Taxonomic Revision of Diatoms belonging to the family Naviculaceae based on morphological and molecular data.

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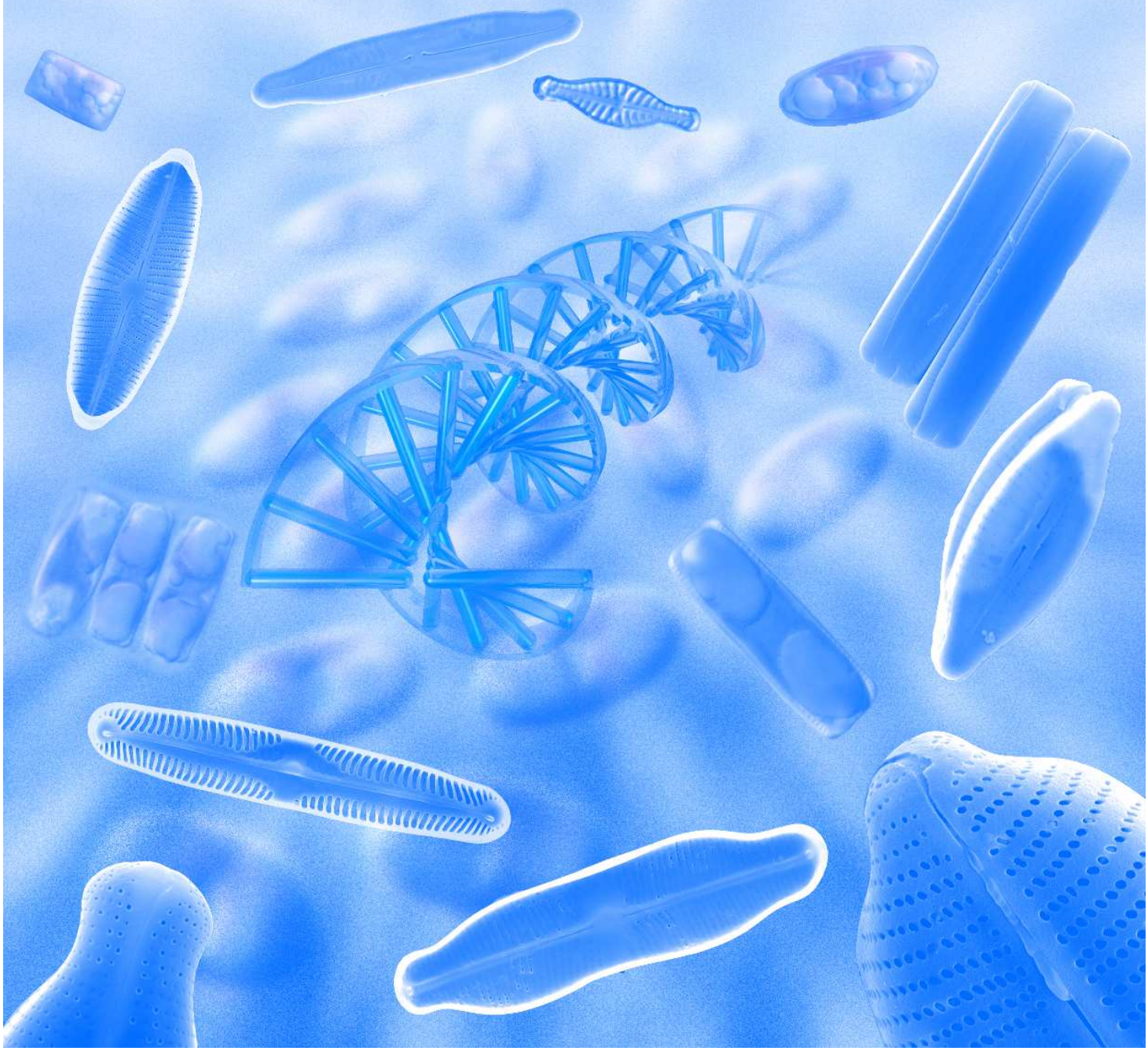

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Summary

The recent taxonomy of diatoms is basically based on investigations of valve morphology, cell components and life cycle (e.g., Round *et al.*, 1990). But the development of PCR has facilitated the use of DNA sequences for inferring phylogenies. Based on morphology, the taxonomy of the family Naviculaceae (*sensu* Krammer & Lange-Bertalot, 1986) has been highly changed (e.g., Round *et al.*, 1990), but little work has been carried out with molecular data for this large and ecologically important group of diatoms.

My thesis was aimed at the investigation of evolutionary relationships within the naviculoid pennates using molecular and morphological data. Ninety-one cultures containing 72 species of 22 genera were isolated and their morphology examined. Sixty-two of these species belong to the Naviculaceae. Phylogenies based on sequences of the nuclear-encoded SSU rRNA gene, the LSU rRNA gene, the chloroplast *rbc*L gene and a combined dataset were compared.

The SSU rRNA gene is the most widely used gene for inferring phylogenetic relationships. The combination of conserved and variable regions in this gene allows studies of most phylogenetic relationships. Because the D1/D2-region of the LSU rRNA gene comprises more highly variable areas than the SSU rRNA gene, a stronger phylogenetic signal for closely related species was estimated. Also the *rbc*L gene was used in this study to obtain clearer information of evolution at lower (order to genus) levels of taxonomic hierarchy in diatoms. But in this study the trees based on the LSU rDNA and *rbc*L gene sequences do not provide stronger supported results for closely related species. The analyses of the combined dataset resulted in trees with higher bootstrap support than the analyses of the single genes, although partition homogeneity test resulted in a very low p-value. The results of the partition homogeneity test should not be used to determine whether or not to combine data sets for phylogenetic analysis.

This study confirms the assumption that the genus *Navicula sensu lato* is a very heterogenous group and my results support the monophyly of *Navicula sensu stricto*. The separation of *Craticula*, *Eolimna*, *Hippodonta*, *Luticola*, *Mayamaea* and *Placoneis* from the genus could be confirmed. "*Navicula*" species, which do not belong to the section *Lineolatae* could be recombined: *Navicula integra* is the type species of a newly described genus *Prestauroneis* Bruder, *gen. nov.* (Type species: *Prestauroneis integra* (W. Smith) Bruder *comb. nov.*); *Navicula brockmannii* is transferred to the genus *Adlafia (A. brockmannii* (Hustedt) Bruder *comb. nov.*) and *Navicula hambergii* is placed within *Placoneis* (*Placoneis hambergii* (Hustedt) Bruder *comb. nov.*). The differences of their sequences indicates that *M. atomus* var.

atomus and *M. atomus* var. *permitis* were not just two varieties of the same species but two different species.

The monophyly of the genera *Cocconeis*, *Craticula*, *Cymbella*, *Encyonema*, *Eunotia*, *Gomphonema*, *Lyrella*, *Mayamaea*, *Placoneis*, *Pleurosigma* and *Sellaphora* is supported by the recent study. But the actual differentiation of the genera *Caloneis* and *Pinnularia* is rejected. The molecular results support groups defined by Krammer & Lange-Bertalot (1985), based on the morphology of the internal openings of the alveoli. A genus that should be subdivided is *Amphora*. Molecular and morphological data strongly support a separation of the subgenus *Halamphora*. But further investigations on the subgenus *Halamphora* is needed because the results of the analysis from SSU rDNA sequences indicate that this is still an artificial group. This study does neither support nor refuse a separation of *Cymbella*, because of the different results in the molecular phylogenies.

This study also resolve several relationships between different genera. *Hippodonta* is shown to be sister to *Navicula sensu stricto*. The family Stauroneidaceae could be recovered and the addition of the newly describes genus *Prestauroneis* to this family is proposed. The results also support to include the genus *Mayamaea* into the suborder Sellaphorineae, which could be recovered in most phylogenies. The marine and freshwater monoraphid genera are clearly separated. The marine genera form the sister clade to the Bacillariales, whereas the freshwater monoraphid genera diverge within the naviculoid pennates. The relationship between the freshwater monoraphid genera and the naviculoid pennates could not be resolved unambiguously but they might be close relatives of *Adlafia brockmannii* and the Cymbellales. The monophyly of the order Cymbellales is strongly supported, but the results contradict the arrangement of the families Cymbellaceae and Gomphonemataceae, because in most trees *Gomphonema* (Gomphonemataceae) diverge within the Cymbellaceae. The order Naviculales and the suborder Naviculineae as used in Round *et al*. (1990) are shown to be heterogenous in all trees.

Zusammenfassung

Die Taxonomie der Diatomeen basiert vor allem auf Untersuchungen der Valvenmorphologie, der Zellkomponenten und des Zellzyklus (z.B. Round *et al.*, 1990). Die Entwicklung der PCR hat zusätzlich die Verwendung von DNA-Sequenzen bei der Ermittlung von Stammbäumen ermöglicht. Die Taxonomie der Familie Naviculaceae (*sensu* Krammer & Lange-Bertalot, 1986) wurde bereits aufgrund morphologischer Untersuchungen stark verändert (z.B. Round *et al.*, 1990), aber es gibt nur wenige molekularbiologische Arbeiten für diese große und ökologisch wichtige Gruppe der Diatomeen.

Ziel meiner Arbeit war die Untersuchung der evolutionären Verhältnisse zwischen naviculoiden Diatomeen unter Verwendung von molekularen und morphologischen Daten. Insgesamt wurden 91 Kulturen, die 72 Arten aus 22 Gattungen enthielten, isoliert und ihre Morphologie untersucht. Zur Familie Naviculaceae gehören 62 dieser Arten. Von allen Kulturen wurden die Sequenzen der im Zellkern vorliegenden SSU rDNA und LSU rDNA sowie des im Chloroplastengenom kodierten *rbc*L Gens bestimmt. Die auf den einzelnen Genen sowie einem kombinierten Datensatz basierenden Phylogenien wurden verglichen.

Zur Bestimmung phylogenetischer Beziehungen wird meist das SSU rRNA Gen verwendet. Durch die Kombination konservierter und variabler Regionen eignet es sich für die Untersuchung der meisten phylogenetischer Beziehungen. Die D1/D2-Region der LSU rDNA beinhaltet mehr hoch variable Regionen als die SSU rDNA, weshalb ein stärkeres phylogenetisches Signal bei nah verwandten Arten erwartet wurde. Auch die Verwendung des *rbc*L Gens sollte eine bessere Auflösung der Evolution auf einem niedrigeren Level (Ordnung bis Gattung) erzielen. Die auf der LSU rDNA und dem *rbc*L Gen basierenden Phylogenien zeigen in dieser Studie aber keine eindeutigeren Ergebnisse für nah verwandte Arten. Die Analyse des kombinierten Datensatzes ergab die am besten durch Bootstrap-Werte unterstützten Phylogenien, obwohl der „partition homogeneity test“ einen sehr niedrigen p-Wert ergab. Dies unterstützt, dass das Ergebnis dieses Testes nicht entscheiden sollte, ob mehrere Datensätze kombiniert analysiert werden oder nicht.

Diese Studie bestätigt die Annahme, dass die Gattung *Navicula sensu lato* eine sehr heterogene Gruppe ist. Zusätzlich unterstützen meine Ergebnisse die Monophylie *Navicula sensu stricto*. Die Abspaltung von *Craticula*, *Eolimna*, *Hippodonta*, *Luticola*, *Mayamaea* und *Placoneis* von der Gattung konnte bestätigt werden. “*Navicula*” Arten, die nicht zur Sektion *Lineolatae* gehören, konnten neu zugeordnet werden: *Navicula integra* ist die Typus-Art der neu beschriebenen Gattung *Prestauroneis* Bruder, *gen. nov.* (Typus-Art: *Prestauroneis*

integra (W. Smith) Bruder *comb. nov.*); *Navicula brockmannii* ist zur Gattung *Adlafia (A. brockmannii* (Hustedt) Bruder *comb. nov.*) und *Navicula hambergii* zur Gattung *Placoneis* (*Placoneis hambergii* (Hustedt) Bruder *comb. nov.*) überführt worden. Die Unterschiede ihrer Sequenzen lassen vermuten, dass es sich bei *M. atomus* var. *atomus* und *M. atomus* var. *permitis* nicht nur um zwei Varietäten sondern um zwei Arten handelt.

Die Monophylie der Gattungen *Cocconeis, Craticula, Cymbella, Encyonema, Eunotia, Gomphonema, Lyrella, Mayamaea, Placoneis, Pleurosigma* und *Sellaphora* konnte im Rahmen dieser Studie bestätigt werden. Dagegen widerlegen die Ergebnisse die derzeitige Trennung der Gattungen *Caloneis* and *Pinnularia*. Stattdessen werden die von Krammer & Lange-Bertalot (1985) definierten Gruppen, die sich vor allem durch die Morphologie ihrer internen Alveolenöffnungen unterscheiden, unterstützt. Die Gattung *Amphora* sollte weiter unterteilt werden. Sowohl molekulare als auch morphologische Daten unterstützen eine Abtrennung der Untergattung *Halamphora*. Es sind jedoch weitere Untersuchungen der Untergattung *Halamphora* notwendig, da die Analyse der SSU rDNA Sequenzen andeutet, dass es sich bei dieser Untergattung noch immer um eine künstliche Gruppe handelt. Auf der Basis dieser Studie kann eine Aufteilung der Gattung *Cymbella* weder widerlegt noch befürwortet werden, da sich die Beziehungen innerhalb dieser Gattung in den einzelnen Phylogenien unterscheiden.

Diese Studie klärt auch einige Beziehungen zwischen verschiedenen Gattungen auf. So zeigen die Ergebnisse, dass *Hippodonta* die Schwestergattung von *Navicula sensu stricto* ist. Die Familie Stauroneidaceae konnte bestätigt und die neu beschriebene Gattung *Prestauroneis* zu dieser Familie hinzugefügt werden. Aufgrund dieser Studie sollte die Gattung *Mayamaea* in die Unterordnung Sellaphorineae eingegliedert werden. Innerhalb der monoraphiden Gattungen zeigt sich eine klare Trennung der marinen und der Süßwasser-Arten. Die marinen Gattungen bilden die Schwestergruppe der Bacillariales, während sich die Süßwasser-Arten innerhalb der naviculoiden Diatomeen abspalten. Das Verhältnis zwischen den monoraphiden Süßwasser-Gattungen und den naviculoiden Diatomeen konnte nicht eindeutig geklärt werden, aber meine Ergebnisse weisen auf eine nahe Verwandtschaft mit *Adlafia brockmannii* und der Ordnung Cymbellales hin. Die Ergebnisse bestätigen die Monophylie der Ordnung Cymbellales, aber sie widersprechen der Einteilung der Familien Cymbellaceae und Gomphonemataceae, da sich *Gomphonema* (Gomphonemataceae) in fast allen Phylogenien innerhalb der Cymbellaceae abspaltet. Die Ordnung Naviculales und die Unterordnung Naviculineae, wie sie in Round *et al.* (1990) eingeteilt wurden, haben sich in allen Phylogenien als heterogen erwiesen.

1. Introduction

> "Few objects are more beautiful than the minute siliceous cases of the diatomaceae: were these created that they might be examined and admired under the higher powers of the microscope?" (Darwin, 1859)

1.1 Diatom systematics

Diatom valves were one of the favourite subjects for study by the early microscopists and the first diatom was described at the beginning of the 1700s (Round *et al.*, 1990). The description of diatom species and their taxonomy has been traditionally based on light-microscopical studies of valve shape and structure. With the introduction of electron microscope techniques, more details of valve structure (e.g., the areolae, processes or tubes) were visible. Although diatom classification depends to a great extent on valve morphology, features of the living cell (e.g., number and form of chloroplasts and pyrenoids) and ecology have also been taken into account (Mereschkowsky, 1903, Cox & Williams, 2000).

Based on their valve morphology, Schütt (1896) separated the diatoms into two main groups: Centric diatoms with a radial symmetry and bilaterally symmetrical pennate diatoms. Later the pennate group was subdivided into species with a raphe slit in at least one valve and those species without a raphe (e.g., Hustedt 1961-1966, Round *et al.*, 1990). The raphe slit is necessary for diatom locomotion. This classification implies that centrics and pennates each represent natural evolutionary lineages. But in fossil records, centric diatoms have been recovered from Jurassic and Late Cretaceous (e.g., Rothpeltz, 1896, Strelnikowa & Martirosjan, 1981, Gersonde & Harwood, 1990, Harwood & Gersonde, 1990), whereas araphid pennate diatoms appear in the Late Cretaceous (e.g., Moshkovitz *et al.*, 1983). Raphid pennate diatoms, which today represent the most diverse group, have been recovered from Tertiary (Strelnikova, 1990). In some phylogenetic analyses the centric diatoms grade into the pennate diatoms (e.g., Kooistra *et al.*, 2003, Sorhannus, 2004). Other molecular phylogenies show two different clades (e.g., Medlin *et al.*, 2000, Medlin & Kaczmarska, 2004). These studies differ in the number of used sequences, in species composition and in the outgroup used. But none of the phylogenies reflect the traditional groups. The centric and the araphid pennate diatoms are shown to be paraphyletic. Only the raphid pennate diatoms and the pennate diatoms are monophyletic in all studies.

Medlin and Kaczmarska (2004) proposed a revised classification based on molecular data, morphological and cytological features:

Subdivision *Coscinodiscophytina* Medlin & Kaczmarska

Class *Coscinodiscophyceae* Round & Crawford, emend. Medlin & Kaczmarska, which comprises the "radial" centrics;

Subdivision *Bacillariophytina* Medlin & Kaczmarska

Class *Mediophyceae* (Jousè & Proshkina-Lavrenko) Medlin & Kaczmarska, which comprises the "multipolar" centrics plus the radial Thalassiosirales;

Class *Bacillariophyceae* Haeckel, emend. Medlin & Kaczmarska, which comprises the pennate diatoms.

Study performed by Guillou *et al.* (1999) and Daugbjerg & Guillou (2001) based on different genes have shown the Bolidophyceae to be the sister group to the diatoms.

1.2 Some problematic genera

1.2.1 *Navicula*

The genus *Navicula* was described by Bory de Saint-Vincent in 1922 based on *Navicula tripunctata* (O. F. Müller) Bory. Within the diatoms, this genus is probably the largest and most diverse because "*Navicula* has traditionally been a dump for all bilaterally symmetrical raphid diatoms lacking particularly distinctive features" (Round *et al.*, 1990, p. 566). Nevertheless, with electron microscopy and the investigation of living cells, the true morphological diversity of the genus became apparent. Therefore, taxonomic revisions of this genus are being made or have been carried out and new genera described or old genera resurrected. Since the description of the genus, the taxonomic treatment of the naviculoid diatoms has undergone major changes.

Today most diatomists agree that *Navicula* (*sensu stricto*) should be used only for species that belong to the section *Lineolatae* (*sensu* Cleve, 1895 and Hustedt, 1930). *Navicula sensu stricto* encompasses approximately 200 species, which predominantly (about 150 species) inhabit freshwater environment (Witkowski *et al.*, 1998). There are still many species named *Navicula* that do not belong to this group, but several older genera have been resurrected (e.g., *Placoneis* Mereschkowsky in Cox, 1987) and new genera were described and separated from *Navicula sensu stricto* because they differ clearly in valve morphology and/or

chloroplast features, e.g., *Eolimna* (Schiller & Lange-Bertalot, 1997), *Hippodonta* (Lange-Bertalot, Metzeltin & Witkowski, 1996) *Luticola* (Mann, in Round *et. al*., 1990) or *Mayamaea* (Lange-Bertalot, 1997). But not all new genera have been accepted by all diatomists. For example the separation of the genus *Hippodonta* is under discussion. In her investigation of the variation of valve morphology, Cox (1999) doubted the correctness of this separation. In her study, she could find examples of all characters used to define the genus *Hippodonta* in other species of *Navicula*, but no cytological or reproductive evidence that would support their separation. Therefore she proposed that the species allocated to *Hippodonta* be recognised as a subgenus of *Navicula* and to enlarge the generic description of *Navicula* to cover this.

1.2.2 *Pinnularia* and *Caloneis*

The genus *Pinnularia* was described by Ehrenberg based on *P. viridis* (Ehrenberg, 1843). In 1894, Cleve described the genus *Caloneis* with *C. amphisbaena* as its type and distinguished the genus from *Pinnularia* on the basis of light microscopy. He already noted that "smaller forms of *Caloneis* with indistinct longitudinal lines closely resemble small *Pinnulariae*, and certain of the panduriform species seem to be closely connected with some marine, panduriform *Pinnulariae"* (Cleve, 1894).

Since then, many diatomists investigating the two genera have tried to find morphological characters to make a clear distinction between the two genera. The separation of the genera *Caloneis* Cleve and *Pinnularia* Ehrenberg is discussed controversial: Some infer from their results, that there is a distinguishing combination of characters to recognise each genus easily (e.g., Krammer & Lange-Bertalot, 1985, Krammer, 2000). In addition to this conclusion Krammer & Lange-Bertalot (1985) mentioned a potential separation in three groups: (1) all species whose alveoli are internally nearly open, as existing in *Pinnularia interrupta*; (2) species with partially closed alveoli, e.g., *Caloneis amphisbaena* and *Pinnularia gibba*; (3) species with nearly closed alveoli, like *Caloneis silicula*.

Other scientists saw great difficulty in distinguishing *Caloneis* from *Pinnularia* and consider it is no longer possible to make a clear distinction. Based on valve morphology and chloroplast features, Cox (1988 b) concluded, that "there is as much or as little similarity between *Pinnularia* and *Caloneis* as they presently stand, as between species within each." Her investigation of the live structure supported three groups, which are different to those mentioned by Krammer and Lange-Bertalot (1985): (1) *Caloneis silicula*, *Caloneis bacillum* and *Pinnularia isostauron*; (2) *Caloneis* based on *C. amphisbaena*; (3) *Pinnularia* based on *P.*

nobilis. Round *et al.* (1990, p. 556) “were unable to find a satisfactory basis for the traditional separation of *Pinnularia* from *Caloneis* … and conclude that if *Pinnularia* is ever split, it will not be along the traditional boundary between the two genera”.

Mann (2001) also doubted the correctness of the traditional *Pinnularia-Caloneis* distinction and comes to the conclusion, that “until we have a clearer idea of relationships within the Pinnulariaceae, especially from gene sequence data, it may be best to accept the unsatisfactory classification that we have, rather than attempt to produce a new one that might be worse” (Mann, 2001, p. 34). But hitherto no extensive phylogenetic analysis based on molecular data has been made.

1.3 Molecular phylogenetics

It has long been evident, that there is useful information about evolutionary history in gene sequences. The wide application of this method began with the appearance of the polymerase chain reaction (PCR) in mid-1980 (Mullis *et al.*, 1986, Mullis and Faloona, 1987, Saiki *et al.*, 1988). Coupled with the direct didesoxynucleotide sequencing of amplified products, the technique became a powerful tool in life sciences. Sequences of several genes were used to reconstruct phylogenies of prokaryotes (e.g., Woese, 1987), single-cell eukaryotes (e.g., Medlin *et al.*, 1997) and higher plants (e.g., Soltis *et al.*, 2000) and animals (e.g., Söller *et al.*, 2000). Interest in phylogeny reconstruction has increased so rapidly that now roughly 4,000 articles that include a phylogenetic tree are published each year (Pagel, 1999).

1.3.1 Nuclear-encoded rRNA genes

Because rRNA genes serve a pivotal role in the protein synthesis machinery they occur universally in prokaryotic and eukaryotic cells without a change in their function. Because helical formation occurs in their secondary structure (Fig.1), which cannot change otherwise the function of the molecule would be lost, different regions evolve at very different rates (Woese, 1987). This combination of conserved and variable regions allows studies of most phylogenetic relationships from studies of deep phylogeny (e.g., Cavalier-Smith, 2004) to microdiversity surveys (e.g., Sáez *et al.*, 2003).

The rRNA genes are combined in multigene families with up to thousands of copies arranged in tandem arrays. Each individual repeat consists of the small subunit rRNA gene (SSU rRNA gene, SSU rDNA), the gene encoding the 5.8S rRNA, the large subunit rRNA gene (LSU rRNA gene, LSU rDNA) and two internal transcribed spacers, known as ITS 1 and ITS 2. The

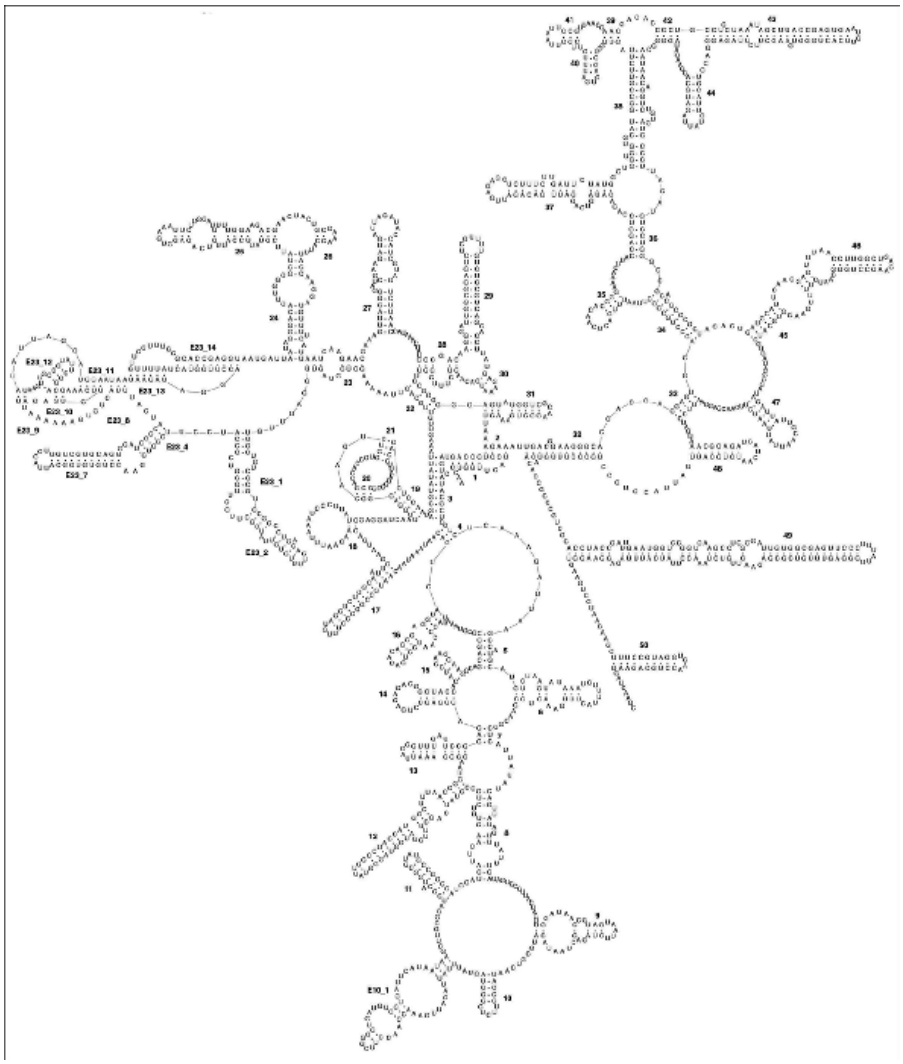


Fig.1: SSU rRNA secondary structure model of *Bacillaria paxillifer*
(The European Ribosomal RNA databank, http://rrna.uia.ac.be/)

internal transcribed spacers are located between the regions coding for small subunit rRNA and 5.8S rRNA, and between the latter and the large subunit rRNA coding region. In addition, an external transcribed spacer (ETS) occurs upstream to the small subunit rRNA gene. These transcription units were separated by an intergenic spacer (IGS). (Long & Dawid, 1980)

The multiple copies of this cluster appear to be highly homogenised within an organism and among different individuals of the same species. The main mechanism for this concerted evolution seem to be gene conversion between sister chromatids after replication and unequal crossing-over between homologous chromosomes (Schlötterer & Tautz, 1994). The high number of homogenized copies avoids the extensive sampling required for most single-copy genes. But some exceptions of the usual gene homogenization are known. For instance in some species of the protist *Plasmodium*, two different types of SSU rDNA exist, whose

expression is linked to different stages of the parasitic life cycle of this organisms (Gunderson *et al.*, 1987, Waters *et al.*, 1989, Qari *et al.*, 1994).

1.3.1.1 Small subunit rRNA gene

The SSU rRNA gene is the most widely used gene for inferring phylogenetic relationships. Thousands of partial and complete sequences (approx. 1800 bp in eukaryotes) from prokaryotes, single-celled and multicellular eukaryotes can be found in internet-available databases like GenBank (http://www.ncbi.nlm.nih.gov/). In diatoms, the gene has been used to study their position within the heterokont algae (e.g., Daugbjerg & Andersen, 1997), to reconstruct the evolution of the major classes (e.g., Medlin & Kaczmarska, 2004) or to assess the monophyly of diatom orders or genera (e.g., Beszteri *et al.*, 2001).

Kooistra & Medlin (1996) calculated a relatively fast substitution rate (1% per 18 to 26 Ma) in the SSU rDNA of diatoms. In the same study it was proven, that the evolutionary rate differs within the diatoms. In particular, the SSU rDNA of pennate taxa evolve more slowly than in the other diatom orders.

1.3.1.2 Large subunit rRNA gene

The LSU rRNA gene comprises more highly variable areas than the SSU rRNA gene (Van der Auwera & De Wachter, 1998). This indicates a stronger phylogenetic signal for closely related species in comparison with the SSU rRNA gene. But it may cause problems for reconstructing deep phylogenies because of saturation effects, the signal might be indistinct. Additionally, highly variable sequences are difficult to align. Because of the large size of LSU rDNA (over 3300 bp) complete sequences of this region are rare. Typically used sequences are derived from several parts of the gene, for example approximately 600 bp from the 5’ end of 26S rDNA (D1/D2 region).

1.3.2 Plastid-encoded protein-coding genes

Not all DNA in eukaryotes is stored within the cell nucleus. Organelles, like mitochondria or chloroplasts, contain their own DNA. Organelle genomes usually consist of a single DNA molecule and each gene is normally present only once. The chloroplast genome contains predominantly protein-coding genes. In protein-coding genes the evolution rate diverges between the different codon positions: The mutation rate at the third position is higher than the rates at the first or second position, because nucleotide changes at the third position in most cases are synonymous mutations. Synonymous mutations have no influence on the

amino acid coded and thus it appears that they depend only on the background mutation rate. But nucleotide changes at the first or second codon position nearly always lead to nonsynonymous substitutions, which result in a change of the encoded amino acid. Therfore the third codon position is downweighted or omitted very often, if protein-coding genes are used for phylogenetic analyses.

1.3.2.1 *rbc*L gene

The enzyme ribulose-1,5-bisphosphate carboxylase (RUBISCO) is responsible for fixation of carbon dioxide in the Calvin cycle. The holoenzyme is formed by a 16-mer structure that includes eight identical chloroplast-encoded large subunit polypeptides and eight small subunit polypeptides. The *rbc*L gene encodes the large subunit of RUBISCO and is located in a single-copy region of the chloroplast genome. It is typically 1428-1434 bp in length and insertions or deletions are extremely rare (Soltis & Soltis, 1998). Although some chloroplast-encoded genes are interrupted by introns, this is not the case for the *rbc*L gene (Clegg, 1993). This positional conservation of coding information permits the unambiguous alignment of *rbc*L sequences.

The relative rate of evolution of SSU rRNA and *rbc*L genes varies among groups. The *rbc*L gene generally evolves about three times faster than SSU rDNA in angiosperms but is slower in Orchidaceae (Soltis & Soltis, 1998). Within the phaeophytes, a slightly faster mutation rate of the *rbc*L gene has been observed (Draisma & Prud'homme van Reine). Compared to SSU rDNA, the *rbc*L gene appears more suited to studies of evolution at lower (order to genus) levels of taxonomic hierarchy in diatoms (Mann *et al.*, 2001).

1.3.3 Gene combination

A gene phylogeny based on a single gene may not agree with the organismal phylogeny because of such biological processes as introgression, lineage sorting and gene duplication (Hillis, 1987, Doyle, 1992, Lutzoni & Vilgalys, 1995). Therefore phylogenetic trees derived from different data sets may also differ. If the primary interest is the phylogeny of organisms rather than genes, this problem of differential phylogenetic history among data sets argues for the use of multiple data sets, often concatenated. Several studies have suggested that data sets should not be combined if the data partitions are heterogenous (e.g., Bull *et al.*, 1993, Huelsenbeck *et al.*, 1996). The incongruence length difference (ILD) test (Farris *et al.*, 1994) or the equivalent partition homogeneity test (Swofford, 1995) have been used to determine whether or not to combine data sets for phylogenetic analysis (e.g. Johnson & Sorensen, 1998,

Hoot *et al.*, 1999). But other studies have found that *P*-values < 0,05 should not preclude dataset combination (e.g., Sullivan, 1996, Davis *et al.*, 1998, Flynn & Nedbal, 1998, Yoder *et al.*, 2001).

Both simulations (e.g., Hillis, 1996, Graybeal, 1998) and empirical studies (e.g., Soltis *et al.*, 1998, Soltis *et al.*, 2000) indicate that additional data can improve phylogenetic inferences of molecular phylogenies. For example, analyses of angiosperm relationships on the basis of gene sequences for *rbc*L, *atp*B and 18S rDNA showed increased resolution and internal support (as measured by bootstrap values), and faster run times when the data sets for these genes were combined rather than analysed separately (Soltis *et al.*, 1998, Soltis *et al.*, 2000). Sorhannus (2001) analysed heterokont phylogeny based on a combined dataset (SSU rDNA, LSU rDNA, *rbc*L gene and morphological data) using one exemplar of each major group. But he did not find greatly increased support among class relationships in his analysis.

1.3.4 Molecular phylogenies of diatoms

Most molecular phylogenies of diatoms have been reconstructed from the nuclear-encoded small subunit (SSU) and the large subunit (LSU) ribosomal RNA genes (Medlin *et al.*, 1991, 1993, Sorhannus *et al.*, 1995, Kooistra & Medlin, 1996, Medlin *et al.*, 1996 a, b, Van der Auwera & De Wachter, 1998, Medlin *et al.*, 2000, Beszteri *et al.*, 2001, Lundholm & Moestrup, 2002, Lundholm *et al.*, 2002 a,b, Kooistra *et al.*, 2003, Behnke *et al.*, 2004, Medlin & Kaczmarska, 2004, Sorhannus, 2004). In addition the internal transcribed spacer regions in the nuclear-encoded ribosomal DNA cistron (Zechmann *et al.*, 1994, Behnke *et al.*, 2004), the mitochondrion-encoded cytochrome c oxidase subunit I (coxA, Ehara *et al.*, 2000), the chloroplast-encoded elongation factor Tu (*tufA*, Delwiche *et al.*, 1995, Medlin *et al.*, 1997), the chloroplast-encoded RNA polymerase alpha subunit (*rpoA*, Fox & Sorhannus, 2003) and the chloroplast-encoded ribulose-1,5-bisphosphate carboxylase large subunit (*rbc*L, Daugbjerg & Andersen, 1997, Daugbjerg & Guillou, 2001, Mann *et al.*, 2001) have been used for studying molecular systematics in diatoms or their relationship within the heterokont algae.

The majority of molecular studies investigating the evolution of diatoms have used species from all classes (e.g., Medlin *et al.*, 1993, Sorhannus *et al.*, 1995, Medlin *et al.*, 1996 a, b, 2000, Kooistra *et al.*, 2003, Medlin & Kaczmarska, 2004, Sorhannus, 2004). But in these studies most orders are represented by three or less species. Few molecular studies have been carried out with focus on some closely related genera (e.g., Zechmann *et al.*, 1994, Beszteri *et al.*, 2001, Lundholm & Moestrup, 2002, Lundholm *et al.*, 2002 a, b, Behnke *et al.*, 2004).

Only two of these studies (Beszteri *et al.*, Behnke *et al.*, 2004) concentrated on species belonging to the Naviculaceae *sensu* Krammer and Lange-Bertalot (1986). Beszteri *et al.* (2001) determined SSU rDNA sequences of six naviculoid species. Their results slightly contradicted the monophyly of the Naviculaceae, because *Gomphonema parvulum* did not cluster within this group. Based on their data Beszteri *et al.* (2001) concluded, that further SSU rDNA sequences from close relatives of *G. parvulum* could possibly reinforce or reject the hypothesis about Naviculaceae being a monophyletic group. In more recent studies based on a large number of sequences (Kooistra *et al.*, 2003, Medlin & Kaczmarska, 2004, Sorhannus, 2004) *G. parvulum* cluster within naviculoid diatoms. But the Naviculaceae did not form a monophyletic group in these studies, because genera like *Surirella* (family Surirellaceae) or *Cocconeis* (family Achnanthaceae) cluster within the Naviculaceae. The study of Behnke *et al.* (2004) concentrated on the genus *Sellaphora* and interclonal relationships of several clones of *S. pupula*. In the SSU rDNA phylogeny shown in this study, the Naviculaceae form a monophyletic group. But this tree did not include species belonging to the families Surirellaceae or Achnanthaceae. This was the first dataset containing a *Navicula sensu stricto* (*N. cryptocephala*) and a *Navicula sensu lato* (*N. pelliculosa*, section *Minusculae*) and the two species were clearly separated in the inferred phylogeny. The greatest number of naviculoid species was present in the dataset used by Sorhannus (2004). Even there only four genera were represented by more than one species. In the shown phylogeny inferred with SSU rDNA sequences only the genus *Gomphonema* (represented by two species) formed a monophyletic clade. *Amphora* (three species), *Eolimna* (two species) and *Navicula sensu stricto* (two species) did not form a monophyletic group.

1.4 Aims of this study

Since the electron microscopy was introduced to diatom research and features of live cells, ecology and molecular data were taken into account, many changes in diatom taxonomy have occurred. The taxonomy of the family Naviculaceae (*sensu* Krammer & Lange-Bertalot, 1986) has been changing greatly. Based on morphology the whole family, as well as many of its genera, have undergone revisions (e.g., Round *et al.*, 1990). But little work has been carried out with molecular data for this large and ecologically interesting group of diatoms.

In order to estimate evolutionary relationships within the Naviculaceae (sensu Krammer & Lange-Bertalot, 1986) and to access the nomenclatural problems I performed phylogenetic analyses of several freshwater species. But a gene tree based on a single gene does not necessarily agree with the true species tree, that represents the actual evolutionary pathway of

the species involved. Therefore three different genes were sequenced for each culture and phylogenies were reconstructed for each gene and a phylogenetic analysis based on a combined data set of all three genes was conducted. Adittionally the morphology of the sequenced species was investigated.

2. Materials and Methods

2.1. Cultures

The cultures used in this study were established within the scope of the AlgaTerra project (http://www.algaterra.net/). The field samples were taken from several terrestrial, freshwater and brackish habitats in northern Germany (Fig.2). Between November 2001 and September 2003 220 samples from 83 different sites were taken.

Fig.2: Sampling sites (map from Stiefel Verlag GmbH, Lenting)

Cultures were initiated from these samples using a DY-IV medium (Andersen *et al.*, 1997) mixed 2:1 with filter-sterilized (pore size: 0,1 µm) water from the sampling sites. After one to four days, clonal cultures were isolated from these initial cultures. Most of these isolates still contain small flagellates. In order to purge these flagellates from the cultures a small number of diatom cells was transferred to fresh medium several times and than grown on agar plates (see recipe below) for one to three weeks. From these plates a small number of diatom cells were transferred to liquid medium. If necessary the entire procedure was repeated several times.

Recipe for agar plates:

- 9 g Agar Agar was diluted in ½ litre deionised water and autoclaved
- Double concentrated DY-IV medium was filter-sterilized (pore size: 0,1 µm)
- Both mixtures were temperated to approximately 60°C and mixed 1:1.

All isolates were grown under a 14/10 light/dark cycle with photon flux densities between 30 and 120 µM photons m^{-2} s^{-1} at 15°C. The clonal cultures were grown in modified DY IV medium (Andersen *et al.*, 1997) enriched with 5%-10% soil-extract (see recipe below). For isolates from alkaline, acid or brackish habitats the media was adjusted by addition of sodium hydroxide, hydrochloric acid or IMR-media (Eppley *et al.*, 1967).

Recipe for soil-extract:

- One l dry nonfertilized garden soil (J.Arthur Bower's African Violet Compost, William Sinclair Horticulture Ltd.) was saturated with bidistilled water and infused for several days at room temperature.
- After autoclaving, the hot water/soil-mixture was filtered through a laboratory paper filter.
- Afterwards, the mixture was filtered several times with with stepwise reduced pore size (10 µm, 5µm, 3µm and 2µm).

The 91 cultures used for this study contain 72 species belong to 22 genera and were isolated from 45 different field samples. Eighty-one cultures contain 62 species belonging to the family Naviculaceae. Because monoraphid species of the family Achnanthaceae cluster within the Naviculaceae in several studies (Kooistra *et al.*, 2003, Medlin & Kaczmarska, 2004, Sorhannus, 2004), I additionally used sequences of species belonging to this family. Three cultures contain *Eunotia* species. Centric and araphid species were used as outgroup. All cultures grown for this study and their place of origin are shown in Table 1.

Tab. 1: List of diatom cultures established and sequenced within the scope of this study.

DNA-				place of origin		
preparation	culture	species	author	GPS	discription	source
1438	AT_196Gel02	*Achnanthidium minutissimum*	(Kützing) Czarnecki	54°10,97N; 10°37,92E	Ukelei See	lake, plankton
1427	AT_212.06	*Amphora* cf. *fogediana*	Krammer	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1264	AT_117.10	*Amphora libyca*	Ehrenberg	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1263	AT_105Gel05	*Amphora normannii*	Rabenhorst	53°09,90N; 08°45,10E	Wümme	river, benthos
1265	AT_117.11	*Amphora pediculus*	(Kützing) Grunow	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1554	AT_221.04	*Amphora* sp.	Ehrenberg ex Kützing	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, plankton
1256 [(1, 3)]	AT_67.02b	*Asterionella formosa*	Hassall	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	river, plankton
1550	AT_177.07	*Caloneis amphisbaena*	(Bory) Cleve	53°04,08N; 08°29,04E	Hasbruch, near hunting lodge	ditch, benthos
1323	AT_220.06	*Caloneis budensis*	(Grunow) Krammer	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	riverside, soil
1446	AT_160Gel04	*Caloneis lauta*	Carter & Bailey-Watts	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1415	AT_212.07	*Cocconeis pediculus*	Ehrenberg	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1418	AT_212Gel11	*Cocconeis placentula*	Ehrenberg	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1318	AT_200.05	*Craticula cuspidata*	(Kützing) D.G. Mann	54°11,69N; 10°36,24E	Krumm See	lake, benthos
1320	AT_219.03	*Craticula cuspidata*	(Kützing) D.G. Mann	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, benthos
1283	AT_5Nav02	*Craticula halophilioides*	(Hustedt) Lange-Bertalot	53°09,65N; 08°43,40E	Maschinenfleet	canal, plankton
1308	AT_36klein	*Craticula halophilioides*	(Hustedt) Lange-Bertalot	53°12,72N; 08°26,85E	Weser, near Rekum	river, benthos
1284	AT_70Gel14a	*Craticula molestiformis*	(Hustedt) Lange-Bertalot	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1493 [(2, 3)]	AT_L1840	*Cyclotella choctawatcheeana*	Prasad		Geeste, near Bremerhaven	river
1414	AT_204Gel02	*Cymbella affinis*	Kützing	54°09,09N; 10°27,45E	Großer Madebroken See	lake, plankton
1423	AT_213.04	*Cymbella affinis*	Kützing	54°19,86N; 10°17,72E	Dobersdorfer See	lake, periphyton
1421	AT_210Gel07	*Cymbella aspera*	(Ehrenberg) Cleve	54°09,98N; 10°25,19E	Trammer See	lake, periphyton
1431	AT_194Gel07	*Cymbella helmckei*	Krammer	54°08,53N; 10°39,70E	Großer Eutiner See	lake, benthos
1317	AT_177.04	*Cymbella naviculiformis*	(Auerswald) Cleve	53°04,08N; 08°29,04E	Hasbruch, near hunting lodge	ditch, benthos

[(1)] DNA and SSU rDNA sequence provided by I. Jung; [(2)] DNA and SSU rDNA sequence provided by B. Beszteri; [(3)] species used as outgroup

Tab. 1: Continued

DNA-				place of origin		
preparation	culture	species	author	GPS	discription	source
1324	AT_221.02	*Cymbella naviculiformis*	(Auerswald) Cleve	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, plankton
1422	AT_210Gel13	*Cymbella proxima*	Reimer	54°09,98N; 10°25,19E	Trammer See	lake, periphyton
1441	AT_214Gel03	*Encyonema caespitosum*	Kützing	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1266	AT_137.13	*Encyonema minutum*	(Hilse) D.G. Mann	53°41,96N; 11°29,15E	Schweriner See	lake, plankton
1267	AT_70Gel18	*Eolimna minima*	(Grunow) Lange-Bertalot	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1268	AT_111Gel09	*Eunotia formica*	Ehrenberg	53°11,39N; 08°47,05E	Hamme, near sluice	river, plankton
1321	AT_219.07	*Eunotia implicata*	Nörpel, Lange-Bertalot & Alles	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, benthos
1269	AT_73Gel02	*Eunotia* sp.	Ehrenberg	53°38,11N; 10°44,56E	Pinnsee	lake, periphyton
1254 [(1, 3)]	AT_185Gel03	*Fragilaria crotonensis*	Kitton	54°08,53N; 10°39,70E	Großer Eutiner See	river, plankton
1410	AT_124.05b	*Fragilaria* sp.	Lyngbye	53°33,00N; 10°55,16E	Schaalsee, Zarrentiner Becken	lake, benthos
1445	AT_108Gel03	*Frustulia vulgaris*	(Thwaites) De Toni	53°10,89N; 08°45,70E	Hamme, near bridge	river, benthos
1424	AT_219Gel10	*Gomphonema acuminatum*	Ehrenberg	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, benthos
1439	AT_196Gel03	*Gomphonema affine*	Kützing	54°10,97N; 10°37,92E	Ukelei See	lake, plankton
1322	AT_219Gel06	*Gomphonema affine*	Kützing	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	river, benthos
1409	AT_109Gel08b	*Gomphonema* cf. *angustatum*	(Kützing) Rabenhorst	53°10,89N; 08°45,70E	Hamme, near bridge	river, plankton
1315	AT_161.15	*Gomphonema* cf. *parvulum*	(Kützing) Kützing	52°57,65N; 08°20,67E	Poggenpohls Moor	puddle, soil
1270	AT_117.09	*Gomphonema micropus*	Kützing	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1271	AT_117Gel21	*Gomphonema micropus*	Kützing	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1313	AT_160Gel27	*Gomphonema productum*	(Grunow) Lange-Bertalot & Reichardt	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1552	AT_195Gel09	*Gomphonema truncatum*	Ehrenberg	54°08,53N; 10°39,70E	Großer Eutiner See	lake, periphyton
1272	AT_124.24	*Hippodonta capitata*	(Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	53°33,00N; 10°55,16E	Schaalsee, Zarrentiner Becken	lake, benthos
1273	AT_104Gel12a	*Luticola goeppertiana*	(Bleisch) D.G. Mann	53°09,90N; 08°45,10E	Wümme	river, plankton
1274	AT_115Gel07	*Mayamaea atomus* var. *atomus*	(Kützing) Lange-Bertalot	53°11,79N; 08°48,11E	Hamme, near Osterholz	river, benthos

[(1)] DNA and SSU rDNA sequence provided by I. Jung; [(3)] species used as outgroup

Tab. 1: Continued

DNA-				place of origin		
preparation	culture	species	author	GPS	discription	source
1275	AT_101Gel04	*Mayamaea atomus* var. *permitis*	(Hustedt) Lange-Bertalot	53°40,20N; 10°50,21E	Schwarze Kuhle	lake, periphyton
1425	AT_111Gel10	*Navicula brockmannii*	Hustedt	53°11,39N; 08°47,05E	Hamme, near sluice	river, plankton
1417	AT_212Gel07	*Navicula capitatoradiata*	Germain	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1310	AT_82.04c	*Navicula cari*	Ehrenberg	53°36,36N; 10°54,02E	Küchensee	lake, periphyton
1279	AT_114Gel08c	*Navicula cryptocephala*	Kützing	53°13,63N; 08°53,22E	Hamme, near Worpswede	river, periphyton
1316	AT_176Gel05	*Navicula cryptocephala*	Kützing	53°04,08N; 08°29,04E	Hasbruch, near hunting lodge	ditch, plankton
1416	AT_212Gel01	*Navicula cryptotenella*	Lange-Bertalot	54°19,86N; 10°17,72E	Dobersdorfer See	lake, benthos
1420	AT_210Gel05	*Navicula cryptotenella*	Lange-Bertalot	54°09,98N; 10°25,19E	Trammer See	lake, periphyton
1435	AT_202Gel03	*Navicula cryptotenella*	Lange-Bertalot	54°09,86N; 10°32,81E	Dieksee	lake, benthos
1280	AT_117Gel05	*Navicula gregaria*	Donkin	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1436	AT_160Gel09	*Navicula hambergii*	Hustedt	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1430	AT_177.13	*Navicula integra*	(W. Smith) Ralfs	53°04,08N; 08°29,04E	Hasbruch, near hunting lodge	ditch, benthos
1278	AT_114Gel06	*Navicula radiosa*	Kützing	53°13,63N; 08°53,22E	Hamme, near Worpswede	river, periphyton
1433	AT_200.04	*Navicula radiosa*	Kützing	54°11,69N; 10°36,24E	Krumm See	lake, benthos
1440	AT_205.02b	*Navicula radiosa*	Kützing	54°09,09N; 10°27,45E	Großer Madebroken See	lake, benthos
1282	AT_124.15	*Navicula reinhardtii*	Grunow	53°33,00N; 10°55,16E	Schaalsee, Zarrentiner Becken	lake, benthos
1411	AT_145.08	*Navicula* sp.1	Bory	54°06,55N; 10°48,68E	Neustädter Binnenwasser	brackish water, plankton
1319	AT_201Gel01	*Navicula* sp.2	Bory	54°11,69N; 10°36,24E	Krumm See	lake, benthos
1434	AT_202.01	*Navicula tripunctata*	(O. F. Müller) Bory	54°09,86N; 10°32,81E	Dieksee	lake, benthos
1276	AT_108Gel01	*Navicula veneta*	Kützing	53°10,89N; 08°45,70E	Hamme, near bridge	river, benthos
1277	AT_110Gel19	*Navicula veneta*	Kützing	53°11,39N; 08°47,05E	Hamme, near sluice	river, benthos
1281	AT_117Gel20b	*Navicula veneta*	Kützing	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1551	AT_177.12	*Neidum affine*	(Ehrenberg) Pfitzer	53°04,08N; 08°29,04E	Hasbruch, near hunting lodge	ditch, benthos

Tab. 1: Continued

DNA-				place of origin		
preparation	culture	species	author	GPS	discription	source
1426	AT_161.03	*Pinnularia acrosphaeria*	Rabenhorst	52°57,65N; 08°20,67E	Poggenpohls Moor	puddle, soil
1286	AT_100Gel01	*Pinnularia anglica*	Krammer	53°40,20N; 10°50,21E	ditch between Plötscher See and Schwarze Kuhle	ditch, periphyton
1314	AT_160Gel30	*Pinnularia mesolepta*	(Ehrenberg) W. Smith	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1429	AT_161.05	*Pinnularia mesolepta*	(Ehrenberg) W. Smith	52°57,65N; 08°20,67E	Poggenpohls Moor	puddle, soil
1287	AT_105Gel08	*Pinnularia microstauron*	(Ehrenberg) Cleve	53°09,90N; 08°45,10E	Wümme	river, benthos
1288	AT_112Gel04	*Pinnularia microstauron*	(Ehrenberg) Cleve	53°11,39N; 08°47,05E	Hamme, near sluice	river, periphyton
1289	AT_113Gel11	*Pinnularia microstauron*	(Ehrenberg) Cleve	53°13,63N; 08°53,22E	Hamme, near Worpswede	river, plankton
1290	AT_69.06	*Pinnularia microstauron*	(Ehrenberg) Cleve	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	river, periphyton
1292	AT_70Gel12b	*Pinnularia obscura*	Krasske	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1311	AT_160Gel10	*Pinnularia rupestris*	Hantzsch	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1285	AT_100.01	*Pinnularia subcapitata*	Gregory	53°40,20N; 10°50,21E	ditch between Plötscher See and Schwarze Kuhle	ditch, periphyton
1442	AT_70.09	*Pinnularia substreptoraphe*	Krammer	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1291	AT_70.10	*Pinnularia viridiformis*	Krammer	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1428	AT_161.02	*Pinnularia viridis*	(Nitzsch) Ehrenberg	52°57,65N; 08°20,67E	Poggenpohls Moor	puddle, soil
1312	AT_160Gel18	*Placoneis elginensis*	(Gregory) E. J. Cox	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1419	AT_220.09	*Placoneis* sp.	Mereschkowsky	53°06,41N; 08°11,23E	Hunte, near Hundsmühlen	riverside, soil
1412	AT_160Gel11	*Stauroneis anceps*	Ehrenberg	52°57,65N; 08°20,67E	Poggenpohls Moor	soil, moss
1294	AT_117Gel17	*Stauroneis gracilior*	Reichardt	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1309	AT_70.12	*Stauroneis kriegerii*	Patrick	53°13,79N; 08°41,06E	Geeste, bridge near Bramel	riverside, moss
1444	AT_101.02	*Stauroneis kriegerii*	Patrick	53°40,20N; 10°50,21E	Schwarze Kuhle	lake, periphyton
1293	AT_117.04	*Stauroneis phoenicenteron*	(Nitzsch) Ehrenberg	53°09,51N; 08°42,57E	Lesum, near river mouth	river, plankton
1437	AT_182.07	*Stauroneis phoenicenteron*	(Nitzsch) Ehrenberg	53°08,06N; 08°53,87E	Wümme, Borgfeld	river, plankton

2.2. DNA Methods

2.2.1 DNA isolation

Culture material was concentrated by filtration and quick-frozen in liquid nitrogen. Nucleic acids were extracted using the Invisorb® Spin Plant Mini Kit (Invitek GmbH, Berlin, Germany). The given protocol was only modified by a duplication of the two washing steps.

2.2.2. PCR

For each culture, the small subunit rRNA coding gene (SSU rDNA), the D1-D2 region of the large-subunit rRNA gene (LSU rDNA) and the middle part of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbc*L) were amplified using the polymerase chain reaction (PCR; Saiki *et al.*, 1988). In the *rbc*L gene sequence of *Rhizosolenia setigera* (GenBank accession number: AF015568) the sequence of the primers F3 and R3 can be found at the position 292-314 and 1028-1051, respectively. The primers and conditions used for PCR are shown in the Tables 2 and 3.

Tab. 2: Primers used for PCR

Gene	Primer	Sequence (5' → 3')	Author
SSU rRNA	1F	AAC CTG GTT GAT CCT GCC AGT	Medlin *et al.* (1988), without polylinker
	1528R	TGA TCC TTC TGC AGG TTC ACC TAC	Medlin *et al.* (1988), without polylinker
LSU rRNA	DIRF	ACC CGC TGA ATT TAA GCA TA	Scholin *et al.* (1994)
	D2CR	CCT TGG TCC GTG TTT CAA GA	Scholin *et al.* (1994)
*rbc*L	F3	GCT TAC CGT GTA GAT CCA GTT CC	Beszteri, unpubl.
	R3	CCT TCT AAT TTA CCA ACA ACT G	Beszteri, unpubl.

Tab. 3: Used PCR programs

Cycle step	SSU an LSU rRNA		*rbc*L	
	Temperature	Time	Temperature	Time
Initial denaturation	94°C	7 min	94°C	10 min
	Cycle		Cycle	
Denaturation	94°C	2 min	94°C	1 min
Annealing	54°C	4 min	56°C	1 min
Elongation	72°C	2 min	72°C	2 min
Cycle repetitions	35		31	
Final elongation	72°C	7 min	72°C	10 min

The PCR-products were purified by MinElute™ PCR Purification Kit (QIAGEN, Germany) according to the manufacturer's protocol. PCR products with multiple bands were purified by excising from a 1% agarose gel.

2.2.3. Sequencing

PCR products were sequenced directly on both strands using Big Dye Terminator v3.1 sequencing chemistry (Applied Biosystems, CA, USA). For the LSU rRNA gene and the *rbc*L-gene the sequencing reactions were made using the same primers already used in the PCR. Because of the length of the SSU rRNA gene, additional internal primers (Table 4) were used. The conditions used for sequencing reaction are shown in table 5. Sequencing products were purified by DyeEx™ Spin Kit (QIAGEN, Germany) and electrophoresed on an ABI 3100 Avant sequencer (Applied Biosystems, CA, USA).

Tab. 4: Additional primers used in the sequencing reactions of the SSU rDNA

Primer	Sequence (5' → 3')	Author
528F	GCG GTA ATT CCA GCT CCA A	Elwood *et al.* (1985)
1055F	GGT GGT GCA TGG CCG TTC TT	Elwood *et al.* (1985)
536R	AAT TAC CGC GGC KGC TGG CA	Elwood *et al.* (1985)
1055R	ACG GCC ATG CAC CAC CAC CCA T	Elwood *et al.* (1985)

Tab. 5: Used program for the sequencing reaction

Cycle step	Temperature	Time
Initial denaturation	96°C	1 min
	Cycle	
Denaturation	96°C	10 sec
Annealing	50°C	5 sec
Elongation	60°C	4 min
Cycle repetitions	25	

2.3. Sequence Analysis

Sequences exported from corrected electropherograms were assembled using SeqMan (Lasergene package, DnaStar, Madison, WI, USA). For the protein-coding *rbc*L-gene, the protein-sequence was checked additionally. The alignment of the SSU rDNA sequences was done with ARB using the secondary structure. The sequences of the D1-D2 region and the *rbc*L Gene were aligned using ClustalX (Thompson *et al.*, 1997) and checked manually using

ProSeq v 2.9 beta (Filatov, 2002). The rRNA genes show hypervariable regions for which it is difficult to obtain an unambiguous alignment. These highly variable sites were excluded from the alignment.

To get three gene trees with the same set of species an alignment was computed for each gene using only the sequences of the cultures established within the scope of this study (Table 1). For each gene a second alignment was made using additional sequences obtained from GenBank (Table 6).

Tab. 6: List of species of diatoms obtained from GenBank and their accession numbers of the used gene sequences

Species		SSU rRNA	LSU rRNA	*rbc*L
Achnanthes	*bongranii*	AJ535150		
Achnanthes	*brevipes*	AY485476		
Achnanthes	*minutissima*	AJ866992		
Achnanthes	sp.	AY485496		
Achnanthes	sp.2	AJ535151		
Achnanthidium	cf. *longipes*	AY485500		
Amphiprora	*alata*	AY485497		
Amphiprora	*paludosa*	AY485468		
Amphora	cf. *capitellata*	AJ535158		
Amphora	cf. *proteus*	AJ535147		
Amphora	*coffeaeformis*	AY485498	AF417682	
Amphora	*montana*	AJ243061		
Amphora	sp.	AB183590		
Anomoeoneis	*sphaerophora*	AJ535153		
Bacillaria	*paxillifer*	M87325	AF417678	
Campylodiscus	*ralfsii*	AJ535162		
Cocconeis	cf. *molesta*	AJ535148		
Cylindrotheca	*closteriva*	M87326		
Cymatopleura	*elliptica*	AJ867030		
Cymbella	*cymbiformis*	AJ535156		
Diadesmis	*gallica*	AJ867023		
Dickieia	*ulvacea*	AY485462		
Encyonema	cf. *sinicum*			AY571754
Encyonema	*triangulatum*	AJ535157		
Entomoneis	cf. *alata*	AJ535160		
Entomoneis	sp.		AF417683	
Eolimna	*minima*	AJ243063		
Eolimna	*subminuscula*	AJ243064		
Eunotia	*minor*			AY571744
Eunotia	*bilunaris*	AJ866995		
Eunotia	cf. *pectinalis* f. *minor*	AJ535146		
Eunotia	*formica* var. *smatrana*	AB085830		
Eunotia	*monodon* var. *asiatica*	AB085831		
Eunotia	*pectinalis*	AB085832		
Eunotia	sp.	AJ535145		

Tab. 6: Continued

Species		SSU rRNA	LSU rRNA	*rbc*L
Fragilariopsis	*cylindrus*	AY672802	AF417657	
Gomphonema	*capitatum*			AY571751
Gomphonema	*parvulum*	AJ243062		
Gomphonema	*pseudaugur*	AB085833		
Gyrosigma	*limosum*	AY485516		
Haslea	*crucigera*	AY485482		
Haslea	*nipkowii*	AY485488		
Haslea	*ostrearia*	AY485523		
Haslea	*pseudostrearia*	AY485524		
Lyrella	*atlantica*	AJ544659		AY571747
Lyrella	*hennedyi*			AY571755
Lyrella	sp.			AY571756
Lyrella	sp.2	AJ535149		
Navicula	*atomus* var. *permitis*	AJ867024		
Navicula	cf. *duerrenbergiana*			AY571749
Navicula	cf. *erifuga*		AF417679	
Navicula	*cryptocephala* var. *veneta*	AJ297724		
Navicula	*diserta*	AJ535159		
Navicula	*lanceolata*	AY485484		
Navicula	*pelliculosa*	AY485454		
Navicula	*phyllepta*	AY485456		
Navicula	*ramosissima*	AY485512		
Navicula	*salinicola*			AY604699
Navicula	*saprophila*	AJ867025		
Navicula	*sclesviscensis*	AY485483		
Navicula	sp.	AY485513		
Navicula	sp.2	AY485502		
Navicula	sp.3	AY485460		
Nitzschia	*amphibia*	AJ867277		
Nitzschia	*apiculata*	M87334		
Nitzschia	*communis*	AJ867278	AF417661	
Nitzschia	*frustulum*	AJ535164	AF417671	
Nitzschia	*sigma*	AJ867279		
Nitzschia	*vitrea*	AJ867280		
Pauliella	*taeniata*	AY485528	AF417680	
Peridinium balticum endosymbiont		Y10566		
Peridinium foliaceum endosymbiont		Y10567		
Petroneis	*humerosa*			AY571757
Phaeodactylum	*tricornutum*	AY485459	AF417681	
Pinnularia	cf. *interrupta*	AJ544658		
Pinnularia	*rupestris*	AJ867027		
Pinnularia	sp.	AJ535154		
Placoneis	cf. *paraelginensis*			AY571753
Placoneis	*constans*			AY571752
Pleurosigma	*intermedium*	AY485489		
Pleurosigma	*planktonicum*	AY485514		
Pleurosigma	sp.	AY485515		

Tab. 6: Continued

Species		SSU rRNA	LSU rRNA	*rbc*L
Pleurosigma	sp.2	AF525664		
Pseudogomphonema	cf. *kamschaticum*			AY571748
Pseudogomphonema	sp.	AJ535152		
Pseudogomphonema	sp.	AF525663		
Rossia	sp.	AJ535144		
Sellaphora	*bacillum*			AY571745
Sellaphora	*laevissima*	AJ544655		
Sellaphora	*pupula*	AJ544649		AY571746
Sellaphora	*pupula* var. *captitata*	AJ535155		
Seminavis	cf. *robusta*			AY571750
Stauroneis	*constricta*	AY485521		
Surirella	*angusta*	AJ867028		
Surirella	*brebissoni*	AJ867029		
Surirella	*fastuosa* var. *cuneata*	AJ535161		
uncultured *Eunotia*-like diatom		AY821975		
Undatella	sp.	AJ535163		

Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford, 1998). In all analyses the data set was rooted using one centric (*Cyclotella choctawatcheea*) and two araphid diatoms (*Fragilaria crotonensis* and *Asterionella formosa*), as the use of several outgroup taxa improves the analyses (Swofford *et al.*, 1996). For maximum likelihood (ML) and distance based tree calculations, likelihood scores of different nucleotide substitution models were compared on a neighbor joining tree using Modeltest 3.0 (Posada & Crandall, 1998). Based on the Akaike Information Criterion (AIC) the best fit model was detected (Table 7). This was used for phylogenetic analyses using ML and neighbor joining (NJ) tree inference with ML distances. Maximum parsimony (MP) and ML trees were obtained in heuristic searches, with 10 random taxa addition sequences. To assess confidence in clades recovered bootstrapping of MP and NJ analyses was made with 1000 replicates. If necessary, a time limit of 15 minutes was set for each replicate. The used PAUP command blocks for all analyses are shown in the appendix.

Tab. 7: Best fit models to perform ML based tree calculations detected by Modeltest based on AIC (modelblocks are shown in the appendix)

aligned sequences	gene			
	SSU rRNA	LSU rRNA	*rbc*L	combination
own cultures	GTR +I +G	TrN +I +G	GTR +I +G	GTR +I +G
own cultures and sequences obtained from GenBank	GTR +I +G	GTR +I +G	GTR +I +G	

For weighting the positions in the dataset of the *rbc*L gene sequences, the entire dataset was transferred into MacClade (Maddison and Maddison, 1989). In MacClade the third position was downweighted and the resulting weight block was added to the dataset. Then the entire weighted dataset was transferred back to PAUP and the phylogenetic analyses were performed.

For the combined dataset 100 replicates of the partition homogeneity test, as implemented in PAUP, were performed.

2.4. Microscopy

For identification and morphological investigations of the cultures, light and electron microscopy were used. Living cells as well as cleaned frustules were examined and photographed by bright field microscopy using a ZEISS Axioplan microscope with a AxioCam MRc digital camera. In addition, electron micrographs of cleaned frustules were taken at 10kV accelerating voltage on a Quanta FEG 200F, a PHILIPS XL30 ESEM or an I.S.I. DS-130.

2.4.1 Purification of the frustules

To remove all organic material, the cells were oxidized with $KMnO_4$ for 12-16 hours. Then HCl was added and the mixture boiled until it turned light yellow. The liquid was discarded and the frustules were washed 4 times with distilled water. The cleaned frustules were stored in distilled water.

2.4.2. Slide preparation

To prepare permanent slides several drops of cleaned frustule material was placed on a coverslip and dried on a heating plate at 60°C. Slides for light microscopy were provided with a drop of a Naphrax/toluene-mixture and the coverslips were placed on this drop. The toluene was evaporated on a heating plate at 200°C.

For electron microscopy the coverslips were attached to aluminium specimen stubs by double-sided adhesive tape. The stubs were platinum-coated with a sputter coater (Emscope SC 500).

3. Results

3.1 Molecular data

For 89 of the 91 established cultures the SSU rRNA gene, the D1/D2-region of the LSU rRNA gene and the *rbc*L gene were sequenced successfully. From *Encyonema minutum* (DNA preparation number 1266) and *Frustulia vulgaris* (DNA preparation number 1445) only the D1/D2-region of the LSU rRNA gene and the SSU rRNA gene respectively could be sequenced successfully. Molecular phylogenies were reconstructed on the base of seven alignments. Four datasets only consists of sequences of the 89 cultures for which all three genes could be sequenced: One alignment for each gene and one dataset combining these alignments. For each gene an additional alignment was made comprising the available sequences from all cultures and sequences obtained from GenBank.

3.1.1 SSU rRNA gene

The SSU rDNA sequences for the sequenced taxa were approximately 1750 nucleotides in length excluding amplification primers, with the exception of *Luticola goeppertiana* (DNA preparation number 1273), which is longer (1904 nucleotides) because of several insertions. One highly variable region in the SSU rDNA alignment could not be aligned unambiguously. This segment of 114 nucleotides was excluded from the analyses. It corresponds with the nucleotides 676 to 790 in the sequence from *Luticola goeppertiana*. The final dataset had 1827 positions in total, of which 442 were parsimony-informative and 196 parsimony-uninformative characters.

The maximum-likelihood (ML) tree based on the sequences from the AlgaTerra cultures is shown in Fig. 3. The condensed regions of this figure are shown in detail in Fig. 4.

The three araphid taxa appeared at the base of the ML tree. *Asterionella formosa* diverged first, followed by the *Fragilaria* species. The three *Eunotia* species formed a monophyletic group (bootstrap support (BS) based on neighbour-joining (NJ) and parsimony (MP) analysis: 100/97), which diverged next.

Navicula sensu stricto and *Hippodonta capitata* were sister groups (clade 1) and formed the basal clade of the raphid pennates. The monophyly of *Navicula sensu stricto* was supported by 96% of both bootstrap analyses. The support for *Hippodonta* being the sister group was 100% in both analyses. *Navicula sensu stricto* was subdivided in three groups (Fig. 4a). The

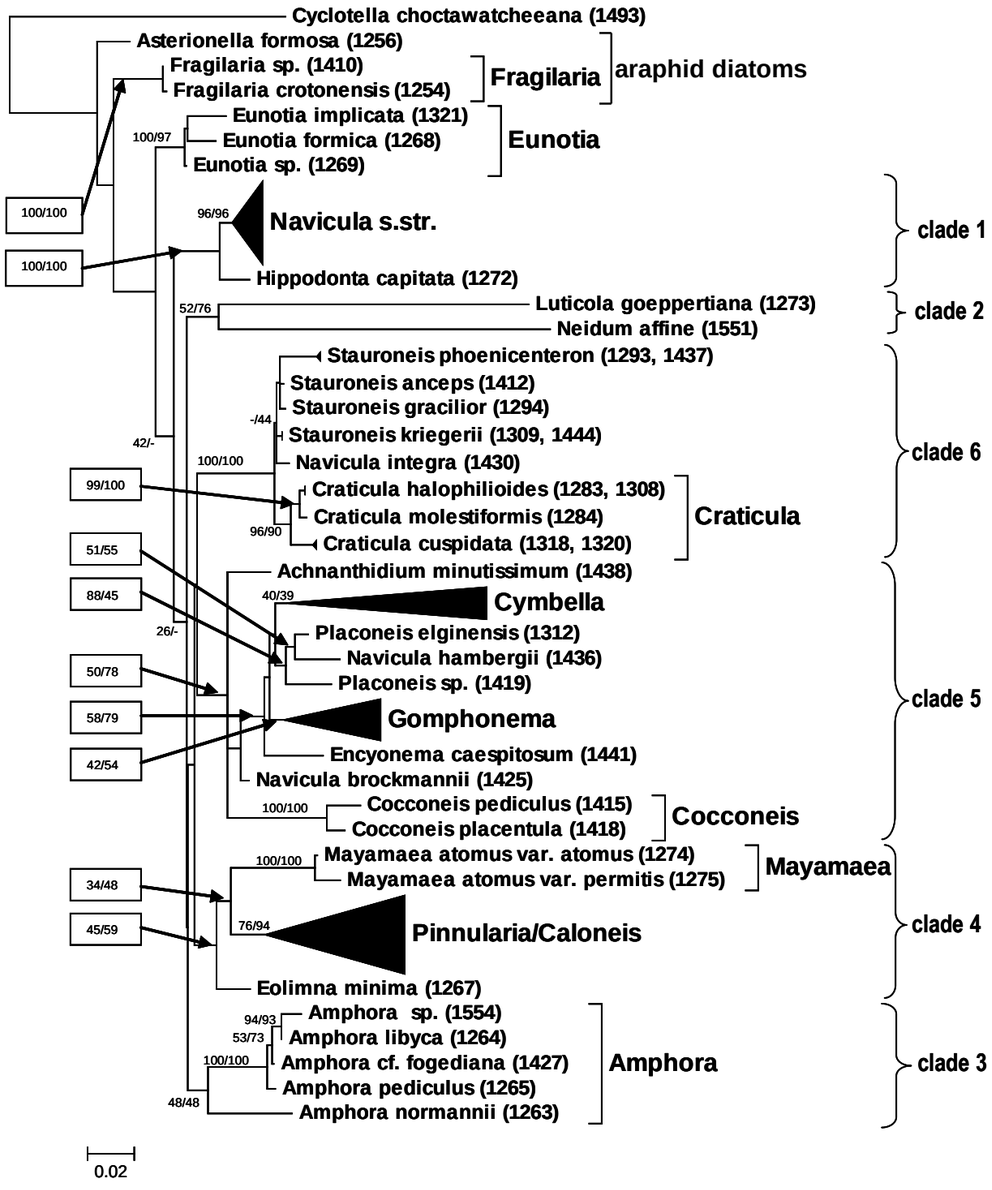


Fig. 3: Phylogeny inferred with the maximum-likelihood (ML) analysis using SSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on neighbor-joining (NJ) analysis using Jukes-Cantor (JC) model and on parsimony analysis have been plotted at the nodes. Collapsed clades are shown in detail in Fig. 4.

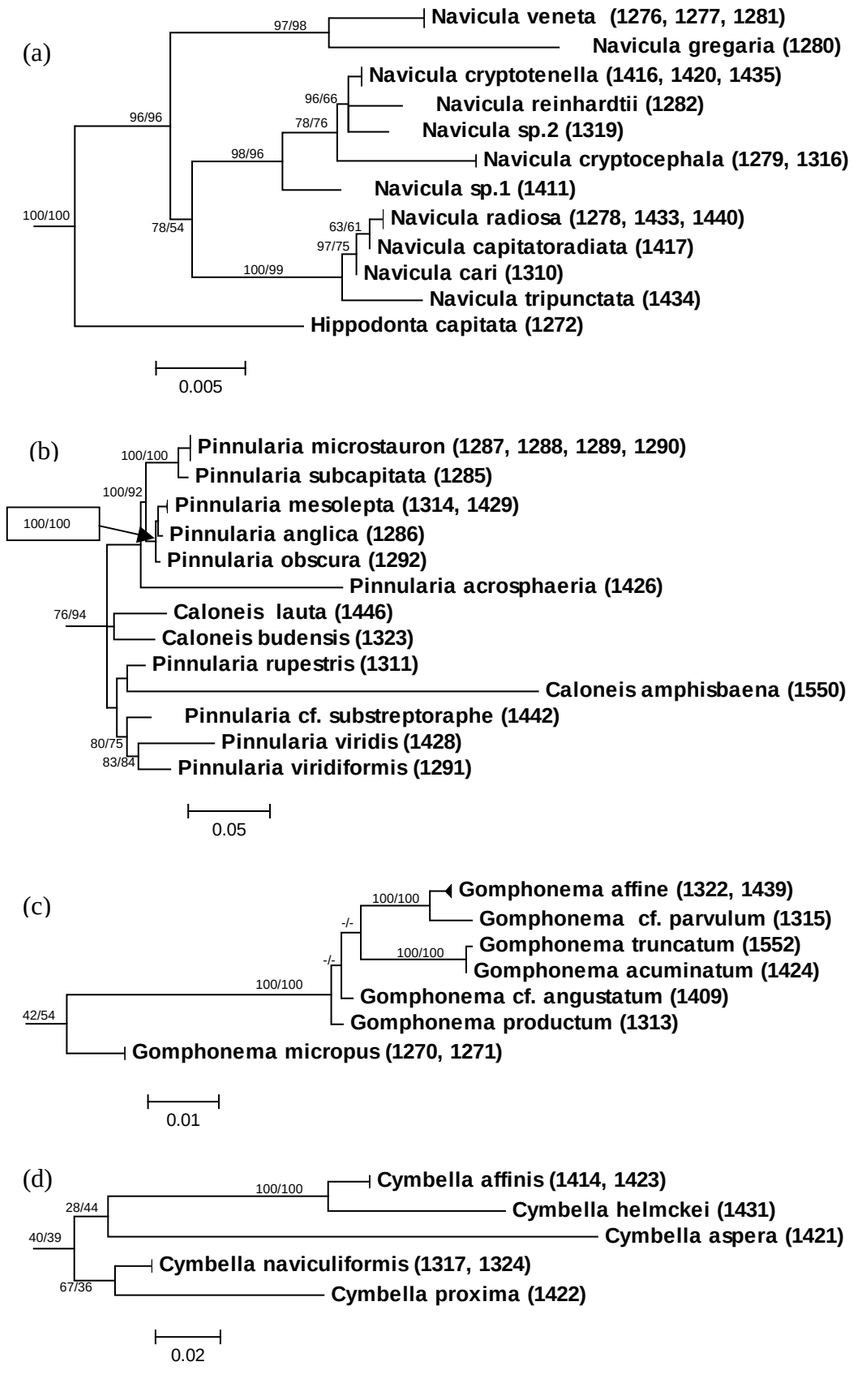


Fig. 4: Details of the ML tree analysis from SSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Pinnularia* and *Caloneis*, (c) *Gomphonema*, (d) *Cymbella*

first consisted of *N. veneta* and *N. gregaria* (BS: 97/98). *N. crytotenella, N. reinhardtii, N. cryptocephala* and the two unidentified *Navicula* species formed the second group, which was supported by 98% and 96% of the bootstrap replicates. The third group contained *N. radiosa, N. capitatoradiata, N. cari* and the type species *N. tripunctata*, supported by bootstrap values of 100 and 99.

Clade 2 in the ML tree (Fig. 3), which comprised *Luticola goeppertiana* and *Neidum affine*, had relatively low bootstrap support (52/76).

The five *Amphora* species formed a monophyletic clade (BS: 48/48), which diverged next (clade 3). In this clade, *A. normannii* is clearly separated from the other *Amphora* species by branch length and maximum BS for the monophyly of the other four *Amphora* species.

Clade 4 in the ML tree includes *Eolimna minima, Mayamaea* and all *Pinnularia* and *Caloneis* species (BS: 45/59). *Eolimna* is at the base of this clade and *Mayamaea* is monophyletic sister group (BS: 34/48) of *Pinnularia* and *Caloneis*. The monophyly of these two genera had strong MP bootstrap support (94) and medium NJ BS (76). Within Pinnularia/Caloneis clade three sub-clades could be distinguished (Fig. 4b). One group contained *P. acrosphaeria, P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron*. *C. lauta* and *C. budensis* formed a second group. The third group consisted of *P. rupestris, C amphisbaena, P. viridis, P*. cf. *substreptoraphe* and *P. viridiformis*.

At the base of clade 5 of the ML tree (Fig. 3) the monoraphid genera *Achnanthidium* and *Cocconeis* and a sub-clade containing *Navicula brockmannii* and the Cymbellales diverge from an unresolved polytomy. In this sub-clade *N. brockmannii* diverges first, followed by *Encyonema caespitosum*. *Gomphonema* (BS: 42/54), *Placoneis*/*Navicula hambergii* (BS: 88/45) and *Cymbella* (BS: 40/39) were monophyletic groups. *Gomphonema* diverges first and *Placoneis*/*N. hambergii* and *Cymbella* were sister groups, but this relationship had no BS. Within the genus *Gomphonema* (Fig. 4c), *G. micropus* is clearly separated by the branch length from the other *Gomphonema* species, which form a strong group (BS: 100/100). The genus *Cymbella* (Fig. 4d) was split into one group containing *C. naviculiformis* and *C. proxima* (BS: 67/36) and another group consisting of *C. aspera, C. helmckei* and *C. affinis* (BS: 28/44).

The clade 6 in the ML tree (Fig. 3) was supported by maximum bootstrap support (100/100). Within this clade the monophyly of *Craticula* was supported by 96% and 90% of NJ and MP bootstrap replicates, respectively. *Stauroneis* and *Navicula integra* cluster together, but this clade had only weak BS (-/44) and the branching order in this group was not fully resolved.

The maximum parsimony (MP) analysis based on the SSU rDNA sequences resulted in 58 most parsimonious trees. The majority-rule consensus tree of these trees is depicted in the Figures 5 and 6.

The three araphid taxa appeared at the base as monophyletic group. Bootstrap values of 96 and 86 from NJ and MP bootstrap analysis support this.

Similar to the ML tree the genus *Eunotia* formed a strongly supported monophyletic group (BS: 100/97). *Luticola goeppertiana* and *Neidum affine* represented the sister group to *Eunotia*, although this relationship had nearly no BS (0/29).

The sister groups *Navicula sensu stricto* and *Hippodonta capitata* diverged next. The MP analysis recovered the same three sub-clades in the *Navicula sensu stricto* as did the ML analysis. The branching order within the *Navicula sensu stricto* (Fig. 6a) is similar to the ML tree (Fig. 4a). But the relationship of *N. cryptotenella*, the unidentified *Navicula* species2 and *N. reinhardtii* was not resolved.

Following the divergence of *Navicula sensu stricto* there was a polytomy of three clades. Clade 1 was the monophyletic *Amphora* clade (BS: 48/48), in which *A. normannii* is separated from the other four *Amphora* species by maximum BS in the same branching order as the ML analysis. The other two groups were more divers and differed from the ML analysis.

Eolimna, *Mayamaea*, *Pinnularia*/*Caloneis* and a clade containing *Stauroneis*, *Craticula* and *Navicula integra* form clade 2 in the basal polytomy (BS: 23/35). The monophyletic *Pinnularia*/*Caloneis* clade BS (76/94) further diverges into two groups (Fig. 6b). One group (BS: 0/45) containing *C. lauta, P. acrosphaeria, C. amphisbaena, P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron.* The second group (BS: 55/44) consisted of *C. budensis, P. rupestris, P. viridis, P*. cf. *substreptoraphe* and *P. viridiformis*. Maximum bootstrap values (100/100) support the polytomy of *Stauroneis*, *Navicula integra* and the well supported monophyletic *Craticula* clade (BS: 99/100). The main difference to the three clades of the ML analysis is that the middle clade of the ML analysis is lost and forms the base of the two clades in the MP analysis.

In the remaining clade 3 (BS: 50/78) *Cocconeis* diverged first. *Achnanthidium minutissimum* diverged next, followed by *Navicula brockmannii* and *Encyonema caespitosum*. A monophyletic clade containing the two *Placoneis* species and *Navicula hambergii* (BS: 88/45) is the sister group to a clade containing *Gomphonema* and *Cymbella* (BS: 14/27). This clade futher diverges into four branches, which are *G. micropus*, a clade (BS: 67/36) containing

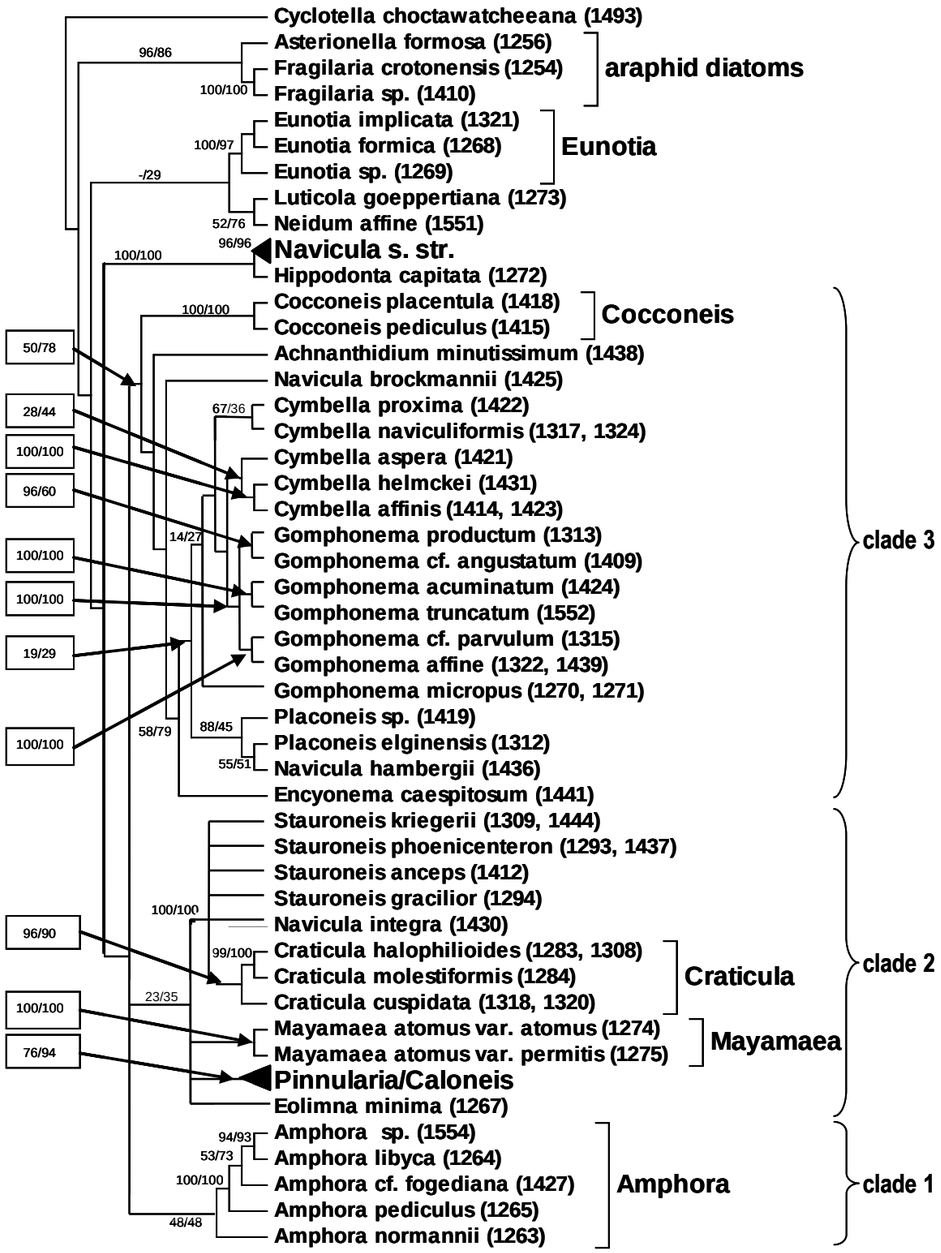
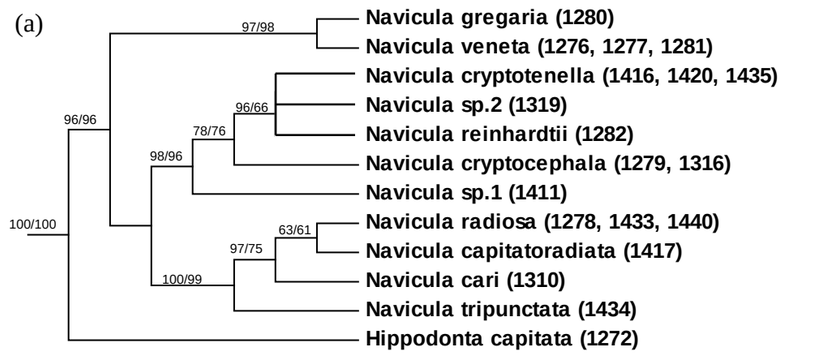


Fig. 5: Majority-rule consensus tree inferred with the parsimony analysis using SSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed regions are shown in detail in Fig. 6.



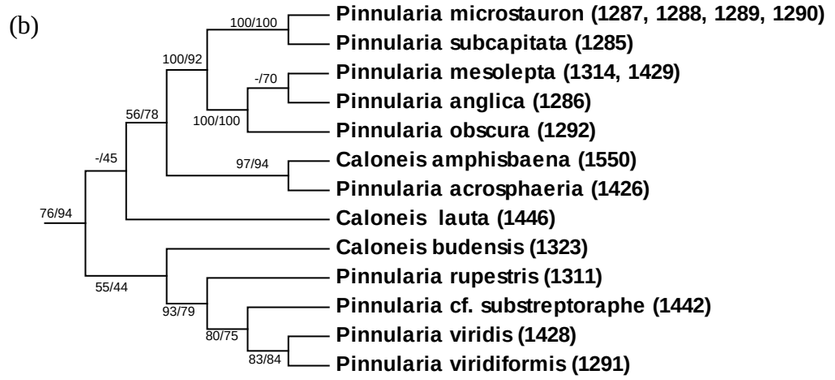


Fig. 6: Details of the parsimony tree analysis using SSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Pinnularia* and *Caloneis*

C. proxima and *C. naviculiformis*, a clade (BS: 28/44) consisting of the remaining three *Cymbella* species and a strongly supported clade (BS: 100/100) containing the other five *Gomphonema* species.

Most of the SSU rDNA sequences obtained from GenBank were similar in length compared to those sequenced within the scope of this study. But there are several sequences missing up to 226 nucleotides at the ends (see Table 9 in the appendix). In this extended alignment the same highly variable region was excluded from the analyses. A MP analysis using this dataset could not be conducted because of the large number of taxa.

The base of the ML tree inferred from the expanded SSU rDNA dataset (Figs. 7-10) was similar to the base of the ML tree based on SSU rDNA sequences from the AlgaTerra cultures (Fig. 3). After a paraphyletic divergence of araphid taxa, the monophyletic *Eunotia* clade (BS: 71) diverged.

The next branch was formed by a monophyletic clade of four monoraphid species (BS: 50) at its base, different *Bacillariales* and the naviculoid *Stauroneis constricta*. The position of this naviculoid diatom within this clade is supported by maximum BS, but is likely a contaminant.

The next branch (naviculoid pennates part 1; Figs. 7, 8) diverges at the base into two sub-clades. Clade 1 (Fig. 8) consists of the well supported monophyletic groups *Haslea* (BS: 92) and *Pleurosigma* (BS: 99) and *Gyrosigma limosum*, which is sister to *Pleurosigma*. Clade 2 includes *Hippodonta*, *Navicula sensu stricto* and *Pseudogomphonema* (BS: 100). *Hippodonta capitata* was found at the base of this clade, *N. diserta* diverged next followed by a polytomy of three clades. The first consists of *N. sclesviscensis, N.cryptocephala* var. *veneta N. veneta, N. gregaria* and two unidentified *Navicula* species (BS: 55). *N. radiosa, N. capitatoradiata, N. cari, N. tripunctata, N. ramosissima* and *N. lanceolata* formed the second group, which was supported by 99% of the bootstrap replicates. The third group (BS: 90) diverges into two clades, which include *Pseudogomphonema* on one hand and *N. crytotenella, N. reinhardtii, N. cryptocephala, N. phyllepta* and three unidentified *Navicula* species on the other hand.

Neidum affine and *Haslea nipkowii* diverge next, although this clade had no BS (Fig. 7).

Detail of the next large clade containing naviculoid pennates part 2 is shown Fig. 9. Only clades at the tip of the tree show strong bootstrap support. The naviculoid pennates part 2 diverge into two clades.

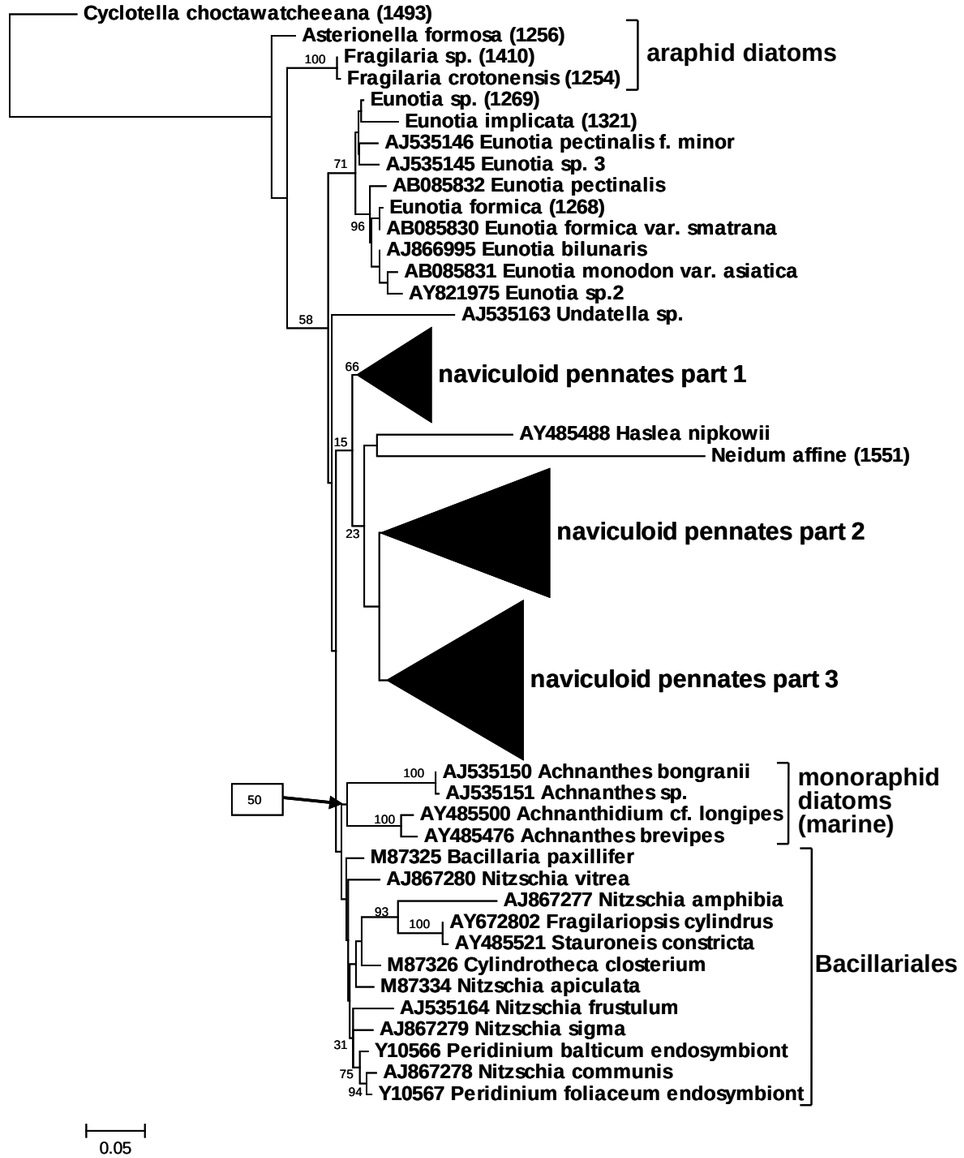


Fig. 7: Phylogeny inferred with the ML analysis using SSU rDNA sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses (JC-model) have been plotted at the nodes. Condensed regions are shown in detail in separate figures.

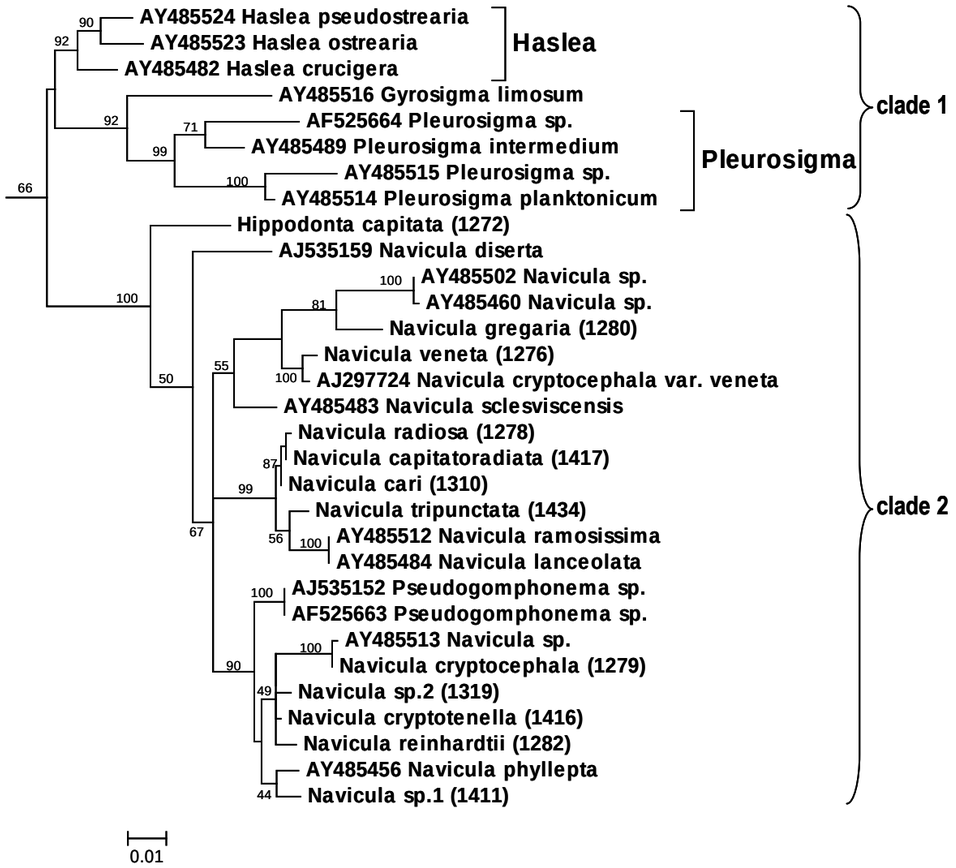


Fig. 8: Naviculoid pennates part 1. Detail of the ML tree analysis from SSU rDNA sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model have been plotted at the nodes.

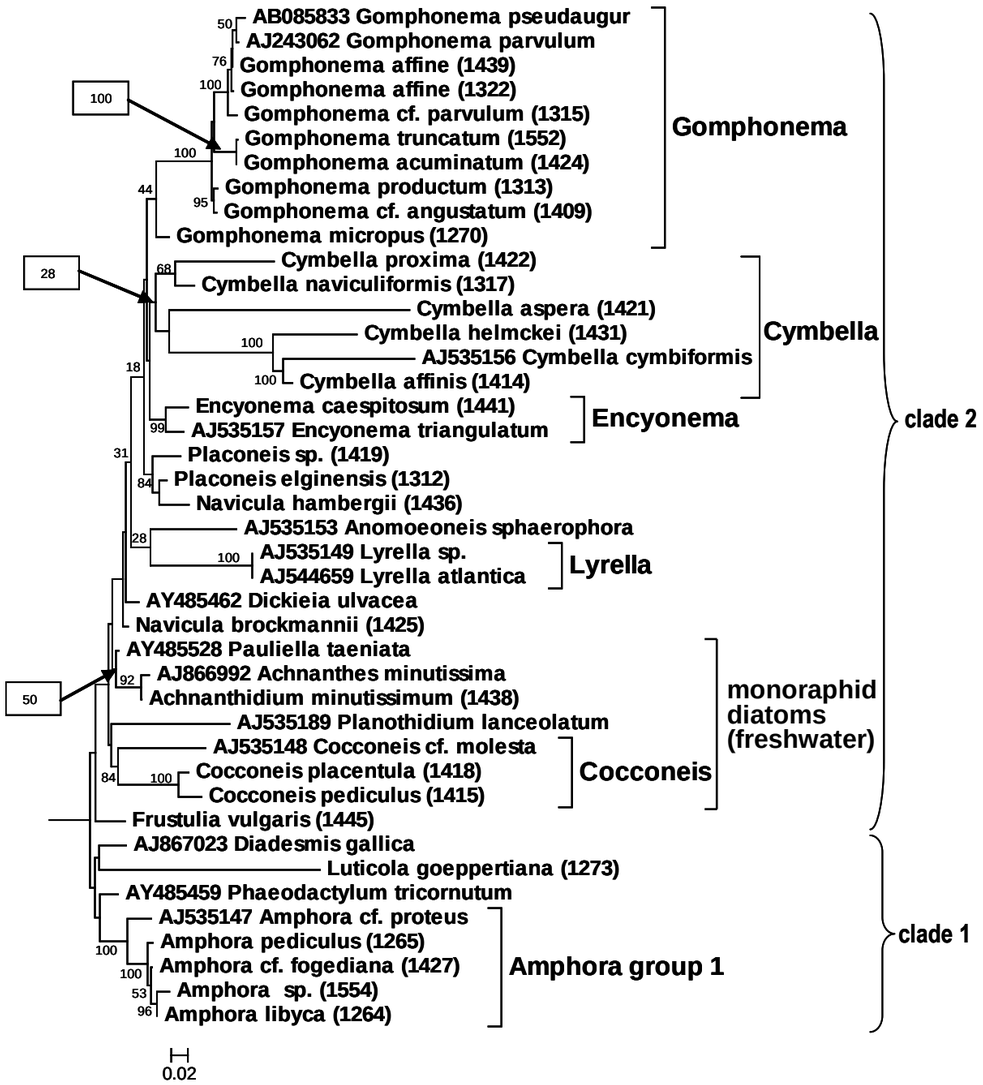


Fig. 9: Naviculoid pennates part 2. Detail of the ML tree analysis from SSU rDNA sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model have been plotted at the nodes.

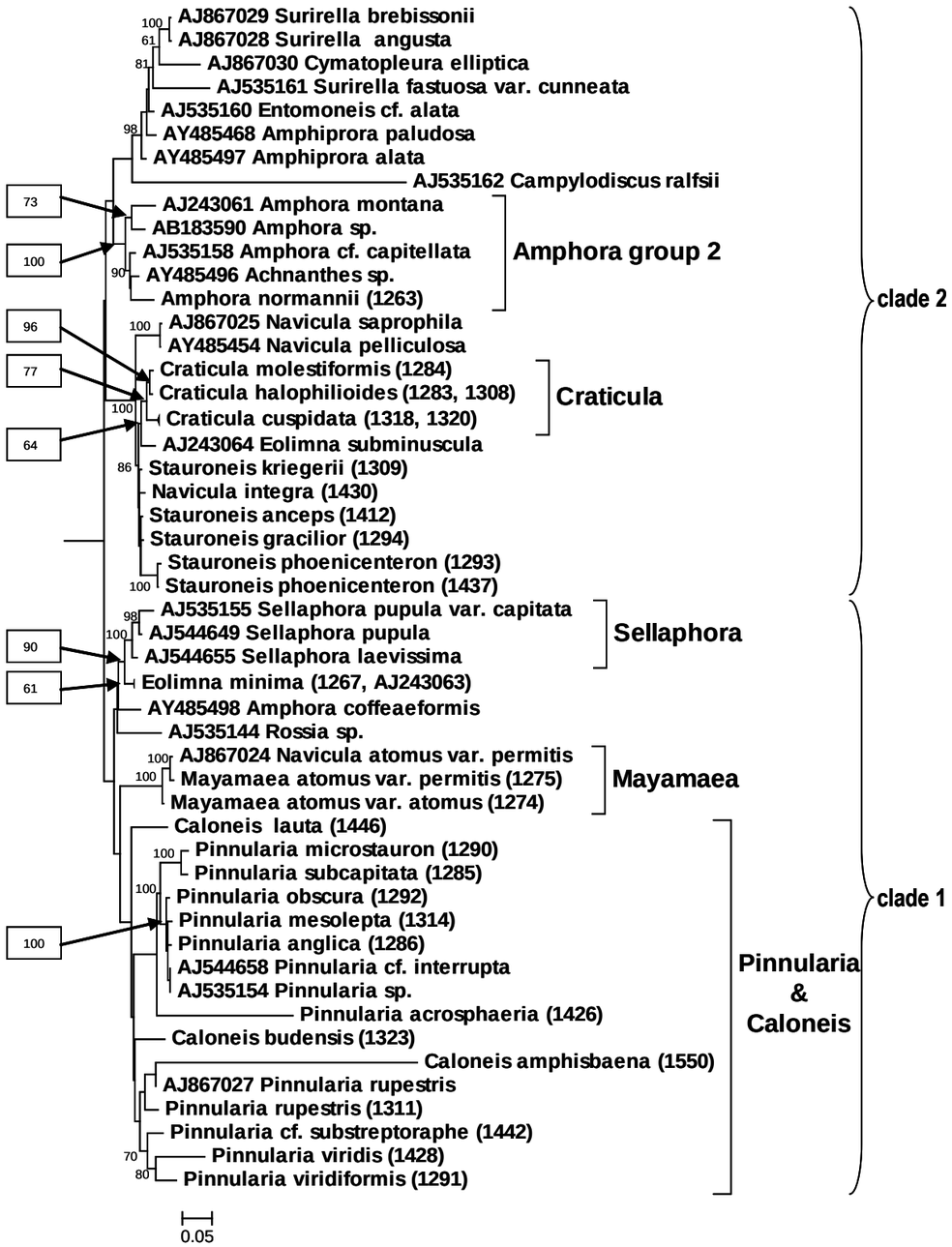


Fig. 10: Naviculoid pennates part 3. Detail of the ML tree analysis from SSU rDNA sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model have been plotted at the nodes.

Clade 1 (Fig 9) consists of *Diadesmis gallica* as sister to *Luticola goeppertiana* and *Phaeodactylum tricormutum* as sister to a strongly supported (BS: 100) monophyletic group containing five *Amphora* species.

Frustulia vulgaris diverge at the base of the clade 2. The next two diverging clades contained monoraphid species. The first clade consists of *Planothidium lanceolatum* and a monophyletic *Cocconeis* (BS: 84) and the other clade (BS: 50) contained *Pauliella taeniata* and *Achnanthidium minutissimum*. *Navicula brockmannii, Dickieia ulvacea* and *Anomoeoneis sphaerophora* with its sister group *Lyrella* diverging between the monoraphids and a clade containing different *Cymbellales,* but there is no BS for their positions. Within the *Cymbellales, Placoneis* and *Navicula hambergii* diverges first. Following this divergence is a very rapid divergences of *Gomphonema*, followed by *Encyonema* and *Cymbella*. A monophyletic clade containing all *Gomphonema* species has low bootstrap support (BS: 44). *G. micropus* branch off at the base of this clade and was separated from the other *Gomphonema* species, which are well supported by maximum BS and by branch length. The genus *Encyonema* form a well supported monophyletic clade (BS: 99) but the monophyly of *Cymbella* has only low BS (28). *Cymbella* further diverges into sub-clades, one containing *C. proxima* and *C. naviculiformis* and another (BS: 28/44) consisting of the remaining four *Cymbella* species.

Navicoloid pennates part 3 is shown in detail in Fig. 10. It diverges into two major clades.

Clade 1 diverges into one branch containing *Rossia, Amphora coffeaeformis, Eolimna minima* and the three *Sellaphora* species and another branch, which includes the monophyletic *Mayamaea* clade and a monophyletic clade containing *Pinnularia* and *Caloneis* species. Within this monophyly, C. lauta diverges first, followed by a clade containing *P. acrosphaeria, P. microstauron, P. subcapitata, P. obscura, P.* cf. *interrupta, P. anglica, P. mesolepta* and one unidentified *Pinnularia* species. Then *C. budensis* diverged and a second larger clade containing *P. rupestris, C. amphisbaena, P. viridis, P.* cf. *substreptoraphe* and *P. viridiformis.*

Clade 2 also diverges into two clades. At the base of one clade, which had maximum BS, *Navicula saprophila* and *N. pelliculosa* diverges. The branching order of the *Stauroneis* species and *Navicula integra* was not totally resolved. *Eolimna subminuscula* appeared as sister to *Craticula*. The second clade contains several *Surirellales* and two *Amphiprora* species on one hand and a strongly supported (BS: 100) *Amphora* group with one *Achnanthes* in between on the other hand. This *Achnanthes* sequence is also likely a contaminant.

The missing BS for several deeper branches within the clades in Figs. 9 and 10 were caused by one clade in the NJ tree (clade LB in Fig. 59 in the appendix) containing the five species with the most nucleotide changes (visible as long branches in the ML tree): *Caloneis amphisbaena*, *Campylodiscus ralfsii*, *Luticola goeppertiana*, *Neidum affine* and *Pinnularia acrosphaeria*. This might be an artefact of long-branch attraction. Therefore these bootstrap results should be interpreted with caution.

3.1.2 LSU rRNA gene

The LSU rRNA sequences for all sequenced taxa were approximately 540 nucleotides in length excluding amplification primers, except for *Luticola goeppertiana*, which was longer (927 nucleotides) because of several large insertions. One highly variable region that contains the largest insertion in the sequence from *L. goeppertiana* was excluded from the analyses. This region covered 262 nucleotides from *L. goeppertiana* and approximately 85 nucleotides from the other taxa. The final datset contained 715 positions, of which 252 were parsimony-informative and 61 parsimony-uninformative characters.

The maximum-likelihood (ML) tree based on the sequences from the AlgaTerra cultures is shown in Fig. 11. The collapsed clades of this figure are shown in detail in Fig. 12.

The phylogeny in Fig. 11 diverges at the base into two large clades. Supported by bootstrap values of 92 and 87 *Amphora* formed a monophyletic group at the base of clade 1. Within this group *A. normannii* was separated from the other *Amphora* species by maximum BS and branch length (Fig. 12a). The genus *Eunotia* diverges next. *Hippodonta capitata* and *Navicula sensu stricto*, interspersed with *Neidum affine* and *Luticola goeppertiana* were pooled in the next clade. *H. capitata* diverged at the base of this clade. Within this group only the last nodes were supported by the bootstrap analyses. The araphid diatoms diverge next (BS: 71/73). *Encyonema caespitosum* diverges at the base of the next clade, which further diverges into three monophyletic groups. The first group is formed by the genus *Gomphonema* (Fig. 12b) and diverge into *G. micropus* on one hand and all other *Gomphonema* species on the other hand (BS: 99/100). The second group contains *Placoneis* and *Navicula hambergii* (BS: 84/42) and is the sister group to the genus *Cymbella* (BS: 31/19). This genus diverges into two groups, but they were not supported by the bootstrap analyses (Fig. 12c).

The polytomy at the base of clade 2 diverges into three branches. One consists of *Eolimna minima* only. The second branch was formed by *Pinnularia* and *Caloneis* (BS: 0/22). This branch further diverges into two clades (Fig. 12d), which includes *C. budensis*, *P. rupestris*, *P. viridis*, *C. amphisbaena*, *P.* cf. *substreptoraphe*, *P. acrosphaeria* and *P. viridiformis*

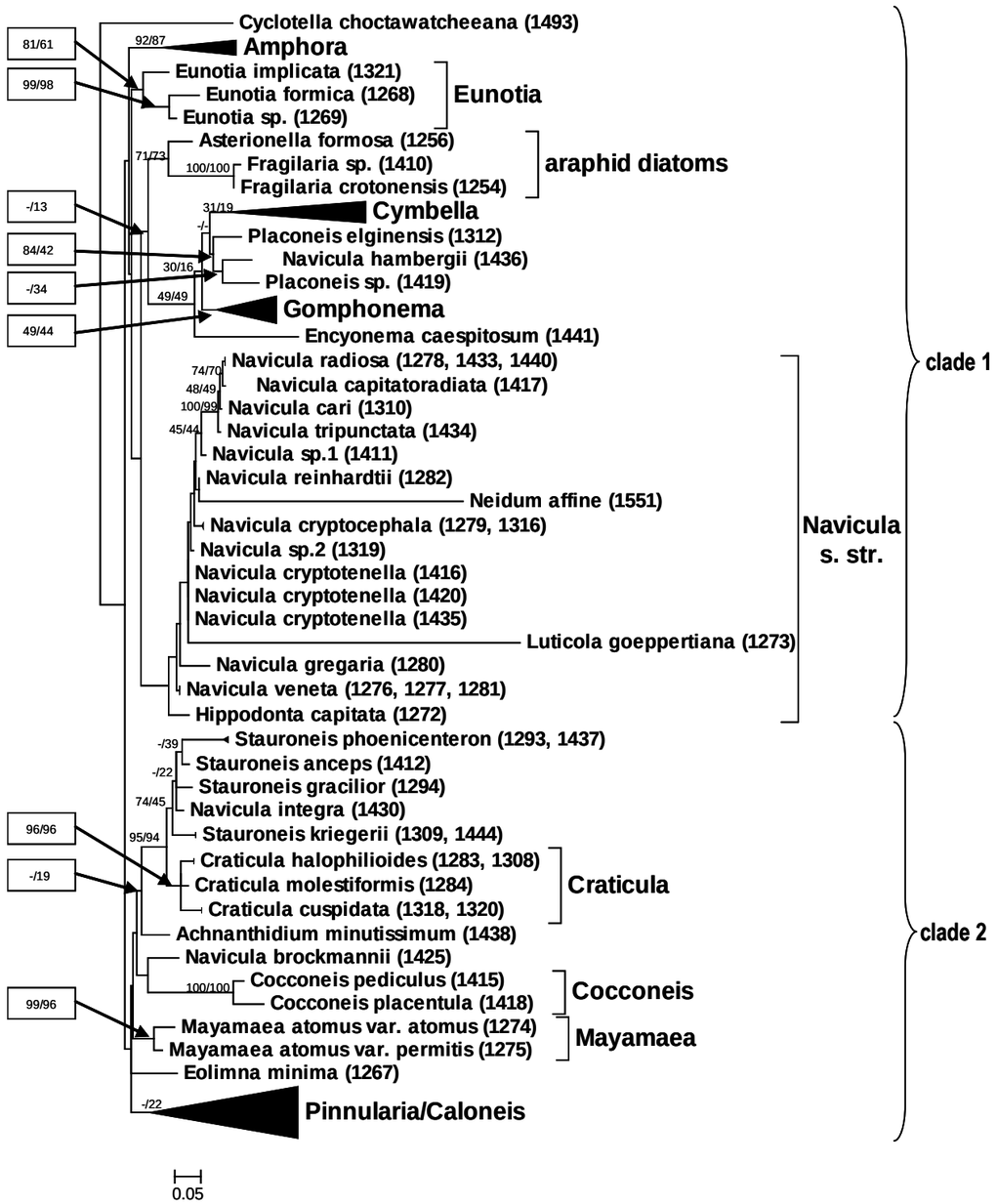


Fig. 11: Phylogeny inferred with the ML analysis using LSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed regions are shown in detail in Fig. 12.

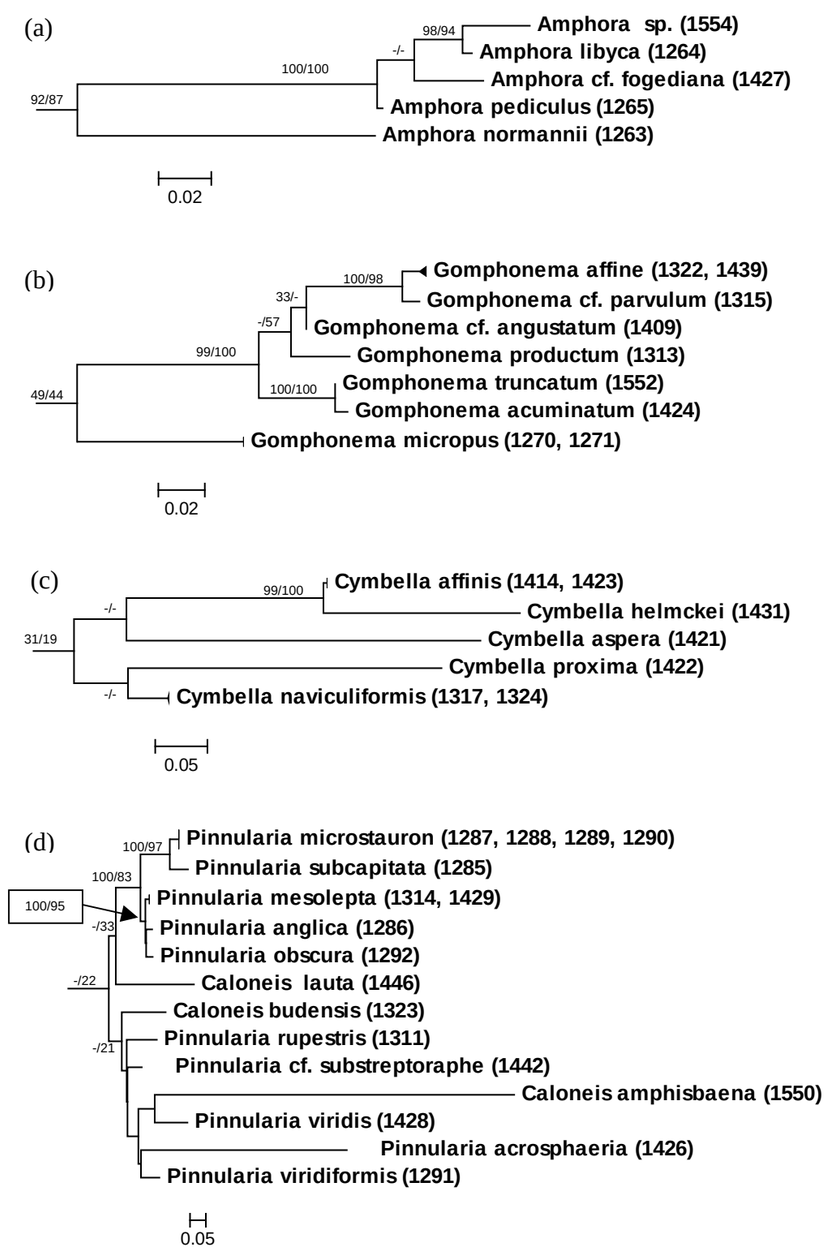


Fig. 12: Details of the ML tree analysis based on LSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Amphora*, (b) *Gomphonema*, (c) *Cymbella*, (d) *Pinnularia* and *Caloneis*

on one hand and *C. lauta*, *P. obscura*, *P. anglica*, *P. mesolepta*, *P. subcapitata* and *P. microstauron* on the other hand. The third group consist of several genera. *Mayamaea* diverges at the base. The next clade consists of *Navicula hambergii* and *Cocconeis* and then *Achnanthidium minutissimum* diverges. The monophyly of *Craticula* was supported of 96% of both bootstrap analyses. *Stauroneis* and *Navicula integra* (BS: 74/45) formed the sister clade (BS: 95/94) to *Craticula*.

The maximum parsimony (MP) analysis based on the LSU rDNA data sequenced within the scope of the study resulted in 98 most parsimonious trees. The majority-rule consensus tree of these trees is depicted in Fig. 13; condensed clades are shown in detail in Fig. 14.

This consensus tree is poorly resolved with a large polytomy at the base. Clades of this polytomy, which consist of a single genus, were formed by *Eolimna minima*, *Mayamaea* (BS: 99/96), *Eunotia* (BS: 81/61), *Amphora* (BS: 92/87) and *Cocconeis* (maximum BS). In clade 1 bootstrap values of 95 and 94 support that *Stauroneis* and *Navicula integra* (BS: 74/45) formed a sister group of the genus *Craticula* (BS: 96/96). Clade 2, which contains *Achnanthidium minutissimum* and *Navicula brockmannii*, had no bootstrap support. *Pinnularia* and *Caloneis* formed a monophyletic clade (BS: 0/22), which further diverges into two groups (Fig. 14a). Each group consist of the same species as described for the ML phylogeny, but the branching order within the clades differs (Figs. 12d and 14a). Also *Hippodonta capitata* and *Navicula sensu stricto* form a monophyletic clade (clade 3, BS: 99/76). The branching order at the base of this clade is not resolved (Fig. 14b) and only the group containing *N. tripunctata*, *N. cari*, *N. capitatoradiata* and *N. radiosa* is supported by high bootstrap values (100/99). Clade 4, which contains *Neidum affine* and *Luticola goeppertiana*, was supported only by 35% of the MP bootstrap replicates. Bootstrap values of 71 and 73 support the clade containing the araphid diatoms. The most diverse clade 5 consists of *Encyonema caespitosum*, all species belonging to *Cymbella*, *Gomphonema* and *Placoneis* and *Navicula hambergii*. From the polytomy at the base of this clade only two groups diverge. One contains *C. helmckei* and *C. affinis* (BS: 99/100), the other consists of the genus *Gomphonema*, whose monophyly is supported by bootstrap values os 49 and 44.

The LSU rDNA sequences obtained from GenBank were similar in length compared to the sequences, which were sequenced within the scope of this study. In this extended alignment the same highly variable region was excluded from the analyses. The calculation of some

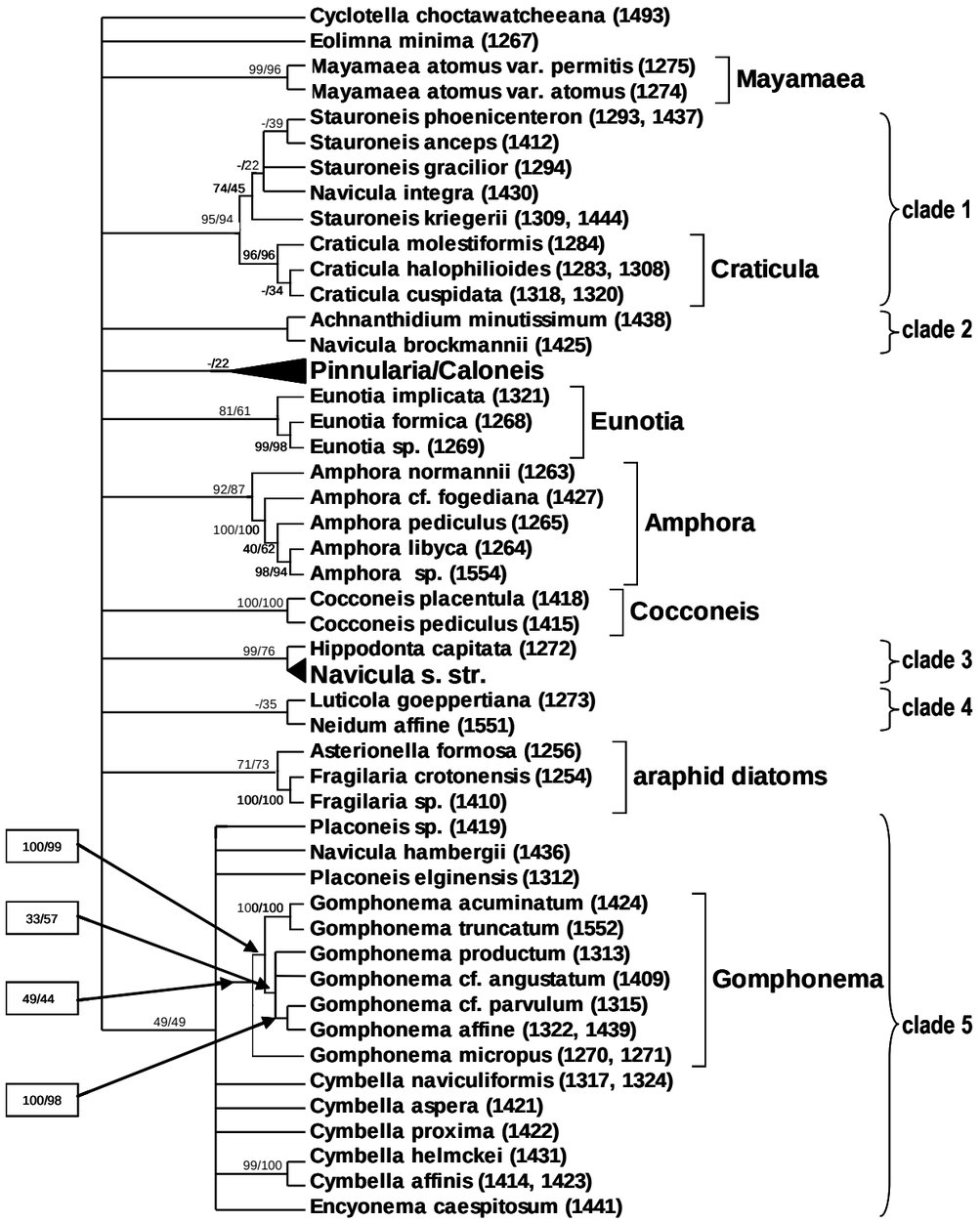
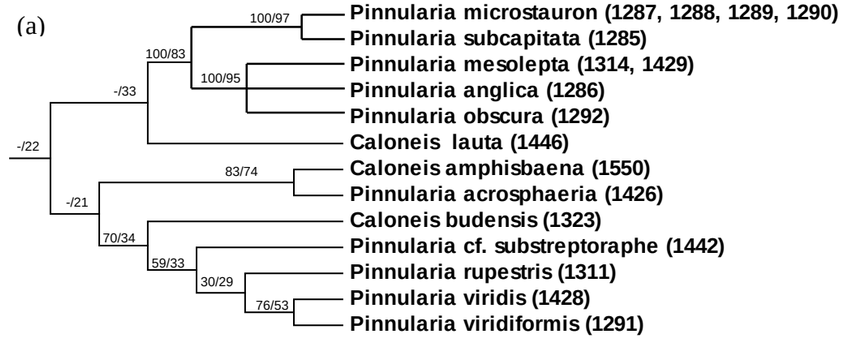


Fig. 13: Majority-rule consensus tree inferred with the parsimony analysis based on LSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed regions are shown in detail in Fig. 14.



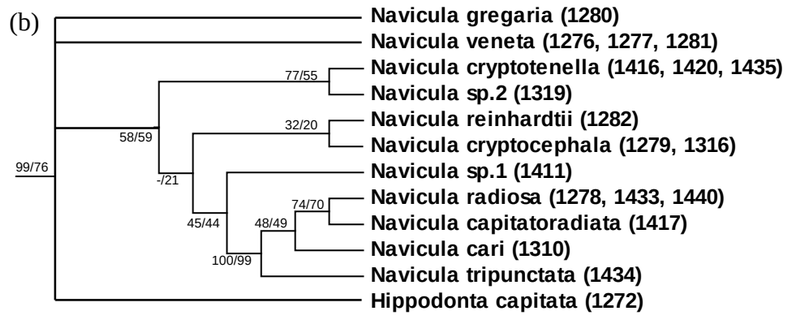


Fig. 14: Details of the parsimony tree analysis based on LSU rDNA sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Pinnularia* and *Caloneis*

replicates in the MP bootstrap analysis needed plenty of time. Therefore a time limit of 15 minutes was set for each replicate. As a result of this time limit 89 of 1000 replicates were terminated. For these replicates it is not certain if the best tree was detected.

The tree resulted from the ML analysis based on the extended is shown in Fig. 15. The collapsed clades are only shown in detail (Fig. 16) if they differ from the equivalent clades shown in Figs. 11 and 12.

In the ML tree clade 1, which contains the monoraphids species, *Navicula brockmannii*, *Craticula*, *Stauroneis* and *N. integra*, diverges first. The branching within this clade was similar to the equivalent clade in Fig. 11. The additional species, *Pauliella taeniata*, clusters with *Achnanthidium minutissimum*.

Mayamaea diverges next, followed by a polytomy of *Eolimna minima*, a clade containing *Pinnularia* and *Caloneis* and clade 2. Although there were no additional *Pinnularia* or *Caloneis* species in this dataset, the branching order of *P. viridis* and *P. rupestris* had changed (Fig. 16a) compared to the equivalent clade shown in Fig. 12d.

Clade 2 diverges further into two sub-clades. At the base of clade 2a the genus *Amphora* was separated by the *Entomoneis* species into two groups (Fig. 16b), which consists of *A. normannii* and *A. coffeaeformis* on one hand (BS: 99/98) and the remaining *Amphora* species on the other hand (BS: 100/100). *Phaeodactylum tricornutum* diverges next (Fig. 16). Within the *Cymbellales*, *Encyonema* (BS: 99/92) diverges first. The next group contains *Placoneis* and *N. hambergii*. In this tree, the genera *Gomphonema* and *Cymbella* were sister groups.

The genus *Eunotia* (BS: 84/73) diverges at the base of clade 2b, followed by the araphid diatoms (BS: 69/77). Within these two clades there were no changes compared to the tree in Fig. 11. The next divergence is a polytomy, from which *Luticola goeppertiana*, a clade containing the *Bacillariaceae* and a clade consisting of *Hippodonta capitata* and *Navicula sensu stricto* interspersed with *Neidum affine*.

The majority-rule consensus tree (Fig. 17) from the extended alignment based on 242 most parsimonious trees. The collapsed clades are only shown in detail (Fig. 18) if they differ from the equivalent clades shown in Figs. 13 and 14.

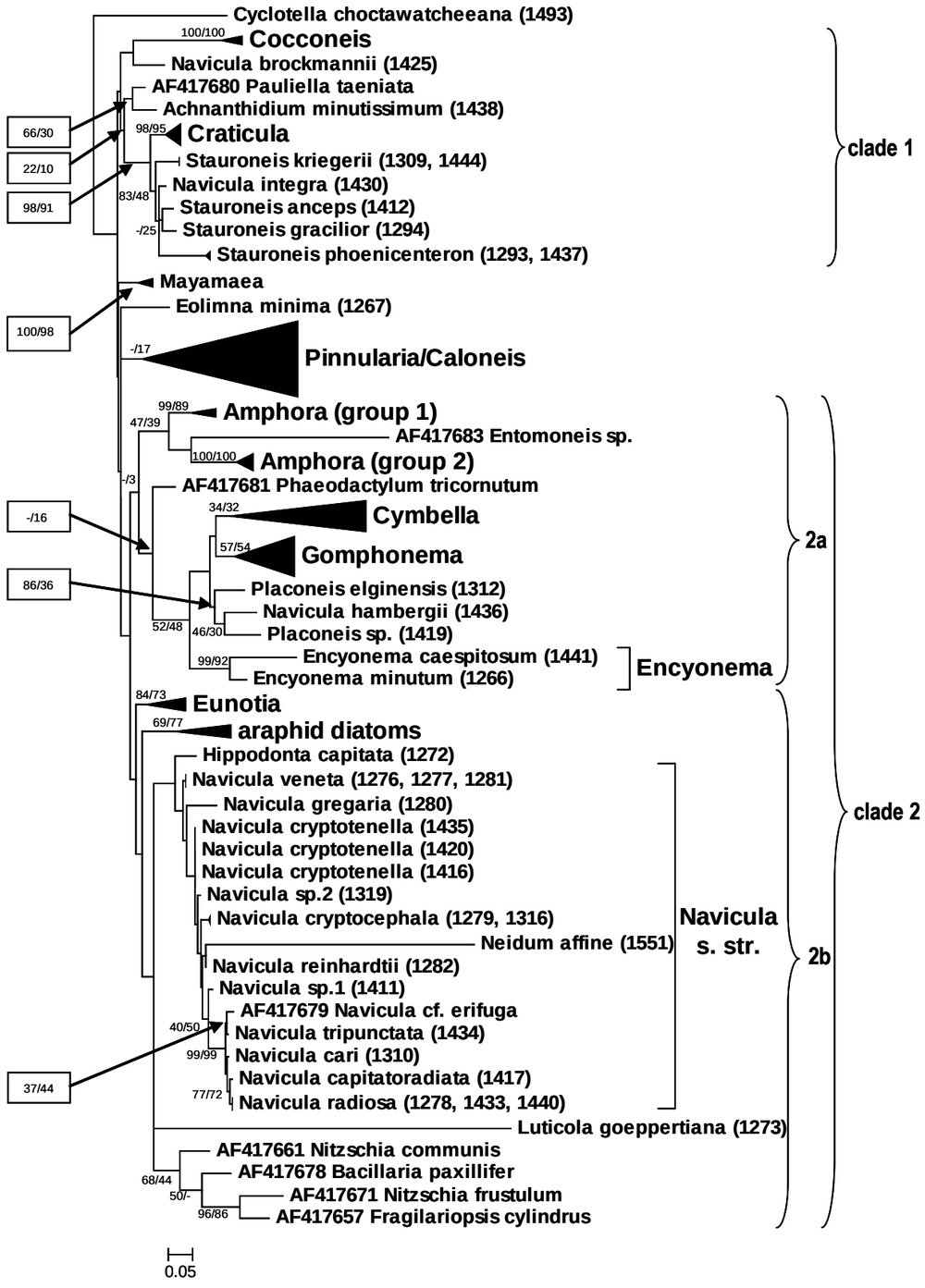


Fig. 15: Phylogeny inferred with the ML analysis using LSU rDNA sequences from GenBank and AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed regions, which differ to the equivalent clades in Figs. 11 and 12, are shown in detail in Fig. 16.

Fig. 16: Details of the ML tree analysis from LSU rDNA sequences from GenBank and AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Pinnularia* and *Caloneis*, (b) *Amphora*

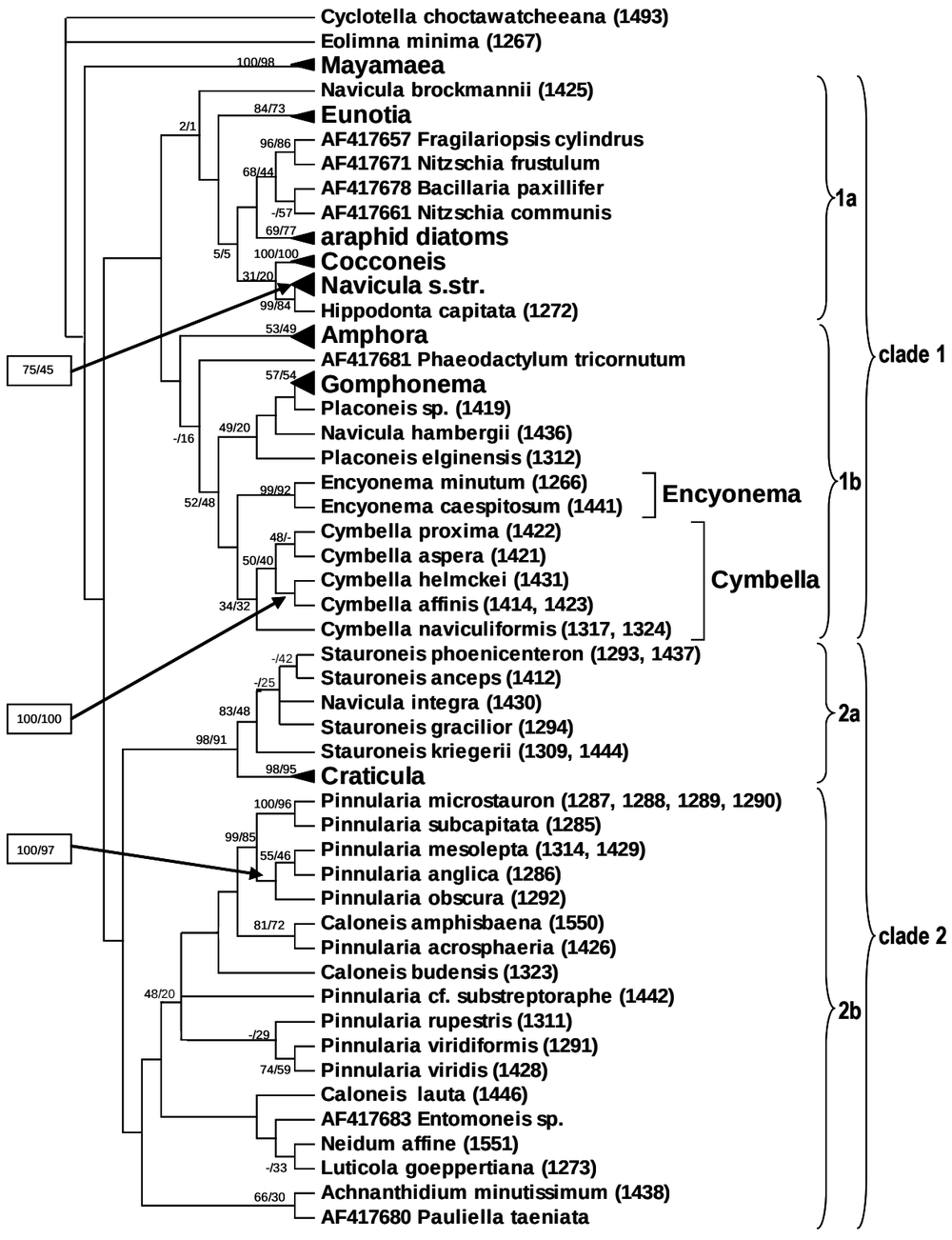


Fig. 17: Majority-rule consensus tree inferred with the parsimony analysis based on LSU rDNA sequences from GenBank and AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed clades, which differ to the equivalent clades in Figs. 13 and 14, are shown in detail in Fig. 18.

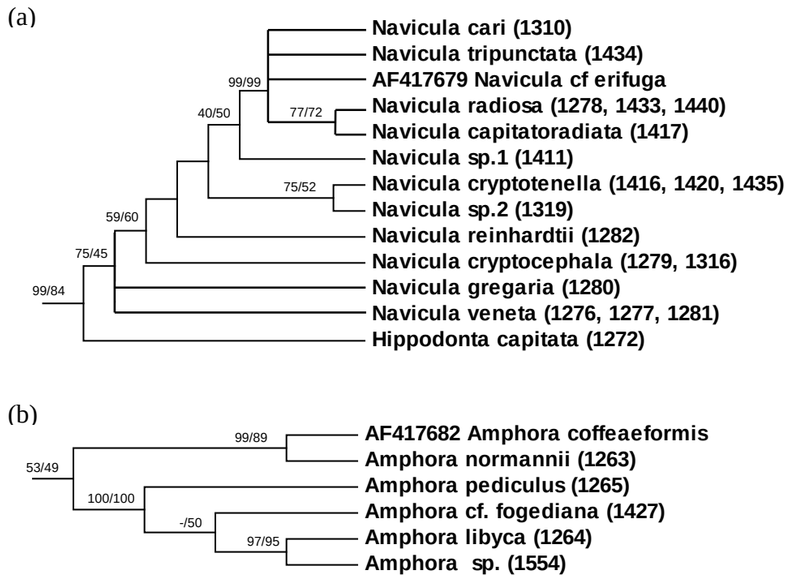


Fig. 18: Details of the parsimony tree analysis based on LSU rDNA sequences from GenBank and AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Amphora*

Eolimna minima and *Mayamaea* were found at the base of the consensus tree. At the next lineage the tree diverges into two main clades. Both of these clades further diverge into two sub-clades.

Navicula brockmannii diverges at the base of clade 1a. Then *Eunotia* diverges (BS: 84/73). The next branch consists of two monophyletic groups, the araphid diatoms on one hand (BS: 69/77) and the *Bacillariaceae* on the other (BS: 68/44). *Cocconeis* was the sister group (BS: 31/20) to a strongly supported clade (BS: 99/84) containing *Hippodonta capitata* and *Navicula sensu stricto* (BS: 75/45). *Navicula sensu stricto* did not diverge into separate groups (Fig. 18a).

The genus *Amphora* diverges at the base of clade 1b. Within this genus two groups could be distinguished (Fig. 18b). One consists of *A. normannii* and *A. coffeaeformis* (BS: 99/89) and the other group contains the remaining *Amphora* species (BS: 100/100). After the divergence of *Phaeodactylum tricornutum* the clade diverges into *Gomphonema* and *Placoneis*/*N. hambergii* on one hand (BS: 49/20) and *Encyonema* and *Cymbella* on the other hand (no BS). *Placoneis*/*N. hambergii* were paraphyletic whereas the other three genera formed monophyletic groups.

Clade 2 consists of the strongly supported clade 2a (BS: 98/91) containing *Craticula* (BS: 98/95) and *Stauroneis*/*N. integra* (BS: 83/48) and a clade 2b containing a mixture of monoraphid and raphid taxa. The branching order within the *Stauroneis*/*N. integra* is not totally resolved. In clade 2b the monoraphid *Pauliella taeniata* and *Achnanthidium minutissimum* diverge first. The next clade contains *Caloneis lauta*, the *Entomoneis* species, *Neidum affine* and *Luticola goeppertiana*. This is followed by a clade of *Pinnularia* and *Caloneis* species. Therefore *Pinnularia* and *Caloneis* were not a monophyletic group in this tree.

3.1.3 *rbc*L gene

The *rbc*L gene sequences for most sequenced taxa are 684 nucleotides in length excluding amplification primers. 15 sequences missing between 3 and 51 nucleotides because of sequencing problems in the regions close to the primer (Table 8). In none of the sequences were insertions or deletions. This permitted an unambiguous alignment. The final dataset had 684 positions in total, of which 210 were parsimony-informative and 49 parsimony-uninformative characters.

Tab. 8: Number of unknown nucleotides in incompletely sequenced *rbc*L sequences

Species	unknown nucleotides close to	
	primer F3	primer R3
Achnanthidium minutissimum (1438)	3	27
Amphora sp. (1554)	3	
Caloneis amphisbeana (1550)		9
Cocconeis pediculus (1415)	21	30
Craticula molestiformis (1284)	3	
Cymbella helmckei (1431)		17
Eunotia sp. (1269)	6	
Gomphonema affine (1439)		27
Gomphonema productum (1409)	24	18
Navicula cryptotenella (1416)	9	12
Navicula cryptotenella (1420)	6	
Navicula radiosa (1433)	19	
Pinnularia rupestris (1311)	39	7
Stauroneis kriegerii (1444)	15	21
Stauroneis phoenicenteron (1293)	24	21

The genus *Eunotia* (BS: 99/95) formed the base of the tree inferred with the ML analysis (Fig.19).

Clade 1 consists of *Navicula sensu stricto* and *Hippodonta capitata* (BS: 88/78). The monophyly of *Navicula sensu stricto* was supported by only 51% and 54% of the bootstrap replicates. *Navicula sensu stricto* was subdivided in three groups (Fig. 20a). The first consists of *N. veneta, N. gregaria* and one unidentified *Navicula* species (BS: 29/0). *N. crytotenella, N. reinhardtii, N. cryptocephala* and the other unidentified *Navicula* species formed the second group which was supported by 86% and 80% of the bootstrap replicates. The third group contained *N. radiosa, N. capitatoradiata, N. cari* and *N. tripunctata* (BS: 83/89).

The araphid diatoms formed a monophyletic clade (clade 2, BS: 85/61), which diverges next.

Clade 3 contains all *Pinnularia* and *Caloneis* species, *Eolimna minima* and *Mayamaea*, but this clade had nearly no bootstrap support. *Eolimna* and *Mayamaea* formed a monophyletic sister group to *Pinnularia* and *Caloneis*. *C amphisbaena* and *C. budensis* diverged at the base of the clade formed by *Pinnularia* and *Caloneis* (Fig. 20b). The other species were subdivided into two clades. One group contained *P. acrosphaeria, P. subcapitata, P. microstauron, P. mesolepta, P. anglica* and *P. obscura.* The other group consists of *C. lauta, P. rupestris, P. viridis, P.* cf. *substreptoraphe* and *P. viridiformis*.

Navicula brockmannii diverges at the base of clade 4. At the next lineage the tree diverges into a sub-clade, which consists of *Encyonema caespitosum* and the monoraphid species and a sub-clade containing the remaining *Cymbellales*. The genus *Cymbella* did not form a

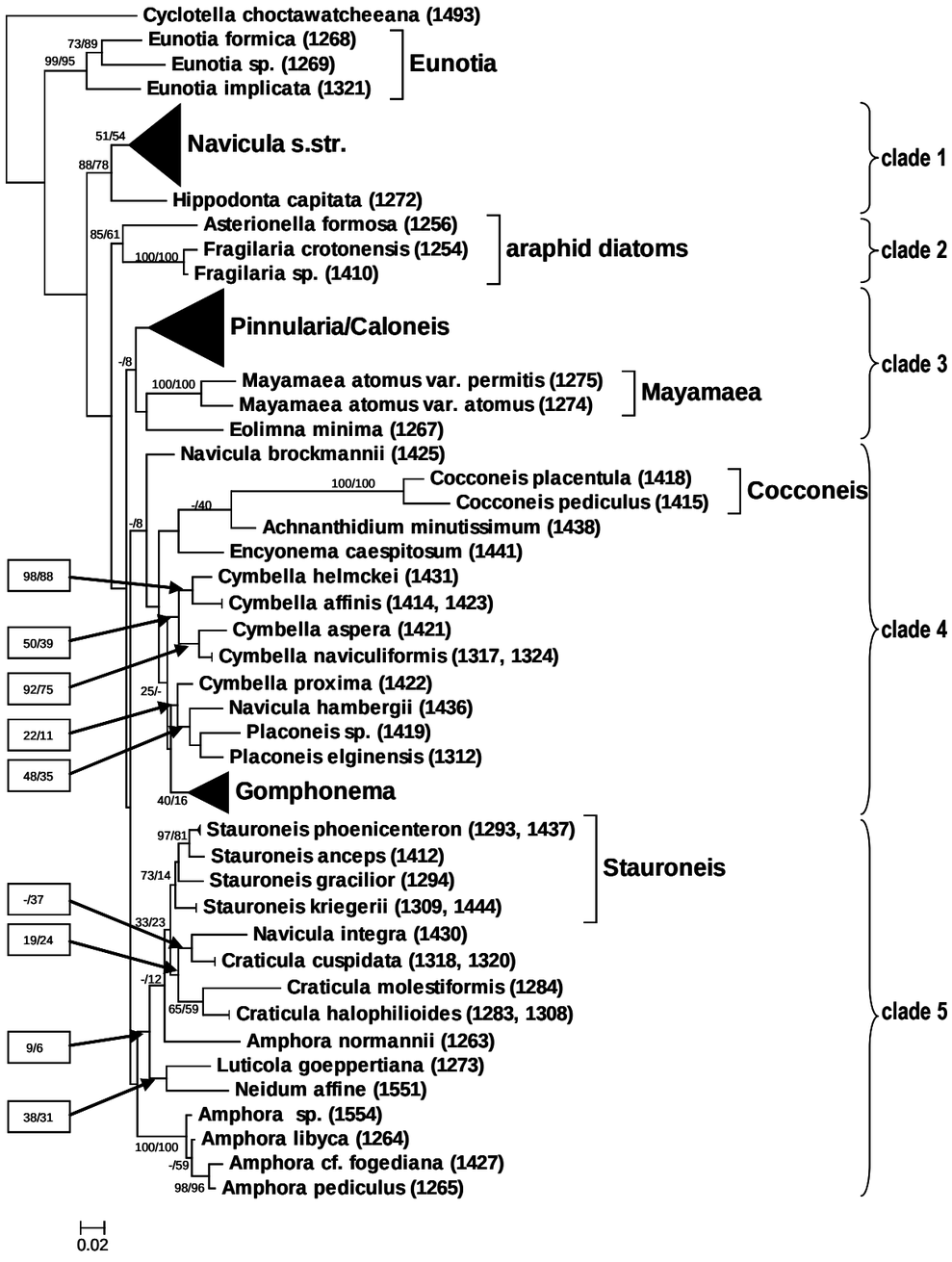


Fig.19: Phylogeny inferred with the ML analysis using *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Collapsed clades are shown in detail in Fig. 20.

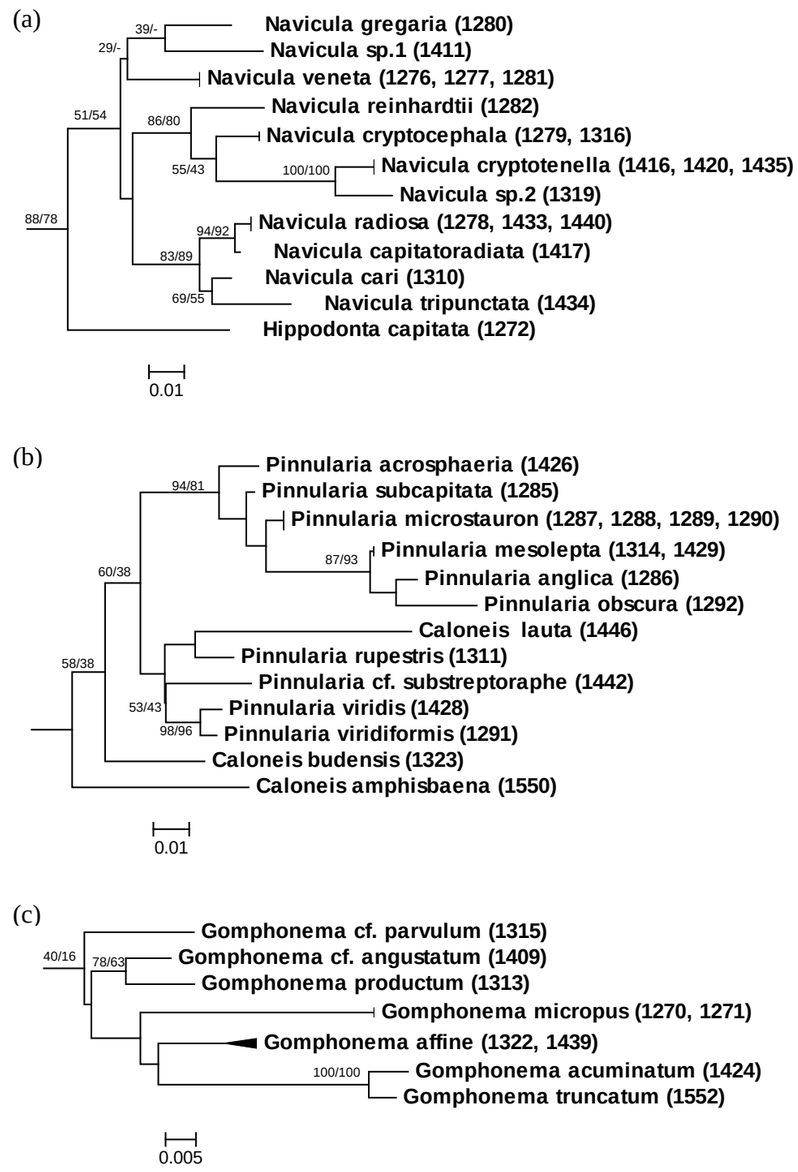


Fig. 20: Details of the ML tree analysis from *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Pinnularia* and *Caloneis*, (c) *Gomphonema*

monophyletic group. *C. helmckei, C. affinis, C. aspera* and *C. naviculiformis* formed a clade (BS: 50/39), but *C. proxima* clusters with *Navicula hambergii* and *Placoneis* (BS: 22/11). Within the monophyletic clade formed by the genus *Gomphonema* (BS: 40/16), the branching order calculated by the ML analyses had nearly no bootstrap support (Fig. 20c).

Four *Amphora* species formed a strongly supported monophyletic clade (BS: 100/100) at the base of clade 5. *Amphora normannii* was separated from the other *Amphora* species by a branch consisting of *Luticola goeppertiana* and *Neidum affine*. At the next divergence the monophyletic *Stauroneis* clade (BS: 73/14) was separated from a clade containing *Craticula* and *Navicula integra* (BS: 19/24). *Craticula* was paraphyletic.

The maximum parsimony (MP) analysis based on the *rbc*L sequences sequenced within the scope of the study resulted in 2 most parsimonious trees.

The genus *Eunotia* (BS: 99/95) diverges at the base of the majority-rule consensus tree (Fig. 21). Then a large polytomy with 34 branches followed. 22 of these branches leaded to single species. Four *Amphora* species formed a clade, which had maximum bootstrap support. Within the genus *Gomphonema* only *G. acuminatum* and *G. truncatum* grouped together (maximum BS). *Cymbella affinis* and *C. helmckei* (BS: 98/88) formed a group as well as *C. aspera* and *C. naviculiformis* (BS: 92/75). The *Pinnularia* species formed two clades, which consists of *P. acrosphaeria, P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron* one hand (BS: 94/81) and *P. rupestris, P. viridis, P.* cf. *substreptoraphe* and *P. viridiformis* on the other hand (BS: 91/54). Other small clades consists of *Craticula halophilioides* and *C. molestiformis* (BS: 65/59), *Stauroneis anceps* and *St. phoenicenteron* (BS: 97/81), *Mayamaea atomus* var. *atomus* and *M. atomus var. permitis* (maximum BS), the araphid species (BS: 85/61) and *Cocconeis placentula* and *C. pediculus* (maximum BS). The largest clade contains *Hippodonta capitata* and *Navicula sensu stricto*. The branching order within *Navicula sensu stricto* was not totally resolved, but two groups could be distinguished. One group contained *N. capitatoradiata, N. radiosa, N. cari* and *N. tripunctata* (BS: 83/89) thither group consists of *N. reinhardtii, N. cryptocephala, N. crytotenella* and an unidentified *Navicula* species (BS: 86/80).

The *rbc*L gene sequences obtained from GenBank were longer, than those sequenced within the scope of this study. They were all cut to a length of 684 nucleotides.

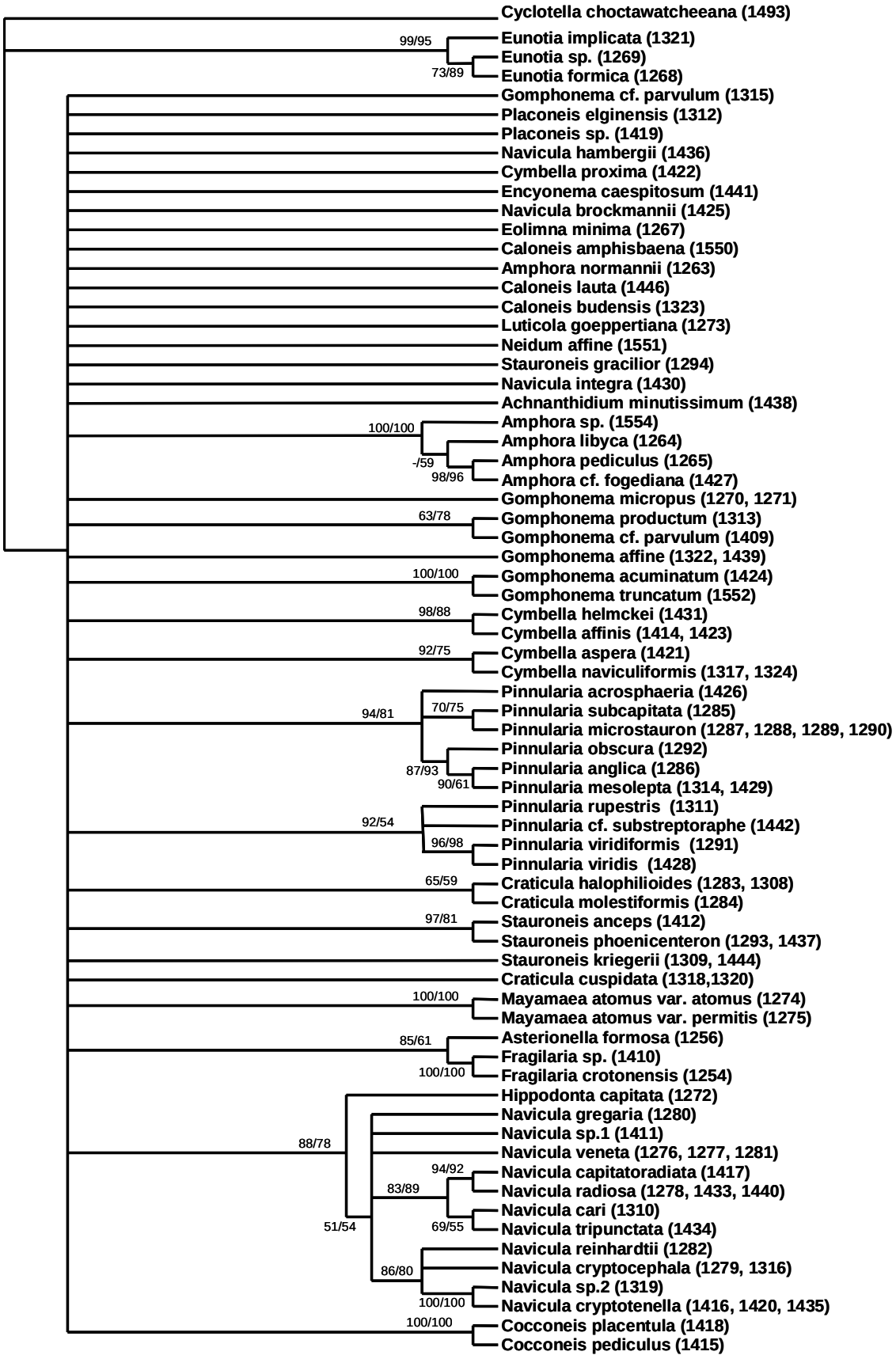


Fig. 21: Majority-rule consensus tree inferred with the parsimony analysis based on *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes.

The phylogeny inferred with the ML analysis using the extended alignment is shown in figure 22. The genus *Eunotia*, which forms a well supported monophyletic group (BS: 99/96), diverges at the base of this tree (Figs. 22 and 23a).

In this tree *Hippodonta capitata* and *Navicula sensu stricto* did not form a monophyletic group. *H. capitata* and *N. salinicola* diverges first. Then clade 1 with *Pseudogomphonema* cf. *kamschaticum* and *Seminavis* cf. *robusta* at the base and a monophyly of the remaining *Navicula sensu stricto* species diverge. Within this *Navicula sensu stricto* two groups could be distinguished.

The next two clades consist of *Petroneis humerosa* and *Lyrella* (clade 2, BS: 96/93) and the monoraphid species (clade 3, BS: 0/46).

At the next lineage the tree diverges into two clades. At the base of clade 4 *Navicula brockmannii* diverges. The next clade consists of the genus *Gomphonema* (BS: 40/16), which further diverges into two groups (Fig. 23b). *Encyonema* cf. *sinicum* and *E. caespitosum* formed a monophyletic group which diverges next. The genus *Cymbella* was paraphyletic. *Navicula hambergii* and *Placoneis* formed a monophyletic group (BS: 33/24) which cluster with *C. proxima* (BS: 16/9). The remaining *Cymbella* species formed the sister group of this clade.

The araphid diatoms diverged at the base of clade 5 (BS: 84/67). Maximum bootstrap values support the monophyly of four *Amphora* species (group 1). After the divergence of *Luticola goeppertiana* and *Neidum affine* (BS: 36/30) the tree diverges into two groups. *Amphora normannii* was at the base of the first group. *Stauroneis* forms a monophyletic clade, which separate *Craticula halophilioides* and *C. molestiformis* from *C. cuspidata* and *Navicula integra*. In the second group *Mayamaea, Sellaphora* and *Eolimna minima* formed the sister group to *Pinnularia* and *Caloneis*. At the base of this clade *C. budensis* diverged first (Fig. 23c). After the divergence of *C. amphisbaena* the other species were subdivided into two clades.

The majority-rule consensus tree (Fig. 24) from the extended alignment based on 105 most parsimonious trees.

The genus *Eunotia* (BS: 99/95) diverges at the base of this tree.

Navicula sensu stricto, Hippodonta capitata, Seminavis cf. *robusta* and *Pseudogomphonema* cf. *kamschaticum* formed clade 1, which diverges next (BS: 66/45). *H. capitata, S.* cf. *robusta* and *P.* cf. *kamschaticum* diverge within *Navicula sensu stricto*.

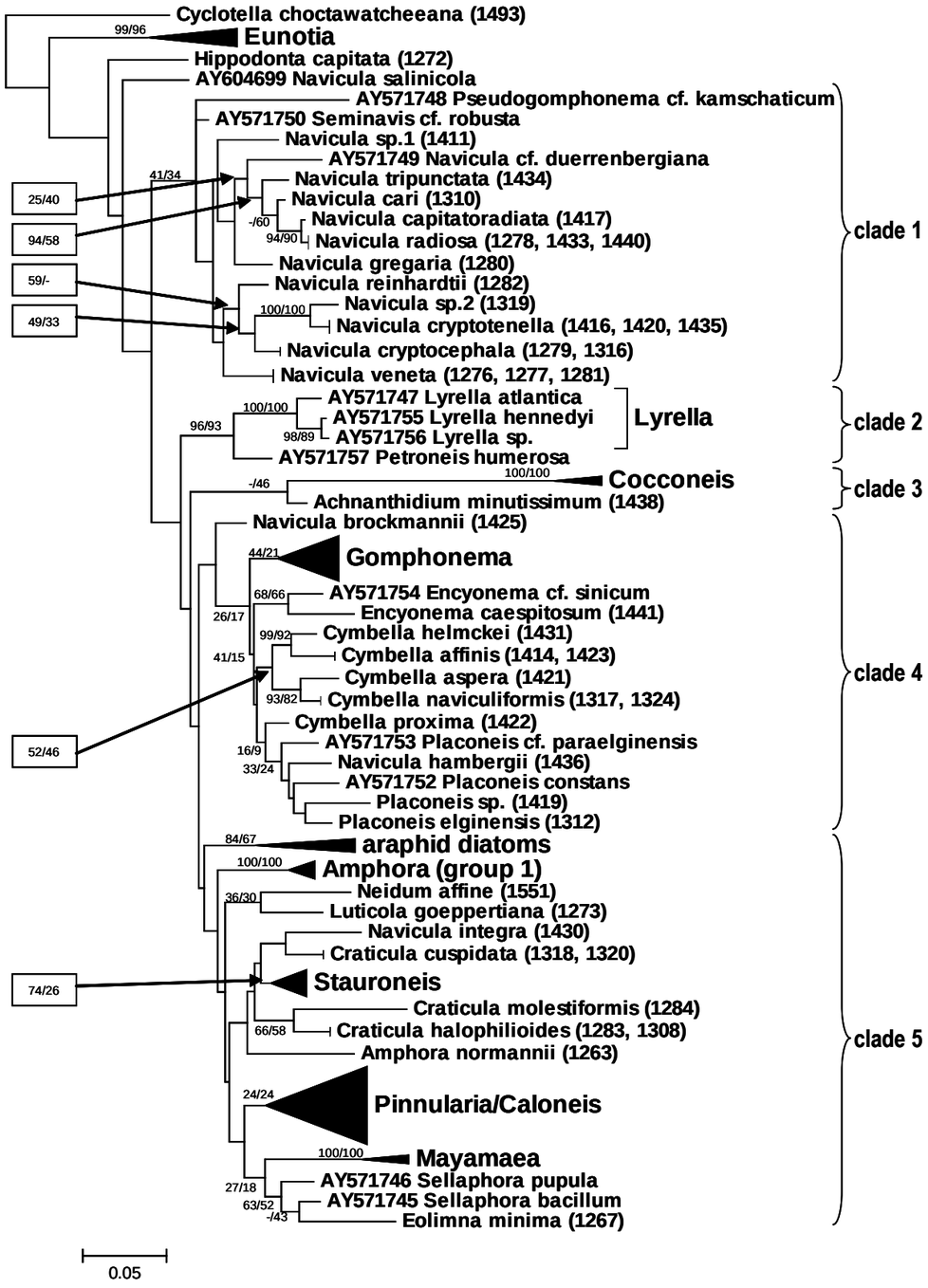


Fig. 22: Phylogeny inferred with the ML analysis using *rbc*L sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Collapsed clades, which differ to the equivalent clades in Figs. 19 and 20, are shown in detail in Fig. 23.

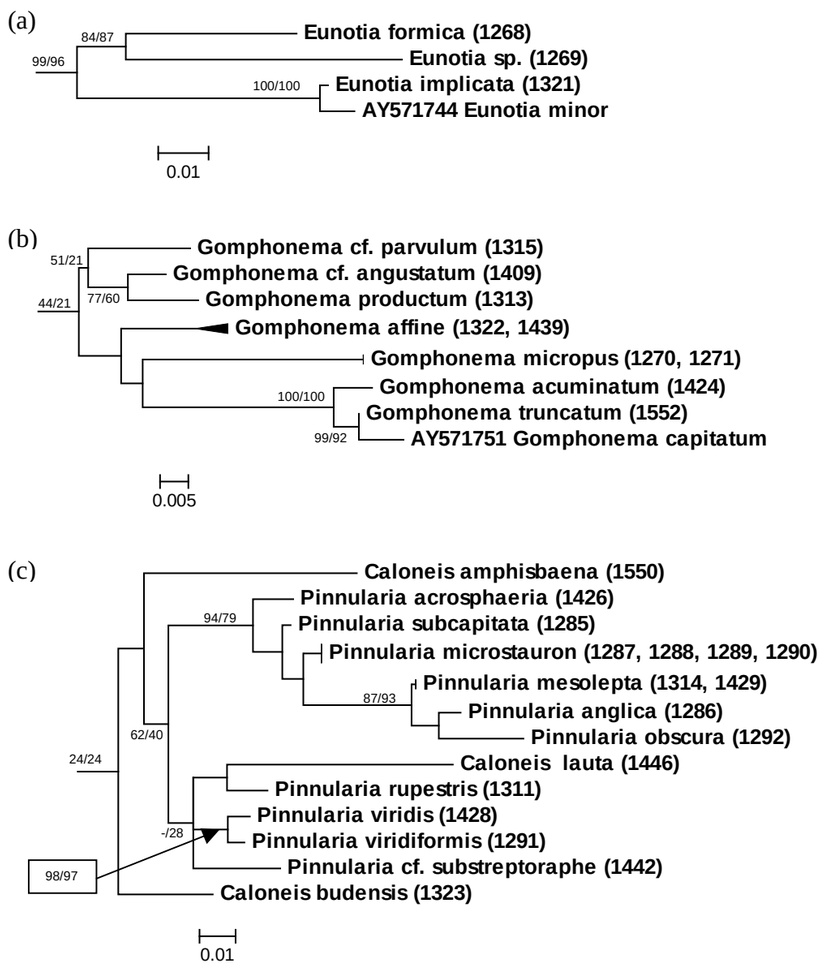


Fig. 23: Details of the ML tree analysis from *rbc*L sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) Eunotia, (b) *Gomphonema*, (c) *Pinnularia* and *Caloneis*

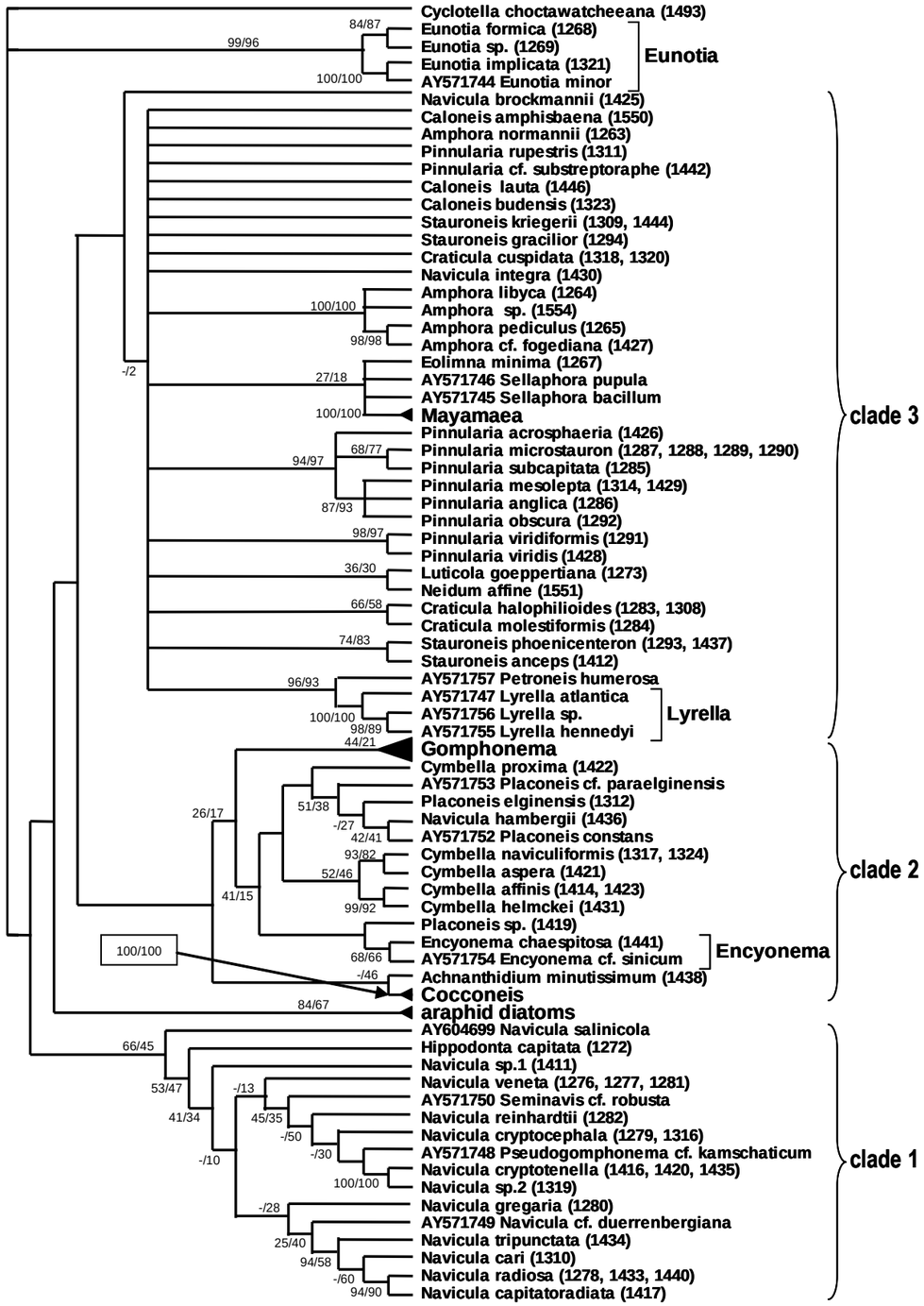


Fig. 24: Majority-rule consensus tree inferred with the parsimony analysis based on *rbc*L sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Collapsed clades, which differ to the equivalent clades in Fig. 21, are shown in detail in Fig. 25.

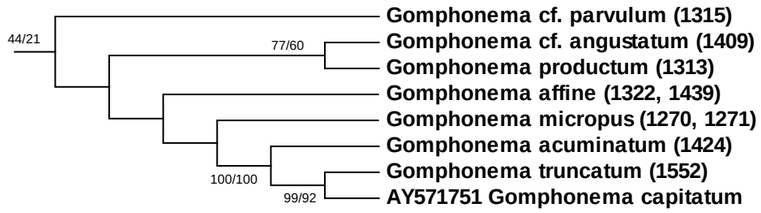


Fig. 25: *Gomphonema* clade of the parsimony tree analysis based on *rbc*L sequences from GenBank and the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes.

The next clade consists of araphid diatoms (BS: 84/67).

At the base of clade 2 the monoraphid diatoms diverge (BS: 0/46). The *Gomphonema* species formed a monophyletic clade (Fig. 25), but it had only weak BS. *Cymbella* and *Placoneis*/*N. hambergii* did not form monophyletic clades, because the unidentified *Placoneis* species clusters with *Encyonema* and *C. proxima* clusters with remaining *Placoneis* species. But none of these clades had bootstrap support.

Navicula brockmannii diverged at the base of clade 3. The other species were pooled in a large polytomy. Four *Amphora* species formed a branch, which had maximum bootstrap support. Another branch contained *Eolimna minima*, *Sellaphora* and *Mayamaea* (BS: 27/18). Two strongly supported clades consist of *Pinnularia* species. *P. acrosphaeria, P. microstauron, P. subcapitata, P. mesolepta, P. anglica* and *P. obscura* one hand (BS: 94/97) and *P.* cf. *substreptoraphe* and *P. viridiformis* on the other hand (BS: 98/97). Other small clades consists of *Luticola goeppertiana* and *Neidum affine* (BS: 36/30), *Craticula halophilioides* and *C. molestiformis* (BS: 66/58), *Stauroneis anceps* and *St. phoenicenteron* (BS: 74/83) and *Petroneis humerosa* and *Lyrella* (BS: 96/93).

3.1.4 Gene combination

The combination of the three alignments, which consists of data sequenced within the scope of this study, resulted in an alignment having 3226 positions in total. 896 of these positions were parsimony-informative and 297 parsimony-uninformative characters.

Results from 100 partition homogeneity test replicates indicated that SSU rDNA, LSU rDNA and *rbc*L gene data were significantly heterogeneous (p=0,01).

The ML phylogeny based on this alignment is shown in Fig. 26. The collapsed clades of this figure are shown in detail in Fig. 27.

The strongly supported (BS: 100/100) monophyletic clade of *Eunotia* diverged at the base of this tree.

Clade 1, which includes *Hippodonta capitata* and the *Navicula sensu stricto*, had maximum bootstrap support. Bootstrap values of 100 and 99 from NJ and MP bootstrap analysis support the monophyly of *Navicula sensu stricto*. The *Navicula sensu stricto* was subdivided in three groups (Fig. 27a). The first consists of *N. veneta* and *N. gregaria* (BS: 100/91). *N. crytotenella, N. reinhardtii, N. cryptocephala* and the two unknown *Navicula* species formed the second group which was supported by 88% and 84% of the bootstrap

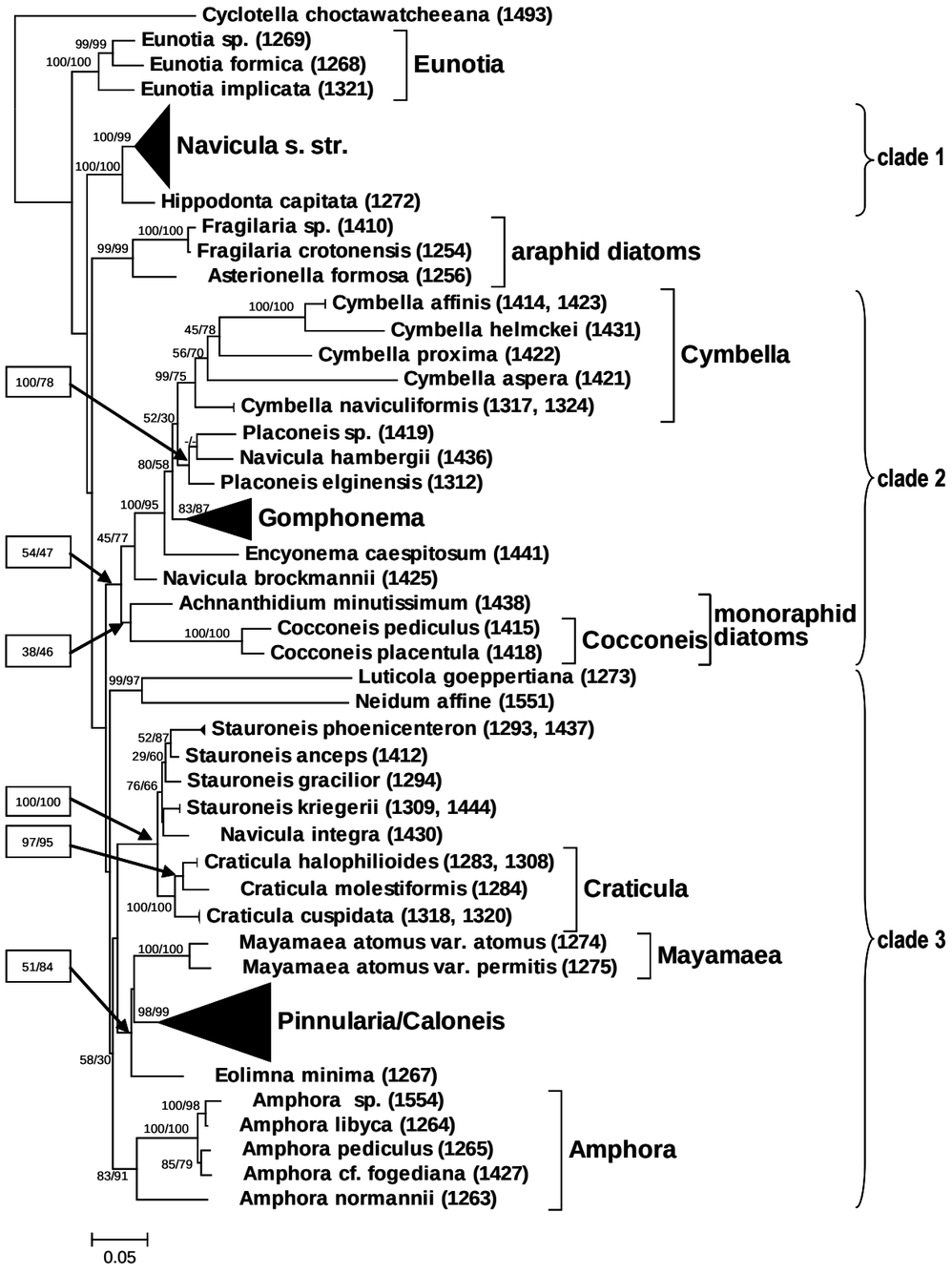


Fig. 26: Phylogeny inferred with the ML analysis using the combined dataset of SSU rDNA, LSU rDNA and *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed clades are shown in detail in Fig. 27.

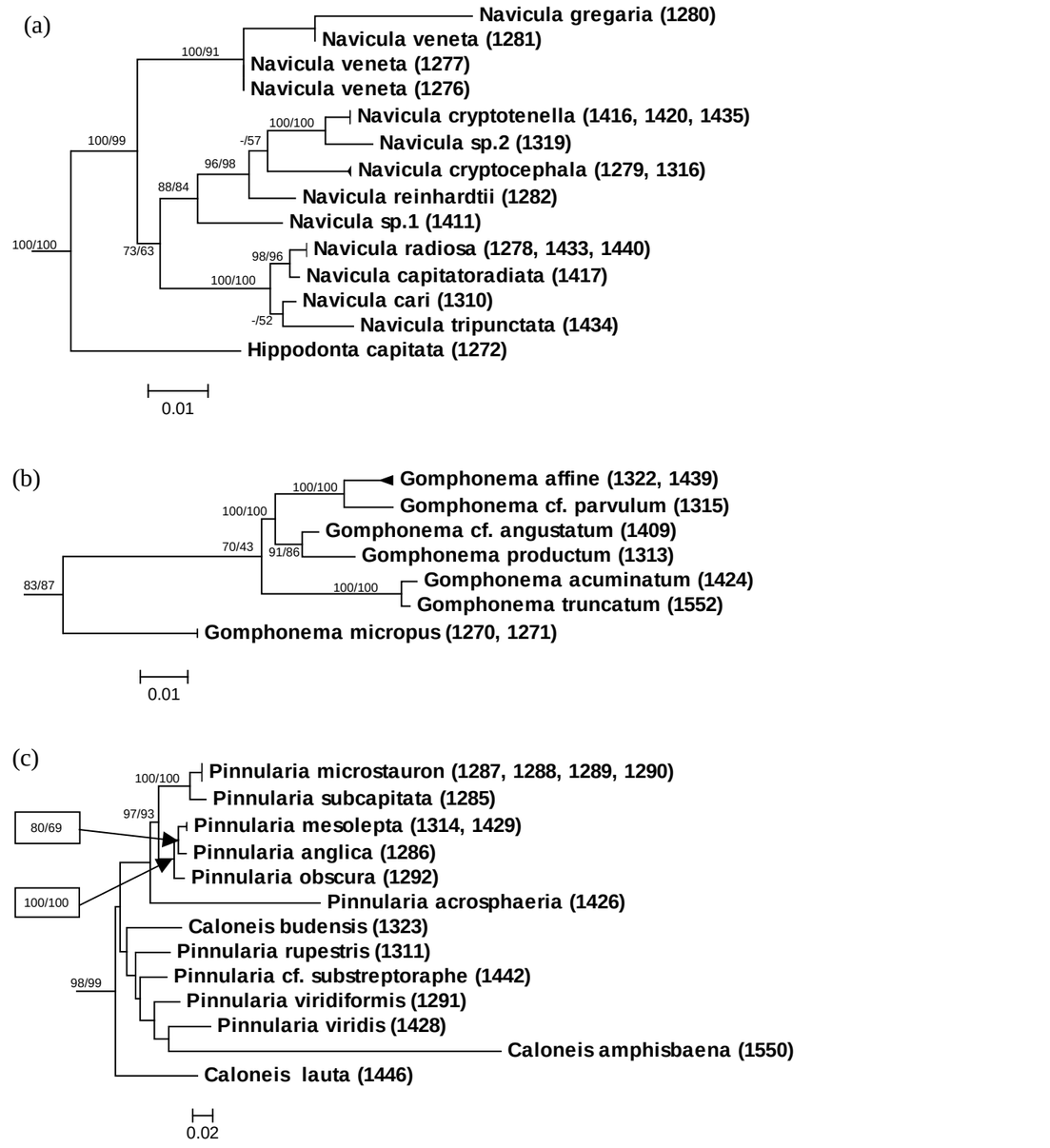


Fig. 27: Details of the ML tree analysis from the combined dataset of SSU rDNA, LSU rDNA and *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Gomphonema*, (c) *Pinnularia* and *Caloneis*

replicates. The third group contained *N. radiosa, N. capitatoradiata, N. cari* and the type species *N. tripunctata* and had maximum bootstrap support.

Although the araphid diatoms were assigned as outgroup they diverge next. They formed a strongly supported monophyletic group (BS: 99/99).

The remaining taxa diverge into two major clades. At the base of clade 2 the monoraphid genera formed a monophyletic group (BS: 38/46). Then *Navicula brockmannii* and *Encyonema caespitosum* diverged. The next clade contained *Gomphonema* species. Within this monophyletic group (BS: 83/87) *G. micropus* diverged first and was separated from the other *Gomphonema* species (Fig. 27b) by branch length and maximum BS. The next divergence separates *Placoneis*/*Navicula hambergii* (BS: 100/78) from *Cymbella* (BS: 99/75).

At the base of clade 3 in the ML tree *Luticola goeppertiana* and *Neidum affine* form a clade, which was supported by bootstrap values of 99 and 97.

Within the next sub-clade (BS: 83/91) *Amphora normannii* was separated from the other *Amphora* species by branch length and maximum bootstrap support from both analyses.

Eolimna minima is at the base of the next sub-clade, which includes *Mayamaea* and a monophyletic group containing *Pinnularia* and *Caloneis*. Within this well supported group (BS: 98/99) *Caloneis lauta* diverged first (Fig. 27c). The other species were subdivided into two clades. One clade consists of *C. budensis, P. rupestris, P. viridis, P.* cf. *substreptoraphe, P. viridiformis* and *C. amphisbaena,* the other contained *P. acrosphaeria, P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron*.

At the next divergence, the monophyletic clade containing the three *Craticula* species (BS: 100/100) and a clade containing *Navicula integra* and four *Stauroneis* species (BS: 76/66) were separated.

The maximum parsimony (MP) analysis based on the combined data set resulted in 6 most parsimonious trees. The majority-rule consensus tree of these trees is shown in Figs 28 and 29.

Neidum affine and *Luticola goeppertiana* formed a well supported clade (BS: 99/97), which diverged from the polytomy at the base of the tree.

The *Eunotia* species formed monophyletic group with maximum bootstrap support and a bootstrap value of 99 from both bootstrap analyses support the monophyly of the araphid diatoms. They diverge next, but the phylogenetic relationship between these two groups is not resolved.

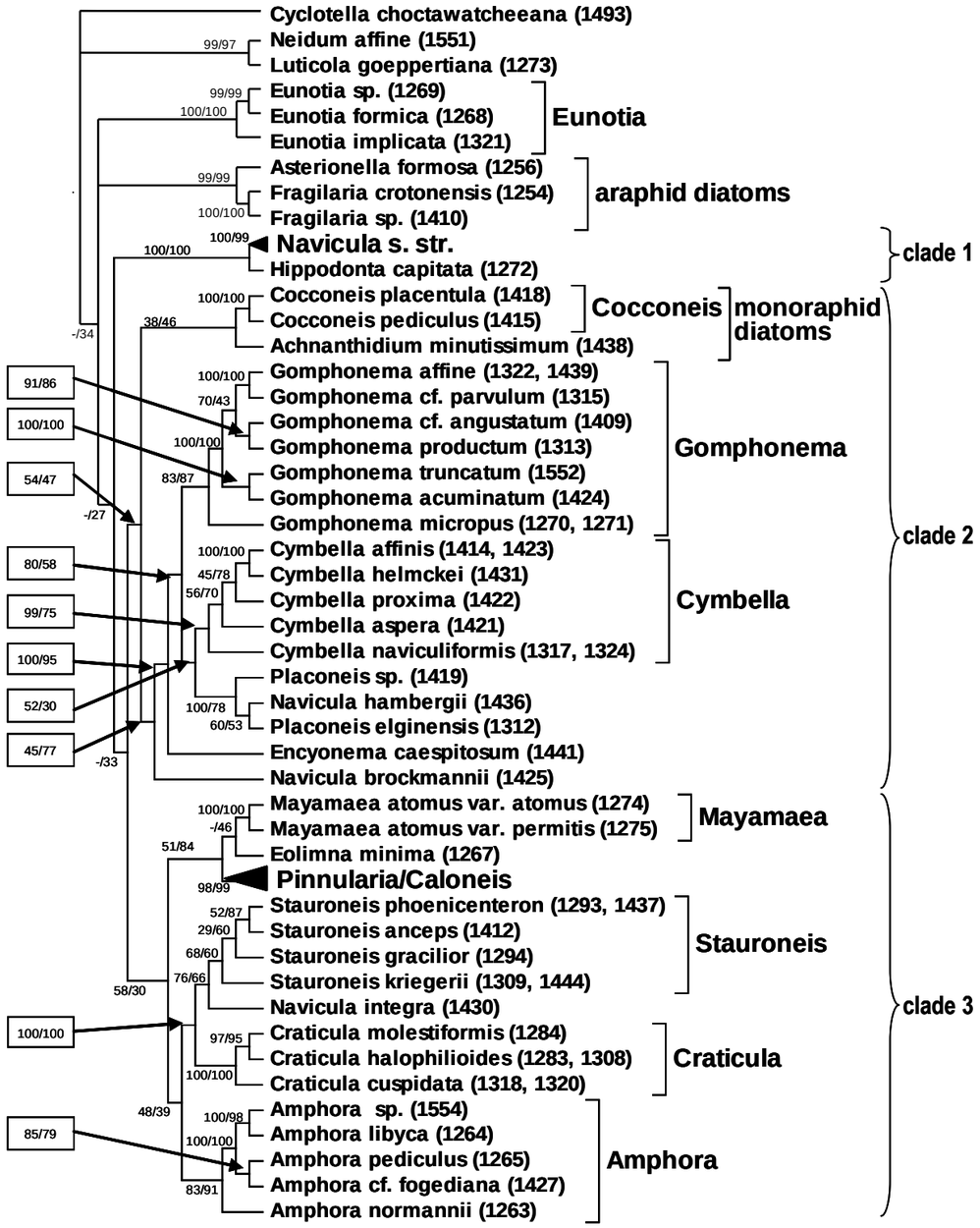


Fig. 28: Majority-rule consensus tree inferred with the parsimony analysis based on a combined dataset of SSU rDNA, LSU rDNA and *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. Condensed regions are shown in detail in Fig. 29.

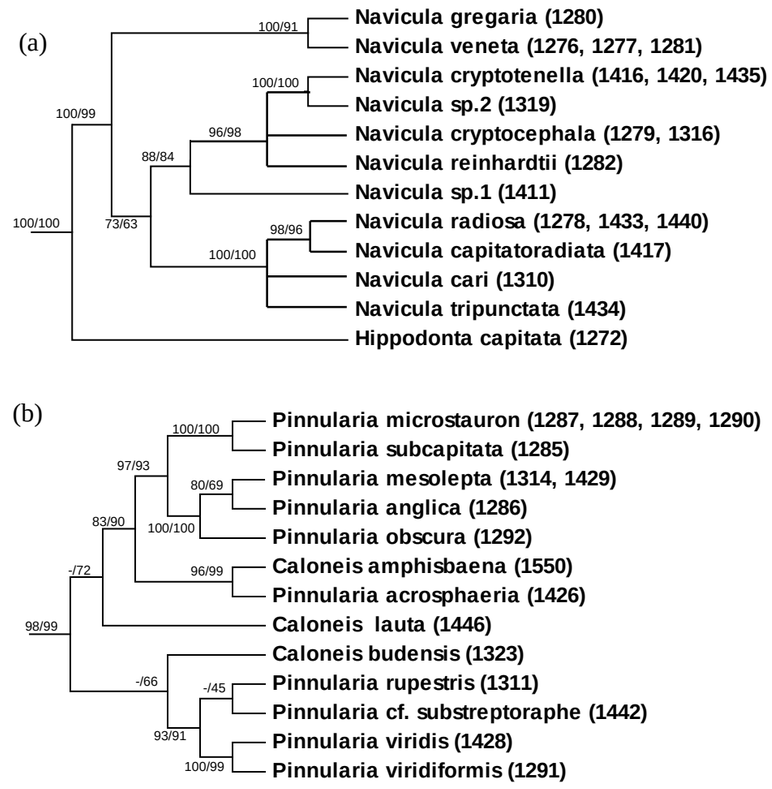


Fig. 29: Details of the parsimony tree analysis based on a combined dataset of SSU rDNA, LSU rDNA and *rbc*L sequences from the AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model and on parsimony analyses have been plotted at the nodes. (a) *Navicula sensu stricto*, (b) *Pinnularia* and *Caloneis*

Navicula sensu stricto and *Hippodonta capitata* were sister groups (BS: 100/100) and formed the basal clade 1 of the raphid diatoms. Bootstrap values of 100 and 99 support the monophyly of *Navicula sensu stricto*. The *Navicula sensu stricto* clade further diverges into three groups Fig. 29a). *N. veneta* and *N. gregaria* formed the first group, which was supported by 100% and 91% of the bootstrap replicates. The second group consisted of *N. crytotenella, N. reinhardtii, N. cryptocephala* and the two unidentified *Navicula* species (BS: 88/84). The third group contained *N. radiosa, N. capitatoradiata, N. cari* and *N. tripunctata*. The phylogenetic relationship within these groups is not totally resolved.

The other raphid diatoms diverge into two large clades, which were supported by bootstrap values between 30 and 58.

Clade 2 consists of the monoraphid diatoms, *Navicula brockmannii* and several *Cymbellales*. A monophyletic clade containing the monoraphid diatoms (BS: 38/46) is the basal branch. *N. brockmannii* diverges next. Within the *Cymbellales* (BS: 100/95) *Encyonema caespitosum* diverges first. The next clade was formed by *Gomphonema* species. Within this monophyletic group (BS: 83/87) *G. micropus* diverges first. Its sister clade consists of *Cymbella, Placoneis* and *Navicula hambergii* (BS: 52/30). The monophyly of *Cymbella* is supported by relativly high bootstrap values (BS: 99/75). The *Placoneis* species and *N. hambergii* form a monophyletic group supported by bootstrap values of 100/78.

The basal divergence of clade 3 consists of *Eolimna, Mayamaea, Pinnularia* and *Caloneis* and supported by bootstrap values of 51 and 84. *Pinnularia* and *Caloneis* formed a strongly supported monophyletic group (BS: 98/99), which further diverges into two clades (Fig. 29b). *C. budensis, P. rupestris, P. viridis, P.* cf. *substreptoraphe* and *P. viridiformis* formed on clade, the other contained *C. lauta, P. acrosphaeria, C. amphisbaena, P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron*. The genus *Amphora* diverges next (BS: 83/91). Maximum bootstrap values support the monophyly of *Craticula*, which diverges next. The sister group of *Craticula* consists of *Navicula integra* at the base of *Stauroneis.*

3.2 Morphological support for molecular data

3.2.1 *Navicula sensu stricto*

With the exception of the tree in Fig. 22, the *Navicula sensu stricto* species were pooled in a monophyletic clade with *Hippodonta capitata* diverging at the base. In one tree (Fig. 8) two *Pseudogomphonema* species appeared within this clade, in other trees *Neidum affine* (Figs.

11, 15) and *Luticola goeppertiana* (Fig. 15) diverged within *Navicula sensu stricto*. In all trees *N. radiosa*, *N. capitatoradiata*, *N. cari* and *N. tripunctata* form a monophyletic group (group 1), to which *N. lanceolata* and *N. ramosissima* were additionally added in Fig. 8 and *N.* cf. *erifuga* in Figs. 15 and 18. The other species were paraphyletic (Fig. 8 and 24) or subdivided in two groups. With the exception of one unidentified species, these groups consists always of *N. veneta* and *N. gregaria* on one hand (group 2) and the remaining species on the other hand (group 3).

The morphological investigations concentrated on the three groups. But no features could be found that were typical for one group and absent in the other groups. All species show the typical features for *Navicula sensu stricto*, such as two plate-like plastids (Figs. 30 a + e, 31 a + d and 33 a, c, e, g) or apically elongated liner poroids (Figs. 30 c, d, g, h; 32 and 34). Differences in the outline of the valves were greater within the groups than between them (Figs. 30 - 34)

3.2.2 *Amphora*

The four species *A. libyca*, *A. pediculus*, *A.* cf. *fogediana* and the unidentified *Amphora* species (1554) formed a monophyletic clade, which had maximum BS in all trees. In the tree in Fig. 9, the SSU rDNA sequence of *A.* cf. *proteus* (obtained from GenBank) diverged at the base of this group (BS: 100). The fifth species isolated within the scope of this study (*A. normannii*) also diverged at the base of this group in some trees (Figs. 3, 5, 12, 13, 26 and 27), but in the ML trees (Figs. 3, 12 and 26) the branch length indicated a separation. In all phylogenies based on *rbc*L sequences (Figs. 19, 21, 22 and 24), *A. normannii* is separated from the other four species. In Fig. 10 *A. normannii* formed a strongly supported monophyletic clade with *A. montana*, *A.* cf. *capitellata* and an unidentified species. *A. coffeaeformis* is separated from this group in Fig. 10, but formed a well supported clade with *A. normannii* in Fig. 16.

All species show the typical asymmetrical valve morphology (Figs. 35 - 37). In an intact frustule both raphe systems lie on the same side (Figs. 35 c; 36 c, d, f; 37 b, d, h). Therefore live individuals normally lie on the ventral side (Figs. 35 a; 36 a). From the five species cultured within the scope of this study, only *A. normannii* has numerous girdle bands (Figs. 35 c; 36 c, d, f; 37 b, d, f, h). The girdle of the other four species have not more than two girdle bands (valvocopula, Fig. 37 b, d, f, h) From the species, of which sequences were obtained from GenBank, *A. coffeaeformis*, *A. montana* and *A.* cf. *capitellata* have numerous girdle bands (Frenguelli, 1938, Krammer & Lange-Bertalot, 1986).

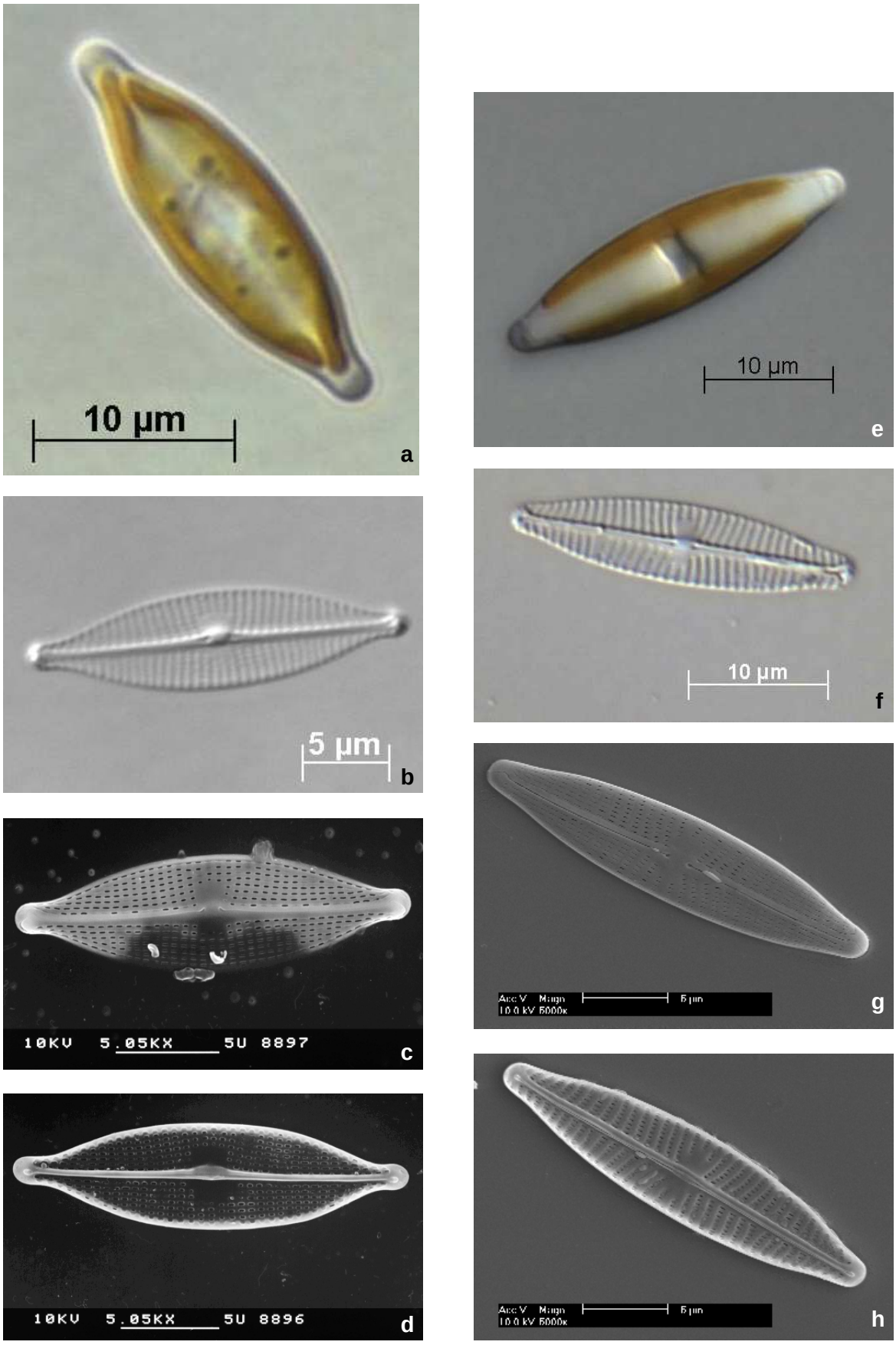


Fig. 30: a - d: *Navicula gregaria*, e - h: *Navicula veneta*.
a + e: Light micrograph of live individual. b + f: Light micrograph of cleaned valve.
c + g: SEM, internal view of a valve. d + h: SEM, external view of a valve.

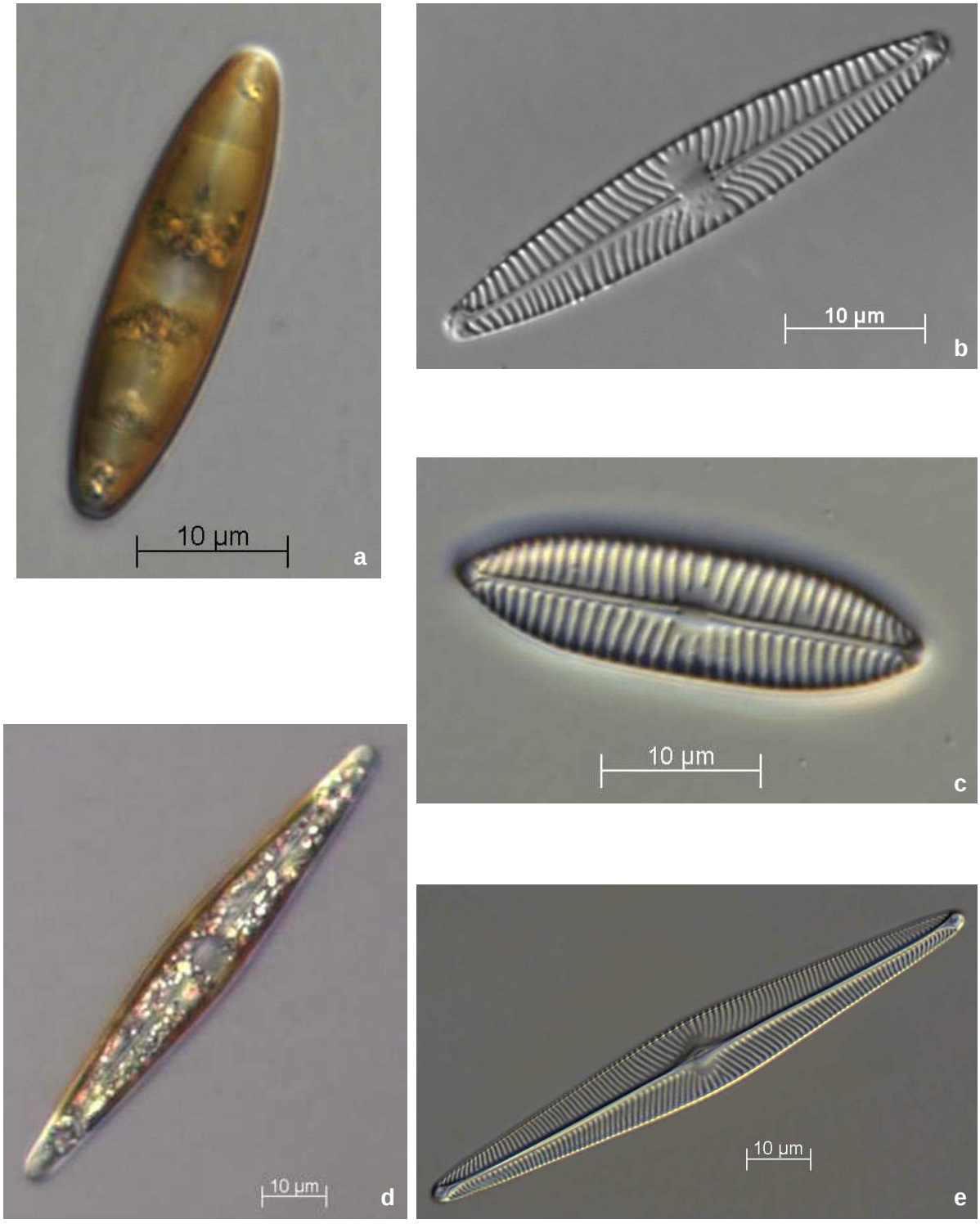


Fig. 31: *Navicula* species
a + b: *N. cari*, light micrograph of live individual (a) and cleaned valve (b);
c: *N. tripunctata* light micrograph of a cleaned valve;
d + e: *N. radiosa*, light micrograph of live individual (d) and a cleaned valve (e).

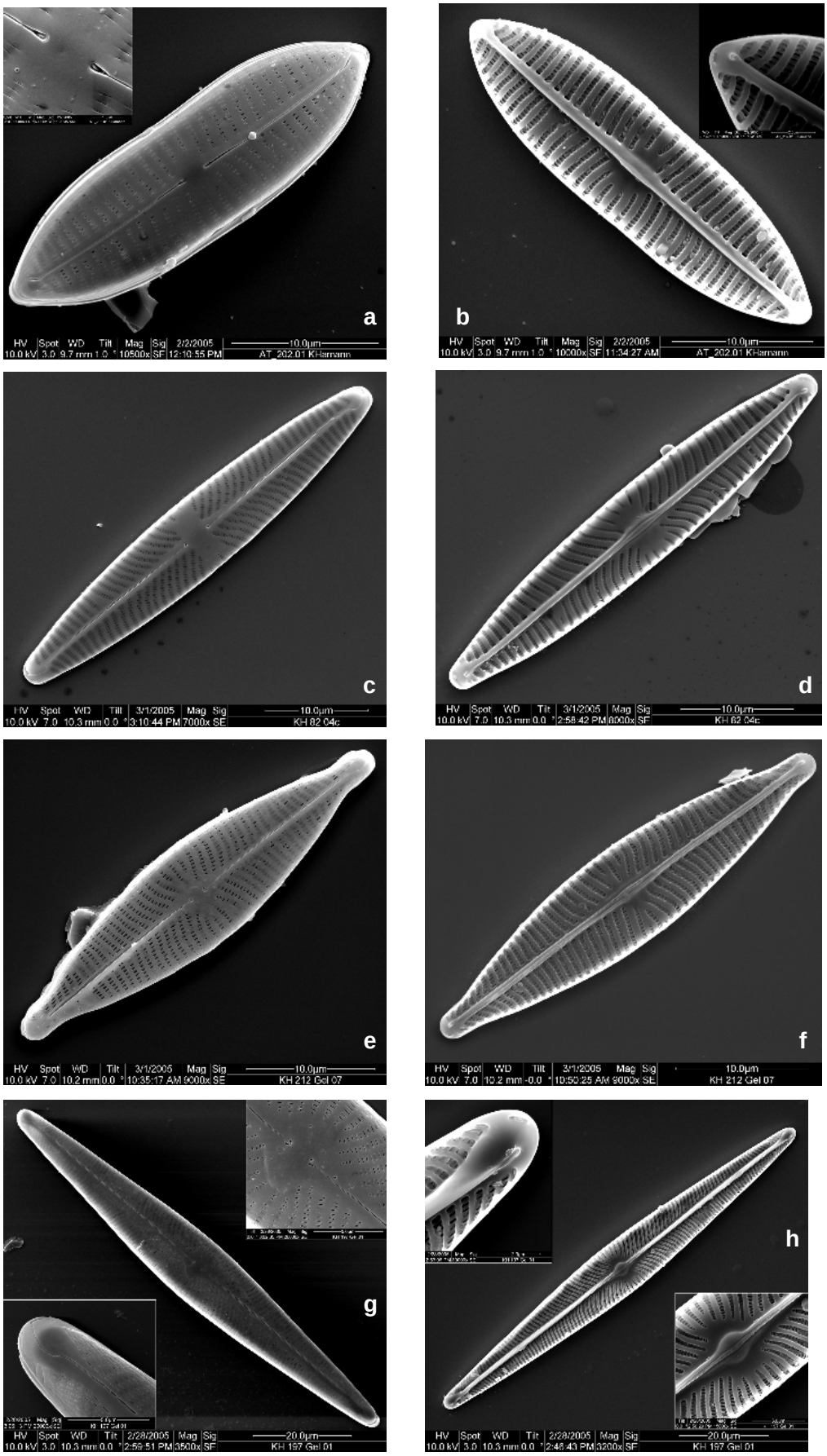


Fig. 32: *Navicula* species, SEM of valve exteriors (a, c, e, g) and interiors (b, d, f, h), some with enlarged midvalve or apice details.

a + b: *N. tripunctata*, c + d: *N. cari*, e + f: *N. capitatoradiata*, g + h: *N. radiosa*

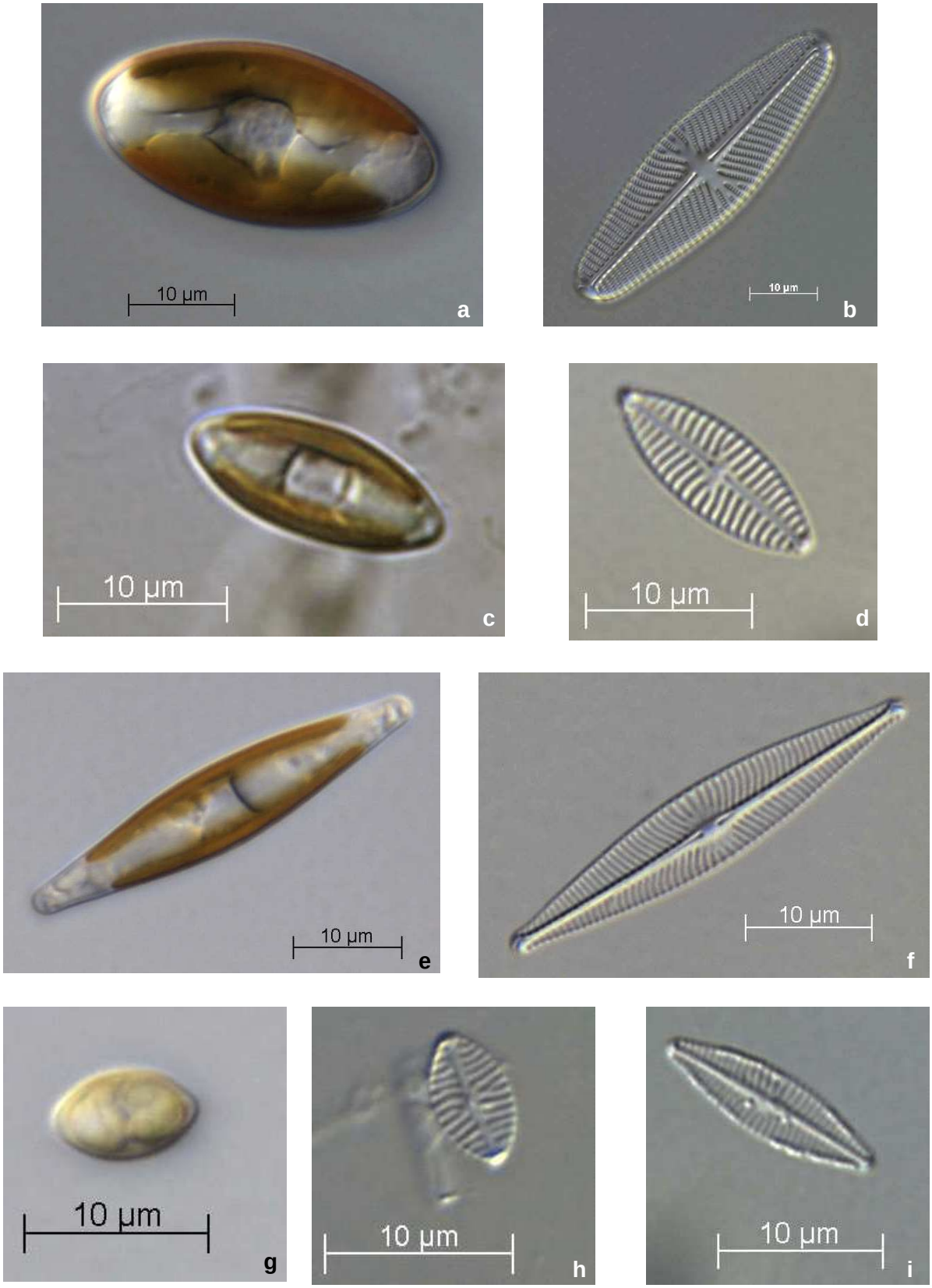


Fig. 33: *Navicula* species, light micrographs of live individuals (a, c, e, g) and cleaned valves (b, d, f, h, i)

a + b: *N. reinhardtii*, c + d: *N. cryptotenella*, e + f: *N. cryptocephala*, g + h: *Navicula* sp.2 (1319), i: *Navicula* sp.1 (1411).

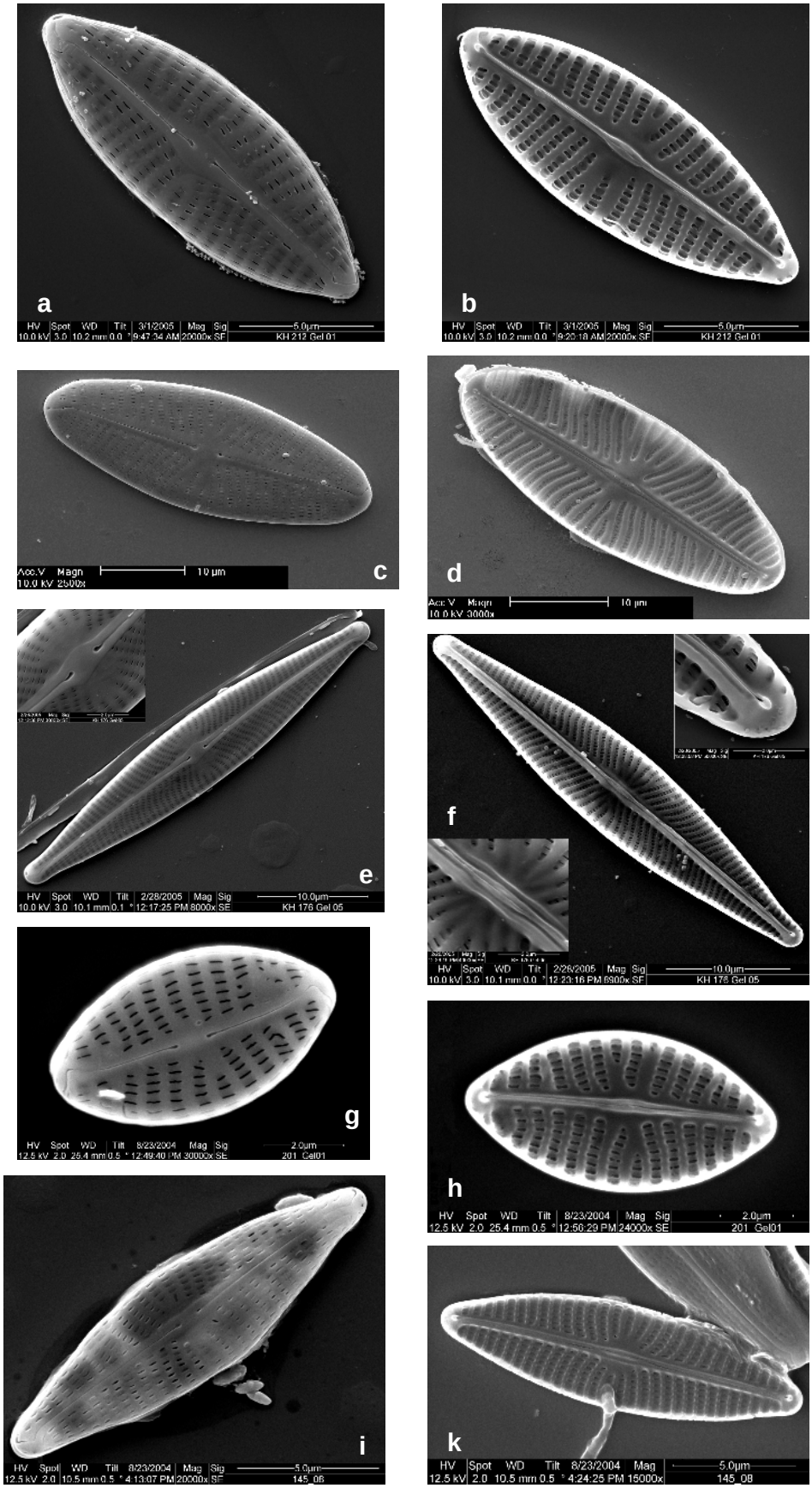


Fig. 34: *Navicula* species (group 3), SEM of valve exteriors (a, c, e, g, i) and interiors (b, d, f, h, k), some with enlarged midvalve or apice details.

a + b: *N. cryptotenella*, c + d: *N. reinhardtii*, e + f: *N. cryptocephala*, g + h: *Navicula* sp.2 (1319), *i + k: Navicula* sp.1 (1411).

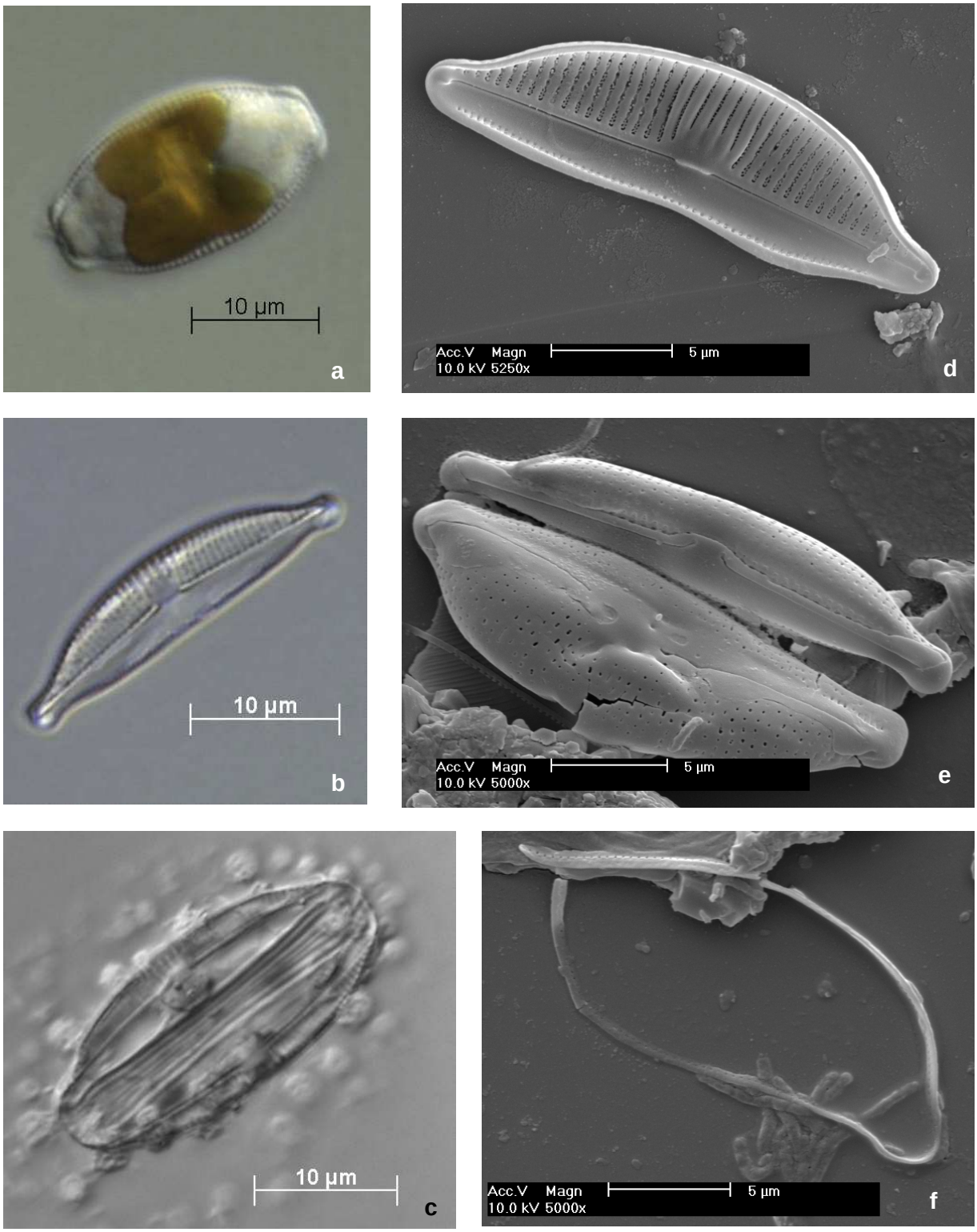


Fig. 35: *Amphora normannii*.

a: Light micrograph of live individual. b, c: Light micrographs of cleaned valve in valve view (b) and ventral view (c). d: SEM, internal view. e: SEM, external view. f: SEM, girdle band

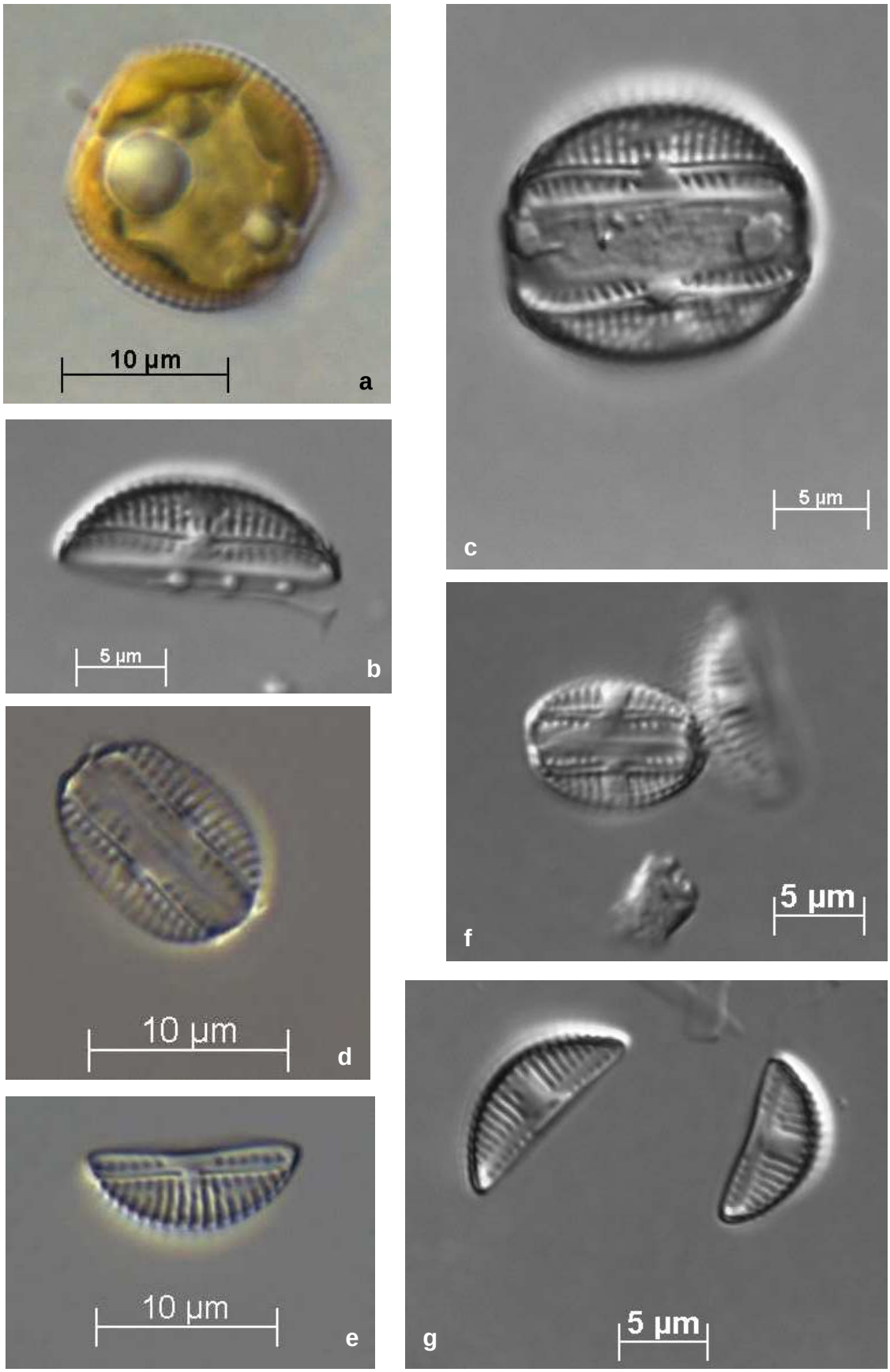


Fig. 36: Light micrographs of *Amphora* species.

a - c: *A. libyca*, live individual (a) and cleaned valves in valve view (b) and ventral view (c).
d + e: *A. cf. fogediana*, cleaned valves in valve view (d) and ventral view (e).
f + g: *A. pediculus*, cleaned valves in valve view (f) and ventral view (g).

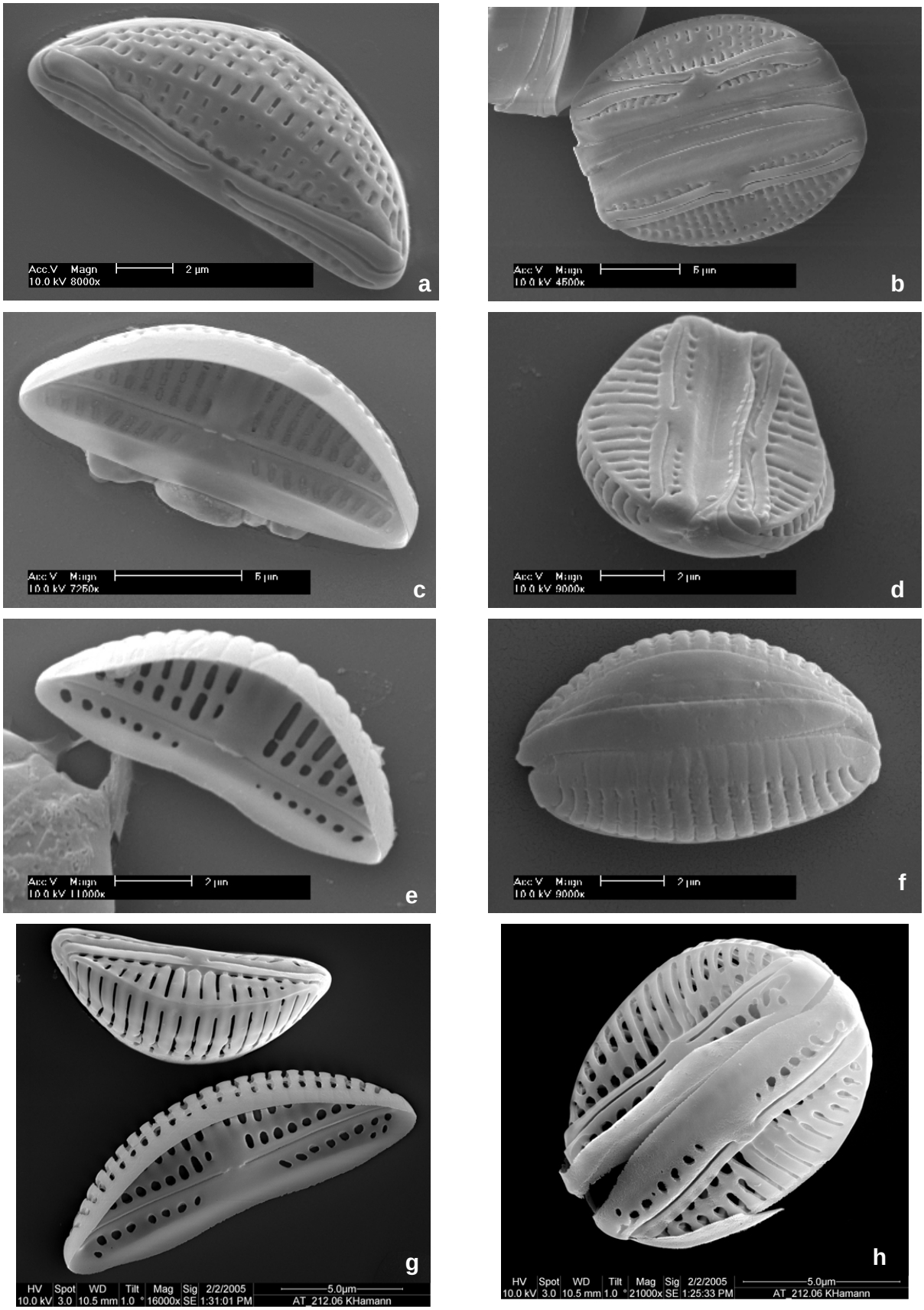


Fig 37: SEM micrographs of *Amphora* species.

a - c: *A. libyca*, frustule exteriors (a: valve view, b: ventral side) and valve interior (c).
d - f: *A. pediculus*, frustule exteriors (d: ventral side, f: dorsal side) and interior (e).
g + h: *A.*cf.*fogediana*, frustule exteriors (g top: valve view, h: ventral side) and valve interior (g below).

3.2.3 *Pinnularia* and *Caloneis*

The position of the three *Caloneis* species and of *P. acrosphaeria* differs in the different phylogenies. But in all trees, *P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron* were members of one clade (group 1), whereas *P. rupestris, P. viridis, P.* cf. *substreptoraphe* and *P. viridiformis* formed a second clade (group 2). Within group 1 *P. obscura, P. anglica* and *P. mesolepta* formed a well supported clade in all trees (BS: 87 – 100).

The morphological investigations of the two *Pinnularia* groups showed clear differences.

The species in group 1 have a filiform or slightly lateral raphe system, but even if the external raphe slit is slightly undulate it never crosses the internal slit (Fig. 38 b, d, f, h, i and l). All species have a large central area, which is often a fascia extended to the margin on one or both sides (Figs. 38 b, d, f, h, i and l and 39). Midvalve, the striae are radial or parallel and become convergent or parallel at the apices (Figs. 38 b, d, f, h, i and l and 39). *P. subcapitata* and *P. microstauron* have two plate-like chloroplasts (Fig. 38 g, k). But *P. obscura, P. anglica* and *P. mesolepta* have a single H-shaped plastid arranged as two large plates along each side of the girdle and a very fine bridge under one valve face (Fig. 38 a, c, e).

All species in group 2 have a lateral raphe system (Fig. 40 b, d, f, g). In some species the undulate external raphe fissure crosses over the internal fissure along the raphe (complex raphe, Fig. 40 f, g). The linear or linear-lanceolate axial area pass into a slightly expanded central area, which is often asymmetric (Fig. 40 b, d, f, g). The striae are radial or parallel at the centre of the valve and convergent or parallel at the apices (Fig. 40 b, d, f, g). Under the light microscope the striae were crossed by a lateral line (Fig. 40 b, d, f, g). As it can be seen in the SEM pictures (Fig. 41), partially closed alveolae are causal for this line. All species have two plate-like chloroplasts (Fig. 40 a, c, e)

The lateral raphe system of *P. acrosphaeria* lies in a wide, linear axial area (Fig. 42 b). The central area is only slightly wider (Figs. 42 b and 43 a). The texture of these areas is very special (Figs. 42 b and 43 a). Midvalve the striae are radial or parallel and become convergent or parallel at the apices (Fig. 42 b and 43 a) and the alveolae are partially closed (Fig. 43 b). As shown in Fig. 42 a , this species has two lobed chloroplasts.

The raphe of *C. lauta* is curved, but not lateral (Fig. 42 d). The axial area is relatively narrow and the central area form a wide fascia (Figs. 42 d and 43 c). The striae are parallel or slightly radial (Figs. 42 d and 43 c). On the external valve face a raised line cross the striae (Fig. 43 c),

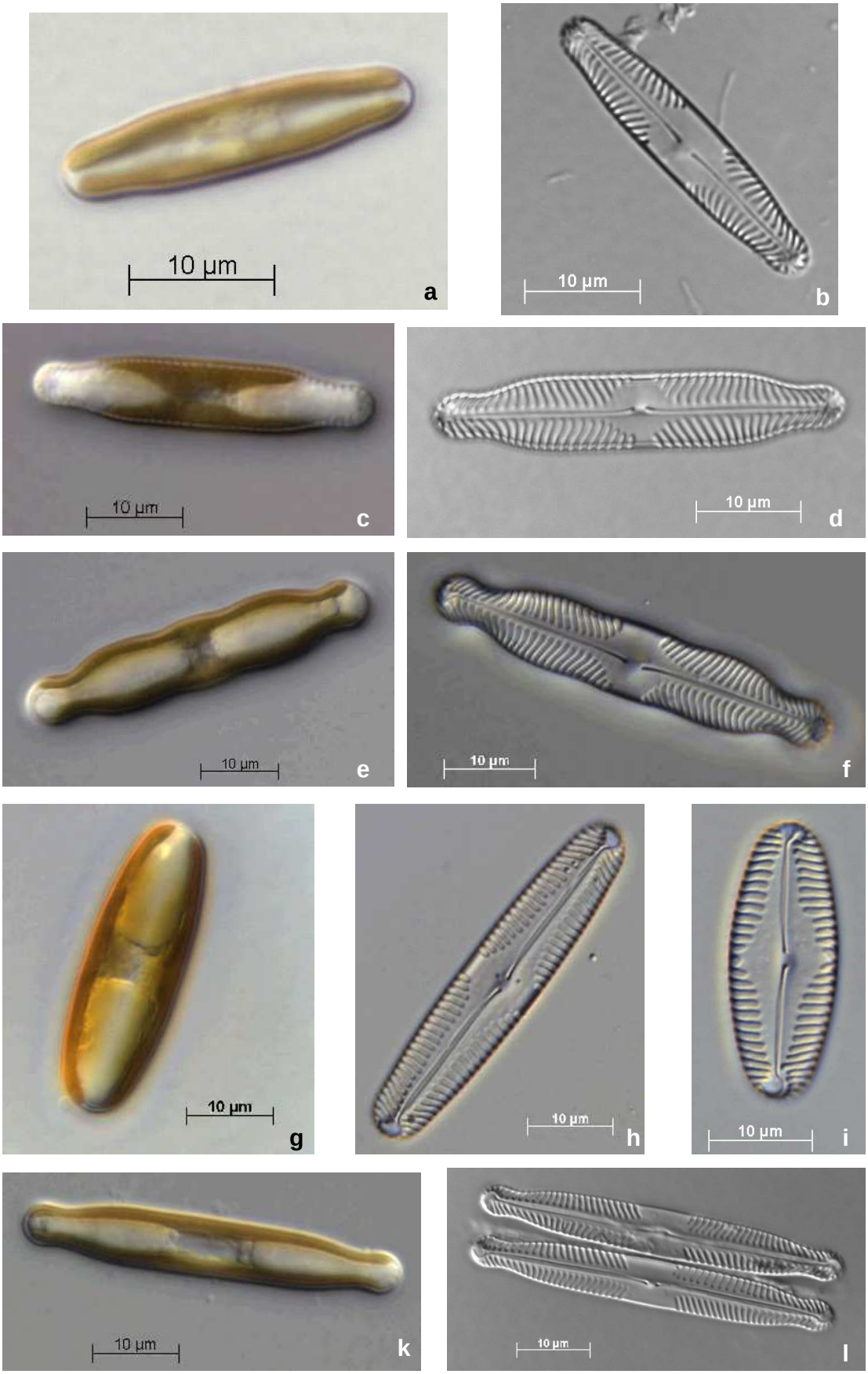


Fig. 38: *Pinnularia* species (group 1), light micrographs of live individuals (a, c, e, g, k) and cleaned valves (b, d, f, h, i, l)

a + b: *P. obscura*, c + d: *P., anglica* e + f: *P., mesolepta* g - i: *P. microstauron*, k + l: *P. subcapitata*.

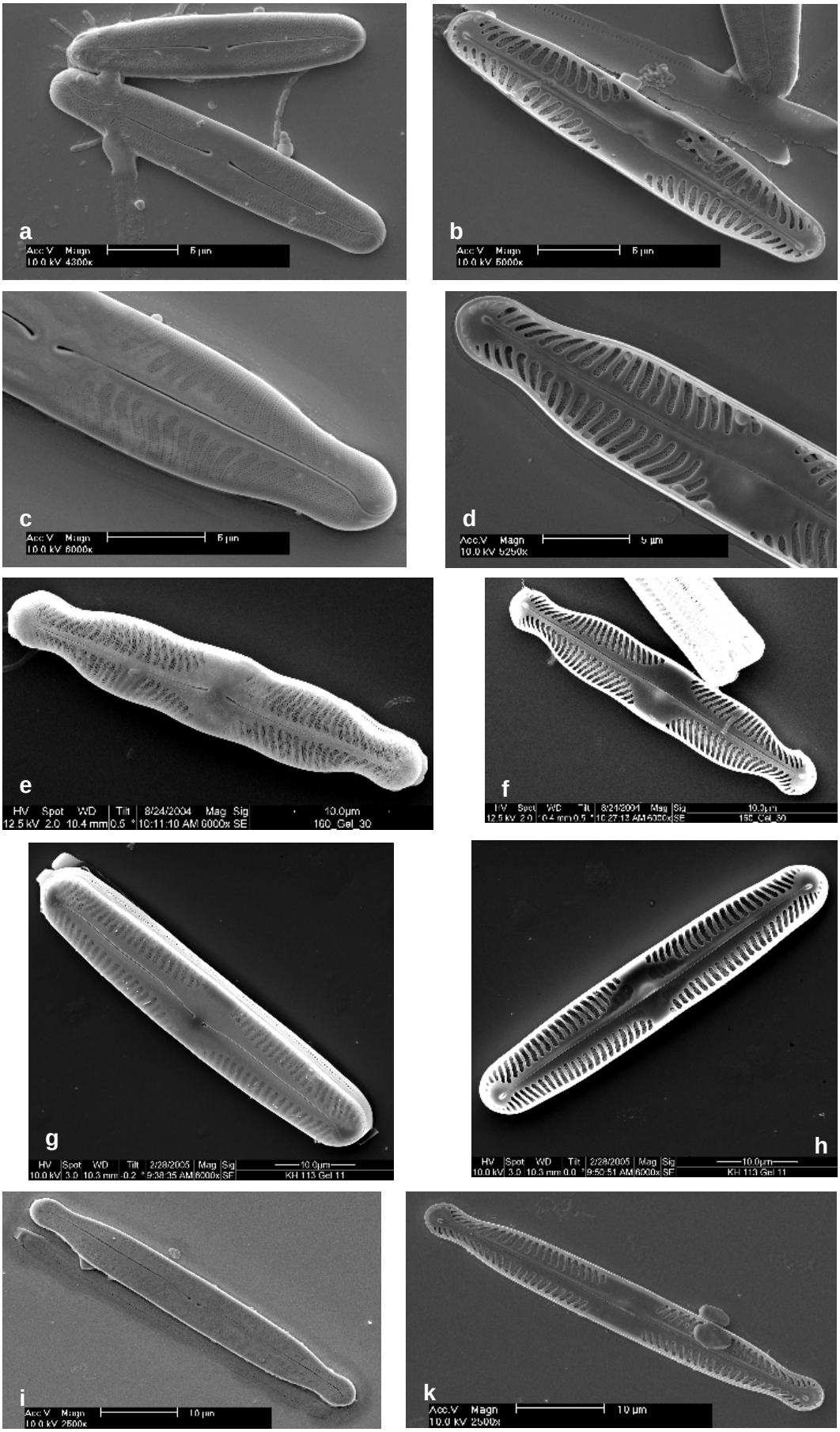


Fig. 39: *Pinnularia* species (group 1), SEM of valve exteriors (a, c, e, g, i) and interiors (b, d, f, h, k). a + b: *P. obscura*, c + d: *P.anglica*, e + f: *P. mesolepta*, g + h: *P. microstauron*, i + k: *P. subcapitata*

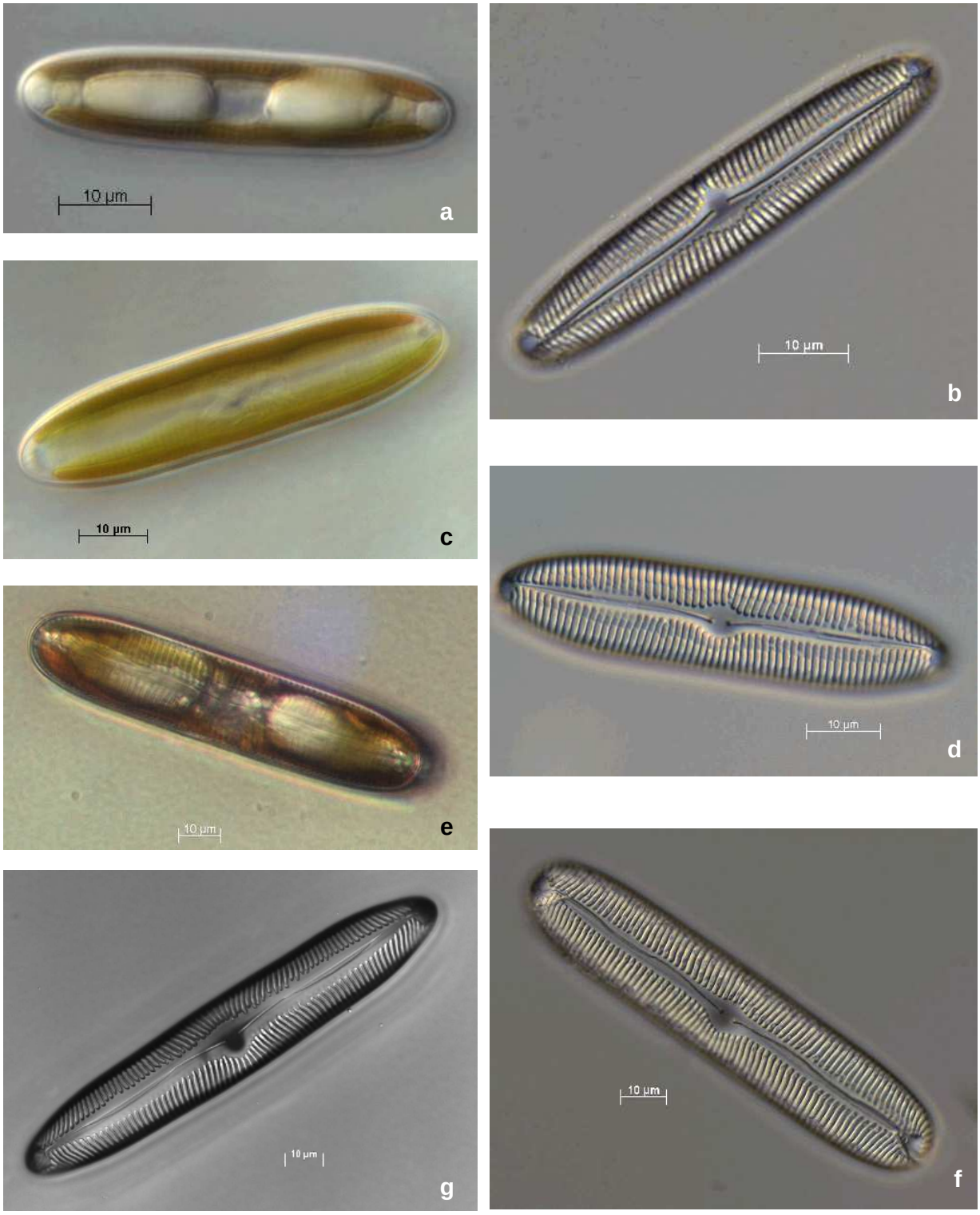


Fig. 40: *Pinnularia* species (group 2), light micrographs of live individuals (a, c, e) and cleaned valves (b, d, f, g)

a + b: *P. rupestris*, c + d: *P. viridiformis*, e + f: *P. viridis*, g: *P.* cf. *substreptoraphe*.

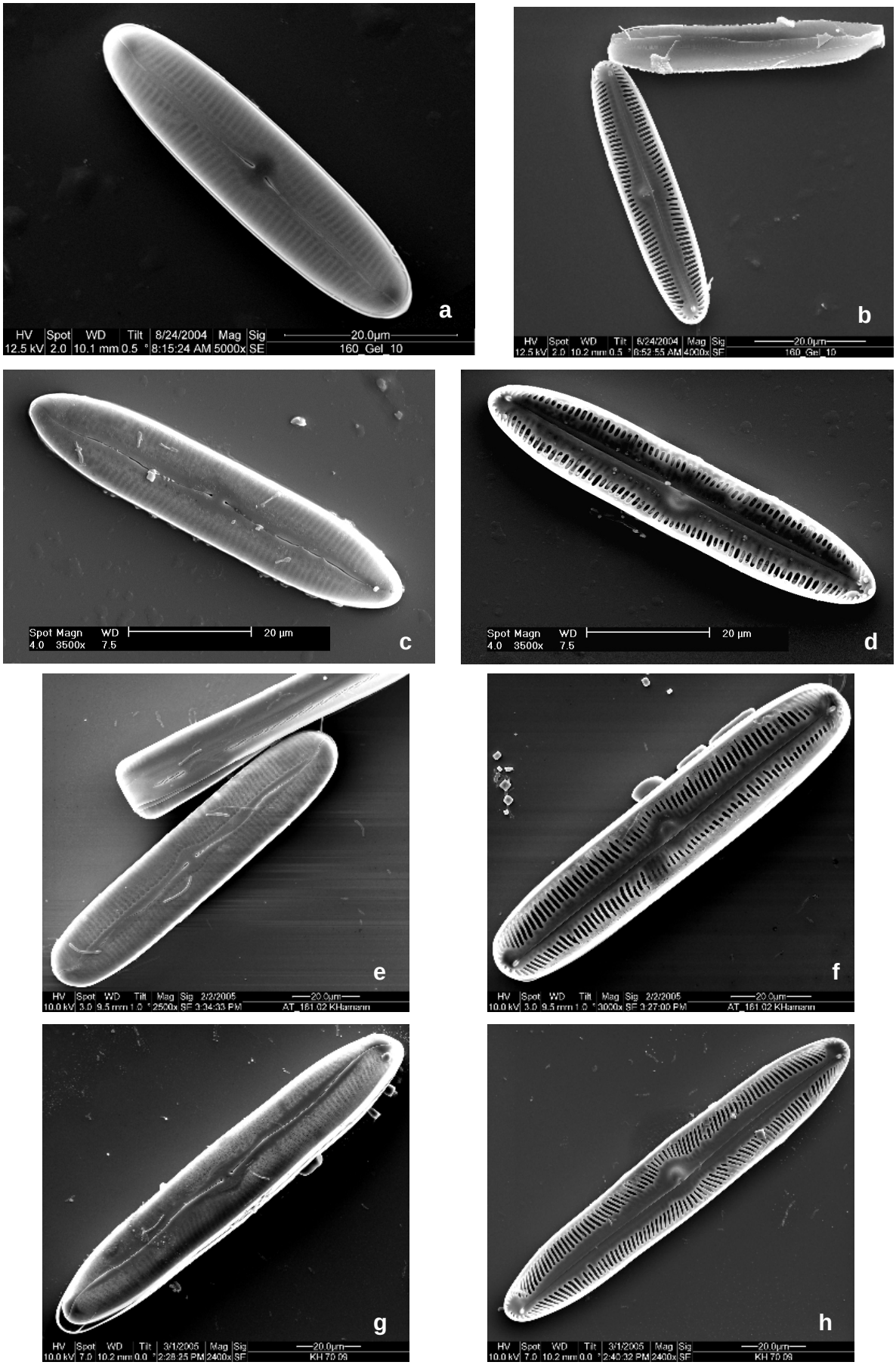


Fig. 41: *Pinnularia* species (group 2), SEM of valve exteriors (a, c, e, g) and interiors (b, d, f, h). a + b: *P. rupestris*, c + d: *P. viridiformis*, e + f: *P. viridis*, g + h: *P.* cf. *substreptoraphe*.

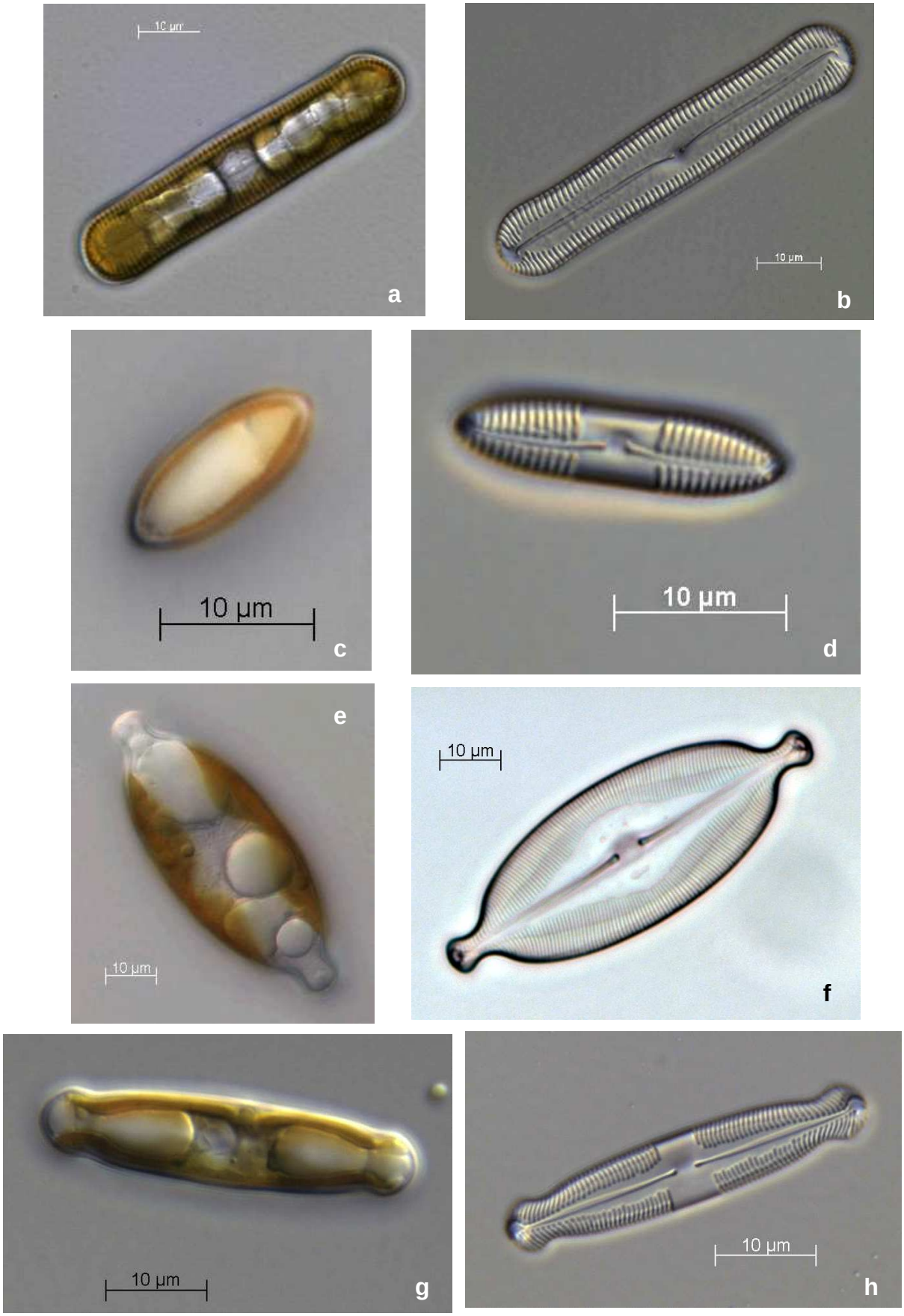


Fig. 42: *Pinnularia and Caloneis* species, light micrographs of live individuals (a, c, e, g) and cleaned valves (b, d, f, h)

a + b: *P. acrosphaeria,* c + d: *C. lauta,* e + f: *C. amphisbaena,* g + h: *C. budensis*.

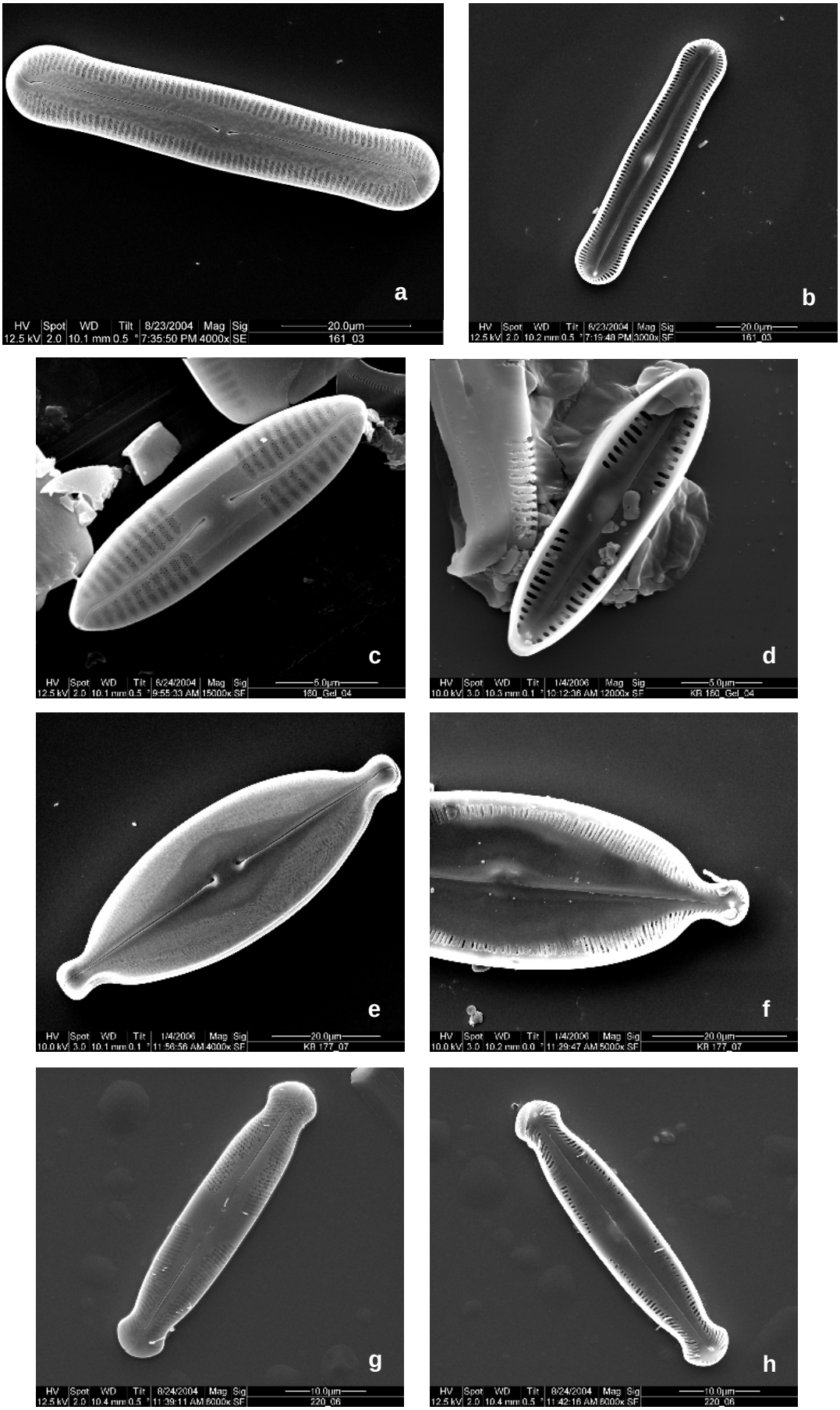


Fig. 43: *Pinnularia and Caloneis* species, SEM of valve exteriors (a, c, e, g) and interiors (b, d, f, h). a + b: *P. acrosphaeria*, c + d: *C. lauta*, e + f: *C. amphisbaena*, g + h: *C. budensis*.

internally the alveolae are partially closed (Fig. 43 d). This species has two plate-like chloroplast, too (Fig. 42 c).

C. amphisbaena has a slightly lateral raphe system and a narrow and linear axial area close to the apices, which passes into a large rhombic-lanceolate central area (Fig. 42 f). The striae are radial at the centre of the valve and become convergent or parallel at the apices (Figs. 42 f and 43 e + f), crossed by a lateral line (Figs. 42 f and 43 e). The alveolae are partially closed (Fig. 43 f). Two lobed, plate-like chloroplast can be observed in live individuals (Fig. 42 e)

C. budensis has a lateral raphe system and the axial area is lanceolately expanded towards the wide fascia midvalve (Fig. 42 h). The striae are parallel or slightly radial at the centre of the valve and convergent at the apices (Figs. 42 h and 43 g + h). They are crossed by a lateral line (Fig. 42 h), because the alveolae are partially closed (Fig. 43 h). Two lobed, plate-like chloroplast can be observed in live individuals (Fig. 42 g).

3.2.4 *Stauroneis, Craticula* and *Navicula integra*

With the exception of the trees based on the *rbc*L sequences, *Stauroneis*, *Craticula* and *N. integra* formed a strongly supported monophyletic clade (BS: 91 – 100). In the ML trees based on the *rbc*L sequences, the monophyletic clades of this group had poor BS (Figs. 19, 22) and in the MP phylogenies based on this gene, this group was merged in a large polytomy (Figs. 21, 24). Within the monophyletic clades, *Craticula* and *Stauroneis* were clearly separated. The only exception was the tree in Fig. 22, where a monophyletic clade of *Stauroneis* divided the *Craticula* species into two groups. In most trees, *N. integra* clusters with *Stauroneis*. Only in the ML phylogenies based on the *rbc*L sequences does *N. integra* cluster with *Craticula*.

Also the morphological investigation showed several similarities. All species within this group has two plate-like chloroplasts, lying one against each side of the girdle (Fig. 44 a, d, g). All frustules were isopolar and tend to lie in valve view, because they are wider transapically than pervalvarly (Fig. 44). The external central raphe endings are expanded and the well developed terminal fissures at the poles curve off to the same side of the valve (Fig. 44 b, c, e, f, h, i). The internal central raphe endings are simple and straight or slightly curved Fig. 45 a, c, e). The girdle composed of several open, porous bands with one or two rows of small round poroids (Fig. 45 b, d, f). The uniseriate striae consist of small round or elliptical poroids which were occluded by hymenes at their internal aperatures (Fig. 45 c and Round *et al.* 1990: p. 592 Fig. i and p. 595, Figs. i. j). In cleaned material these hymenes were often eroded (Fig. 45 c).

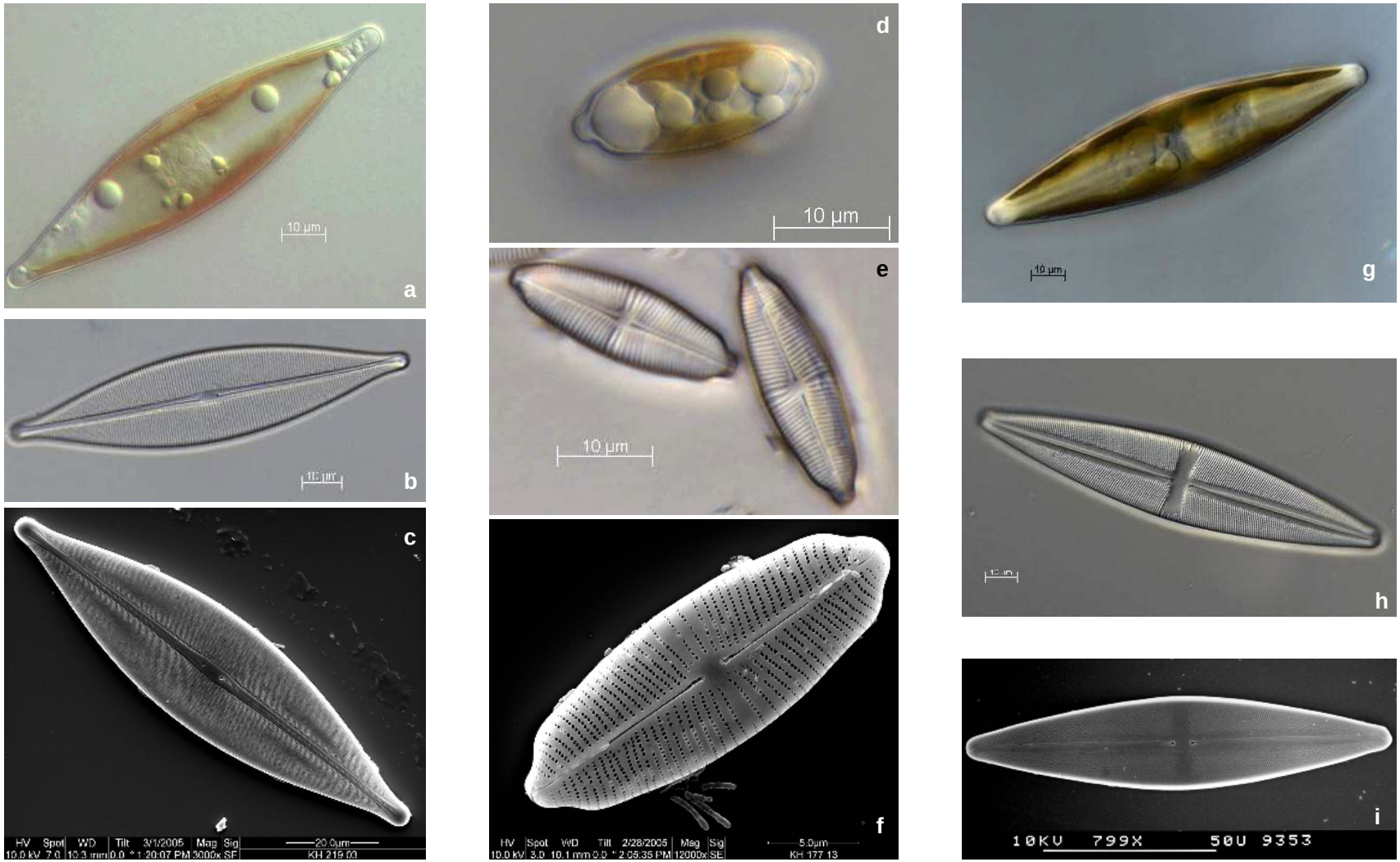


Fig. 44: *Craticula cuspidata*, *Navicula integra* and *Stauroneis phoenicenteron*
a - c: *C. cuspidata*, light micrograph of a live individual (a) and of a cleaned valve (b) and SEM of valve exterior (c).
d - f: *N. integra*, light micrograph of a live individual (d) and of a cleaned valve (e) and SEM of valve exterior (f).
g- i: *St. phoenicenteron*, light micrograph of a live individual (g) and of a cleaned valve (h) and SEM of valve exterior (i).

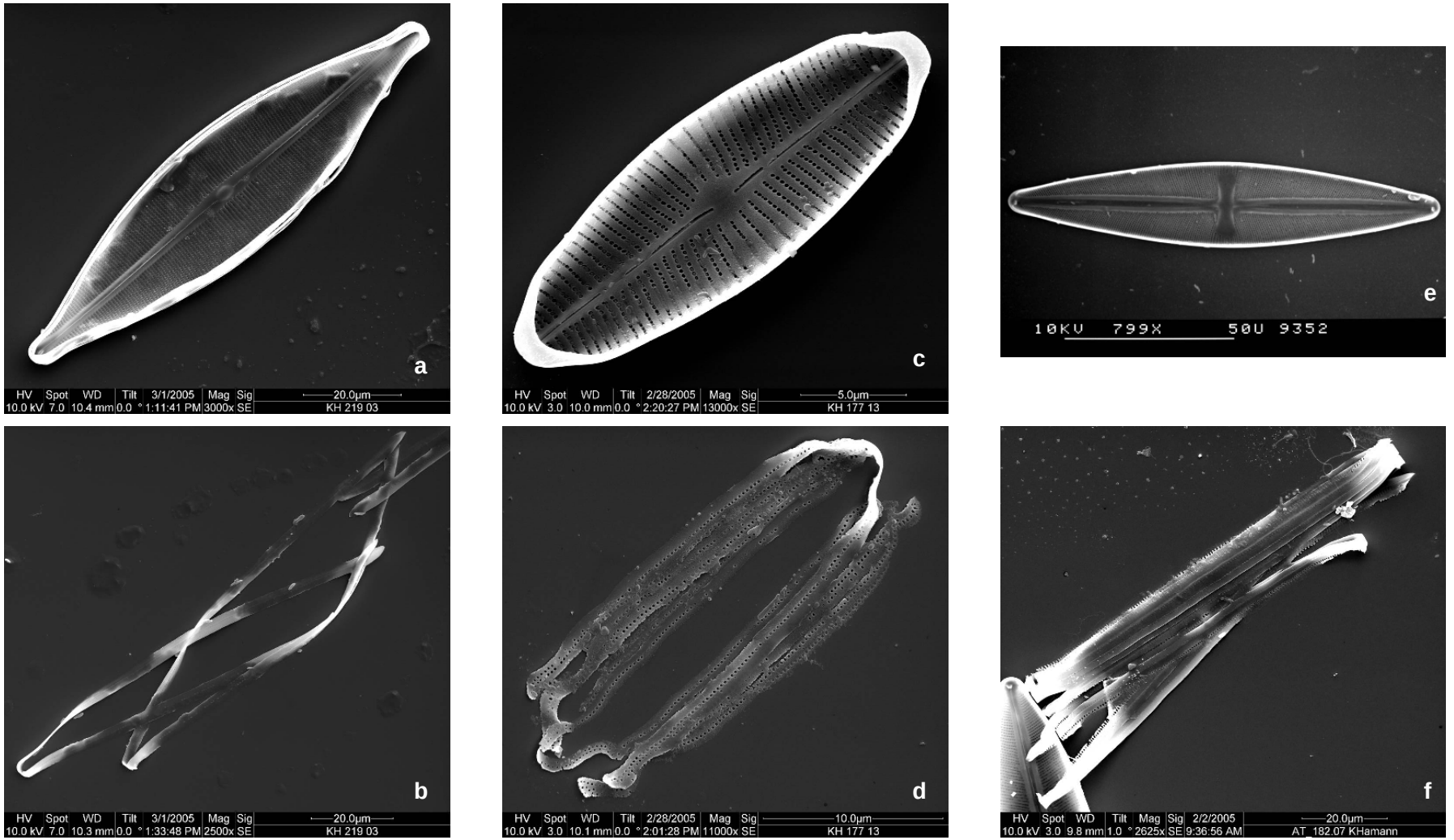


Fig. 45: *Craticula cuspidata*, *Navicula integra* and *Stauroneis phoenicenteron*
a - b: *C. cuspidata*, SEM of valve interior (a) and girdle bands (b).
c - d: *N. integra*, SEM of valve interior (d) and girdle bands (e).
e - f: *St. phoenicenteron*, SEM of valve interior (g) and girdle bands.

The most obvious feature of the genus *Stauroneis* is its stauros (Figs. 44 e + f, 45 e + f and 46). The stauros extends from the raphe-sternum to the valve margins, with decreasing thickness.

All species belonging to the genus *Craticula* had parallel and equidistant striae (Figs. 44 a + b, 45 a + b and 47). The areolae are aligned longitudinal in straight lines parallel to the raphe system.

N. integra has a lanceolate or lanceolate-elliptical valve with subrostrate apices and an additional undulation in the valve margin before the apices (Figs. 44 a – c and 45 c). At the apices are pseudosepta (Fig. 45 c) The striae are radiate and midvalve more distant (16 – 18 striae/ 10 µm) (Figs. 44 a – c and 45 c). The costae separating the striae are thickened at the centre of the valve, producing a stauros-like structure (Figs. 44 e and 45 c).

3.2.5 *Gomphonema*

In most gene trees, the species *Gomphonema* formed a monophyletic clade. Only in the tree in Fig. 21 did the genus merged in a large polytomy and in Fig. 5, *G. micropus* is separated from the other *Gomphonema* species by *Cymbella*. With the exception of the trees based on *rbc*L sequences, *G. micropus* diverges at the base of the group. Whereas the entire group had only low or moderate BS between 16 and 87, the clade without *G. micropus* was supported by high bootstrap values of 99 or 100.

Nearly all morphological features of *G. micropus* can be found in one or more of the other *Gomphonema* species cultured within the scope of this study. All species belonging to this genus have a single H-shaped chloroplast (Figs. 48 a and 49) and heteropolar valves. All species cultured have a single stigma, which internally opens in a slit (Figs. 48 d and 50). Generally the striae are uniseriate, but in some species they can become biseriate close to the raphe (Figs. 48 c + g and 50). Also the separation of the areolas in the alveolus by a small strut can be found in several species (Fig. 48 d and 50 b, c, f). But *G. micropus* is the only species where the areolae externally open in small round poroids (Fig. 48 e – g). In all other cultured species, they are C- or kidney-shaped (Fig. 51). In all species, the external central raphe endings are expanded and internally they deviate (Figs. 48 d and 50). Internally the raphe slit ends in a helictoglossae at both poles. Externally the raphe fissure is hooked. The only exception can be found in *G. micropus* with a smooth curved terminal fissure (Figs. 48 e + f and 51).

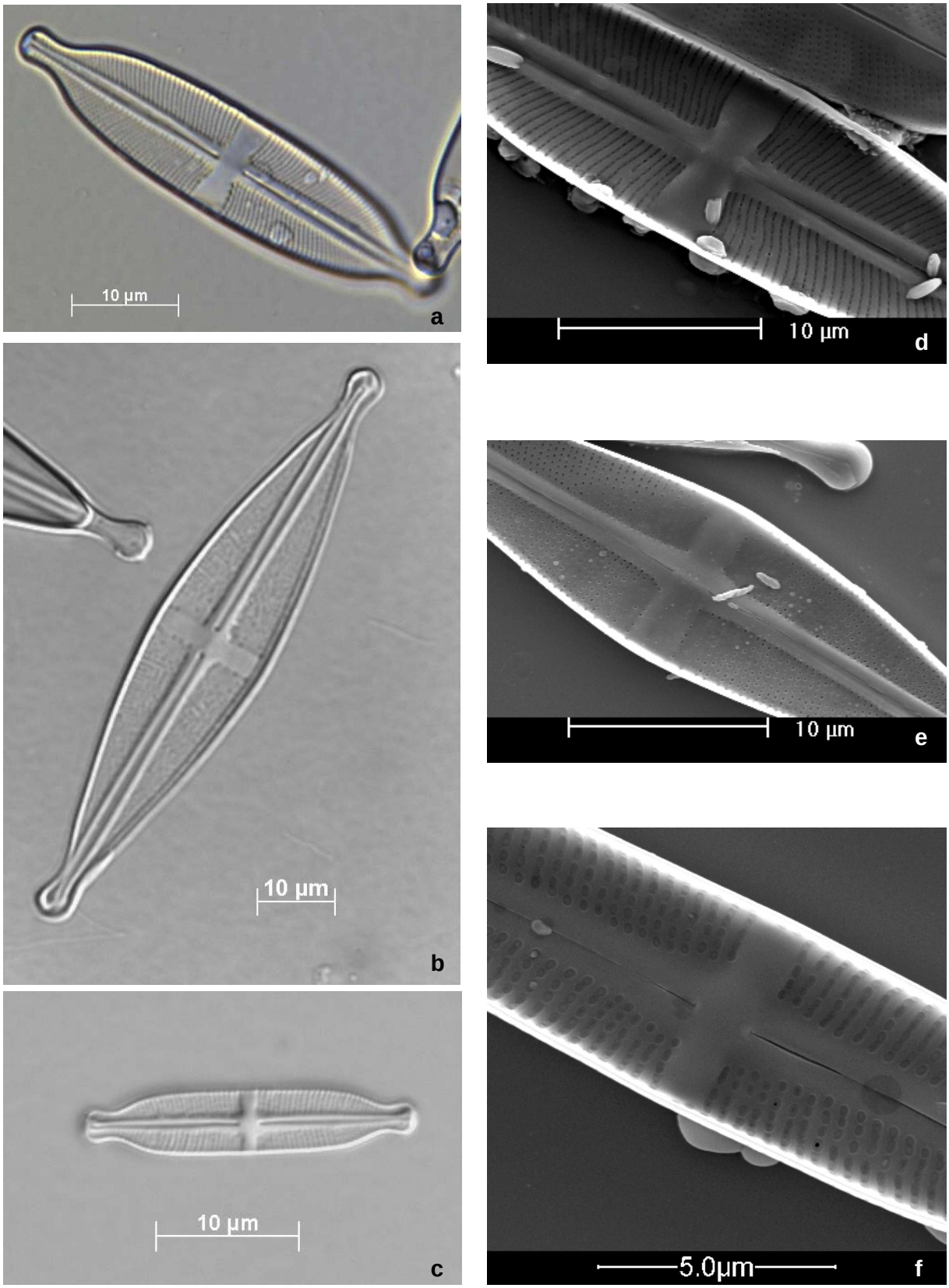


Fig. 46: *Stauroneis* species, light micrograph of cleaned valve (a – c), SEM of internal view of the valve centre (d – f).

a + d: *St. anceps*, b + e: *St. gracilior*, c + f: *St. kriegerii.*

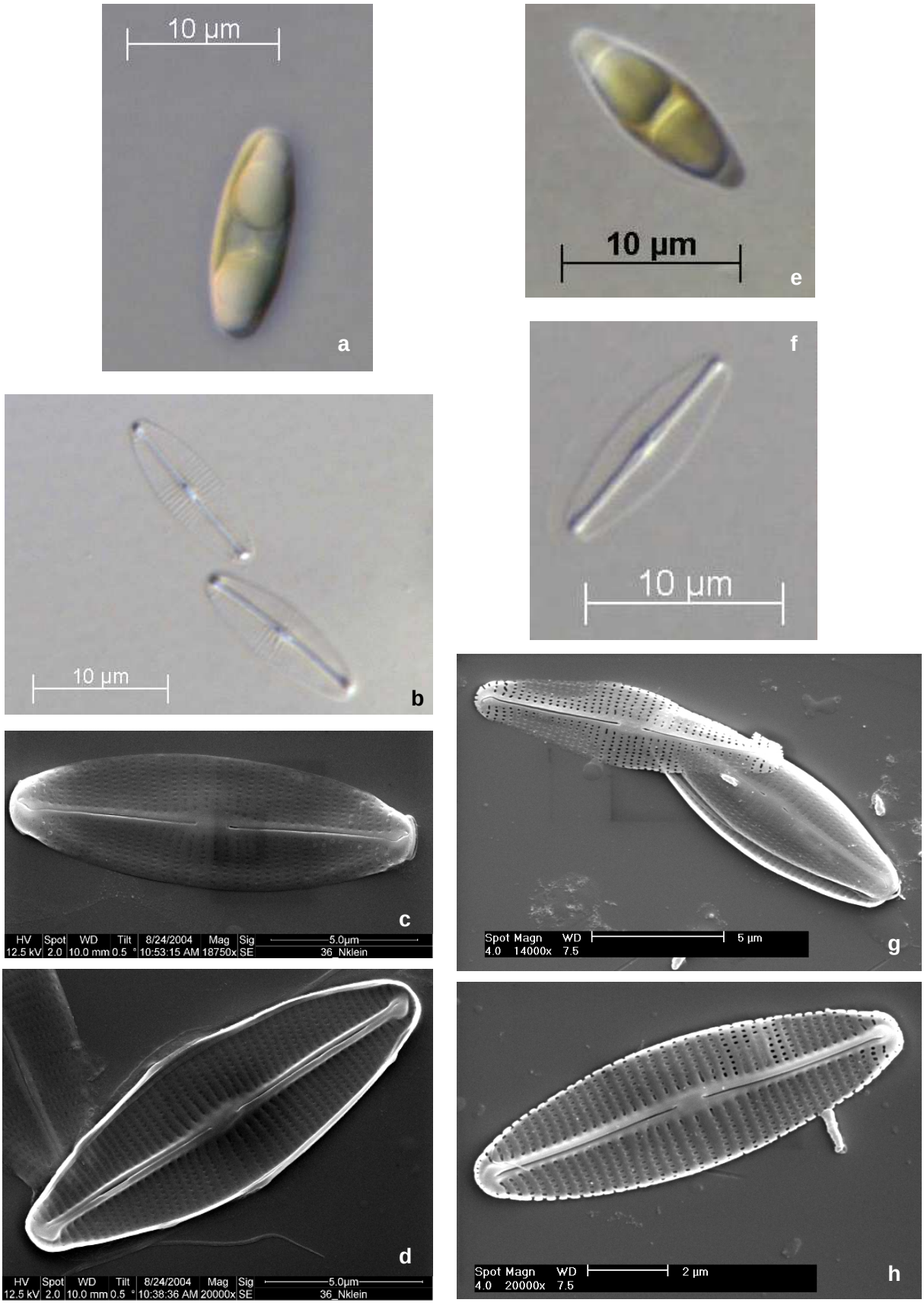


Fig 47: a - d: *Craticula halophilioides*, e - h: *Craticula molestiformis*.

a + e: light micrograph of live individuals, b + f: light micrographs of cleaned valves, c + g: SEM of valve exteriors, d + h: SEM of valve interiors.

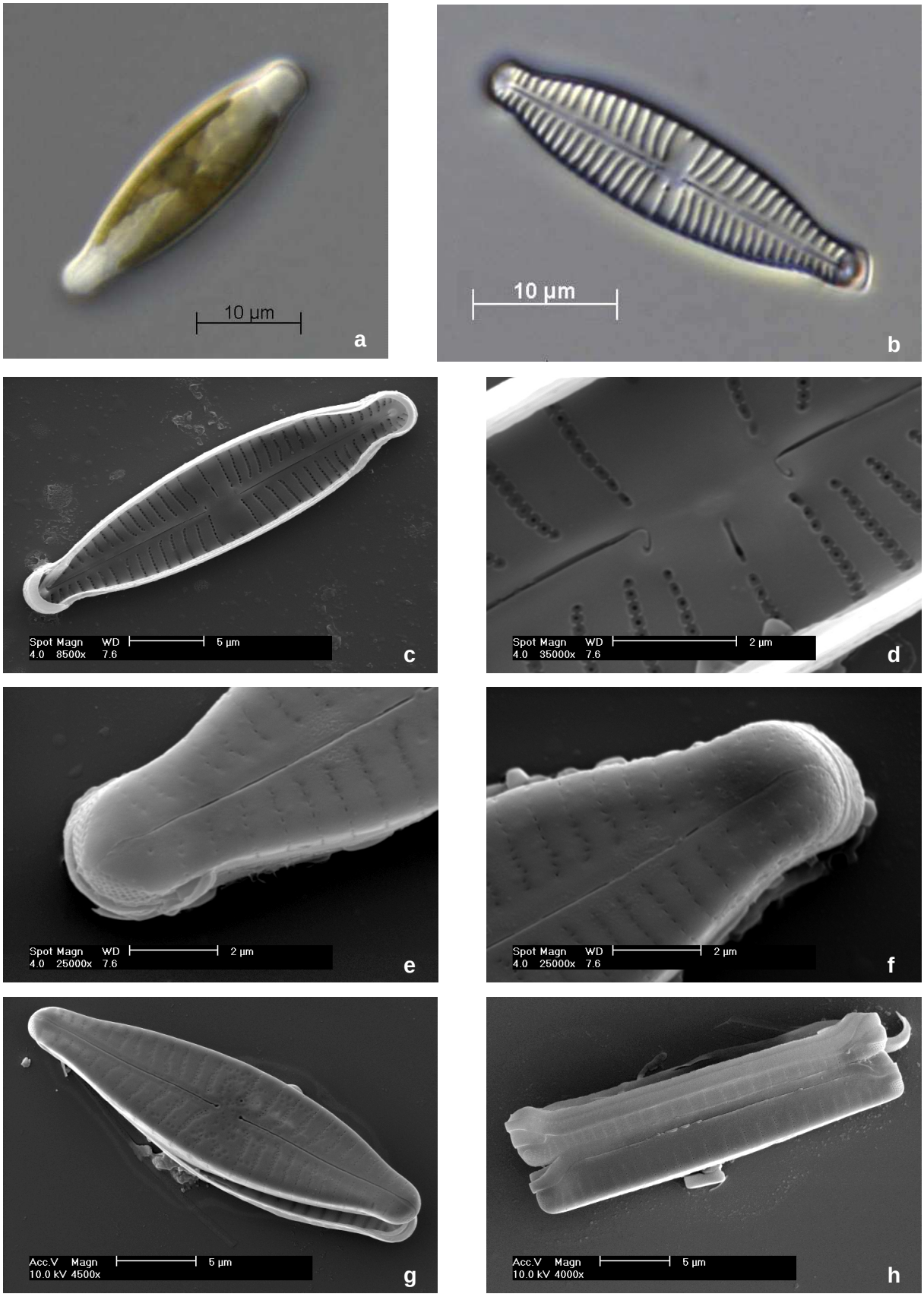


Fig. 48: *Gomphonema micropus*.
a + b: light micrograph of live individual (a) and cleaned valve (b) in valve view,
c + d: SEM of valve interior of the whole valve (c) and midvalve detail (d),
e - h: SEM of valve exteroir of base (e) and head pole (f), whole valve (g) and girdle (h).

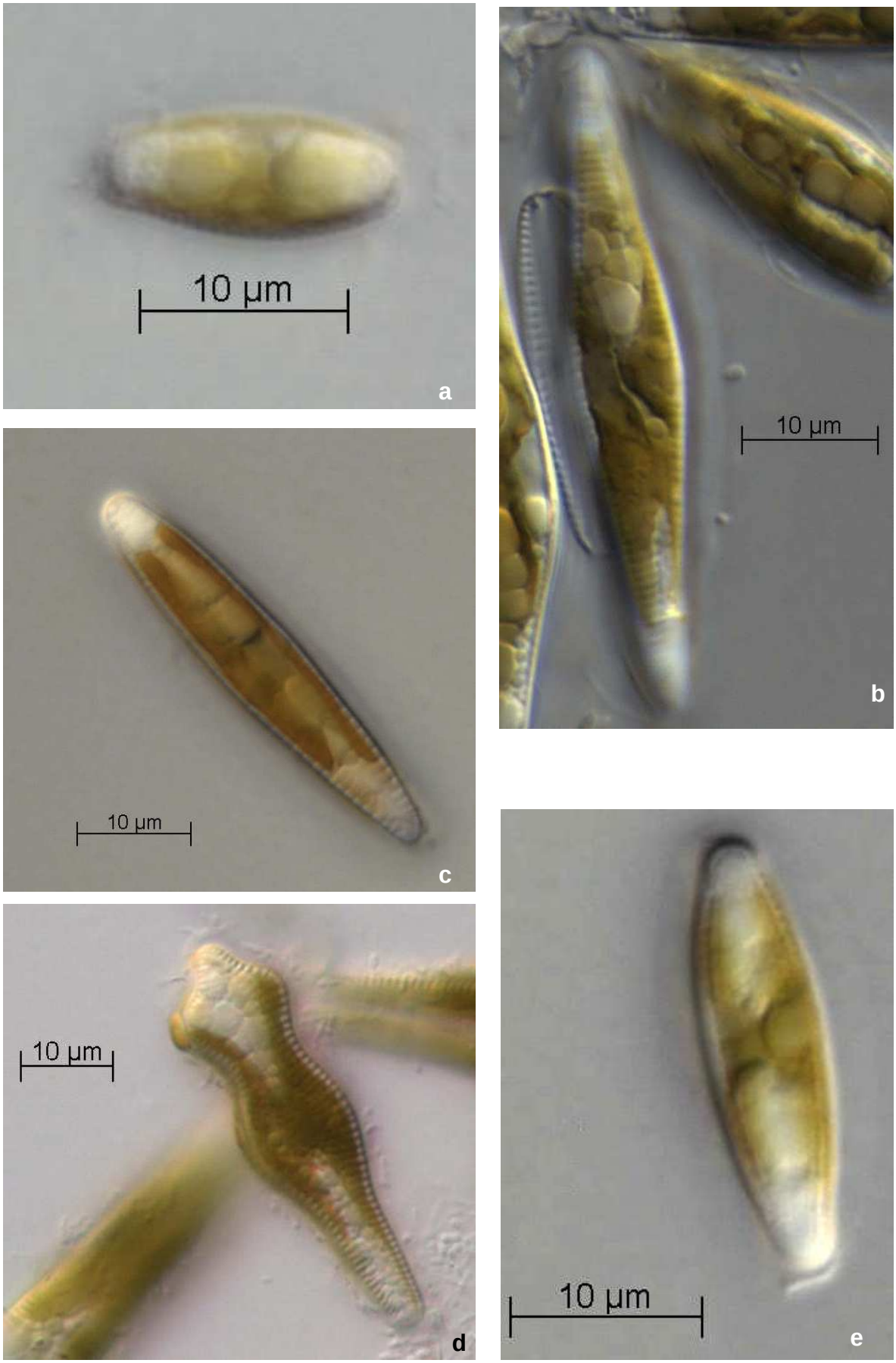


Fig 49: *Gomphonema* species, light micrographs of live individuals.
a: *G.* cf. *angustatum*, b: *G. affine*, c: *G.* cf. *parvulum*, d: *G. acuminatum*, *e*: *G. productum*.

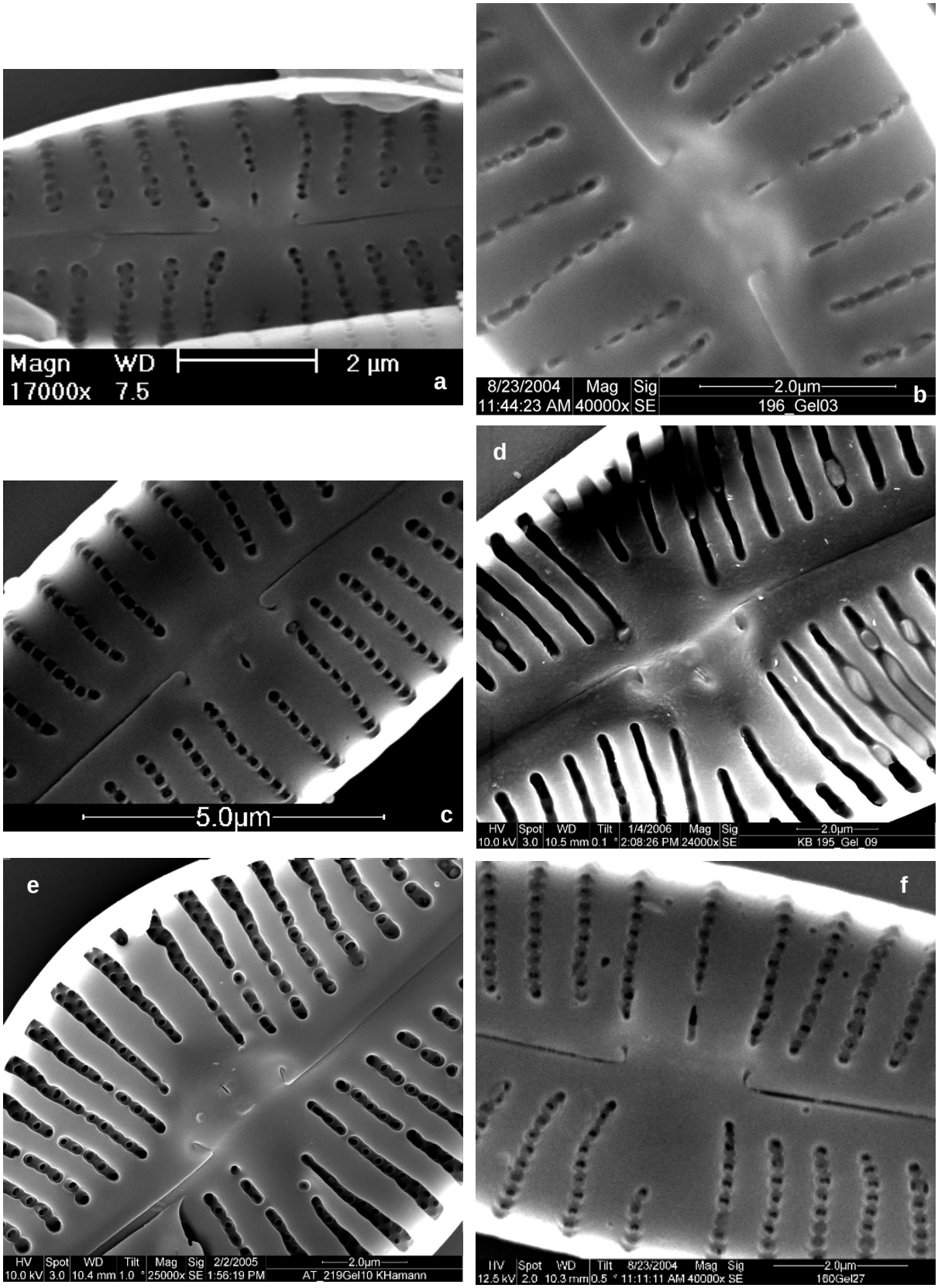


Fig 50: *Gomphonema* species, SEM of valve interiors showing midvalve.

a: *G.* cf. *angustatum*, b: *G. affine*, c: *G.* cf. *parvulum*, d: *G. truncatum*, e: *G. acuminatum*, *f*: *G. productum*.

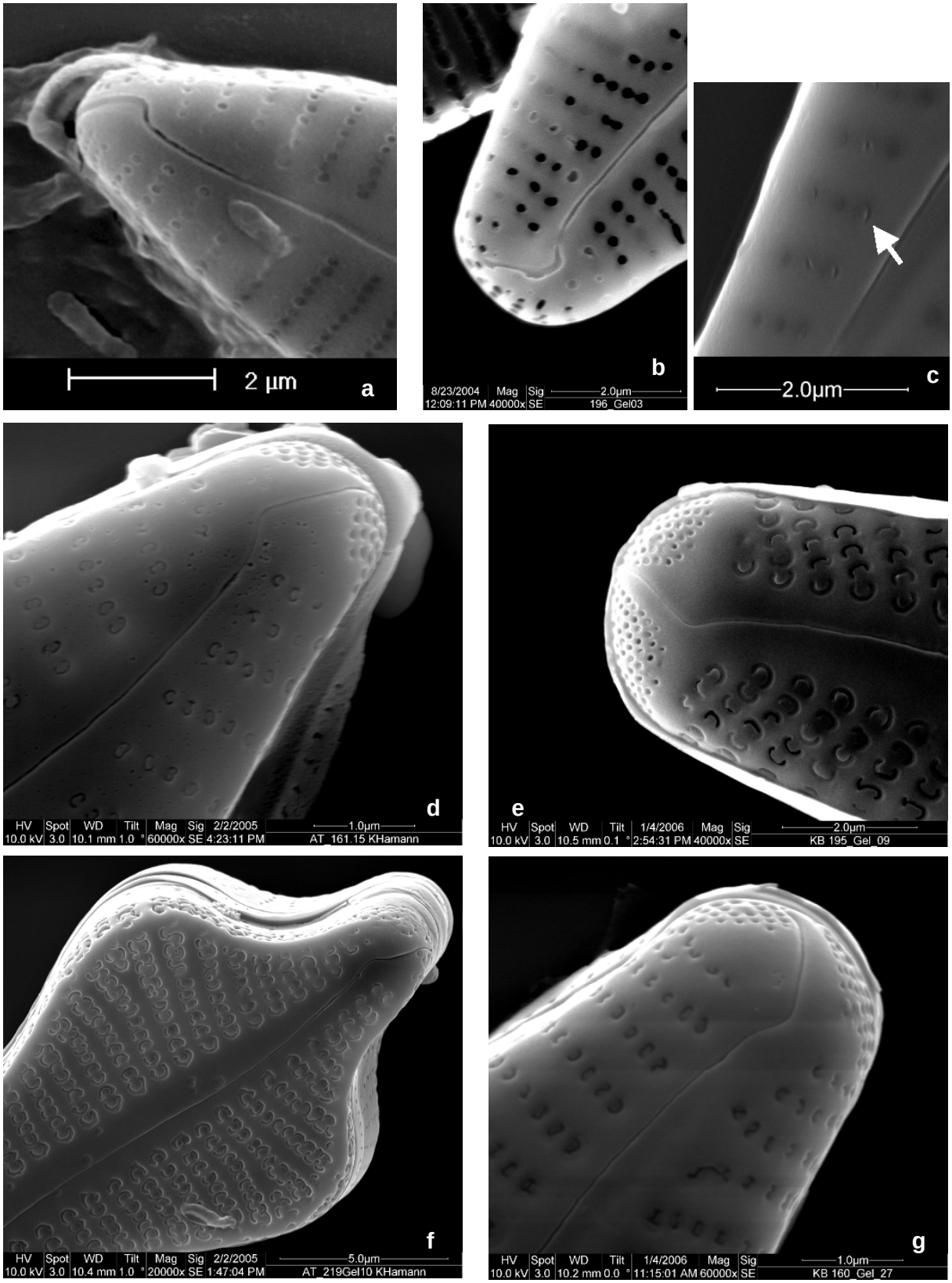


Fig 51: *Gomphonema* species, SEM of valve exteriors showing the polar raphe curvature and the areolae.

a: *G.* cf. *angustatum*, head pole; b + c: *G. affine*, head pole of with corroded areolae (b) and uncorroded areolae (c); d: *G.* cf. *parvulum*, base pole; e: *G. truncatum*, base pole; f : *G. acuminatum*, head pole; g: *G. productum*, base pole.

3.2.6 *Placoneis* and *Navicula hambergii*

The species belonging to the genus *Placoneis* and *N. hambergii* formed a monophyletic clade in all phylogenies inferred with the ML analysis (Figs. 3, 9, 11, 15, 19, 22 and 26). These clades were supported by bootstrap values from 24 to 100. The MP analyses of SSU rDNA sequences (Fig. 5) and the combined dataset (Fig. 28) resulted in a monophyly of these species, too. In the MP phylogenies based on the sequences of the LSU rDNA (Figs. 13, 17) and *rbc*L (Figs. 21, 24) these species were not monophyletic, but still closely related.

Morphological investigations of *Navicula hambergii* and *Placoneis elginensis* indicated that the two species are near relatives. The single chloroplast, with a central bridge from which lobes project into the four quadrants of the cell (Fig. 52 a, b, h, i), is typically for species belonging to the genus *Placoneis*. The striae are radiate (Fig. 52 c and k). At the centre of the valve the striae are irregularly abbreviated (*P. elginensis*, Fig. 52 c + g) or alternately longer and shorter (*N. hambergii*, Fig. 52 k + o). With SEM it can be seen, that, externally, the striae consist of small round poroids (Fig. 52 g + o). Internally, the striae poroids are almost square and closed by vola-like occlusions (Fig. 52 d + l). Both species have a straight raphe with slightly expanded external central endings and at both poles the hook-like raphe fissures curve to the same side (Fig. 52 c, g, k, o). The internal central raphe endings of both species are hooked (Fig. 52 f + n) and the internally helictoglossae at the polar raphe endings are strait and knob-like (Fig. 52 e + m).

3.2.7 *Cymbella*

In all trees with the exception of the tree in Fig. 21 *Cymbella* is most closely related to *Placoneis, Gomphonema* and *Encyonema*. If there is no polytomy in this part of the tree, *C. aspera, C. helmckei* and *C. affinis* always belong to a monophyletic clade. But the position of *C. naviculiformis* and *C. proxima* differs in the different trees. In some gene trees, the branch with these two species formed a monophyletic clade with the other *Cymbella* species (Figs. 4, 9, 12,). In Fig. 5 this branch is separated from the other *Cymbella* species by *Gomphonema*. In the trees based on the *rbc*L sequences *C. proxima* appeared at the base of *Placoneis,* whereas in the trees based on the combined data set this species diverged within *Cymbella* and *C. naviculiformis* formed the base of the clade.

The morphological investigations of this genus concentrated on differences between *C. aspera, C. helmckei* and *C. affinis* on one side and *C. naviculiformis* or *C. proxima* on the other side. All species have the typical features of the genus *Cymbella*, such as dorsiventral

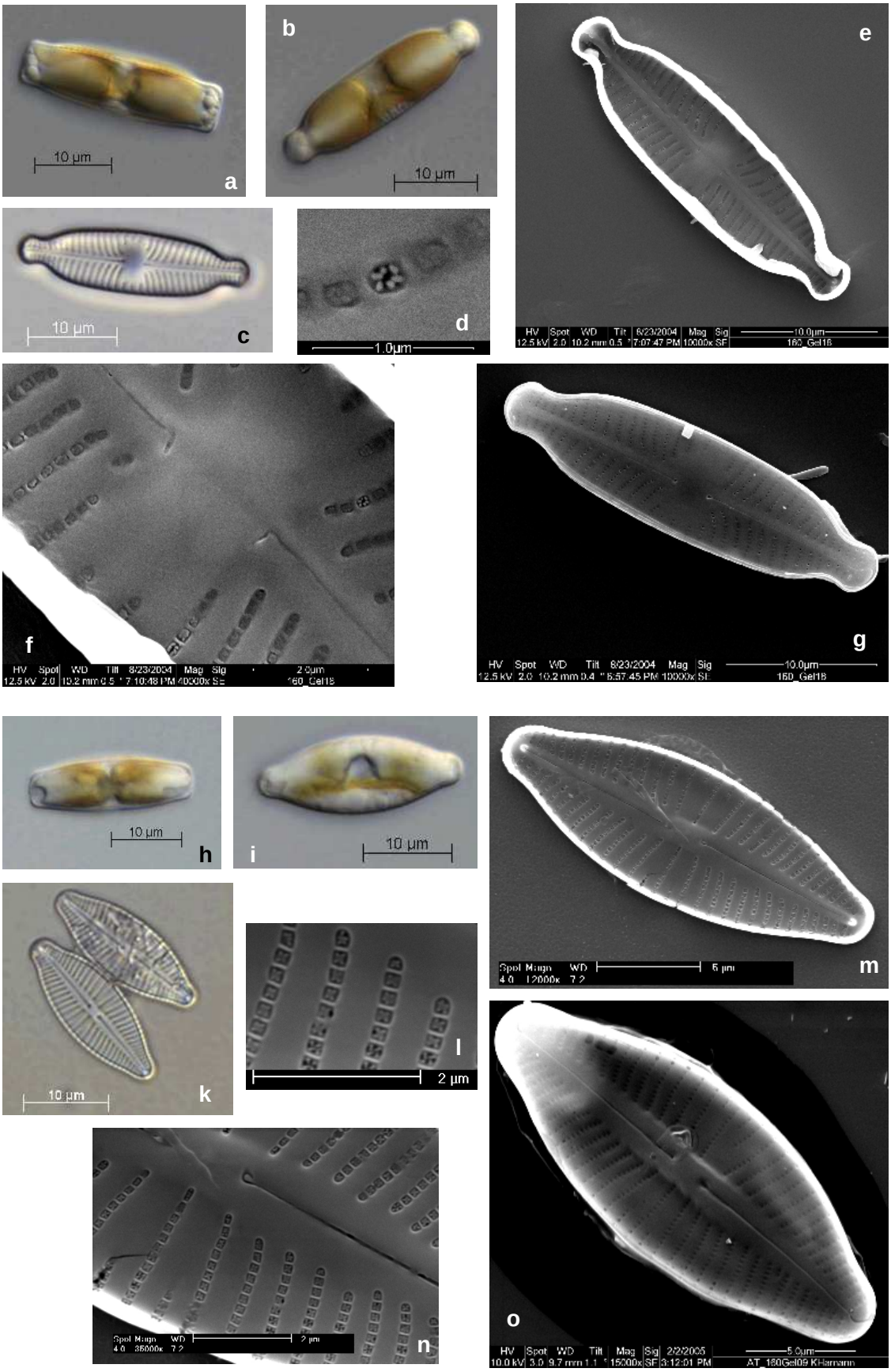


Fig. 52: *Placoneis paraelginensis* (a – g) and *Navicula hambergii* (h – o)

Light micrographs of a live cell (a + h: girdle view, b + i: valve view) and cleaned valve (c + k).

SEM showing valve interiors (d + l: detail areolae, e + m: total view, f + n: detail central raphe endings) and exterior (g + o: total view).

valves, uniseriate striae and dorsal deflected terminal raphe fissures (Fig. 53). Contrary to the other four species, stigmata (Fig. 55) and apical pore fields (Fig. 54) are absent in *C. naviculiformis*. Internally the raphe ends straight in a helictoglossa (Fig. 56 e) in *C. naviculiformis*. In the other species, the internally polar raphe slit is curved (Fig. 56 a – d). *C. proxima* did not show obvious differences to *C. aspera*, *C. helmckei* and *C. affinis*.

3.2.8 *Navicula brockmannii*

In most phylogenies, *N. brockmannii* was closely related to the monoraphid diatoms and the *Cymbellales*. Exceptions were only the trees based on LSU rDNA sequences (Figs. 11, 13). In Fig. 11 *N. brockmannii* and the monoraphid species were most closely related to a clade consisting of *Craticula* and *Stauroneis* and within the large polytomy in Fig. 13 only a relationship of *N. brockmannii* and *Achnanthidium minutissimum* was shown. In all phylogenies, *N. brockmannii* is always clearly separated by several genera from *Navicula sensu stricto*.

In contrast to species belonging to *Navicula sensu stricto* (see 3.2.1), *N. brockmannii* had only a single chloroplast (Fig. 57 a). The valves were linear with parallel or slightly convex margins and broad rostrate or subcapitate ends (Fig. 57 b + c). The raphe was filiform with scarcely expanded central pores (Fig. 57 b - d) and laterally strongly deflected terminal fissures (Fig. 57 b + e). The helictoglossae at the internal polar raphe endings are straight and knob-like (Fig. 57 f). The axial area was linear and narrow and slightly widened close to the central area, which was variable in size and form because of irregularly abbreviated striae (Fig. 57 b + c). The striae were radiate, getting parallel towards the poles (Fig. 57 b + c). At the centre of the valve the striae were less dense (25 – 27/10µm) than towards the valve ends (30 – 32/10µm). The striae run continuously from the valve surface down onto the mantle (Fig. 57 e + g) and consists of uniseriate rows of round areolae, which where externally closed by hymenes (Fig. 57 c + d). One or two rows of areolae could be found on the girdle bands (Fig. 57 g).

3.2.9 Varieties of *Mayamaea atomus*

The two varieties *M. atomus* var. *atomus* and *M. atomus* var. *permitis* formed strongly supported (BS: 96 – 100) monophyletic clades in all phylogenies shown above. The differences between the sequences, which were visualised by the branch length in the ML phylogenies (Figs. 3, 10, 11, 15, 19, 22 and 26), were almost as many as between the two well defined *Cocconeis* species.

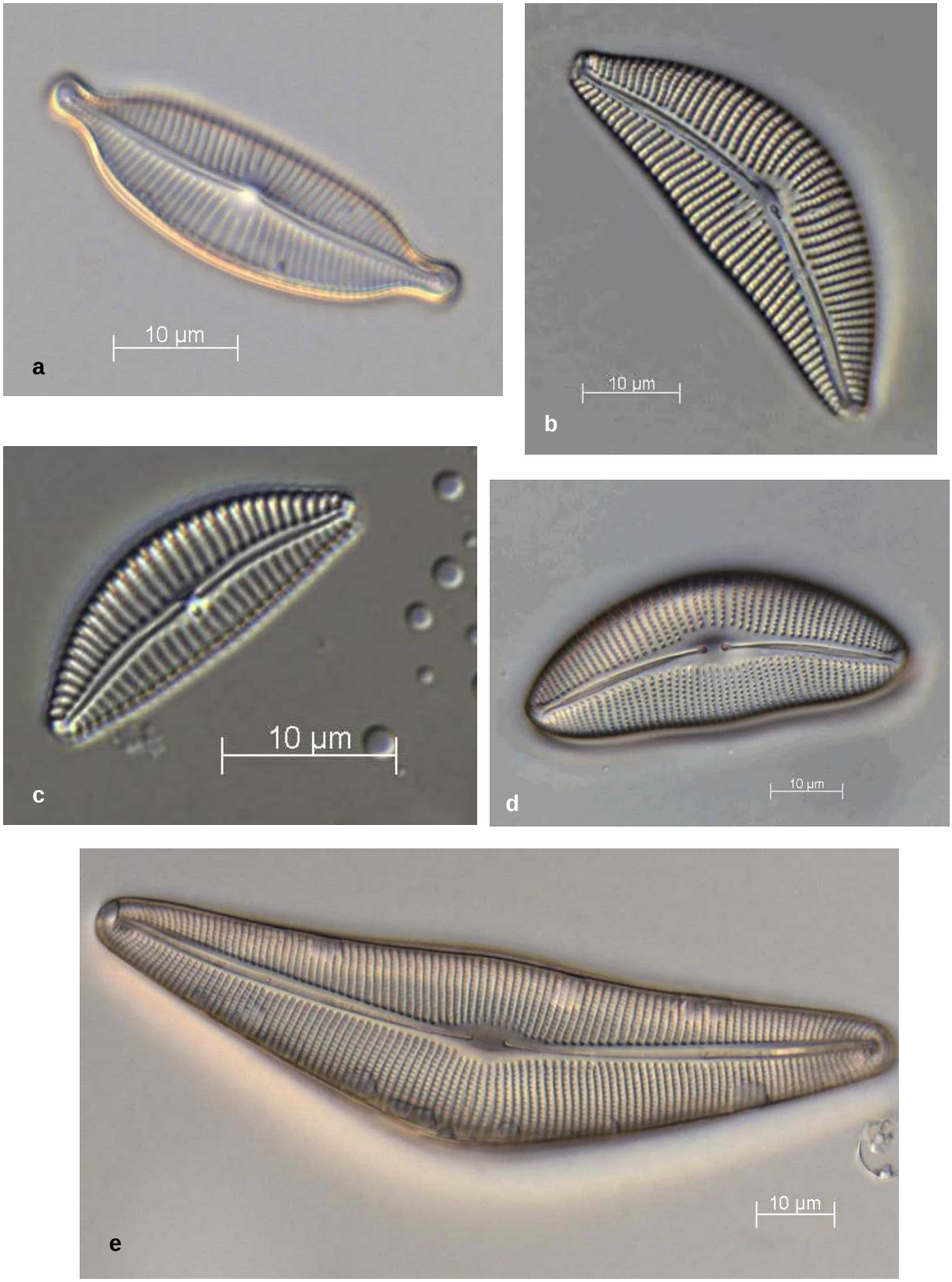


Fig. 53: *Cymbella* species, light micrographs of cleaned valves.
a: *C. naviculiformis*, b: *C. proxima*, c: *C. affinis*, d: *C. aspera*, e: *C. helmkei*.

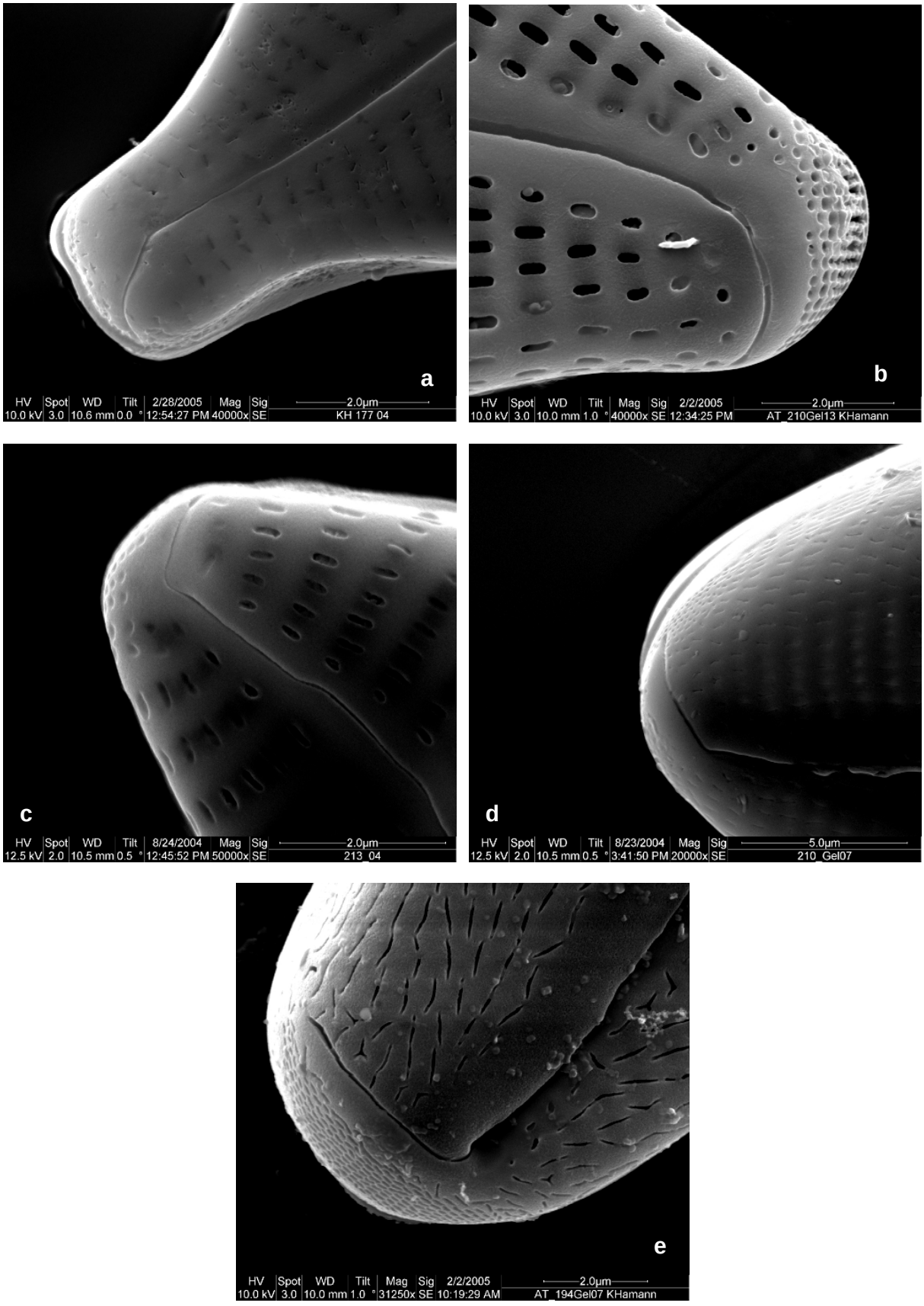


Fig. 54: *Cymbella* species, SEM of external polar raphe endings.
a: *C. naviculiformis*, b: *C. proxima*, c: *C. affinis*, d: *C. aspera*, e: *C. helmkei*.

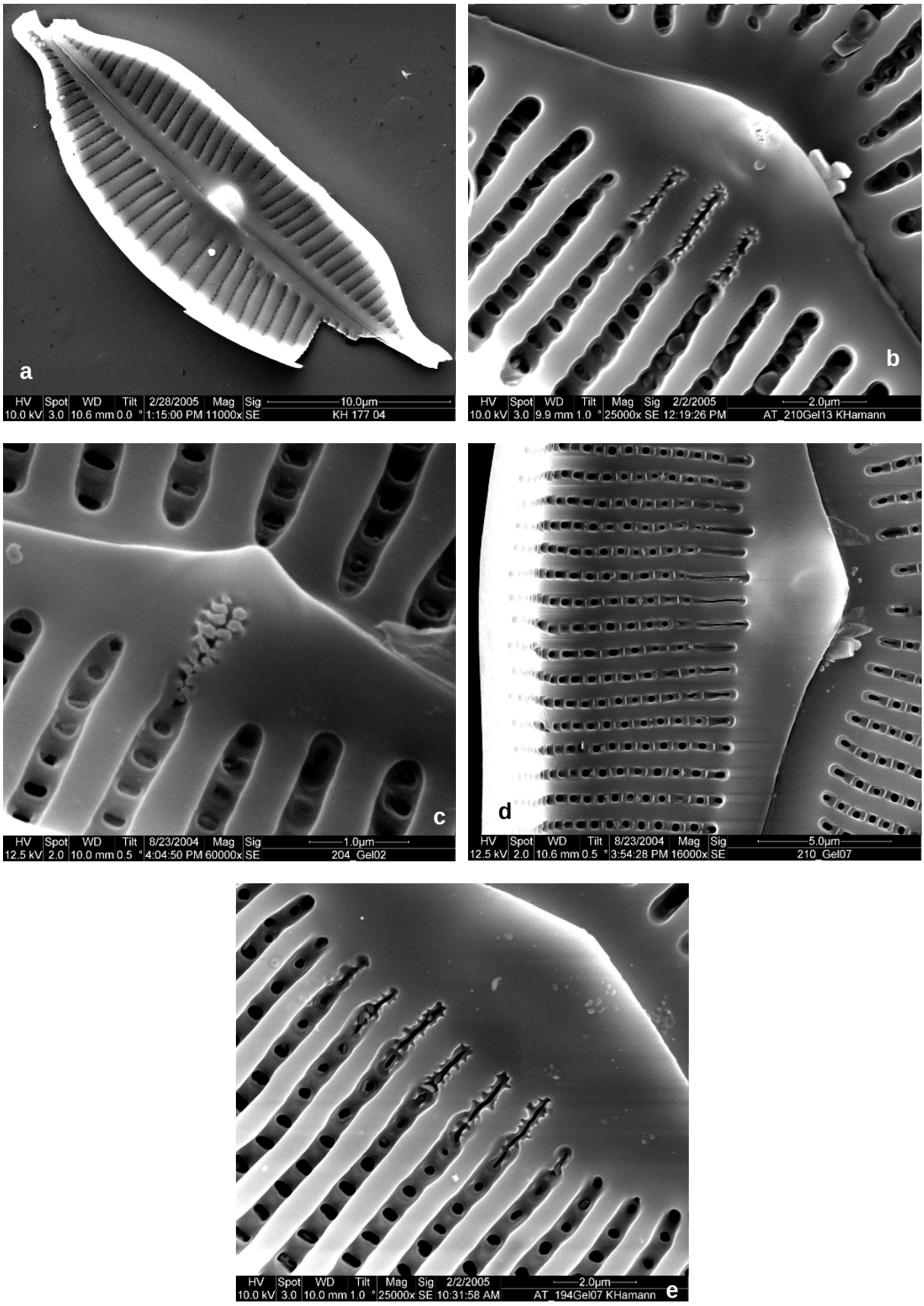


Fig. 55: *Cymbella* species, SEM of midvalve interior.
a: *C. naviculiformis*, b: *C. proxima*, c: *C. affinis*, d: *C. aspera*, e: *C. helmkei*.

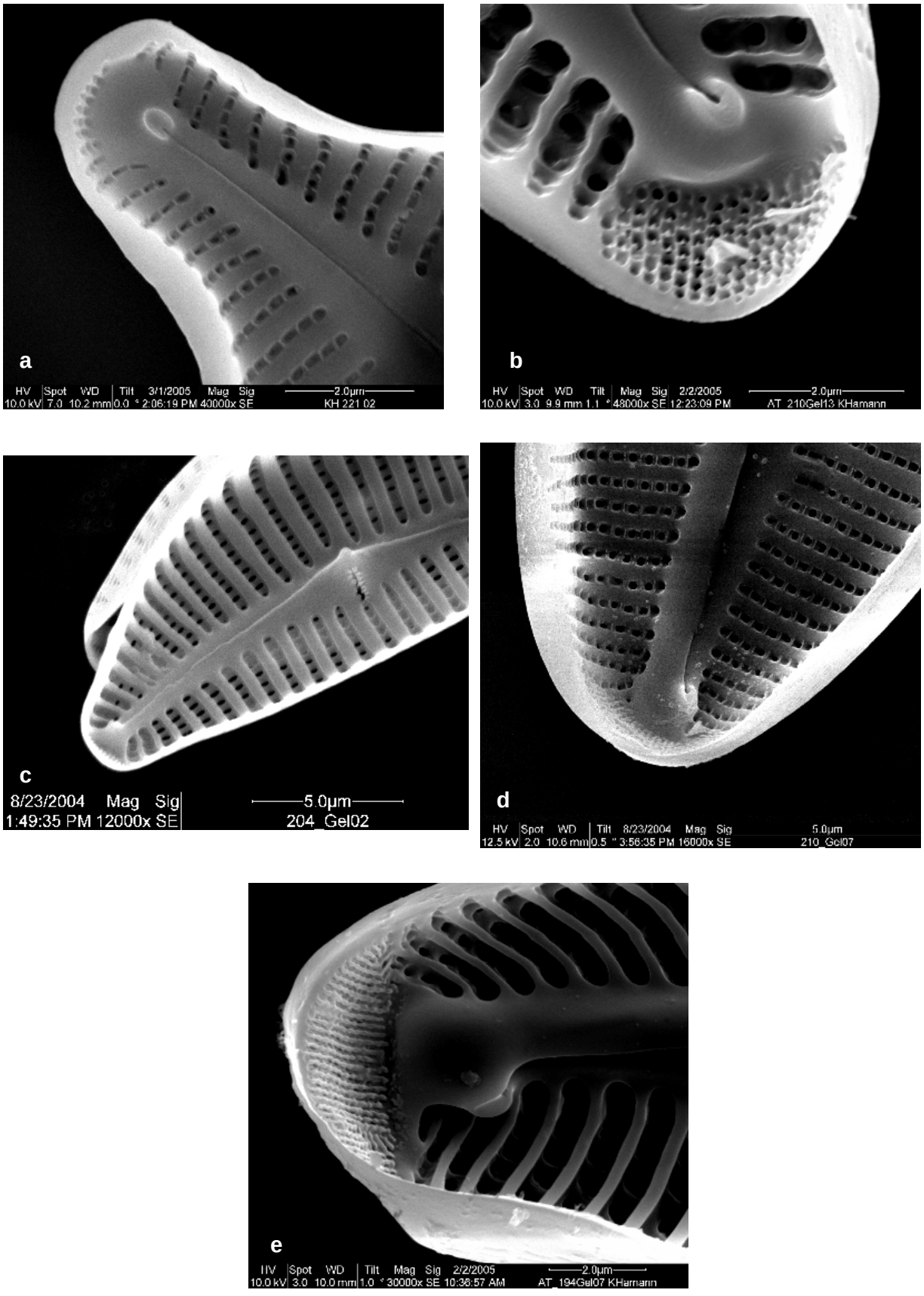


Fig. 56: *Cymbella* species, SEM of valve interior showing the helictoglossae.
a: *C. naviculiformis*, b: *C. proxima*, c: *C. affinis*, d: *C. aspera*, e: *C. helmkei*.

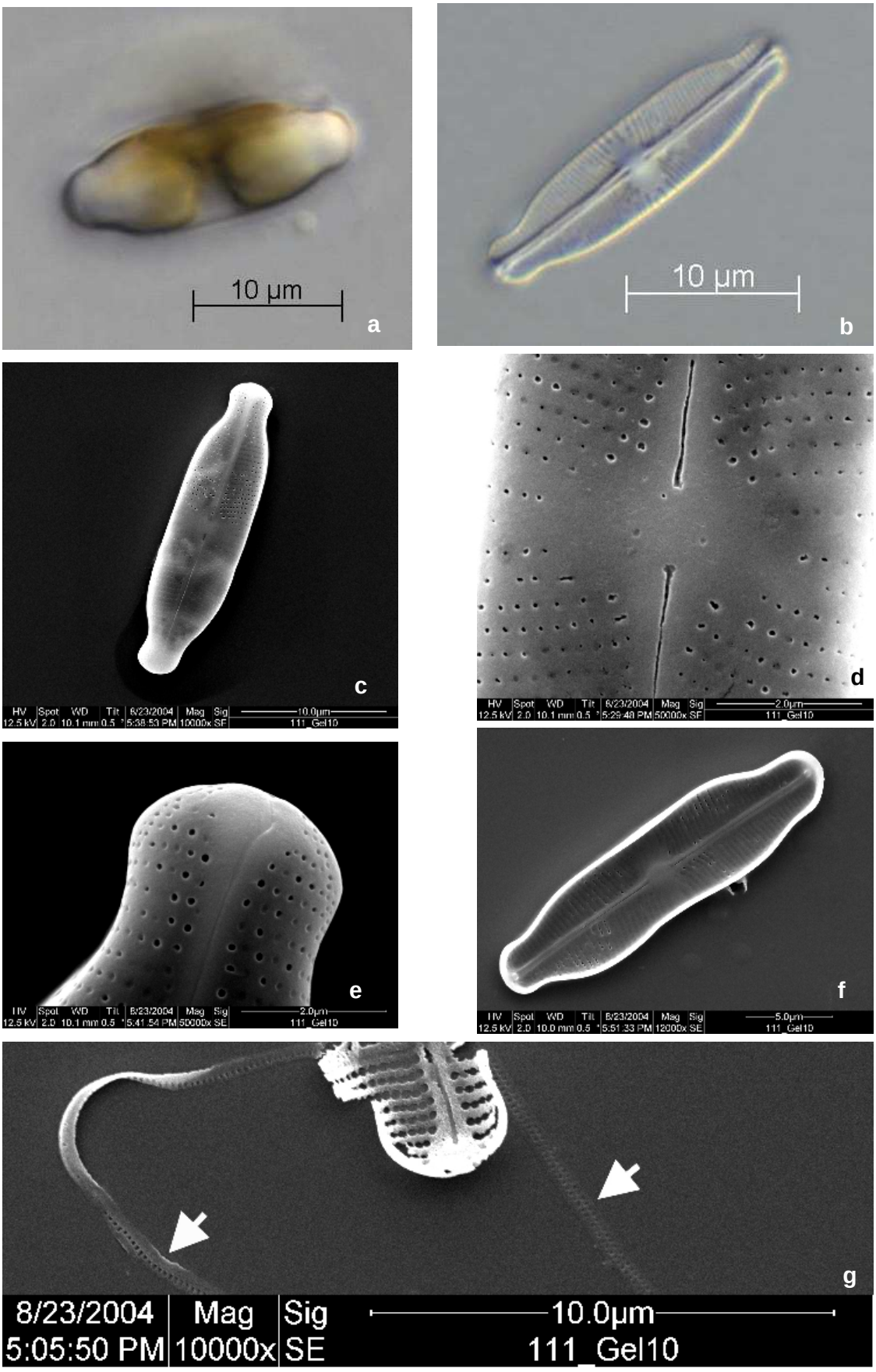


Fig. 57: *Navicula brockmannii*.

a+b: Light micrographs of live individual (a) and cleaned valve (b), c - e: SEM, external view. f: SEM, internal view. g: SEM, girdle bands

Both varieties of *M. atomus* (Fig. 58) had radiate striae, which consists of uniseriate rows of round areolae. The filiform raphe slit lies in a heavily silicified median costa. The raphe is slightly curved and the terminal fissures curved to the same side. The two varieties differ in size and density of striae and areolae. *M. atomus* var. *atomus* (Fig. 58 a - c) had a medium size of length/width =10 µm/4 µm and 20-24 striae/10 µm with approximately 40 areolae/10 µm. The variety *permitis* (Fig. 58 d - f) had 35 striae/10µm with approximately 60 areolae/10 µm and reached a medium size of length/width =7,5/3 µm.

Micrographs of sequenced species that are not present above are shown in the appendix (Figs. 60 – 70).

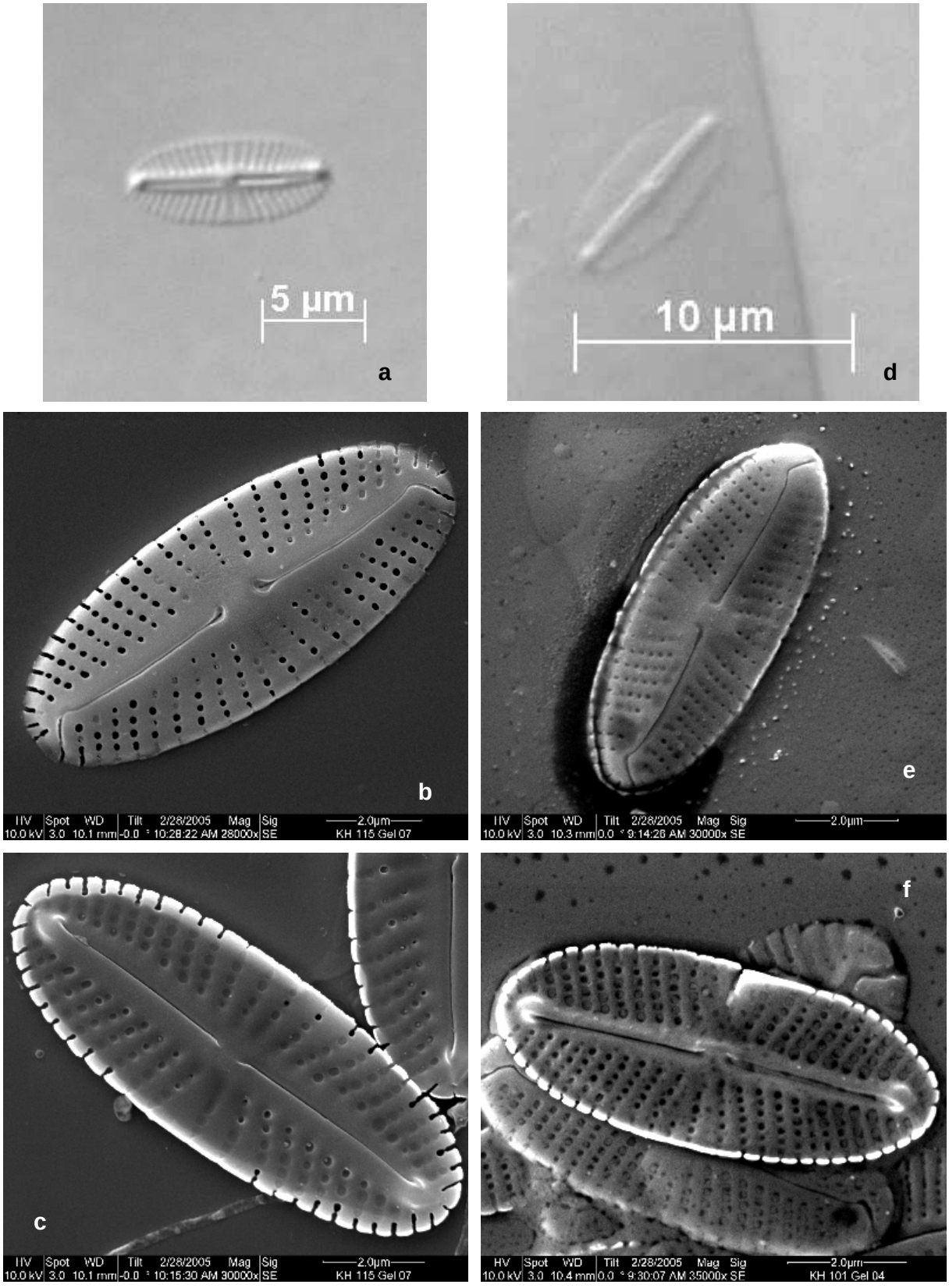


Fig. 58: *Mayamaea atomus* varieties.

a - c: *M. atomus* var. *atomus*, d - f: *M. atomus* var. *permitis*

a + c: light micrographs of a cleaned valve, b + e: SEM of valve exterior and c + f: SEM of valve interior.

4. Discussion

The recent taxonomy of naviculoid pennates is basically based on investigations of valve morphology, cell components and life cycle (e.g., Round *et al.*, 1990). But the development of the PCR has facilitated the use of DNA sequences for inferring phylogenies and several studies dealing with diatoms had been carried out (e.g., Medlin *et al.*, 1996 a, b, Medlin *et al.*, 2000, Kooistra *et al.*, 2003, Medlin & Kaczmarska, 2004, Sorhannus, 2004). With molecular phylogenetics, the *Bolidophyceae* were recovered as the sister group to the diatoms (Guillou *et al.*, 1999) and a revised classification with new subdivisions and classes was proposed (Medlin & Kaczmarska, 2004). There are several studies, which concentrate on the relationships of the diatoms with other heterokonta (e.g., Medlin *et al.*, 1997, Guillou *et al.*, 1999) or on the relationship of the different diatom classes (e.g., Sorhannus *et al.*, 1995), but there are only two studies with focus on the naviculoid pennates (Beszteri *et al.*, 2001, Behnke *et al.*, 2004). But in both studies, each genus is only represented by one or two species. The recent study concentrates on naviculoid pennates. The 91 isolated and sequensed cultures covered 22 genera and 72 species. 62 of these species belong to the Naviculaceae covering 16 genera. With the addition of sequences obtained from GenBank the number of naviculoid species rises up to 66, 76 and 109 in the dataset of *rbc*L gene, LSU rDNA and SSU rDNA sequences, respectively.

4.1 Comparison of the gene trees

A gene tree constructed from DNA sequences does not necessarily agree with the true species tree that represents the actual evolutionary pathway of the species involved. This is well known from several simulations (e.g., Pamilo & Nei, 1988, Hillis, 1996, Graybeal, 1998) and empirical studies (e.g., Soltis *et al.*, 1998, Soltis *et al.*, 2000). Most molecular phylogenies of diatoms based on the SSU rDNA (e.g., Medlin *et al.*, 1996 a, b, Medlin *et al.*, 2000, Kooistra *et al.*, 2003, Medlin & Kaczmarska, 2004). Those studies based on other gene sequences (e.g., Behnke *et al.*, 2004) deal with a different set of taxa, which make it very difficult to compare the phylogenies. Because the same set of cultures was used for all genes in this study, the phylogenies based on sequences of the nuclear SSU rDNA, LSU rDNA and the chloroplast *rbc*L gene could be easily compared. For the same reason, the sequences could be additionally analysed in a combined dataset.

For all datasets molecular phylogenies were inferred with maximum likelihood (ML) and maximum parsimony (MP) analyses. The only exception is the dataset of SSU rDNA

sequences, which contains sequences obtained from GenBank. For this dataset the maximum parsimony analysis could not be conducted. The time to conduct the MP analysis extremely increased, because of the large number of species in this dataset.

4.1.1 Phylogenies based on the AlgaTerra cultures

4.1.1.1 Phylogenies based on SSU rDNA sequences

The resulting phylogenetic trees based on the SSU rDNA dataset of AlgaTerra cultures (Figs. 3 – 6) show only few differences. Most relationships between and within the different genera in the ML tree could be recovered in the MP tree. The polytomies in the MP phylogeny does not contradict the branching order in the ML tree. *Luticola goeppertiana* and *Neidum affine* form a clade in both gene trees, but this clade diverges at different positions in the two phylogenies. The very long branches of these two species in the ML tree show that their sequences differ very much from all other sequences. Especially in MP analyses these rapidly evolving lineages are inferred to be closely related, regardless of their true evolutionary relationships or diverge very early in the tree (e.g., Felsenstein, 2004, Salemi & Vandamme, 2003). This phenomenon in phylogenetic analyses is known as “Long Branch attraction”. Long Branch attraction is most commonly in maximum parsimony analyses but it is also known for ML or distance methods (e.g., Felsenstein, 2004, Salemi & Vandamme, 2003). The problem arises when the DNA of two (or more) lineages evolves rapidly. These rapidly evolving lineages are inferred to be closely related, regardless of their true evolutionary relationships or diverge very early in the tree. In bootstrap trees, this misinterpretations will be supported with high bootstrap values. Therefore the close relationship of the two genera might also be a result of Long Branch attraction. But although their morphology differ clearly (e.g number of chloroplasts, absence or existence of a stigma, raphe endings) and they were placed in different families in Round *et al.* (1990), the two genera belongs to the same suborder Neidiineae. Because *Neidum* and *Luticola* are the only representatives of this suborder in this phylogeny, their close relationship in the tree might reflect their true relation. The second clade that changed its position, consisted of *Stauroneis*, *Craticula* and *Navicula integra* (clade 5 in the ML tree, clade 3 in the MP tree). In both trees, this clade is closely related to the same two clades, *Mayamaea*, *Eolimna*, *Pinnularia* and *Caloneis* on one hand and the monoraphid genera, *Navicula brockmannii* and the *Cymbellales* on the other hand. In the MP tree, *Stauroneis*, *Craticula* and *Navicula integra* are the sister group of the former clade, in the ML phylogeny the latter is the sister group. Compared to the classification in Round *et al.* (1990) the relationship in the MP tree is more likely, because these species were

placed in the same order (Naviculales). On the other hand the order Naviculales could not be supported by the molecular phylogenies.

The deep divergences had no or only poor bootstrap support, but at lower level (genus to species) many clades were well supported. Several genera, which were established on the base of morphological data, could be recovered as monophyletic groups. Most of these groups were supported by high bootstrap values (≥90). This is true for *Fragilaria*, *Eunotia*, *Navicula sensu stricto*, *Craticula*, *Cocconeis* and *Mayamaea*. The monophyly of *Amphora*, *Cymbella*, *Gomphonema* and *Placoneis* (with *Navicula* [*Placoneis*] *hambergii*, see 4.2.6) had medium or low bootstrap support and the controversially discussed genera *Pinnularia* and *Caloneis* form a monophyletic clade, which was supported relatively well (for detailed discussion on these genera see 4.2.3).

Eunotia diverges first after the outgroup, followed by a clade containing *Navicula sensu stricto* and *Hippodonta capitata.* The close relationship of the two genera correspond with the discussion whether or not to separate *Hippodonta* from *Navicula sensu stricto*. The strongly supported monophyly of *Navicula sensu stricto* suggest a separation. All other new described genera that were segregated from *Navicula sensu stricto* (*Craticula*, *Eolimna*, *Luticola*, *Mayamaea* and *Placoneis*) and all "*Navicula*" species, that do not belong to the section *Lineolatae* (*N. integra*, *N. hambergii* and *N. brockmannii*, for detailed discussion see 4.2.4, 4.2.6 and 4.2.8, respectively) did not cluster with the *Navicula sensu stricto. Craticula* is most closely related to *Stauroneis* and *N. integra.* The close relationship of *Craticula* and *Stauroneis* agree with the assumption made by Round *et al.* (1990) and the results of the phylogenetic analysis of morphological data conducted by Cox and Williams (2000). The cymbelloid genera (*Cymbella*, *Placoneis*, *Encyonema* and *Gomphonema*), *N. brockmannii* and the monoraphid genera *Cocconeis* and *Achnanthidium* form a clade (clade 4). *Eolimna minima* and *Mayamaea* are most closely related to *Pinnularia*/*Caloneis* (clade 3). *Amphora* diverges at the base of the whole group (clade 2).

4.1.1.2 Phylogenies based on LSU rDNA sequences

The analyses of LSU rDNA alignments resulted in less supported phylogenies (Figs. 11 – 14) as compared to those based on the SSU rDNA. The tree inferred with the parsimony analysis using the sequences of the AlgaTerra cultures (Figs. 13 + 14) had several large unresolved polytomies, but with the exception of the position of *N. affine* and *L. goeppertiana* they do not contradict the branching order in the ML tree (Figs. 11 + 12). In the MP tree, the two species form a clade, which diverge from the basal polytomy, whereas in the ML tree they diverge

within the *Navicula sensu stricto*. The integration in the *Navicula sensu stricto* had no bootstrap support, whereas the clade consisting of *Hippodonta capitata* and *Navicula sensu stricto* was well supported (BS: 99/76). Therefore and because of the clear morphological differences, it is unlikely that *Neidum* and *Luticola* belong to the *Navicula sensu stricto*. Similar to the results of the analyses of the SSU rDNA sequences their close relationship might represent their true relationship or might be caused by Long Branch attraction.

Although the branching order of the ML tree differs from those of the trees based on SSU rDNA sequences, several groups could be recovered. Similar to the SSU rDNA gene tree *Amphora, Craticula, Cocconeis, Cymbella, Eunotia, Fragilaria, Gomphonema, Mayamaea Placoneis* (with *Navicula* [*Placoneis*] *hambergii*, see 4.2.6) and *Pinnularia*/*Caloneis* form a monophyletic clade. But only *Craticula*, *Cocconeis, Fragilaria* and *Mayamaea* were supported by high bootstrap values (≥90). The four cymbelloid genera form a monophyletic clade with an identical branching order compared to the SSU rDNA gene tree. *Craticula* and *Stauroneis* with *N. integra* were sister groups and *Hippodonta* is sister to *Navicula sensu stricto*. The close relationship of *Eolimna minima, Mayamaea* and *Pinnularia*/*Caloneis* could be recovered, even though the branching order of the genera differs. The most unexpected difference to the SSU rDNA gene tree is that the araphid taxa diverge within the raphid, although they were assigned as outgroup (see PAUP commands in the appendix). This is probably, because only one centric diatom was included as outgroup to pull the araphids out of the raphid diatoms. The ML tree consists of two large clades. In clade 1, *Amphora* diverged first followed by *Eunotia*. Then *Hippodonta* and *Navicula sensu stricto* diverges, followed by the araphid taxa and finally the *Cymbellales*. In clade 2 *Pinnularia*/*Caloneis* and *Eolimna minima* form the base and *Mayamaea* diverges next. The next sub-clade contains *N. brockmannii* and *Cocconeis*. *Achnanthidium minutissimum* is sister to the *Craticula*/*Stauroneis*/*N. integra*-clade.

4.1.1.3 Phylogenies based on *rbc*L gene sequences

For the datasets of *rbc*L sequences the maximum parsimony analyses resulted in a poorly resolved phylogenetic tree (Figs. 19 + 20). Only *Hippodonta capitata* and *Navicula sensu stricto* and the two araphid genera form clades, which contain two genera. *Eunotia*, *Cocconeis* and *Mayamaea* were monophyletic. All other genera were merged in a large polytomy. Most branches in the ML tree had only low bootstrap support (Fig. 21).

Nevertheless, in the ML tree based on *rbc*L sequences, several clades from the SSU rDNA gene tree could be recovered. *Cocconeis, Eunotia, Fragilaria, Gomphonema, Mayamaea,*

Navicula sensu stricto, Placoneis (with *Navicula* [*Placoneis*] *hambergii*, see 4.2.6) and *Pinnularia*/*Caloneis* form monophyletic clades, again. Additionally *Stauroneis* is monophyletic because in this gene tree *N. integra* diverges within *Craticula*. Three clades containing the same species but in different branching order could be recovered. The first clade consists of *Eolimna minima*, *Mayamaea* and *Pinnularia*/*Caloneis* (clade 3 in Fig. 19). *Stauroneis, Craticula* and *N. integra* form a second recovered clade (within clade 5 in Fig. 19) and the third clade contains the monoraphid species, *N. brockmannii* and the Cymbellales (clade 4 in Fig. 19). But in this gene tree, the Cymbellales are not a monophyletic group, because *Encyonema* form a clade with the monoraphid species. But it is unlikely, that this clade represents the true relationship of *Encyonema*, because of the strong morphological support for the Cymbellales and the monophyly of this order in most phylogenies. Analogue to the phylogenies based on the SSU rDNA sequences, *Hippodonta capitata* is sister to *Navicula sensu stricto* and they diverge close to the base of the tree after *Eunotia*. The araphid diatoms diverge next, followed by *E. minima*, *Mayamaea* and *Pinnularia*/*Caloneis*. Therefore this is the second gene tree where they diverge within the naviculoid pennates. *Luticola goeppertiana* and *Neidum affine* form a clade that splits the genus *Amphora*. The branches of *L. goeppertiana* and *N. affine* in the ML tree are not very long. Therefore this clade could not be caused by Long Branch attraction. Together with *Stauroneis, Craticula* and *N. integra* these species form the sister clade to the group containing the *Cymbellales*.

4.1.1.4 Phylogenies based on the combined dataset

The analyses of the combined dataset resulted in the best supported trees (Figs. 26 – 29), but the deep divergences still has only weak bootstrap support. Most relationships of the ML tree could be recovered in the MP tree. The different positions of the clade containing *Luticola goeppertiana* and *Neidum affine* could be explained by Long Branch attraction. Additionally the araphid diatoms and the genus *Amphora* diverged at different positions. In the ML tree the araphids diverge after *Navicula sensu stricto*, but in the MP phylogeny they form a clade with *Eunotia* and diverge before *Navicula sensu stricto*. In both trees, *Amphora* is most closely related to *Craticula*/*Stauroneis*/*N. integra*, but in the ML tree *Amphora* diverges before the divergence of *Eolimna minima*, *Mayamaea* and *Pinnularia*/*Caloneis* and in the MP tree after this group.

The ML tree is very similar to the ML tree based on SSU rDNA sequences. Most differences are found in the deeper divergences. Like in the phylogeny based on the *rbc*L gene, the araphid pennates diverge after *Eunotia* and *Hippodonta* and *Navicula sensu stricto*. Similar to

the separate analyses of the LSU rDNA and *rbc*L gene sequences, the use of a single centric species might cause the problems to find the real position of the araphid pennates. The next clade contained the monoraphid taxa, *N. brockmannii* and the *Cymbellales*. Then *Luticola goeppertiana* and *Neidum affine* diverge (again with long branches), followed by *Amphora*. *Craticula, Stauroneis* and *N. integra* forming the sister clade of *Eolimna minima, Mayamaea* and *Pinnularia*/*Caloneis.*

4.1.1.5 General results of the analyses of the AlgaTerra cultures

The used D1/D2-region of the LSU rDNA comprises more highly variable areas than the SSU rRNA gene (Van der Auwera & De Wachter, 1998), which makes it even more difficult to align. A stronger phylogenetic signal for closely related species in comparison with the SSU rRNA gene and problems for reconstructing deep phylogenies were estimated. The latter expectation was proven by the MP phylogeny with its large polytomy at the base of the tree. But in this study the trees based on the LSU rDNA sequences do not provide stronger supported results for closely related species. Compared to the SSU rDNA gene trees, the bootstrap values are lower at all levels. Therefore the use of the D1D2-region does not result in more detailed information of the relationships between the species used in this study compared to the SSU rDNA.

A part of the *rbc*L gene was the second sequence additionally used in this study to obtain clearer information of evolution at lower (order to genus) levels of taxonomic hierarchy in diatoms. But in the tree resulted from the analyses of the *rbc*L dataset the bootstrap supports at all levels were low compared to the SSU rDNA gene trees. It is known that in protein-coding trees the three codon positions evolve at different rates. Therefore, these dataset set was additionally analysed with differently weighted positions, but the resulting tree differs only slightly (see Fig. 71 in the appendix). In this study only 684 bp of the *rbc*L gene, which has a total length of 1428 – 1434 pb, were used. This might be the reason, that the results fall short of the expectations.

With the combination of the sequences in a single dataset the information of all genes was combined. From several studies it is known that an increased number of nucleotides (e.g., Saito & Nei, 1986) and the use of different genes that have evolved independently (e.g., Pamilo & Nei, 1988). The analysis of the combined dataset should result in trees with an increased resolution and internal support (as measured by bootstrap values) because the number of nucleotides increased and the nuclear-encoded rDNA evolved independently from the plastid-encoded *rbc*L gene. From other studies it is known that the analyses of combined

data sets run faster times compared to the separate datasets (Soltis *et al.*, 1998, Soltis *et al.*, 2000). In the recent study, the analyses of the combined dataset ran faster and resulted in trees with higher bootstrap support than the analyses of the single genes. Especially the divergences at genus and species level were supported by increased bootstrap values. But the deep divergences, where the most differences between the different gene trees appeared, still have only poor bootstrap support. The partition homogeneity test of the combined dataset resulted in a very low p-value of 0,01. If the test have been used to determine whether or not to combine data sets for phylogenetic analysis, this p-value denotes separate analyses. But the resulting best supported tree of the combined analyses in this study agree with other studies, that have found that *P*-values < 0,05 should not preclude dataset combination (e.g., Sullivan, 1996, Davis *et al.*, 1998, Flynn & Nedbal, 1998, Yoder *et al.*, 2001).

Most differences between the trees are located at deeper branches. In all phylogenies, the deep branches had no or only extremely low bootstrap support. Therefore, based on the molecular data, these divergences could not be resolved unambiguously. Although the trees based on the different datasets differ, many relationships could be recovered in the analyses of each dataset. This is a strong support that these relationships in the phylogenetic trees agree with the true species tree.

Hippodonta capitata diverges at the base of *Navicula sensu stricto* in all trees. The well supported monophyly of *Navicula sensu stricto* in most trees support a separation of the two genera as promoted by Witkowski *et al.* (1998) and Round (2001). Although the two genera appeared as sister groups, the results refutes the idea of Cox (1999, 2002) enlarging the generic description of *Navicula sensu stricto* to cover both genera. The results strongly support the concept of *Navicula sensu stricto* (*Navicula* section *Lineolatae*), because all other "*Navicula*" species, that do not belong to the section *Lineolatae* (*N. brockmannii*, *N. hambergii* and *N. integra*, for discussion see 4.2.8, 4.2.6 and 4.2.4, respectively) and all new described genera, that were segregated from *Navicula sensu stricto* (*Craticula*, *Eolimna*, *Mayamaea* and *Placoneis*), did not cluster with the *Navicula sensu stricto.* This is also true for *Luticola*, with the exception of the ML tree inferred using *rbc*L gene sequences.

As proposed by Round *et al.* (1990) on the base of plastid behaviour, sexual reproduction and some aspects of the valve morphology, *Craticula* is closely related to *Stauroneis* in all trees. This also agree with Cox and Williams (2000), who conducted a phylogenetic analysis of several naviculoid diatoms with a stauros based on morphological data.

Eolimna minima and *Mayamaea* are closely related to *Pinnularia*/*Caloneis* in all trees, but the branching order of the three genera differs in the different trees. A similar result was found by Behnke *et al.* (2004). In their ML phylogeny of SSU rDNA sequences *E. minima* and several *Sellaphora* species form the sister clade to *Pinnularia* cf. *interrupta* and *Navicula pelliculosa*.

Placoneis diverged within the *Cymbellales* as presumed by Round *et al.* (1990) based on frustule and protoplast characters. All genera Round *et al.* (1990) summarised in the order *Cymbellales*, which were present in this study, form a monophyletic clade in the different phylogenies. But the relationships within this order differ between the phylogenetic trees inferred in this study and the classification shown in Round *et al.* (1990). *Encyonema* was describes by Kützing (1833) and later added to *Cymbella* by Cleve-Euler (1948). Round *et al.* (1990) restored the genus *Encyonema* and placed it together with *Cymbella*, *Placoneis*, *Brebissonia* and *Gomphocymbella* in the family Cymbellaceae. But in all phylogenetic trees *Gomphonema*, which was placed in the family Gomphonemataceae, diverge within the family Cymbellaceae. This result advises a revision of the involved families *Cymbellaceae* and *Gomphonemataceae* on the base of a detailed morphological and molecular investigation of all genera. The close relationship of the cymbelloid lineage and the freshwater monoraphid taxa, which was shown by Medlin and Kaczmarska (2004), could be recovered, although both studies used a totally different set of taxa.

Eunotia form a monophyletic clade, which diverges at the base of the naviculoid pennates in most trees. This contradicts the position of this genus found by Medlin and Kaczmarska (2004), where *Eunotia* diverges between two clades containing naviculoid taxa. But the results of other analyses (Medlin *et al.*, 2000, Sorhannus, 2004) in which the Eunotiales fell at the base of all raphid diatoms, are supported.

In all trees, the position of *N. affine* and *L. goeppertiana* differs. The long branches of this species in both rDNA ML trees indicate that the rDNA evolves more rapidly in these species. Especially for the sequences of *L. goeppertiana* this was obvious in the alignment, because of the large insertions. That these species belong to *Navicula sensu stricto* as it is shown in the LSU rDNA gene tree inferred with ML is refused by the mainly well supported monophyly of *Navicula sensu stricto* in all other trees. Additionally the valve morphology of both species deviates from the generic description of *Navicula sensu stricto*. Beside other differences in both species the raphe structure does not fit to the generic description of *Navicula sensu stricto* (Round *et al.*, 1990). The two species also form a clade in the *rbc*L gene trees, where they do not have long branches and in the trees based on the combined data set this clade is

well supported (BS: 99/97). Because the two genera are the only representatives of the suborder Neidiineae, the clade reflects this relationship. But it should be expected, that the relationship of the two genera is more distant, than the trees in this study show.

4.1.2 Phylogenies based on enlarged datasets

4.1.2.1 Phylogenies based on SSU rDNA sequences

The ML tree (Figs. 7 – 10) based on the enlarged dataset with additional sequences obtained from GenBank shows a similar relationship of the genera compared to the tree based on the smaller dataset. Similar to this phylogeny the deeper divergences had very low bootstrap support.

Most diatom sequences available at GenBank are sequences of the SSU rDNA. From the huge amount of available sequences, I choose all naviculoid pennates, several Bacillariales and *Eunotia* species. The nomenclatures of two of these additional sequences were obviously wrong: AY485521 *Stauroneis constricta* must be a *Fragilariopsis* species and AY485496 *Achnanthes* sp. must be an *Amphora* species, because both species belongs to monophyletic clades with maximum bootstrap support.

Eunotia form a monophyletic clade, which diverges at the base of all raphid pennates. The enlarged dataset contains naviculoid and nitzschioid taxa and the phylogeny contradicts the position of *Eunotia* found by Medlin and Kaczmarska (2004) and support the results of Medlin *et al.* (2000) and Sorhannus (2004). This result also agree with the classification in Round *et al.* (1990), in which the Eunotiaphycidae (contain *Eunotia* and related species) and the Bacillariophycidae (contain all ather raphid pennates) were combined in one class.

The *Bacillariales* form a clade with the marine *Achnanthes* species, which diverges between *Undatella* sp. and the other naviculoid pennates. This separation of monoraphid genera contradict the order Achnanthales, as mentioned in Round *et al.* (1990). In contrast to the tree of Medlin and Kaczmarska (2004) the naviculoid pennates form a monophyletic clade, with the exception of *Undatella* sp. This clade was subdivided into four sub-clades, of which only the first one is supported by bootstrap analysis (BS: 66).

The first sub-clade (naviculoid pennates part 1, Figs. 7 + 8) contains *Haslea, Gyrosigma* and *Pleurosigma* as sister to *Hippodonta*, *Navicula sensu stricto* plus *Pseudogomphonema*. Equivalent to the phylogenies based on sequences from AlgaTerra cultures, *H. capitata* diverges at the base of the *Navicula sensu stricto*. A close relationship of the two genera *Pseudogomphonema* and *Navicula sensu stricto* was already proposed based on

morphological analyses (e.g. Medlin & Round, 1986). The molecular data suggest that *Pseudogomphonema* should not be separated from *Navicula sensu stricto,* although these two genera differ in their valve symmetry. It is clear that the asymmetry is a derived character from within the *Navicula sensu stricto*. The close relationship of *Gyrosigma* and *Pleurosigma* agree with the placement in one family by Round *et al.* (1990). All genera in this sub-clade belong to the suborder Naviculineae sensu Round *et al.* (1990), but not all genera summerized in this subgenus by Round *et al.* (1990) appeared in this sub-clade.

Haslea nipkowii did not cluster with the other *Haslea* species in the first sub-clade, but form a clade with *Neidum affine*. But in Damsté *et al.* (2004) and Poulin *et al.* (2004) the genus is monophyletic and the affiliation of *H. nipkowii* is also suppoted by morphological and biochemical data.The result of the recent study might be caused by Long Branch attraction.

Clade 1 of the sub-clade naviculoid pennates part 2 (Fig. 9) contains *Amphora* subgenus *Amphora* as sister to *Phaeodactylum tricornutum* and *Luticola goeppertiana* as sister to *Diadesmis gallica*. The close relationship of *Luticola* and *Diadesmis* agree with the combination of the two genera in the family Diadesmidaceae by Mann (in Round *et al.*, 1990). But *Amphora* and *Phaeodactylum* were placed in different orders by Round *et al.* (1990). All monoraphid genera, with the exception of the marine *Achnanthes* species, could be found in clade 2. They form two clades with *Cocconeis* and *Planothidium* on one hand and *Pauliella* and *Achnanthidium* on the other hand. The divergence in two groups agree with the separation of two families in Round *et al.* (1990), although *Planothidium* and *Pauliella* were not mentioned there. But that the monoraphids diverge within several genera placed in the Naviculales contradict the seperation of these genera in an other order (Achnanthales). Similar to the phylogenie based on the SSU rDNA sequences of AlgaTerra cultures, the genera *Cymbella*, *Encyonema*, *Gomphonema* and *Placoneis* form a monophyletic clade, only the relationships between these genera differs in the two phylogenies. Bur again the families Cymbellaceae and Gomphonemataceae could not be recovered in the molecular phylogeny. In this phylogeny *Anomoeoneis*, which also belongs to the order Cymbellales (Round *et al.*, 1990), and *Lyrella*, which belongs to the order Lyrellales (Round *et al.*, 1990), form the sister clade of the other Cymbellales. *Anomoeoneis* and *Lyrella* were each represented by only one species and their clade had only low bootstrap support. Therefore the result of this analysis does not suffice to suggest any chages in the classification of them. A close relationship of the two orders was also found in the study conducted by Behnke *et al.* (2004).

The sub-clade naviculoid pennates part 3 (Fig. 10) contains all *Amphora* species of the subgenus *Halamphora*, but they did not form a monophyletic clade (for detailed discussion of relationships within the genus *Amphora* see 4.2.2). This dataset contains one species (*Undatella* sp.) that is assumend to be closely related to *Amphora* by Round *et al.* (1990). But none of the Amphora species is closely related to *Undatella* sp.. Most species in clade 1 of this sub-clade belong to the suborder Sellaphorineae (sensu Round *et al.*, 1990). Based on this phylogeny the genus *Mayamaea*, which was not mentioned in Round *et al.* (1990), should be placed to the same suborder. That *Mayamaea* forms the sister clade to *Pinnularia*/*Caloneis* suggests the addition of *Mayamaea* to the family Pinnulariaceae. The well supported monophyly of *Eolimna minima* and *Sellaphora* agree with Behnke *et al.* (2004), who concluded that *E. minima* "could be regarded as belogning to the Sellaphoraceae, or even to *Sellaphora* itself " (p. 206). In clade 2 *Navicula pelliculosa*, *N. saprophila*, *Stauroneis*/*N. integra*, *Eolimna subminuscula* and *Craticula* form a strongly supported monophyletic clade (BS: 100). *N. pelliculosa* and *N. saprophila* does not belong to *Navicula sensu stricto* and the results support the separation of these species from the genus. But the position of *N. pelliculosa* contradicts the assumption of Round *et al.* (1990) and Behnke *et al.* (2004) that this species belongs to *Sellaphora* or at least to the suborder Sellaphorineae. Based on the recent phylogeny *N. pelliculosa*, *N. saprophila* and *E. subminuscula* belong to the family Stauroneidaceae together with *Craticula*, *Stauroneis* and *N.* [*Prestauroneis*] *integra*. The sister clade to the Stauroneidaceae consists of a highly supported monophyletic *Amphora* subgenus *Halamphora* clade and a second clade containing several Surirellales.

As described above, this phylogeny supports several classifications made in Round *et al.* (1990) and several families as well as suborders and orders could be recovered as monophyletic clades. But the results clearly show, that the order Naviculales as described in Round *et al.* (1990) is a heterogenous group.

4.1.2.2 Phylogenies based on LSU rDNA sequences

Although only nine sequences could be obtained from GenBank the trees resulting from the analyses of the enlarged LSU rDNA dataset differs strongly from those inferred with the smaller dataset. Some relationships between closely related genera, such as the monophyly of the Cymbellales, could be recovered. But the branching order of the different groups differs strongly from those found in all other trees. These deep divergences have no support by bootstrap values and could not be explained by morphological data. Therefore the use of the D1/D2-region of the LSU rRNA gene appears problematic.

Similar to the the tree based on the enlarged SSU rDNA dataset, the Baccillariales form a monophyletic clade. But in the phylogenies based on the LSU rDNA sequences this clade diverge within the naviculoid diatoms. With the addition of *Amphora coffeaeformis,* the seperation of the two subgenera of *Amphora* becomes more obvious. Similar to its position in the tree based on the enlarged SSU rDNA dataset, *Phaeodactylum* diverges close to *Amphora*.

4.1.2.3 Phylogenies based on *rbc*L gene sequences

The enlarged dataset of *rbc*L sequences contains 15 additional sequences obtained from GenBank. The two phylogenies (Figs. 22 – 25) inferred with this dataset show great differences in the deep branches, compared with each other and with the trees based on the *rbc*L sequences from the AlgaTerra cultures. The resolution of the MP tree based on the enlarged dataset is better compared to the MP tree based on the *rbc*L sequences from the AlgaTerra cultures, but it still contains several unresolved polytomies.

The additional species of the genera *Encyonema*, *Eunotia*, *Gomphonema* and *Placoneis* form monophyletic clades with the other species of their genera. *Lyrella* form the sister clade to *Petroneis* (clade 2), which agree with the family Lyrellaceae erected by Mann (in Round *et al*., 1990). Similar to the tree based on the enlarged SSU rDNA dataset, they are relativly close related to the monoraphid species and the Cymbellales in the ML tree. But the diverge before the entire group. Equivalent to the tree based on the enlarged SSU rDNA dataset, *Sellaphora* forms a monophyletic clade with *Eolimna minima* and is closely related to *Mayamaea* and *Pinnularia*/*Caloneis*. *Navicula sensu stricto* is paraphyletic, because of *N. salinicola*. Both phylogenies suggest that *Pseudogomphonema* and *Seminavis* should not be separated from *Navicula sensu stricto*. The three genera also share several morphological feature, like uniseriate striae containing apically elongate, slit-like poroids or the raphe structure, with simple, straight internal central raphe endings, expanded external central raphe endings and internal raphe fissures, that open laterally (e.g., Medlin & Round, 1986, Round *et al*., 1990, Danielidis & Mann, 2002). For *Seminavis* it is additionally known, that apart from creating an asymmetrical shape of the vegetative cell, almost all characteristics exhibited by the live cell and auxospores agree with what is found in *Navicula sensu stricto* (e.g., Mann & Stickle, 1989, Chepurnov *et al*., 2002).

4.1.3 General relationships of the genera

The phylogenies based on the different datasets differ. Especially the branching order of the early divergences differs strongly and could not be resolved in this study. The results show,

that it could be difficult to detect the relationships of genera, which are represented by only a single species. They diverge within closly related genera (e.g., *Pseudogomphonema*) or change their position in the different phylogenies, especially if their DNA evolves rapidly (e.g., *Luticola, Neidum*). But several relationships on different levels could be determined based on all or at least most trees.

The results of this study support the monophyly of the genera *Cocconeis*, *Craticula*, *Cymbella*, *Eunotia*, *Gomphonema*, *Mayamaea*, *Navicula sensu stricto* and *Placoneis* (with *N. hambergii*, see 4.2.6), because they form monophyletic clades in all or at least most trees. A monophyletic group containing both, *Caloneis* and *Pinnularia*, is also supported. Additionally, in all phylogenies based on enlarged datasets containing two or more species of these genera, *Encyonema*, *Lyrella*, *Pleurosigma* and *Sellaphora* (with *Eolimna minima*) are monophyletic. But the monophyly of the genus *Amphora* is rejected (see 4.2.2).

Navicula sensu stricto and *Hippodonta capitata* are sister groups in most phylogenies. The close relationship of the two genera correspond with the discussion whether or not to separate them. The strongly supported monophyly of *Navicula sensu stricto,* which appear in most phylogenies, approve a separation. In all phylogenies based on enlarged datasets containing sequences from *Pseudogomphonema* or *Seminavis* species, these species diverge within *Navicula sensu stricto*. In contrast to *Navicula sensu stricto Pseudogomphonema* and *Seminavis* exhibit asymmetrical valves. On the other hand all three genera share several morphological features, like apically elongate, slit-like poroids, the raphe structure or the two plastids, lying along each side of the girdle. This suggests, that different valve symmetry alone does not approve separating genera. Reichardt (1992) came to the same result while comparing the morphology of *Navicula sensu stricto* and *Rhoikoneis*.

The genera *Craticula* and *Stauroneis*, which were summarised in the family Stauroneidaceae by Mann (in Round *et al*., 1990) and appear as close relatives in a phylogenetic analysis based on morphological data (Cox & Williams, 2000), are found to be close relatives in the recent study, too. The results also suggest to add *Navicula* [*Prestauroneis*] *integra* to this family, because this species is associated with this genera in all phylogenies.

The close relationship of *Pinnularia*/*Caloneis* and *Sellaphora*/*Eolimna minima* as proposed with the suborder Sellaphorineae by Mann (in Round *et al*., 1990) could be recovered in most phylogenies. The results also support to include the genus *Mayamaea* to this suborder.

The recent study support the monophyly of the order Cymbellales erected by Mann (in Round *et al*., 1990). But the results contradict the arrangement of the families Cymbellaceae and

Gomphonemataceae, because in most trees *Gomphonema* (Gomphonemataceae) diverge within the Cymbellaceae.

The order Naviculales and the suborder Naviculineae as used in Round *et al*. (1990) are shown to be heterogenous in all trees.

The monoraphid genera diverge within the naviculoid pennates in all phylogenies. They are close relatives of *Navicula* [*Adlafia*] *brockmannii* in most trees and and diverge at the base of the Cymbellales in several phylogenies, but the relationship between the monoraphid genera and the naviculoid pennates could not be resolved unambiguously.

4.2 Relationships within the genera

4.2.1 *Navicula sensu stricto*

J. B. M. Bory de Saint-Vincent (1922) described the genus *Navicula* based on *N. tripunctata* (O.F.Müller) Bory. In the beginning all diatoms with a central raphe on both valves that lack other light microscopic characteristics of the frustule were assigned to this genus. But with further investigations, the morphological diversity of the genus became apparent. Today, it is widely accepted that *Navicula* (*sensu stricto*) should be used only for species that belong to the section *Lineolatae* (sensu Cleve, 1895 and Hustedt, 1930).

This study confirms the assumption that the genus *Navicula sensu lato* is a very heterogeneous group and the results support the monophyly of *Navicula sensu stricto*. All "*Navicula*" species, that do not belong to the section *Lineolatae* (*sensu* Cleve, 1895 and Hustedt, 1930) did not cluster with the *Navicula sensu stricto* (see 4.1.3). In the molecular phylogenies the *Navicula sensu stricto* are divided into three sub-clades, but the morphological investigations shows no obvious differences between these sub-clades. Therefore a further separation of this genus is not reasonable. But this result does not contradict Witkowski *et al.* (1998). Based on morphological investigations of freshwater and marine *Navicula sensu stricto* species, they reasoned that *Navicula sensu stricto* is still a heterogeneous group and distinguishes six different groups. But the five groups, which were segregated from *Navicula sensu stricto*, contain mainly marine and few brackish-water taxa. Therefore none of these taxa is part of this study.

4.2.2 *Amphora*

The genus *Amphora* was described by Ehrenberg in 1844 (in Kützing, 1844). The genus embraced all species whose raphe systems of both valves lie on the same side of the cell. That

Amphora is an artificial genus has been known for over 100 years and Cleve (1895) subdivide the genus into six subgenera. Three of these subgenera contain freshwater species: *Amphora*, *Halamphora* and *Oxyamphora*. But this subdivision did not induce the creation of new genera from *Amphora*. Only in 1990, did Mann establish the genus *Seminavis* (in Round *et al.*, 1990), which covered several marine species previously assigned to *Amphora*. The species cultured within the scope of this study belong to the subgenera *Amphora* (*A. libyca*, *A. pediculus*, *A.* cf. *fogediana* and the unidentified *Amphora* species) and *Halamphora* (*A. normannii*). Most species whose sequences were obtained from GenBank are assigned to the subgenus *Halamphora* and only *A.* cf. *proteus* belongs to *Amphora* subgenus *Amphora*. The most obvious morphological difference between the two subgenera is the organisation of the girdle. The girdle of the species belonging to the subgenus *Amphora* consists only of the valvocopula (Fig. 36 c, d, f and Schoeman and Archibald, 1986: Figs. 70 – 86), whereas the girdle of the species belonging to the subgenus *Halamphora* contains additionally numerous girdle bands (Fig. 35 c and Krammer and Lange-Bertalot, 1986: Fig.151: 1 – 6, 18 – 27).

The results of the phylogenetic analyses support a partition of the genus *Amphora*. In all phylogenies those species, which belong to the subgenus *Amphora*, formed a monophyletic clade with maximum bootstrap support. In most phylogenies based on the sequences of the AlgaTerra cultures (Figs. 3, 5, 12, 13, 26 and 27) *A. normannii* diverged first in a monophyletic clade of all *Amphora* species. Although this monophyly is supported by bootstrap values up to 92, the branch length in the ML trees (Figs. 3, 12 and 26) indicated a separation. In all trees based on *rbc*L sequences (Figs. 19, 21, 22 and 24), *A. normannii* and the subgenus *Amphora* did not form a monophyletic group. An explicit separation of the two subgenera occurs with the addition of SSU rDNA and LSU rDNA sequences obtained from GenBank. In the consensus tree inferred with the parsimony analysis based on LSU rDNA sequences, the six *Amphora* species formed a monophyletic clade (Figs. 17, 18). But the bootstrap support for this clade was relatively low (53/49), whereas the monophyly of each subgenus was supported by high bootstrap values (>95). In the ML phylogeny (Figs. 15, 16) of this alignment the two subgenera were separated by *Entomoneis*. In the ML phylogeny of the SSU rDNA sequences (Figs. 7 - 10), the two subgenera appeared in two different clades. The subgenus *Amphora* was most closely related to *Phaeodactylum*, *Diadesmis* and *Luticola* (Fig. 9). The subgenus *Halamphora* did not form a monophyletic group (Fig. 10). *A. coffeaeformis* was most closely related to *Rossia*, *Eolimna minima* and *Sellaphora*, but most species of this subgenus formed a sister group to several *Surirellales*. In fact, the sequence of *A. coffeaeformis* missed 29 bases at the beginning and 23 bases at the end of the sequence, but

these were relatively conserved regions. Therefore this could not be the reason for the clear separation of this species from the subgenus *Amphora*. The GenBank sequence AY485496 belongs definitely to an *Amphora* species and not an *Achnanthes* species. As mentioned above (see 4.1.2) there must be a contamination or confusion somewhere.

Molecular and morphological data strongly support a separation of the species belonging to the subgenus *Halamphora* from the genus *Amphora*. But further investigations on the subgenus *Halamphora* is needed because the results of the analysis from SSU rDNA sequences indicate that this is still an artificial group.

4.2.3 *Caloneis* and *Pinnularia*

As pointed out in the introduction, the separation of the two genera is controversial because the morphological distinction of *Pinnularia* and *Caloneis* is very problematic. Round *et al.* (1990) and Mann (2001) doubted the correctness of the traditional *Pinnularia-Caloneis* distinction. Based on her investigation on live material, Cox (1988 b) proposed three new groups: (1) *Caloneis silicula, Caloneis bacillum* and *Pinnularia isostauron*; (2) *Caloneis* based on *C. amphisbaena* and (3) *Pinnularia* based on *P. nobilis*. Krammer & Lange-Bertalot (1985) interpreted a different separation, based on the formation of the internal alveoli aperture: (1) all species whose alveoli are internally nearly open, as existing in *Pinnularia interrupta*; (2) species with partially closed alveoli, e.g., *Caloneis amphisbaena* and *Pinnularia gibba*; (3) species with nearly closed alveoli, like *Caloneis silicula*. Nevertheless they preferred the traditional *Pinnularia-Caloneis* distinction.

This study also rejects this traditional distinction of the two genera, because in none of the trees did *Pinnularia* or *Caloneis* form separated monophyletic groups. But in most phylogenetic trees, the two genera were merged into a monophyletic clade. In all trees two groups consisting of several *Pinnularia* species appeared, which could be characterised by different valve morphology.

One group contained *P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron*. *P. interrupta*, whose SSU rDNA sequences was obtained from GenBank, belongs to this group, too. All species in this group have a filiform or slightly lateral raphe system, where the external raphe slit never crosses the internal slit. Midvalve, the striae are radial or parallel and become convergent or parallel at the apices. All species have a large central area, which often form a fascia extending to the margin on one or both sides. Whereas *P. subcapitata, P. microstauron* and *P. interrupta* have two plate-like chloroplasts, *P. obscura, P. anglica* and *P. mesolepta* have a single H-shaped plastid. Although the three

species exhibit a single chloroplast were very close related, the number of plastids could not alone be used as distinctive feature.

The second group consisted of *P. rupestris, P. viridis, P.* cf. *viridiformis* and *P. viridiformis.* All species in this group have a lateral raphe system and in some species the undulate external raphe slit crosses the internal slit several times. The linear or linear-lanceolate axial area enlarges into a slightly expanded central area, which is often asymmetric. But in contrast to the first group, the central area does not form a fascia. The striae are radial or parallel at the centre of the valve and become convergent or parallel at the apices. Under the light microscope the striae were crossed by a lateral line, which is caused by partially closed alveolae. All species have two plate-like chloroplasts.

The position of *P. acrosphaeria* and of the three *Caloneis* species differs in the different phylogenetic trees. Based on the valve morphology, *P. acrosphaeria* is more closely related to the second group. With this group, *P. acrosphaeria* shares partially closed alveoli and the absence of a fascia. But only the ML phylogenies based on LSU rDNA sequences support this relationship. In most trees, *P. acrosphaeria* diverged at the base of the first group. In the MP phylogenies based on the rDNA sequences or the combined data set *P. acrosphaeria* and *C. amphisbaena* form a clade. In the ML phylogenies based on these data, the two species had very long branches in contrast to the other species. Therefore this grouping might be caused by Long Branch attraction. Our data does not clarify the position of *P. acrosphaeria.* The species might belong to the second group, which is supported by the morphology and the ML phylogenies based on LSU rDNA sequences. But it could also diverge at the base of the first group or be the only representative of a sister group.

In most phylogenetic trees, *C. amphisbaena* belongs to the second group. This is also supported by valve morphology because *C. amphisbaena* shares partially closed alveoli and the absence of a fascia with this group. Therefore it is possible that *C. amphisbaena* should be included in this group.

C. budensis shows morphological features of both groups. Like the species belonging to the first group, *C. budensis* has a fascia. But the alveolae are partially closed, which is a typical feature of the species in the second group. *C. lauta* shows the same character combination. In most phylogenies, the two taxa diverge early within the *Pinnularia*/*Caloneis* clade. In the ML tree based on SSU rDNA sequences, the two species form a sister clade to the two other groups. But in most trees they diverge independently, often at the base of one or the other

groups. The results indicate that the two *Caloneis* species belong to an additional group, which might be primary within the *Pinnularia*/*Caloneis* clade.

These molecular results support the groups defined by Krammer & Lange-Bertalot (1985). The first group containing *P. obscura, P. anglica, P. mesolepta, P. subcapitata* and *P. microstauron*. *P. interrupta* is equivalent to their group 1, which includes all species whose alveoli are internally nearly open, as existing in *Pinnularia interrupta*. The second group consisted of *P. rupestris, P. viridis, P.* cf. *viridiformis, P. viridiformis* and *C. amphisbaena* is identical with their group 2, which contains species with partially closed alveoli, e.g., *Caloneis amphisbaena* and *Pinnularia gibba*. *C. budensis* and *C. lauta* represent typical *Caloneis* species, which are the members of their group 3.

4.2.4 *Navicula integra*

N. integra is not a member of *Navicula sensu stricto* and Mereschkowsky (1903) include this species in the genus *Placoneis*. But the species was not yet renamed and Cox (1987) doubted the correctness of this combination because *N. integra* did not have the kind of chloroplast typical for this genus.

In all phylogenetic trees shown above the species is clearly separated from *Navicula sensu stricto* and *Placoneis*. With the exception of the two MP phylogenies based on the *rbc*L sequences *N. integra* formed a monophyletic group with *Craticula* and *Stauroneis*. In most trees, this grouping had strong bootstrap support. The position of *N. integra* within this clade differs. In most trees, the species appears within or at the base of *Stauroneis*, but in the phylogenies inferred with ML analyses using *rbc*L sequences *N. integra* diverged within *Craticula*. *N. integra* shares several morphological features with *Craticula* and *Stauroneis*, such as number, form and position of the chloroplasts, the formation of the raphe and the composition of the girdle. But the morphology of *N. integra* also prohibits its inclusion into one of these genera. Parallel and equidistant striae with longitudinal aligned areolae forming straight lines parallel to the raphe system are typical for the genus *Craticula*. The striae of *N. integra* are radiate and at the centre of the valve more distant with thickened costae separating them. This produces a stauros-like structure. But the species has no stauros, which is the most defining feature of the genus *Stauroneis*. Hustedt (1961-1966) placed *N. integra* in *Navicula* section *Microstigmaticae*. Other species of this section were transferred to the genera *Parlibellus* (Cox, 1988 a) and *Proschkinia* (Karayeva, 1978). Based on the morphological investigations an affiliation of *N. integra* to either of these genera could be refuted. For

instant, *Parlibellus* and *Proschkinia* have a wide girdle region with numerous girdle bands but *N. integra* is wider transapically than pervalvarly.

It must therefore be describes a new genus, for which the name *Prestauroneis* has been chosen.

Prestauroneis Bruder, *gen. nov.*

Type species: *Prestauroneis integra* (W. Smith) Bruder *comb. nov.* (Figs. 44 d-f and 45 c-d)

Basionym: *Pinnularia integra* W.Smith (1856, p. 96).

Synonym: *Navicula integra* (W.Smith) Ralfs (in: Pritchard, 1861, p. 895).

The two plate-like chloroplasts lie one against each side of the girdle. The frustules were isopolar and tend to lie in valve view, because they are wider transapically than pervalvarly. The valves are lanceolate or lanceolate-elliptical with subrostrate apices and an additional undulation in the valve margin before the apices. Pseudosepta are at the apices. The striae are radial at the centre of the valve and become nearly parallel at the apices. They are uniseriate and consist of small round or elliptical poroids, which were occluded by hymenes at their internal aperatures. Midvalve the striae are more distant and the costae separating them are thickened, producing a stauros-like structure.The external central raphe endings are expanded and the well developed terminal fissures at the poles curve off to the same side of the valve. The internal central raphe endings are simple and slightly curved. The girdle composed of several open, porous bands with one or two rows of small round poroids.

4.2.5 *Gomphonema*

Whereas the phylogenetic trees show clearly that the genus *Gomphonema* and the genera *Cymbella*, *Placoneis* and *Encyonema* were near relatives, some relationships within the genus are ambiguous. *G. acuminatum* and *G. truncatum* formed the only constant group in all trees. The position of the other species within this genus differs between the different phylogenies. The position of *G. micropus* changed most in the different phylogenies. With exception of the tree in Fig. 21, the other species were always within a well supported monophyletic clade. *G. micropus* diverged at the base of the genus in most trees. But it was also found in the middle of the genus in the trees based on *rbc*L sequences or separated from the genus by *Cymbella* in Fig. 6. That *G. micropus* belongs to the monophyletic group in most phylogenies and the position of this species in the trees based on *rbc*L sequences support the monophyly of the whole genus. On the other hand, the separation of *G. micropus* and the other *Gomphonema* species by *Cymbella* in one tree indicate a division. The long branches between *G. micropus*

and the rest of the genus in most ML trees and the low bootstrap values for the whole group indicate a separation, too. The morphological investigations of the *Gomphonema* species result in only one feature, which could be found exclusively in *G. micropus*. In this species the external openings of the areolae form small round poroids, whereas they are C- or kidney-shaped in all other *Gomphonema* species. This could be interpreted as a reason to separate the genus in two groups. But it could also be the basic form of the feature, which appeared at the base of the genus and evolved to the characteristic found in the other species.

On the base of these results a separation of the genus could not be proposed. With the exception of *G. micropus* the monophyly of this genus could be clearly shown. To resolve the relationship of *G. micropus* to the other species further investigations with additional species are necessary.

4.2.6 *Placoneis* and *Navicula hambergii*

Although it was already known that *N. hambergii* does not belong to *Navicula sensu stricto* (e.g., Krammer and Lange-Bertalot, 1986), the species has not yet renamed. Only Metzeltin *et al.* (2004, p. 8) noted that "*Navicula hambergii* belongs very probably to *Placoneis*".

The phylogenetic trees generated in the recent study show clearly that *N. hambergii* does belong to the genus *Placoneis* because it diverged at the base of or within the genus in most trees. Altogether the monophyly of *N. hambergii* and *Placoneis* was well supported, although this was not found in all phylogenies. In the four trees inferred with the parsimony analysis based on LSU rDNA and *rbc*L sequences (Figs. 13, 17, 21, 24) the genus *Placoneis* is not monophyletic. In two trees of these trees (Figs. 13, 21) *N. hambergii* and the *Placoneis* species diverge from a polytomy. As discussed above this is the result of the relatively few parsimony informative positions. Mereschkowsky described the genus *Placoneis* in 1903 and used *P. exigua* as a typical species. With this genus he separated a group of species from *Navicula sensu lato*, which have a single, asymmetrical chloroplast. Cox (1987) re-erected the genus and chose *P. gastrum* as type species, because "delineation and nomenclature of *P. exigua* are confused" (Cox, 1987, p. 153). In the same paper and a second investigation (Cox, 2003) she adds several morphological features from SEM investigations to the description of the genus. One of the most important features of the genus *Placoneis* is the single chloroplast with a central bridge and lateral lobes, which lies under the valves. The cells are symmetrical and parallel or elliptical sided in their central region. The striae are radiate near the centre of the valve, becoming more parallel at the apices. They are composed of small round poroids, which were internally closed by volae. The usually straight raphe slits lie in a narrow axial

area. Externally, the central raphe endings are straight and slightly expanded and the polar raphe endings curve to the same side. The internal central raphe endings are usually deflected to the same side and at the internal polar end small helictoglossae are present. All these features were found in *N. hambergii*.

Because of the results of the molecular and morphological analyses a new combination must be made:

Placoneis hambergii (Hustedt) Bruder *comb. nov*. (Fig. 52 h-o)
Basionym: *Navicula hambergii* Hustedt (1924, p 562, pl. 17: fig. 2).

4.2.7 *Cymbella*

The molecular phylogenies showed different relationships within the genus *Cymbella*. In most trees they form a monophyletic clade, but in the phylogenies based on the *rbc*L sequences *C. proxima* is separated. In some trees, *C. naviculiformis* and *C. proxima* form a sub-clade within the monophyletic clade, but in other trees only *C. naviculiformis* is separated from the other species. The morphological investigations show no constant feature which support a separation of *C. proxima*, but *C. naviculiformis* shows obvious differences. This corresponds with Krammer's (1982) subgenera *Cymbella* and *Cymbopleura*. Because of the different results in the molecular phylogenies, which had only relatively low bootstrap supports, this study does neither support nor refuse a separation of the subgenera.

4.2.8 *Navicula brockmannii*

It was already known that *N. brockmannii* was not a member of *Navicula sensu stricto* (e.g., Krammer and Lange-Bertalot, 1986), but the species was not yet renamed.

In all phylogenetic trees shown above, the species is clearly separated from *Navicula sensu stricto*. But *N. brockmannii* does not belong to one of the genera present in the tree because it never diverges within another genus. In most trees the monoraphid genera and the *Cymbellales* were the nearest relatives. The only exceptions were the phylogenies based on LSU rDNA sequences. The morphological investigations show, that *N. brockmannii* does not belong to any of the genera present in this study. But the morphology of this species fits well to the diagnosis of the recently established genus *Adlafia* (Moser *et al.*, 1998). The species in under 25 µm long. The valve has a linear outline and broad rostrate or subcapitate ends. The raphe is filiform with scarcely expanded central pores and the terminal fissures are strongly deflected laterally. The axial area is linear and narrow and slightly widened close to the central area, which is variable in size and form but not widening to the margins. The striae are

dense (25 – 32/10µm) radiate, getting parallel towards the poles, but in contrast to the diagnosis of *Adlafia* the direction does not change abruptly. They run continuously from the valve surface down onto the mantle and consists of rows of round areolae, which where externally closed by hymenes. The girdle bands have a uniseriate or biseriate row of areolae.

Because there is only a minor difference between the morphology of *N. brockmannii* and the diagnosis of the genus *Adlafia,* I transfer *N. brockmannii* to the genus:

Adlafia brockmannii (Hustedt) Bruder *comb. nov*. (Fig. 57)

Basionym: *Navicula brockmannii* Hustedt (1934, p. 382, fig. 11).

4.2.9 Varieties of *Mayamaea atomus*

Although the two varieties formed a strongly supported monophyletic clade in all phylogenetic trees generated in the course of this study, the difference between the sequences of the two varieties is relatively large. This is most obvious in the phylogeny inferred with the ML analysis using the combined dataset (Figs. 26, 27). The two varieties are more distant to each other than for instant the well defined species belonging to *Amphora* subgenus *Amphora*. In the morphological investigations differences size and density of striae and areolae were detected. The smaller *M. atomus* var. *permitis* showed a higher density of striae and areolae. In our cultures these differences were consistent, but Mayama and Kobayasi (1988) found continuity in the size and striation density for their Japanese populations. Based on these results and the absence of any ecological differences they reject a separation of the two types. In contrast to the findings of Mayama and Kobayasi (1988) the comparison of the sequences indicates that *M. atomus* var. *atomus* and *M. atomus* var. *permitis* were not just two varieties of the same species but two different species. Mayama and Kobayasi (1988) did not observe the density of the areolae. Therefore this might be the feature for the differentiation of the two forms. To clarify this problem further molecular and morphological investigations including the Japanese populations are necessary.

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Appendix

Used PAUP command blocks:

Outgroup in all analyses

Outgroup 1493_CYCLOTELLA_CHOCTAWATCHEEA
1254_FRAGILARIA_CROTONENSIS 1256_ASTERIONELLA_FORMOSA;

Maximum Likelihood analyses

set cri=L;
"MODELBLOCK";
Hsearch start=NJ Timelimit=144000;
savetrees format=phylip brlens=yes file=NAME_AIC_ML.trees;

Modelblocks:

- SSU rDNA sequences from AlgaTerra cultures:
  Lset Base=(0.2765 0.1690 0.2453) Nst=6 Rmat=(1.0531 3.1951 1.2328 0.7719 5.6023) Rates=gamma Shape=0.5864 Pinvar=0.4892;
- SSU rDNA sequences from AlgaTerra cultures and GenBank:
  Lset Base=(0.2644 0.1619 0.2453) Nst=6 Rmat=(1.3518 3.4650 1.3715 1.2991 6.4325) Rates=gamma Shape=0.4599 Pinvar=0.3493;
- LSU rDNA sequences from AlgaTerra cultures:
  Lset Base=(0.3020 0.1525 0.2403) Nst=6 Rmat=(1.0000 2.5417 1.0000 1.0000 5.8760) Rates=gamma Shape=0.6160 Pinvar=0.2261;
- LSU rDNA sequences from AlgaTerra cultures and GenBank:
  Lset Base=(0.2833 0.1713 0.2460) Nst=6 Rmat=(0.8410 2.5640 1.2117 0.8021 4.9980) Rates=gamma Shape=0.5576 Pinvar=0.2085;
- *rbc*L gene sequences from AlgaTerra cultures:
  Lset Base=(0.2971 0.1400 0.1490) Nst=6 Rmat=(0.6610 2.7121 1.3598 0.7100 3.8746) Rates=gamma Shape=0.6289 Pinvar=0.5455;
- *rbc*L gene sequences from AlgaTerra cultures and GenBank:
  Lset Base=(0.3128 0.1285 0.1443) Nst=6 Rmat=(0.5876 2.6700 1.1400 0.6432 3.5812) Rates=gamma Shape=0.6738 Pinvar=0.5429;

- combined sequences from AlgaTerra cultures:
  Lset Base=(0.2847 0.1625 0.2262) Nst=6 Rmat=(0.9032 3.0043 1.4657 0.8179 5.2164) Rates=gamma Shape=0.5134 Pinvar=0.4521;

Parsimony analyses

[consensus parsimony tree]

set criterion=parsimony increase=auto;
hsearch addseq=random;
contree/ majrule=yes LE50=yes treefile=NAME_parcon.trees;

[Parsimony bootstrap tree]

set cri=par increase=auto;
bootstrap nreps=1000 search=heu keepall=yes treefile=NAME_PARbootNEW.trees;
savetrees from=1 to=1 savebootp=nodelabels maxdecimals=0
file=NAME_PARbootBaumNEW.trees;

[Parsimony bootstrap tree for SSU rDNA sequences from AlgaTerra cultures and GenBank]

log file=6PAR_BS15.log;
set cri=par increase=auto;
bootstrap nreps=1000 search=heu keepall=yes
treefile=6_keepall_PARBS_Timelimit_15.trees /Timelimit=900 Dstatus=300;
savetrees from=1 to=1 savebootp=nodelabels maxdecimals=0
file=6_keepall_PARBSBaum_Timelimit_15.trees;

Neighbor joining analyses

[NJ bootstrap tree]

set cri=dis;
dset dis=JC;
bootstrap nreps=1000 search=NJ keepall=yes treefile=NAME_NJJCboot.trees;
savetrees from=1 to=1 savebootp=nodelabels maxdecimals=0
file=NAME_NJJCbootBaum.trees;

Partition homogeneity test (combined dataset)

set increase=auto;

log file=4_PHT100.log;

charpartition genes=18s:1-1828, *rbc*L:1829-2513, 28s:2514-3229;

hompart partition=genes nreps=100 seed=123 search=heu;

end;

Weightblock (MacClade output used for the *rbc*L gene dataset from AlgaTerra cultures)

BEGIN CODONS;

GENCODE UNIVNUC;

ENDBLOCK;

BEGIN ASSUMPTIONS;

OPTIONS DEFTYPE=unord PolyTcount=MINSTEPS ;

WTSET = 1.00: 1 5 10 11 16 17 19 20 22 24 26 27 28 29 33 34 35 37 38 40 41 42 43 47 49 50 51 53 55 56 61 62 64 65 66 67 68 70 71 73 74 76 77 78 79 80 81 82 83 85 86 88 89 91 92 94 95 97 98 106 107 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 130 131 132 133 134 136 137 139 140 142 143 147 148 149 150 151 152 154 155 156 157 158 160 161 163 164 166 167 169 170 172 173 174 176 181 182 184 185 187 188 190 191 193 194 195 196 197 198 199 200 202 203 205 206 208 209 214 215 217 218 219 220 221 222 223 224 226 227 229 230 232 233 234 235 236 237 238 239 241 242 244 245 246 247 248 253 254 256 257 259 260 261 262 263 265 266 268 269 271 272 273 274 275 277 278 280 284 285 286 287 288 289 290 291 292 293 294 295 296 298 299 301 302 303 304 305 307 308 310 311 312 313 314 315 316 317 319 320 322 323 325 326 328 329 331 332 334 335 337 338 339 340 341 343 344 346 347 348 349 350 351 352 353 354 355 356 357 358 359 361 362 363 364 368 369 371 373 374 375 377 379 380 381 382 386 388 389 391 392 394 395-398\3 404 406 407 409 410 412 413 414 418 419 420 421 422 424 425 427 428 432 433 434 439 440 445 449 454 455 461 463 469 470 472 478 485 486 491 493 494 496 497 508 509 510 512 514 515 517 518 519 520 521 526 527 529 530 532 533 538 539 541 542 543 550 551 556 557 558 562 563 564 568 569 570 571 572 577 578 582 583 584 585 586 587 588 589 590 595 596 598 599 601 602 604 605 610 611 613 614 616 617 619 620 621 622 623 625 626 628 629 631 632 634 635 637 638 640 641 642 643 644 645 646 647 649 650 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 673 674 675 676 680 682, 2.91: 2 9 48 52 69 281 309 366 372 387-390\3 416 447 488 505 534 544 545, 10.00: 3 7 14 23 25 31 39 44 45 57 63 96 105 135 141 145 159 204 210 243 249-

252\3 258 276 297-300\3 318 365 376 393 401 411 423 442 451 452 453 459 464 468 473 479 511 546 560 566 575 580 592 594 603 607 618 639 652 654 671 683, 1.18: 6 192 267 477 487 492 507, 1.34: 12 72 99 211 399 531 553 597 624-627\3, 1.50: 13 21 75 144 178 216 522 579, 1.02: 15, 2.13: 18 103 108 153 212 270 383 403 441 540 648, 3.70: 30 90 129 175 225 255 408 435 456 457-460\3 475 483 498 523 525 535 565 574 576 591 636 670 677, 1.14: 36 228 480 552, 2.44: 54 162 171 186 201 213 231 342 415 417 429 446 476 482 495 528 548 633, 5.28: 58 60 84 180-183\3 189 250 264 279 306 330-333\3 378 384 430 436 458 465 466 537 554 561 600 678, 1.23: 87 100 240 324 336 345 397 474 573, 1.90: 93 490 549 615 672, 1.60: 102 138 321 327 405 444 547 630 681, 1.28: 165 207 612, 1.05: 168 462 499 501, 1.41: 177 370 396 400 402 438 450 504 516 606 651, 1.08: 282 360 555 567, 1.73: 426 502 513 559 609 684;

ENDBLOCK;

Tab. 9: Number of unknown nucleotides in SSU rDNA sequences obtained from GenBank

GenBank accession number	Species	unknown nucleotides close to	
		primer 1F	primer 1528R
AB085830	*Eunotia formica* var. *smatrana*	66	-
AB085831	*Eunotia monodon* var. *asiatica*	104	-
AB085832	*Eunotia pectinalis*	79	18
AB085833	*Gomphonema pseudaugur*	66	25
AJ243061	*Amphora montana*	6	-
AJ243062	*Gomphonema parvulum*	7	-
AJ243063	*Eolimna minima*	8	-
AJ243064	*Eolimna subminuscula*	6	-
AJ535144	*Rossia* sp.	65	-
AJ535145	*Eunotia* sp.	34	-
AJ535149	*Lyrella* sp.	34	-
AJ544649	*Sellaphora pupula*	22	-
AJ544655	*Sellaphora laevissima*	32	-
AJ544659	*Lyrella atlantica*	34	-
AJ866992	*Achnanthes minutissima*	202	25
AJ866995	*Eunotia bilunaris*	202	25
AJ867023	*Diadesmis gallica*	202	25
AJ867024	*Navicula atomus* var. *permitis*	202	25
AJ867025	*Navicula saprophila*	202	25
AJ867027	*Pinnularia rupestris*	202	25
AJ867028	*Surirella angusta*	202	25
AJ867029	*Surirella brebissoni*	202	25
AJ867030	*Cymatopleura elliptica*	202	25
AY485460	*Navicula* sp.	35	22
AY485462	*Dickieia ulvacea*	35	29
AY485468	*Amphiprora paludosa*	33	54
AY485476	*Achnanthes breviceps*	27	10
AY485482	*Haslea crucigera*	-	59
AY485483	*Navicula sclesviscensis*	-	64
AY485484	*Navicula lanceolata*	35	27
AY485488	*Haslea nipkowii*	-	80
AY485489	*Pleurosigma intermedium*	-	100
AY485496	*Achnanthes* sp.	101	53
AY485497	*Amphiprora alata*	64	61
AY485498	*Amphora coffeaformis*	29	23
AY485500	*Achnanthidium* cf. *longipes*	97	74
AY485502	*Navicula* sp.	-	27
AY485512	*Navicula ramonissima*	35	27
AY485513	*Navicula* sp.	62	58
AY485514	*Pleurosigma planktonicum*	-	13
AY485515	*Pleurosigma* sp.	-	56
AY485516	*Gyrosigma limosum*	72	62
AY485521	*Stauroneis constricta*	139	14
AY485524	*Haslea pseudostrearia*	149	14
AY485528	*Paulielle taeniata*	96	63
AY672802	*Fragilariopsis cylindrus*	-	2
AY821975	uncultured *Eunotia*-like diatom	146	62

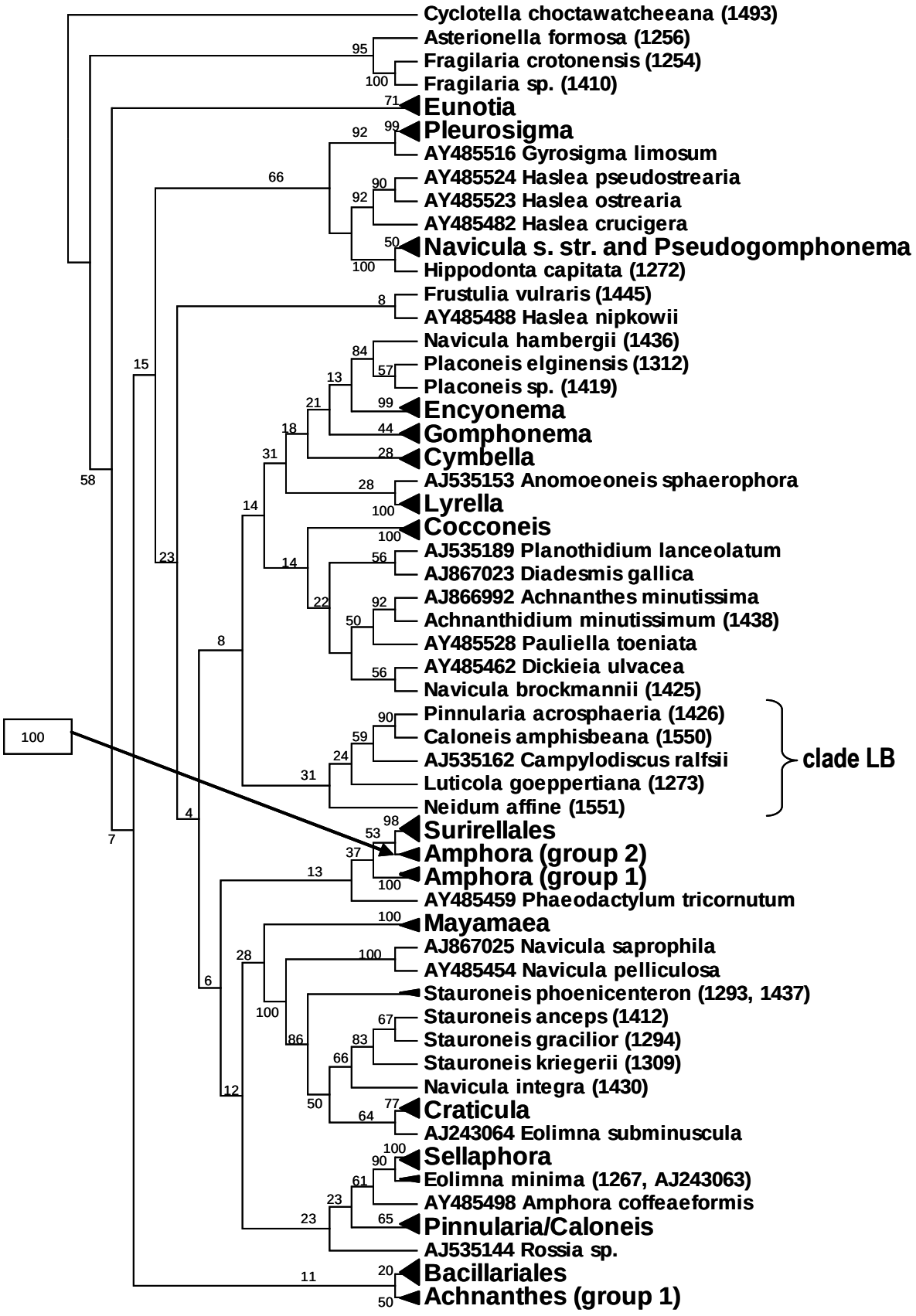

Fig. 59: Neighbor joining tree based on SSU rDNA sequences from GenBank and AlgaTerra cultures. Bootstrap values obtained from 1000 replications based on NJ analyses using JC model have been plotted at the nodes. The marked clade LB is in all probability caused by Long Branch Attraction.

Additional microraphs

The figures 60 – 70 show micrographs of sequenced species, which were not present in the results. The species are shown in alphabetical order.

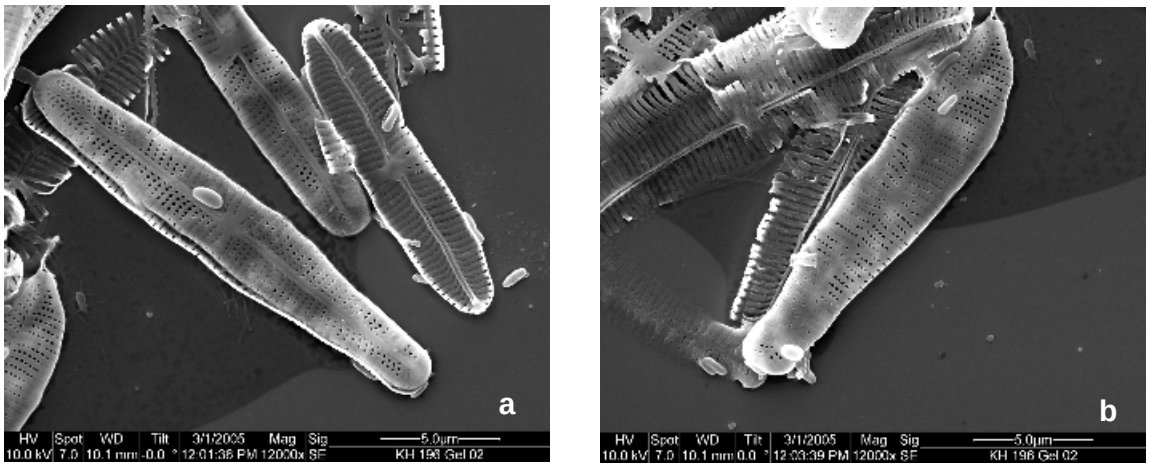

Fig. 60: *Achnanthidium minutissimum*
SEM, raphid (a) and rapheless (b) valve (deformed valves, old culture).

Fig. 61: *Amphora* sp. (1554). Light micrograph of live individual.

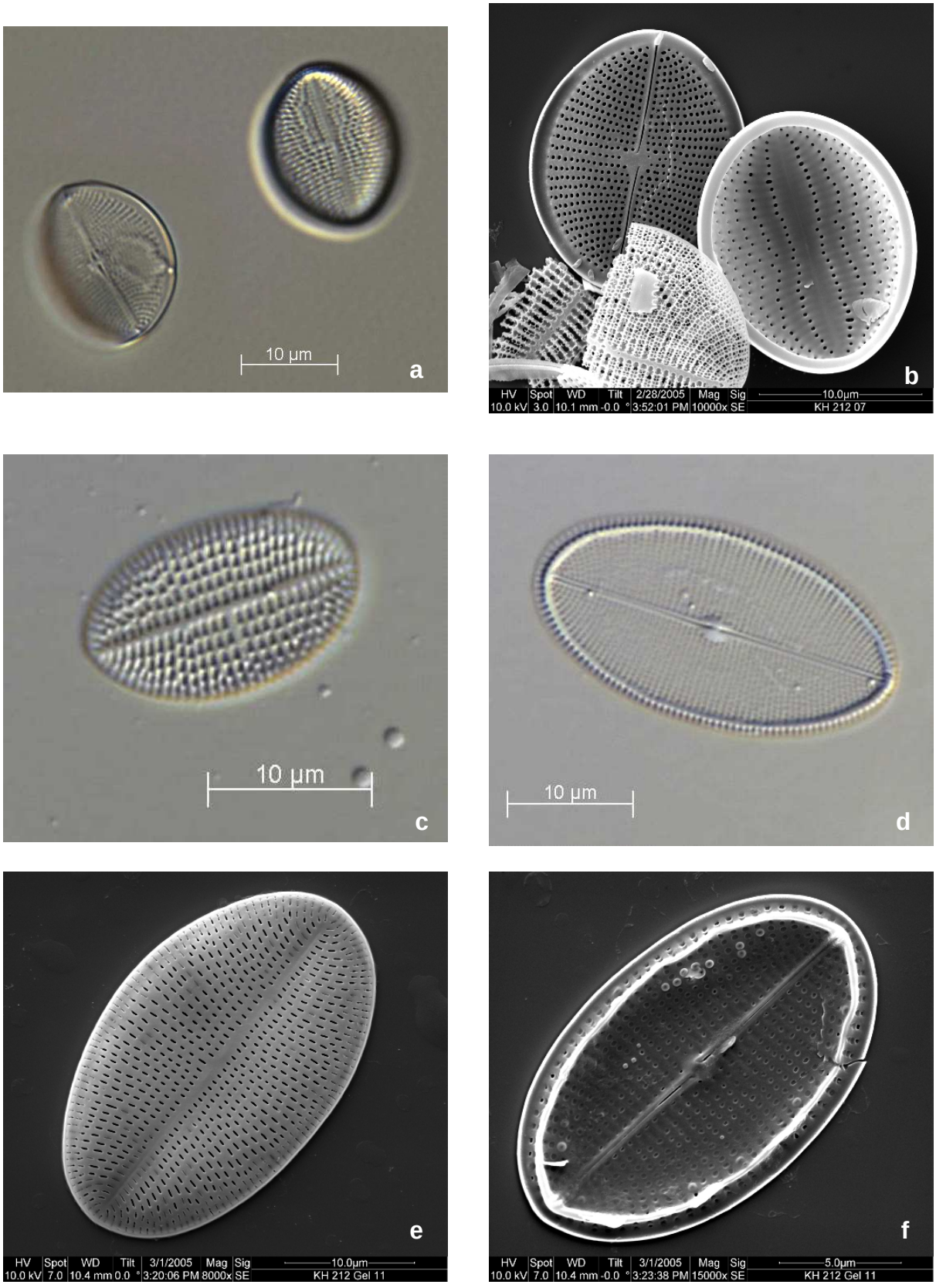

Fig. 62: *Cocconeis* species.
a + b: *C. pediculus.* Light micrograph of cleaned valves (a) and SEM, internal view (b).
c - f: *C. placentula*. Light micrographs of cleaned valves (c + d) and SEM, external view (e + f).

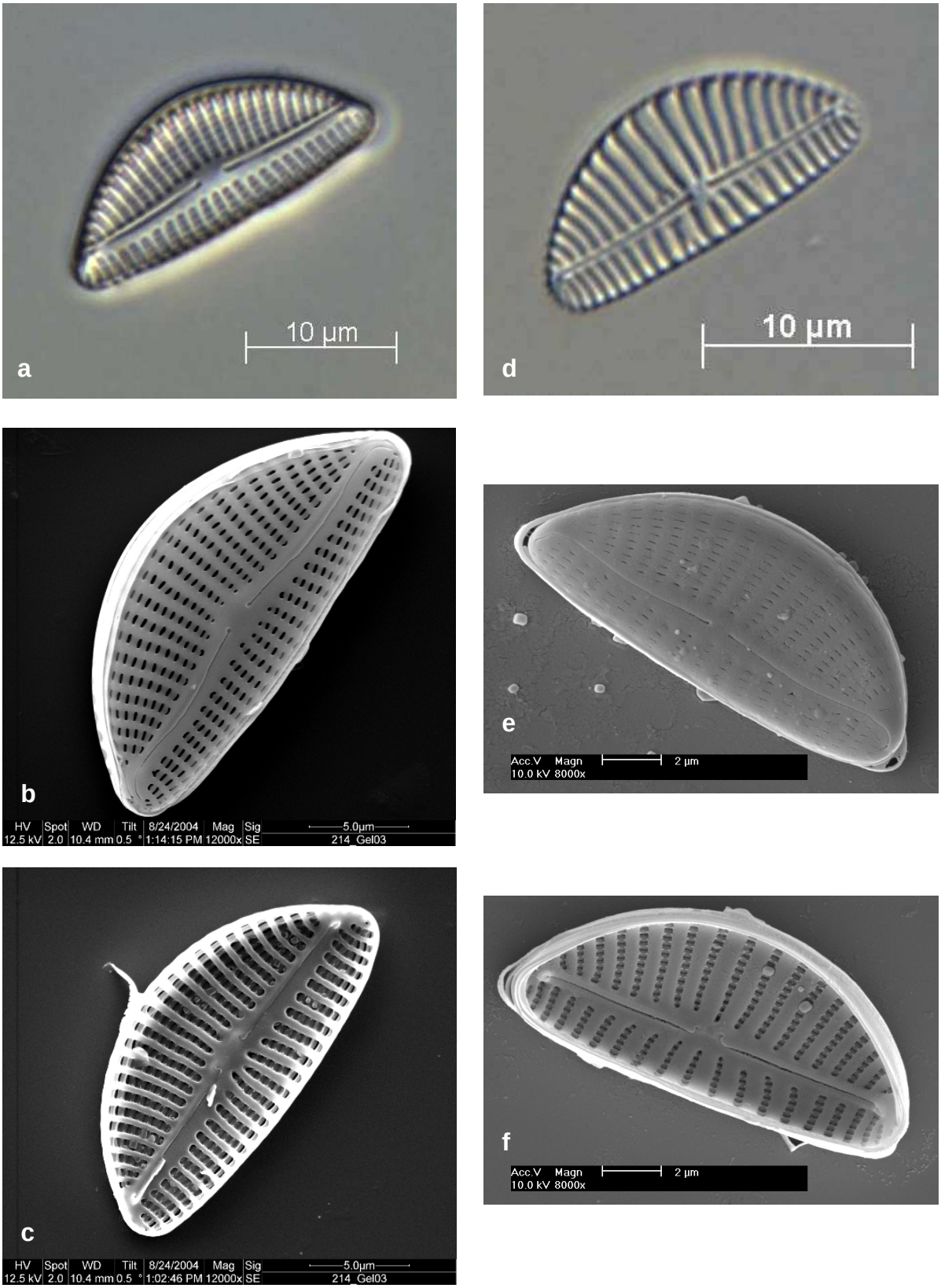

Fig. 63: a – c: *Encyonema caespitosum*, d – f: *E. minutum*
a + d: Light micrographs of cleaned valve, b + e: SEM, external view, c + f: SEM, internal view.

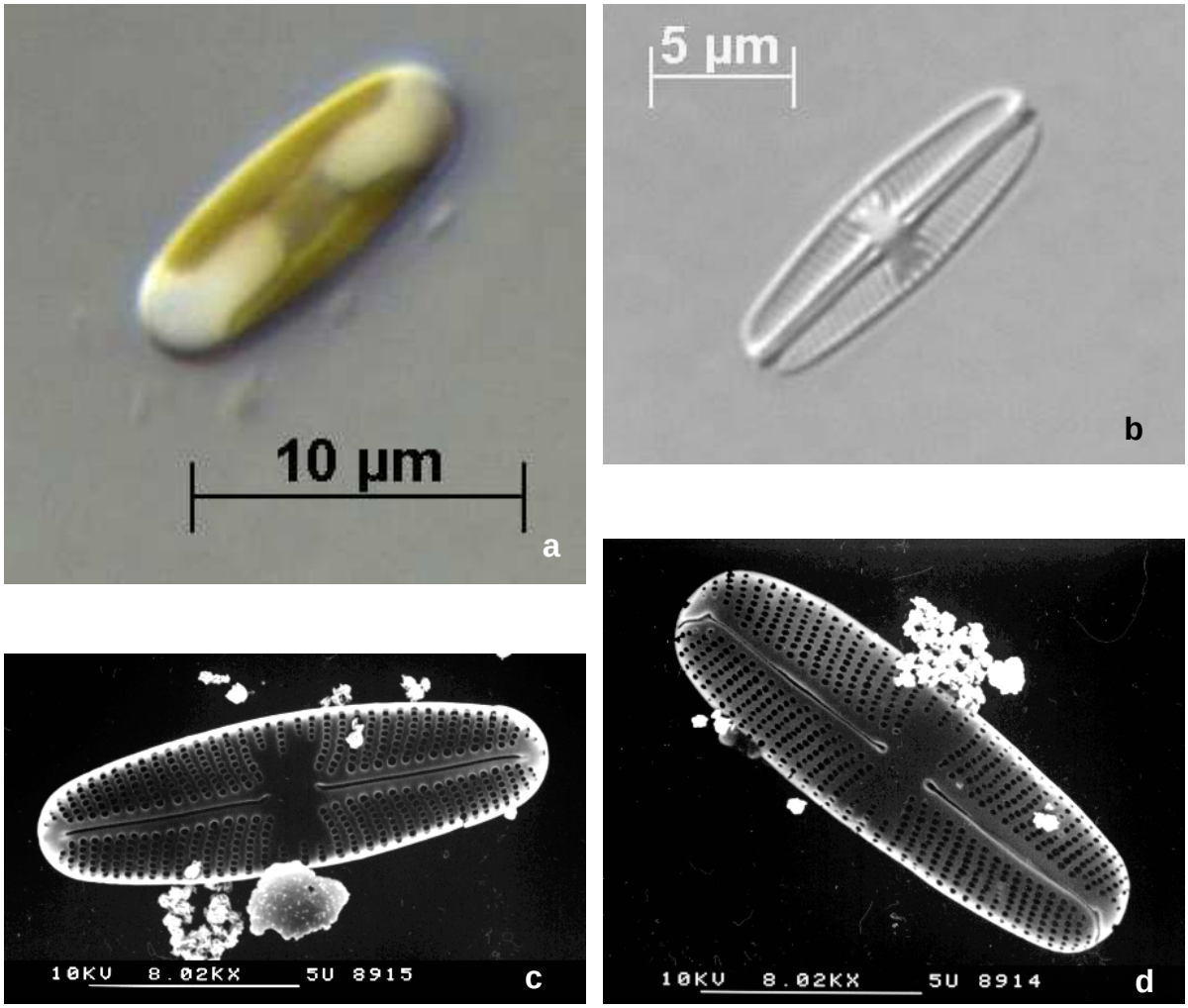

Fig. 64: Eolimna minima

a + b: Light micrographs of live individual (a) and cleaned valve (b), c + d: SEM, external (c) and internal (d) view.

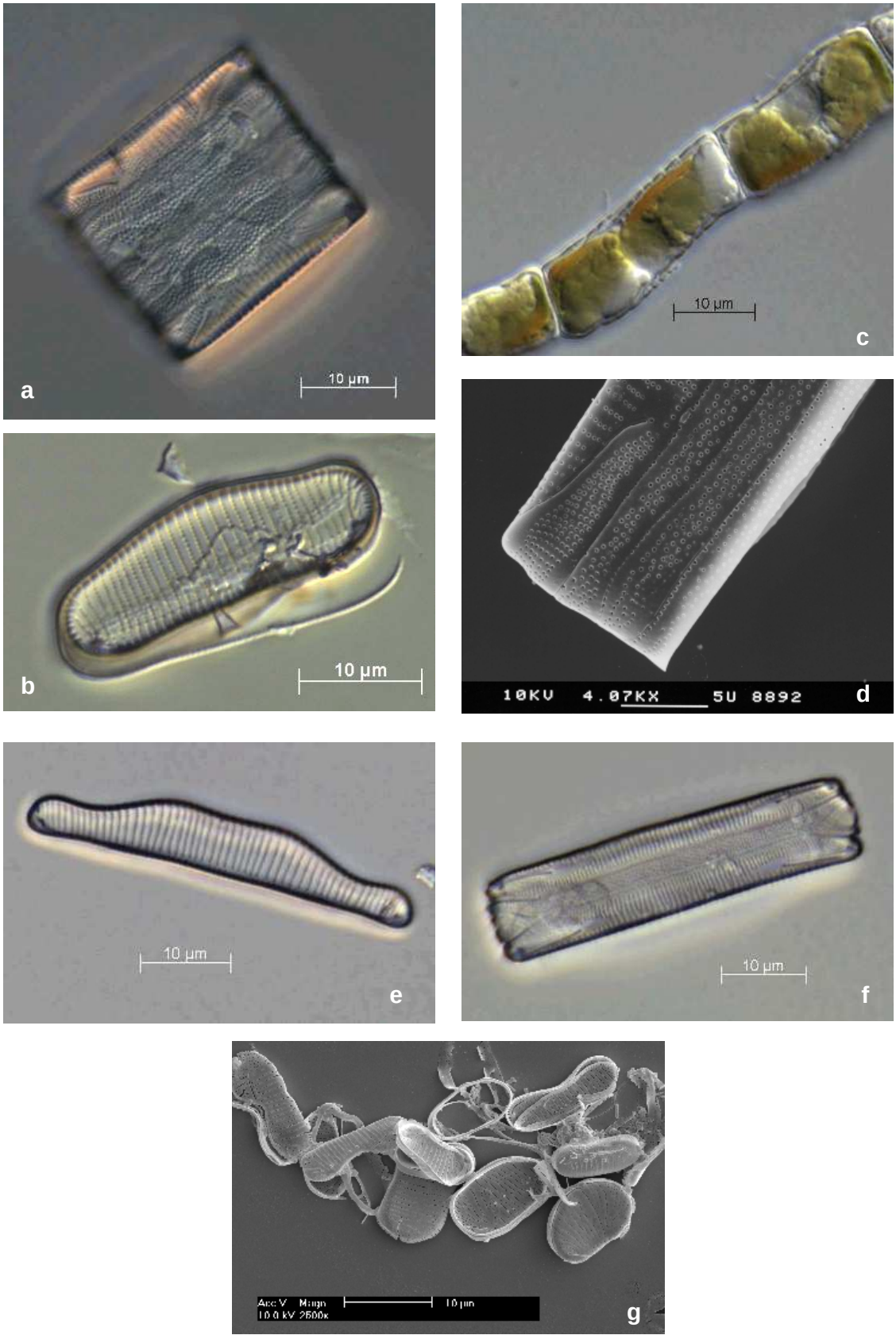

Fig. 65: *Eunotia* species

a – d: *E. formica*. Light micrographs of cleaned valves in girdle (a) and valve view (b) and live individual (c), SEM showing the raphe (d).
e + f: *E. implicata*. Light micrographs of cleaned valves in valve (e) and girdle view (f).
g: *Eunotia* sp. SEM showing several strongly deformed valves.

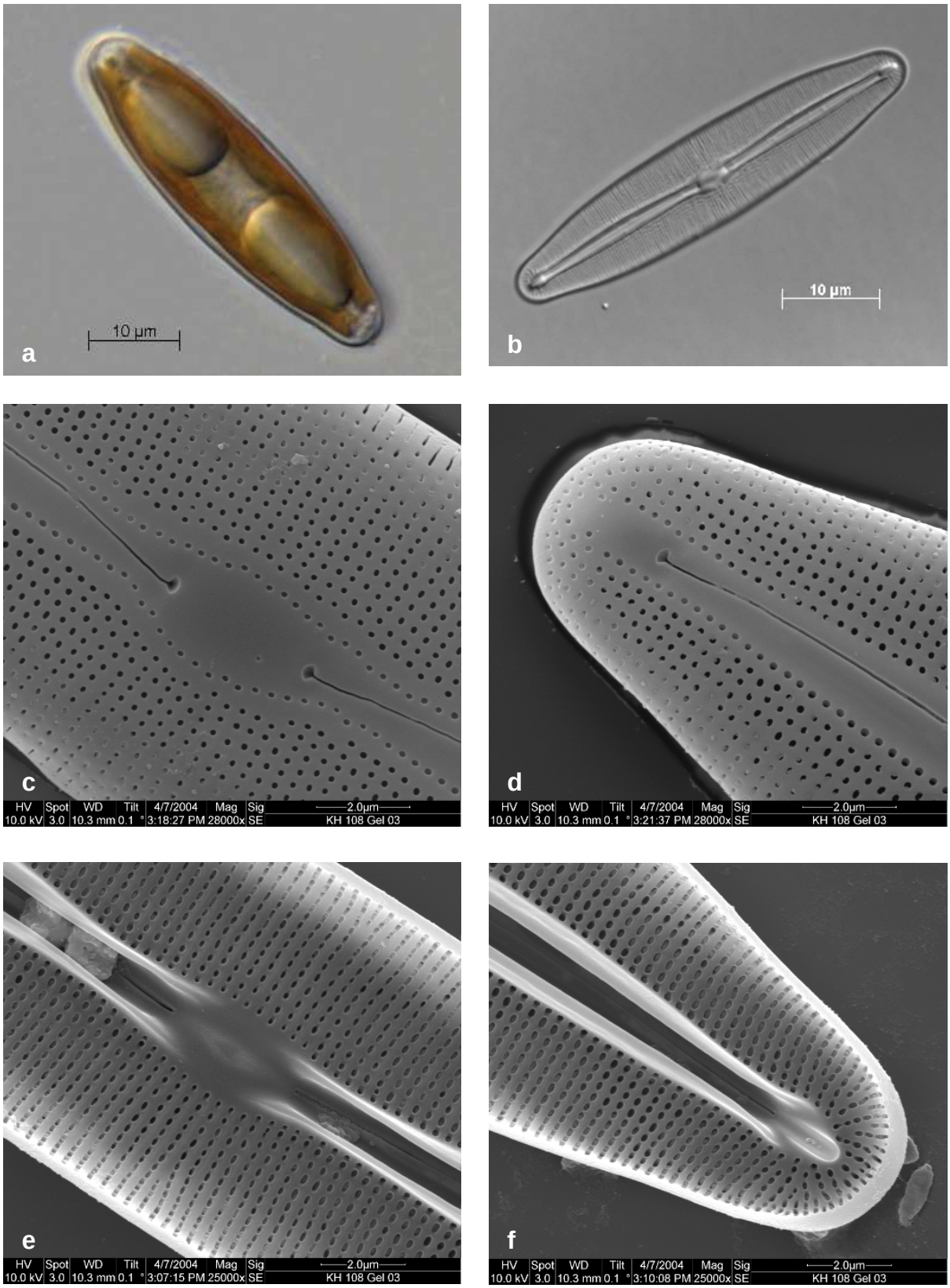

Fig. 66: *Frustulia vulgaris*
a + b: Light micrographs of live individual (a) and cleaned valve (b),
c - f: SEM, external (c + d) and internal (e + f) view.

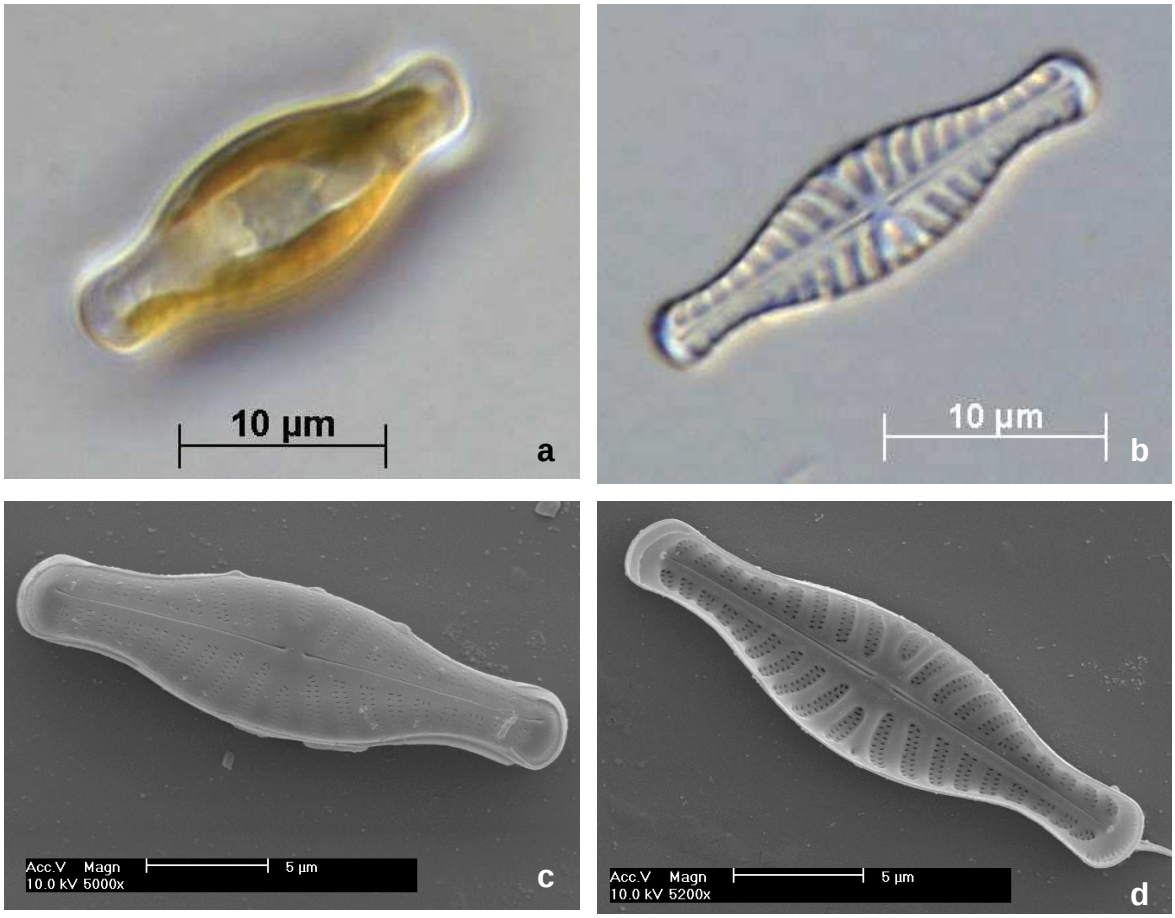

Fig. 67: *Hippodonta capitata*

a + b: Light micrographs of live individual (a) and cleaned valve (b), c + d: SEM, external (c) and internal (d) view.

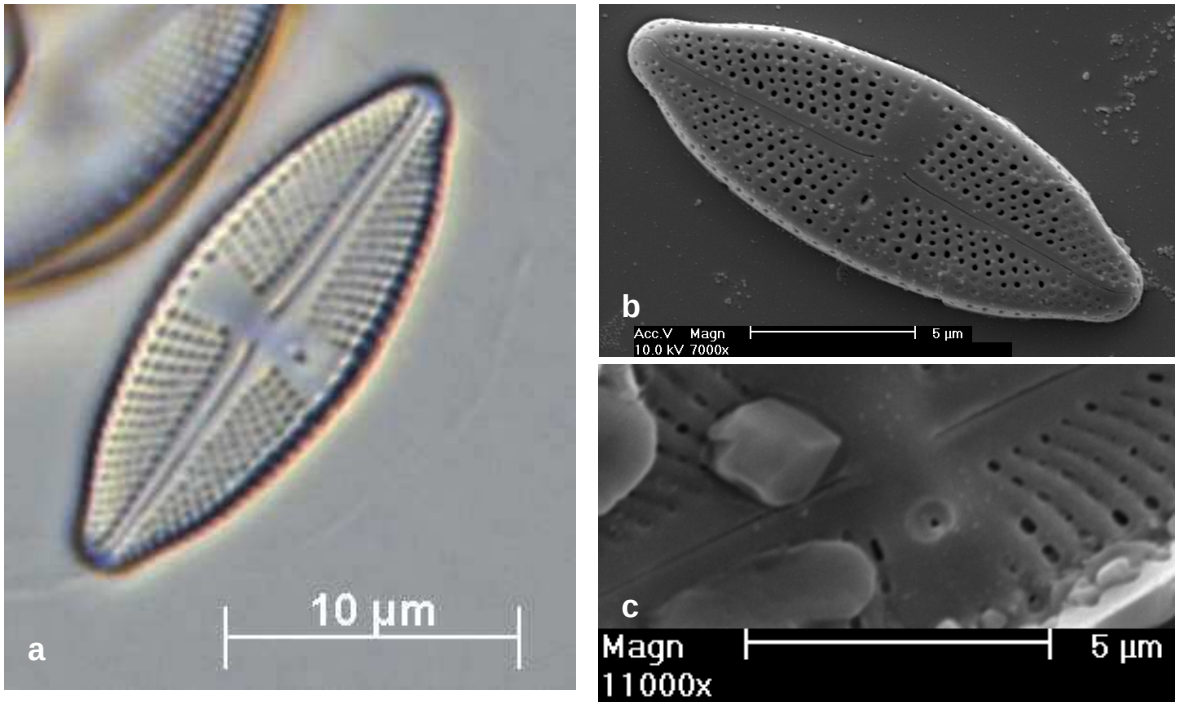

Fig. 68: *Luticola goeppertiana*

a : Light micrograph of cleaned valve, b + c: SEM, external view (b) and detail of the internal stigma aperture (c).

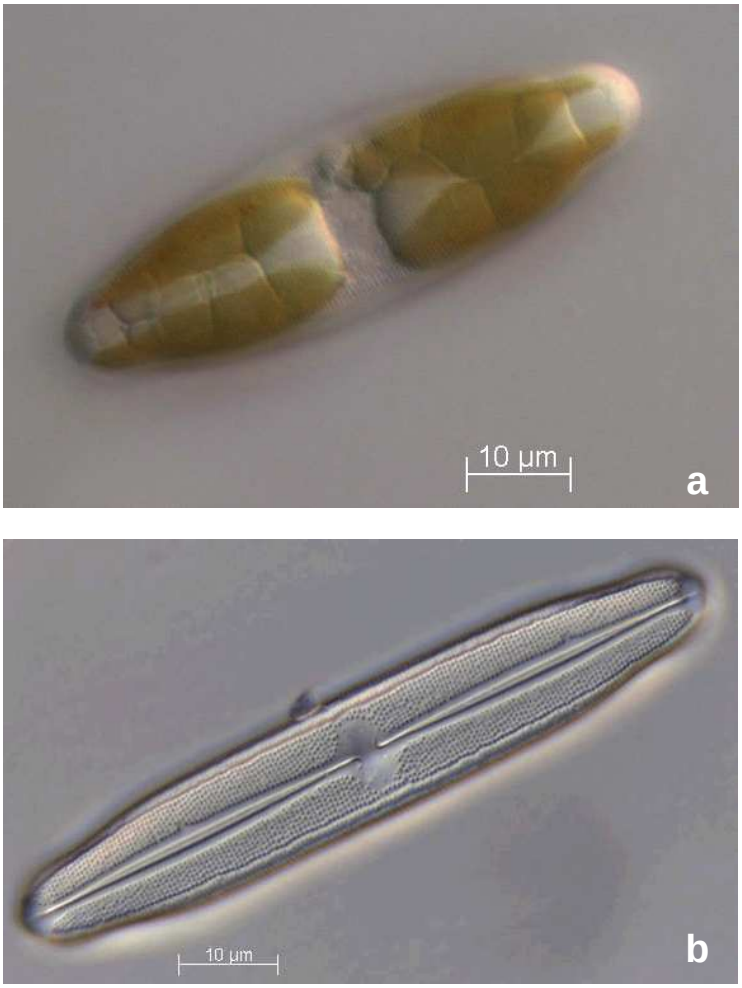

Fig. 69: *Neidum affine*
Light micrographs of live individual (a) and cleaned valve (b).

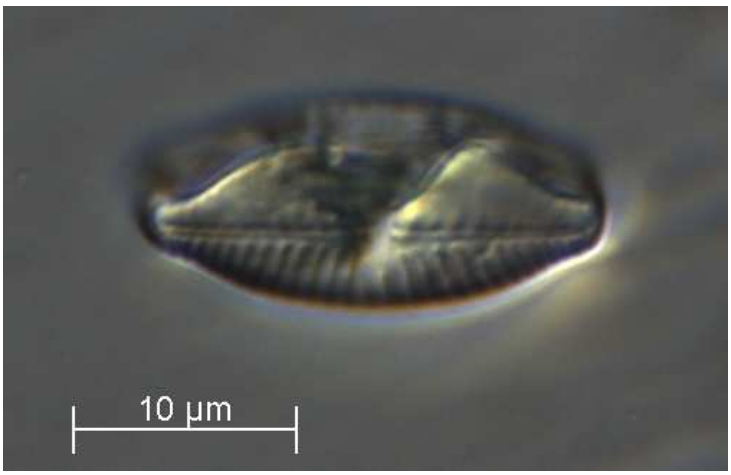

Fig. 70: *Placoneis* sp. (1419)
Light micrograph of cleaned valve.

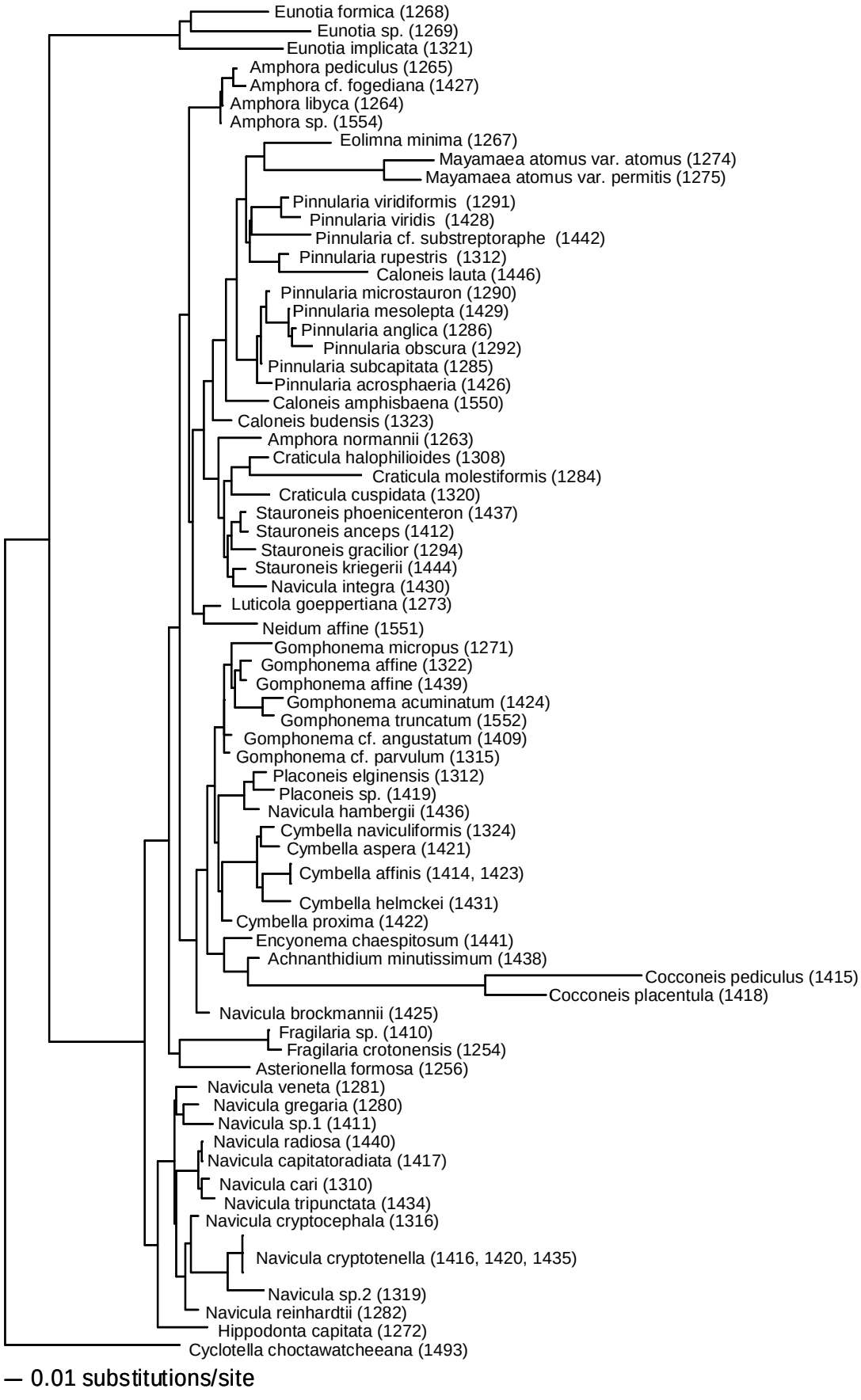

Fig. 71: Phylogeny inferred with the ML analysis using a weight block obtained from MacClade based on *rbc*L sequences from AlgaTerra cultures.

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