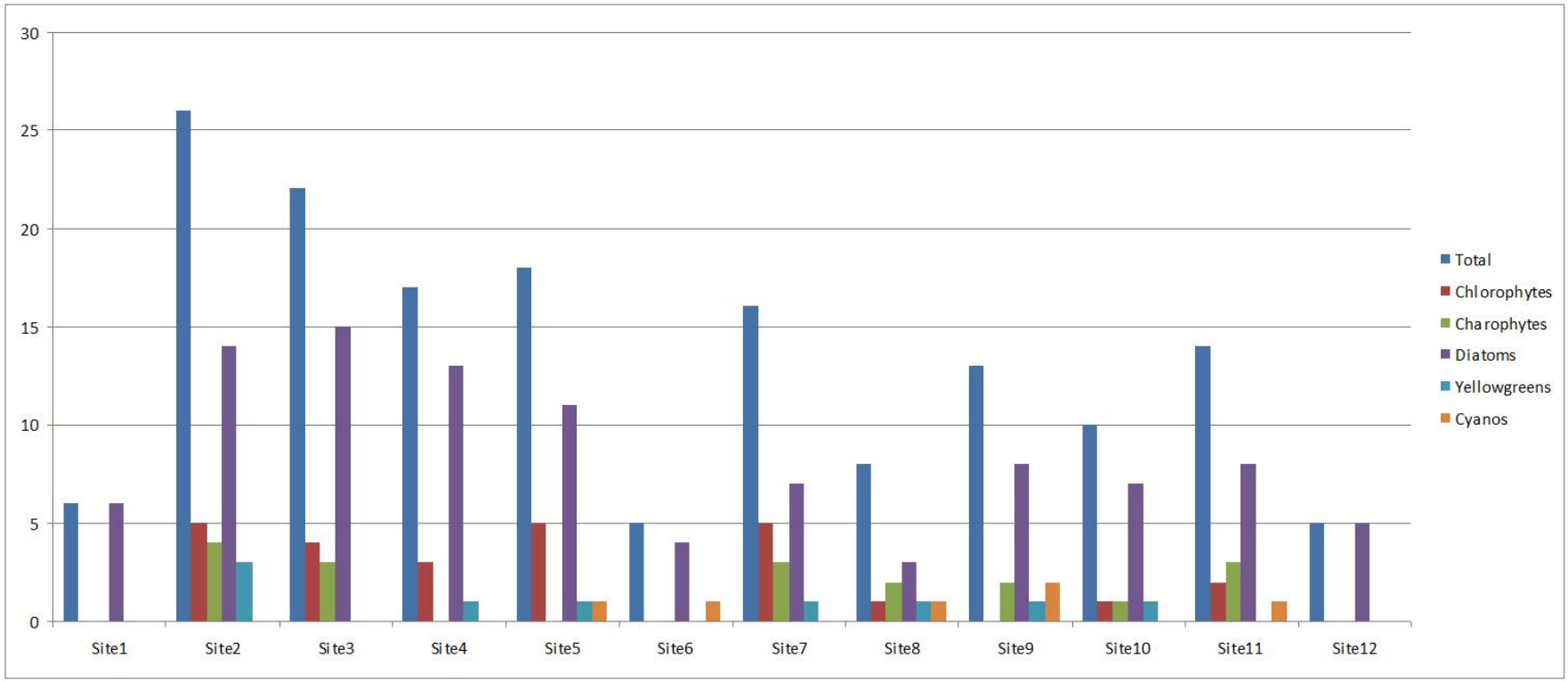


Figure 9. Number of taxa (vertical axis) at each site in the Styx catchment.

Discussion

Diversity

The species found in this study suggest a mix of range restriction and cosmopolitanism in the algal communities in the Styx catchment. Cosmopolitan species such as *Cocconeis placentula* and *Tabellaria flocculosa* (Kilroy et al. 2007) were present as well as range-restricted species like *Actinella indistincta,* previously found in Tasmania and Stewart Island (Sabbe et al. 2001).

As there has been little work done on algal diversity in this river system in the past, it is difficult to determine any changes that may have occurred over time in the composition of the algal community at each of these sites.

One study that does provide some information about the presence of algae at these sites was conducted in 1989 by the Christchurch Drainage Board (Robb 1989). At this time filamentous green algae and *Nitella hookeri* were well represented throughout the catchment area. The study also showed the presence of *Spirogyra* sp. and red algae, in particular *Batrachospermum.* Although the other species mentioned were found during this study, *Batrachospermum* was not found at any of the sites. The absence of this and other species of red algae may be an indication of an increasingly disturbed river system, as *Batrachospermum* in particular is known to prefer undisturbed sites (e.g. Entwisle et al. 1997).

One of the taxa not found in this river system was *Didymosphenia geminata* (didymo), an invasive freshwater diatom that is currently found in a number of South Island rivers. Although it has not been found at any of the sites, invasion by this species is a risk, especially at sites such as Smacks Creek at Gardiners Rd, where environmental characteristics such as moderate flows and high light availability make it a suitable site for *D. geminata.* This site is also the most diverse and is likely to be the worse affected by an invasion by this species.

Distribution

The two methods used to determine patterns in distribution, DCA and CCA, have strengths and weaknesses in analysing these data. DCA allows us to ask questions about how sites are structured in species space, and rare species can be downweighted, but actual environmental data cannot be incorporated into the analysis.

CCA enables us to look for relationships with environmental parameters in the data, but we did not have environmental data for all the sites in this study so 10 taxa had to be dropped from the analysis. Rare species cannot be downweighted so they will have a disproportionate influence on the results, such as *Phoridium* sp.

In Fig. 7, the clarity gradient is approximately opposed to small and stony substrata, but closely correlated with macrophytes. This is not surprising since sediment decreases clarity, and macrophytes would tend to bind and catch sediment.

The flow gradient is largely orthogonal to other gradients.

There are few species in the area of the chart corresponding to high flows and small substrates. Site 6 falls in this category (Fig. 7) and had low diversity (Fig. 9).

Therefore, will sedimentation (e.g. from development) more greatly affect algal communities at faster flowing sites than at slower flowing sites? One hypothesis to explain the distribution of algae in the Styx catchment is that algal communities are likely to be more resilient to sedimentation in sites with slower flows, and that the presence of macrophytes may increase this resilience.

There are several obvious groupings of species in Fig. 8:
Group 1: Clone 28 (*Nitella*?) and Clone 31 (*Nitella* sp.)

Group 2: *Gomphonema truncatum*, *Scenedesmus acustus*, *Cosmarium subcucumis*, *Nitella hookeri*, *Mayamaea atomus*, *Gomphonema minutum*, *Actinella brasiliensis*, *Tabellaria flocculosa*, *Chlamydomonas noctigama*, *Hantzschia amphioxys*, *Desmodesmus aldavei*

Group 3: *Chlamydomonas* cf. *macrostellata*, *Rhopalodia gibba*, *Thalassiosira* cf. *weissflogii*, Cf. *Oscillatoria*, Cf. *Leptolyngbya*

Species in groups 1 and 3 do not appear to be linked to any particular site, whereas species in group 2 were closest to site 3 (Styx River at Styx Mill Conservation Resrve), which has high diversity (Fig. 9). There were few species in the area near site 8 (Kaputone Stream at Belfast Rd), and this site has one of the lowest diversity of algae (Fig. 9).

Identification problems

There is some guesswork involved in accurately identifying the taxa present in clones from each of the samples. There is no way of knowing which taxa the cloned sequences come from without further molecular work. The relationship between the cloned sequences and other known sequences was used to determine the closest relative to each of the cloned sequences. Analysis of clones involved making educated guesses about which taxa each clone belonged to. Identification of the taxa in each sample was aided by morphology from photos as well as sequences from cultures, for which we know the taxa of each.

Another problem in surveying algae is an inability to survey any habitat exhaustively for microscopic organisms. It is unlikely that all the taxa present at each site were found during this study. There are also limitations with surveying sites at a single time point, as algae often bloom and die successionally during a season, so late- or early-blooming taxa may have been missed.

There were a number of strains that had no close relatives in the GenBank database. Of the 26 clone sequences, 27% (n = 7) were clearly allied to a known strain with a short branch; 38% (n = 10) could be placed in a clade but were not particularly close to anything; and 35% (n = 9) did not have any close relatives. This indicates that there are a number of novel lineages in the algal species in the Styx catchment (and therefore in New Zealand generally, or even in this type of habitat) as over half the clone sequences could not be allied with a known strain. This suggests that there is still much to learn about algal diversity and distribution in this area.

The species data collected in this study provide a baseline for monitoring of algal communities in the Styx catchment. This information is useful to determine the current state of algal communities, but future monitoring needs to be done on a regular basis if algae are to be used as environmental indicators of the state of the Styx River catchment.

What I gained from this research project

- Learnt molecular techniques such as DNA extraction and cloning
- Learnt to use light and scanning electron microscopes
- Learnt to use new computer programs – Sequencher and MEGA4
- Leant how to store botanical samples for future use (i.e. drying, preserving, mounting samples on slides, accessioning into herbarium)
- Improved time management skills
- Learnt about regular record keeping
- Learnt about the diversity of algae

Acknowledgements

I thank the Styx Living Laboratory Trust, Lincoln University, Landcare Research, and especially my supervisors Phil Novis and Kelly Walker for the guidance they have given me throughout this project. Nicola Bolstridge provided valuable technical assistance. Molecular work was funded by the Foundation for Research, Science and Technology through the "Defining New Zealand's Land Biota" Outcome-Based Investment, which also supported Phil Novis' contributions.

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Appendix 1. Samples (n = 53) collected at study sites in Styx River catchment

Sites	Coordinates	pH	EC	Sample no.	CHR no.	Other sample
Styx Headwaters	E2476569, N5748545	6.5		1		Dried charophyte
Styx Headwaters				2	608856	
Styx Headwaters				3		Dried charophyte
Styx Headwaters				4	608857	
Styx Headwaters				5		Water sample
Smacks Creek on Gardiners Rd	E2476847, N5749519	6.2	110	6	608858	
Smacks Creek on Gardiners Rd				7	608859	
Smacks Creek on Gardiners Rd				8	608860	
Smacks Creek on Gardiners Rd				9		Dried charophyte
Smacks Creek on Gardiners Rd				10	608861	
Smacks Creek on Gardiners Rd				11		Water sample
Smacks Creek on Gardiners Rd				12	608862	
Smacks Creek at Willowbank Wildlife Reserve	E2477273, N5749264	6.8	90	13	608863	
Smacks Creek at Willowbank Wildlife Reserve				14	608864	
Smacks Creek at Willowbank Wildlife Reserve				15	608865	
Smacks Creek at Willowbank Wildlife Reserve				16		Water sample
Smacks Creek at Willowbank Wildlife Reserve				17		Dried charophyte
Styx Mill Conservation Reserve	E2478044, N5749373	6.8	110	18	608866	
Styx Mill Conservation Reserve				19	608867	
Styx Mill Conservation Reserve				20	608868	
Styx Mill Conservation Reserve				21		Water sample
Styx River at North Rd	E2479041, N5748843	7	110	22	608869	
Styx River at North Rd				23	608870	
Styx River at North Rd				24	608871	
Styx River at North Rd				25	608872	
Styx River at North Rd				26		Water sample
Styx River at Radcliffe Rd	E2481715, N5749022	6.6	110	27		Dried charophyte
Styx River at Radcliffe Rd				28	608873	
Styx River at Radcliffe Rd				29	608874	
Styx River at Radcliffe Rd				30		Water sample
Styx River at Radcliffe Rd				31	608875	
Kaputone Stream at Belfast Rd	E2480820, N5750485	6.7	130	32	608876	

Kaputone Stream at Belfast Rd				33	608877	
Kaputone Stream at Belfast Rd				34	608878	
Kaputone Stream at Belfast Rd				35		Water sample
Styx River at Redwood Spring	E2479468, N5749165	6.9	120	36		Dried charophyte
Redwood Spring		6.9	80	37	608879	
Styx River at Redwood Spring				38		Water sample
Kaputone Stream at Ouruhia Domain	E2481783, N5751732	6.9	130	39	608880	
Kaputone Stream at Ouruhia Domain				40	608881	
Kaputone Stream at Ouruhia Domain				41	608882	
Kaputone Stream at Ouruhia Domain				42		Water sample
Kaputone Stream at Belfast Rd Bridge	E2481818, N5750827	6.7	130	43	608883	
Kaputone Stream at Belfast Rd Bridge				44	608884	
Kaputone Stream at Belfast Rd Bridge				45		Water sample
Styx River at Spencerville Rd Bridge	E2484950, N5753120	6.9	120	46	608885	
Styx River at Spencerville Rd Bridge				47	608886	
Styx River at Spencerville Rd Bridge				48		Water sample
Styx River at Spencerville Rd Bridge				49	608887	
Styx River at Spencerville Rd Bridge				50	608888	
Styx River at Teapes Rd	E2483970, N5751250	7.5	110	51	608889	
Styx River at Teapes Rd				52		Dried charophyte
Styx River at Teapes Rd				53		Water sample

Appendix 2. Primer sequences

Primer name	Sequence
Nr4F	GTTTCCTTTTCGTAGCTGAAGC
PR3R	CCCCAWGGGTGDCCWARWGTWCCW CCACC
Nr1F	ATGGTTCCACAAACAGAAAC
Nr6R	TTAGCTGTGAAACCACCTGTTA
PNK1F	TTCCAAGGTCCTCCTCATGGTAT
PNK2R	CAATCACMGCRTGCATAGCACGGT
PNK4F	TAACTGGTGGCTTTACTGCA
Kkdown	AATTCAAATTTAATTTCTTTCC
HRF2	AAAAGTGACCGTTATGAATC
HRR4	TGGTCAACACCAGCC
HRF5	CACAACCATTCATGCG
HRR2	CCAATAGTACCACCACCAAAT