Phylogenetic, taxonomic and biogeographical studies in the Pithophoraceae (Cladophorales, Chlorophyta)

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Chapter

7

Molecular phylogeny, taxonomy and niche evolution of the *Aegagropila*-clade (Cladophorales, Chlorophyta), including the description of *Aegagropilopsis* gen. nov. and *Pseudocladophora* gen. nov.

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Abstract

The *Aegagropila*-clade is a unique group of cladophoralean algae occurring in brackish or freshwater environments. The clade is sister to the species-rich, primarily marine *Cladophora*- and *Siphonocladus*-lineages. Phylogenetic analyses of partial LSU and SSU nrDNA sequences reveal four main lineages within the *Aegagropila*-clade, and allow clarification of the taxonomy. The earliest diverging lineage consists of two marine '*Cladophora*' species, for which the name *Pseudocladophora* gen. nov. is proposed. The next lineage occurs essentially in brackish and semi-terrestrial habitats, and consists of all known *Wittrockiella* species and *Cladophorella calcicola*. Two other lineages are restricted to freshwater. One of them shows a strong tendency for epizoophytism, and consists of *Basicladia* species and *Arnoldiella conchophila*. The other one includes *Aegagropila linnaei*, the genus *Pithophora* and a small number of tropical *Cladophora* species. These *Cladophora* species are transferred to *Aegagropilopsis* gen. nov. The family name Pithophoraceae is proposed for the *Aegagropila*-clade. Previously, polypyramidal pyrenoids had been suggested to be a character for this lineage, but we show the presence of both polypyramidal and bilenticular pyrenoids in members of the Pithophoraceae. The Pithophoracae show interesting patterns of niche evolution, and generally occur in habitats characterized by unstable environmental conditions where competition is low. The heterotrichous habit of *Wittrockiella* and *Arnoldiella*/*Basicladia* might be an adaptation to these harsh conditions.

Introduction

The Cladophorales (Ulvophyceae) is a species-rich order of green algae with a siphonocladous organisation that is widespread from tropical to polar regions. The classification of the group is highly confused, and the Cladophorales are notorious for taxonomic difficulties at all levels from species to orders. In recent years, molecular data have contributed greatly to develop a better understanding of the evolution of this group. Three main clades have been discovered in molecular phylogenies of the Cladophorales (Fig. 1): the *Siphonocladus*-clade and the *Cladophora*-clade are species-rich, predominantly marine lineages that have a sister relationship (Leliaert *et al.* 2003), and the *Aegagropila*-clade, which consists mainly of freshwater species and is sister to the *Cladophora*- and *Siphonocladus*-clades (Hanyuda *et al.* 2002, Yoshii *et al.* 2004). Members of the *Siphonocladus*-clade (=Siphonocladales s.s.) are distributed mainly in the tropics and are comprised of forms with highly specialized thallus architecture such as pseudoparenchymatic clusters, blades and three-dimensional networks. Members of the *Cladophora*-clade (=Cladophorales s.s or Cladophoraceae sensu Wille) extend their distribution into cold temperate and (ant)arctic waters and consist of branched or unbranched filaments. The *Aegagropila*-clade has not yet received a formal taxonomic rank despite robustness in previous phylogenetic studies (Hanyuda *et al.* 2002, Yoshii *et al.* 2004). Hanyuda *et al.* (2002) suggested that the presence of loraxanthin, chitin and polypyramidal pyrenoids are diagnostic characters of the *Aegagropila*-clade. A fourth lineage, Okellyaceae, is sister to the other three clades (Fig. 1) and includes *Okellya curvata* (Printz) Leliaert & Rueness, an unbranched, marine microfilamentous species occurring in temperate subtidal habitats (Leliaert *et al.* 2009a).

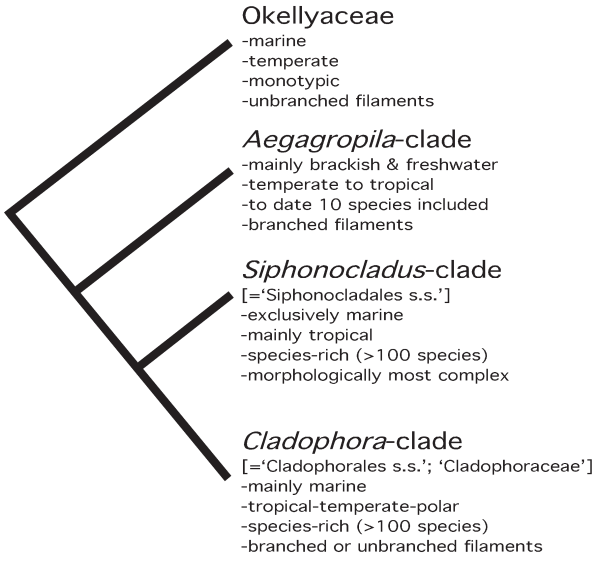

Figure 1. Schematic phylogenetic tree of the Cladophorales with gross information for the four main lineages on habitat, species numbers and morphology.

The *Aegagropila*-clade encompasses six monotypic or species-poor genera and a small number of freshwater *Cladophora* species (Hanyuda *et al.* 2002, Yoshii *et al.* 2004, Rindi *et al.* 2006). Although molecular evidence clearly indicates the polyphyletic nature of the genus *Cladophora*, no nomenclatural changes have been proposed yet based on recent molecular data, except the recent transfer of *C. kosterae* van den Hoek and *C. okamurae* (S. Ueda) van den Hoek to the genus *Basicladia* (Garbary 2010) and the transfer of *Cladophora amphibia* to *Wittrockiella* (Boedeker & Hansen 2010). Species of

Cladophora are distributed in the three main clades of the Cladophorales (Fig. 1). The lectotype species of *Cladophora* is *C. oligoclona* (Kützing) Kützing (Setchell & Gardener 1920, van den Hoek 1963), a synonym of *C. rivularis* (L.) Hoek, which is a member of the *Cladophora*-clade (Leliaert & Boedeker 2007). Thus, taxonomic changes with regards to the *Cladophora* species in the *Aegagropila*-clade and the *Siphonocladus*-clade are required. *Cladophora* species (including earlier synonyms) that are part of the *Aegagropila*-clade, as well as candidate *Cladophora* species for the *Aegagropila*-clade (based on morphological or ecological data) that were shown to have other affiliations by molecular data, are listed in Table 1.

Both from an evolutionary and an ecological perspective this group is very interesting, since it constitutes a freshwater/brackish lineage within the predominantly marine Cladophorales, and most taxa occupy highly specialized niches. Its members occur in narrow niches such as on the carapaces of freshwater turtles (some members of *Basicladia* Hoffmann & Tilden), on freshwater snails and bivalves (monotypic *Arnoldiella* Miller and several *Basicladia* species), on and endophytically in saltmarsh plants and mangrove pneumatophores (some members of *Wittrockiella* Wille), or on marine intertidal snails (*Cladophora conchopheria* Sakai). Recently, an aerophytic unicellular organism occurring on tree bark has been described and included in this lineage based on DNA sequence data (monotypic *Spongiochrysis hawaiiensis*, Rindi *et al.* 2006).

The majority of the species in the *Aegagropila-clade* occurs in freshwater environments, the genus *Wittrockiella* occurs in brackish habitats, and *Cladophora horii* van den Hoek (Fig. 2A) and *C. conchopheria* (Fig. 2B) are the only marine species. *Wittrockiella* currently encompasses three species, *W. lyallii* (Harvey) van den Hoek, Ducker & Womersley (Fig. 2C), *W. salina* V.J. Chapman (Fig. 2D) and *W. amphibia* Boedeker & Hansen (Fig. 2F). A close relationship between *W. salina* and the (sub)tropical, semi-terrestrial species *Cladophorella calcicola* Fritsch (Fig. 2E) has been speculated by van den Hoek *et al.* (1984), but no molecular data are available to date. The genus *Basicladia* consists of seven species, mainly occurring on freshwater turtles. Culture experiments have shown that other substrates can be colonized as well (Proctor 1958), and the recently included species *B. okamurae* (S. Ueda) Garbary (Figs. 2G & H) and *B. kosterae* (van de Hoek) Garbary (Figs. 2G & I) are only sporadically encountered on turtles. The diminutive and poorly known *Arnoldiella conchophila* Miller has been found on the shells of freshwater bivalves (Miller 1928), freshwater gastropods (Kargupta 1994, Keshri & Hazra 2009) and a range of other substrates (Cox Downing 1970). More than 35 taxa of *Pithophora* Wittrock, a genus easily recognized by the characteristic akinetes (Figs. 2K & L), have been described, but numbers are most likely inflated due to plastic morphological characters (Ernst 1908, Mothes 1930, Fott 1971, Pankow & Täuscher 1980). This mainly (sub)tropical freshwater genus only occurs unattached in stagnant, nutrient-rich waters and can form extensive floating masses that can be local nuisances (Entwisle & Price 1992, Lembi 2003). The freshwater species *Aegagropila linnaei* Kützing (Fig. 2P) is currently regarded as the sole member of its genus (van den Hoek 1963, Hanyuda *et al.* 2002), but a number of potentially closely related species has been identified (Boedeker *et al.* 2010b). *Aegagropila linnaei* is the best known representative of the lineage and gained considerable scientific, cultural (in Japan) and economic (aquarium trade) fame due to the peculiar lake balls formed under specific conditions (Kurogi 1980, Niiyama 1989, Boedeker *et al.* 2010a).

Table 1. List of (former) *Cladophora* species that are members of the *Aegagropila*-clade, and candidate members that were shown by molecular data to have a different systematic affiliation.

former *Cladophora* species that are members of the *Aegagropila*-clade			
Cladophora species	*Cladophora* section/subgenus	current name	reference for placement in *Aegagropila*-clade
Cladophora horii C. Hoek & Chihara 2000	section *Rugulosae*[a]	still the same	Leliaert *et al.* 2003
Cladophora conchopheria Sakai 1964	section *Glomeratae*[a]/section *Opacae*[b]	still the same	Hanyuda *et al.* 2002
Cladophora kosterae C. Hoek 1963	section *Basicladia*[c]	*Basicladia kosterae* (C. Hoek) Garbary 2010	Yoshii *et al.* 2004
Cladophora okamurae S. Ueda1932	section *Basicladia*[c]	*Basicladia okamurae* (S. Ueda) Garbary 2010	Hanyuda *et al.* 2002 (as *Chaetomorpha okamurae*)
Cladophora aegagropila (L.) Rabenhorst 1868	section *Aegagropila*[c]/subgenus *Aegagropila*[b]	*Aegagropila linnaei* Kützing 1843	Hanyuda *et al.* 2002
Cladophora sp. ('Tateyama-Marimo')	/	still the same (undescribed species)	Hanyuda *et al.* 2002, Yoshii *et al.* 2004
Cladophora lyallii Harvey	/	*Wittrockiella lyallii* (Harvey) C. Hoek, Ducker & Womersley 1984	Hanyuda *et al.* 2002
Cladophora amphibia Collins 1907	/	*Wittrockiella amphibia* (Collins) Boedeker & Hansen 2010	Yoshii *et al.* 2004 (as *W. paradoxa*)
Cladophora oedogonia (Montagne) Montagne 1856	/	*Pithophora oedogonia* (Montagne) Wittrock 1877[d]	Hanyuda *et al.* 2002 (for the genus *Pithophora*)
Cladophora roettleri (Roth) Kützing 1849	/	*Pithophora roettleri* (Roth) Wittrock 1877[d]	Hanyuda *et al.* 2002 (for the genus *Pithophora*)
candidate members of the *Aegagropila*-clade that were shown by molecular data to have different affiliations			
Cladophora species	*Cladophora* section/subgenus	systematic placement	reference
Cladophora catenata (L.) Kützing 1843	section *Aegagropila*[e]/subgenus *Aegagropila*[b, f]	*Siphonocladus*-clade	Hanyuda *et al.* 2002, Leliaert *et al.* 2007a
Cladophora echinus (Biasoloetto) Kützing 1849	section *Aegagropila*[c]	Longi-Articulatae clade (*Cladophora*-clade)	Leliaert *et al.* 2009c
Cladophora patentiramea (Montagne) Kützing[g]	subgenus *Aegagropila*[b]	(*Siphonocladus*-clade)[g]	(Hanyuda *et al.* 2002, Leliaert *et al.* 2007)
Cladophora sibogae Reinbold	subgenus *Aegagropila*[b]	*Siphonocladus*-clade	Leliaert *et al.* 2003, Leliaert *et al.* 2007
Cladophora socialis Kützing	subgenus *Aegagropila*[b]	*Siphonocladus*-clade	Hanyuda *et al.* 2002, Leliaert *et al.* 2007
Cladophora battersii C. Hoek 1963	section *Rupestres*[c]	*Cladophora*-clade	Leliaert *et al.* 2009c
Cladophora pygmaea Reinke 1888	section *Chamaethamnion*[c]	*Cladophora*-clade	Leliaert *et al.* 2009c
classic freshwater *Cladophora* species[h]	sections *Glomeratae* & *Cladophora*[c]	*Cladophora*-clade	Marks & Cummings 1996, Hanyuda *et al.* 2002

[a]van den Hoek & Chihara 2000, [b]Sakai 1964, [c]van den Hoek 1963, [d]Pankow & Täuscher (1980) reduced *Pithophora* to two species, however disagreement over the number of species exists. Originally, Wittrock (1877) had also transferred *Cladophora acrosperma* Kützing, *C. sumatrana* van Martens and *C. zelleri* van Martens to *Pithophora* in addition to the two species listed here, [e]van den Hoek 1982, [f]as *C. fuliginosa* Kützing, [g]probably a synonym of *C. coelothrix* Kützing (see van den Hoek & Chihara 2000), [h]*C. fracta* (Müller ex Vahl) Kützing, *C. globulina* (Kützing) Kützing, *C. glomerata* (L.) Kützing, *C. rivularis* (L.) C. Hoek.

Figure 2. Morphological variety in the *Aegagropila*-clade. **A**: *Cladophora horii* (D78). Scalebar=200 µm. **B**: *Cladophora conchopheria* (N71), filaments on shell of the marine gastropd *Lunella coronata*. Scalebar=100 µm. **C**: *Wittrockiella lyalli* (H67), heterotichous filaments with secondary rhizoids. Scalebar=1 mm. **D**: *Wittrockiella salina* (B92), heterotichous filaments in culture. Scalebar=100 µm. **E**: *Cladophorella calcicola* (K92), clump of heterotrichous filaments in culture. Scalebar=200 µm. **F**: *Wittrockiella amphibia* (N73), heterotrichous filaments with densely pigmented upright shoots. Scalebar=200 µm. **G**: *Cladophora kosterae* (J79, branched plant on left) and *Cladophora okamurae* (J78, unbranched filaments on right), growing together on dead wood. Scalebar=2 mm. **H**: *Cladophora okamurae* (J78), rhizoidal stratum inside surface layer of wood substrate giving rise to upright shoots. Scalebar=200 µm. **I**: *Cladophora kosterae* (J56), filaments on shell of the freshwater bivalve *Anodonta anatina*. Scalebar=1 mm. **J**: *Basicladia ramulosa* (J85), unbranched basal parts of filaments with very long cells, growing on carapax of the freshwater turtle *Chelodina longicollis*. Scalebar=200 µm. **K**: *Pithophora oedogonia* (K01), filaments with intercalary and terminal akinetes. Scalebar=200 µm. **L**: *Pithophor*a cf. *roettleri* (K93), two filaments consisting mainly of germinating akinetes diving rise to new branches. Scalebar=200 µm. **M**: *Pithophora* cf. *polymorpha* (K97), sterile filament without akinetes showing branches being subterminally inserted and delayed cell wall formation. Scalebar=200 µm. **N**: *Cladophora sterrocladia* (G91), filaments with unbranched basal parts and opposite branches. Scalebar=2 mm. **O**: *Cladophora clavuligera* (L70), filament showing serial insertion of branches. Scalebar=100 µm. **P**: *Aegagropila linnaei* (C01), filaments showing subterimal insertion of branches. Scalebar=200 µm.

Previous molecular phylogenetic studies, based on SSU rDNA sequence data, only partly resolved the relationships within the *Aeagropila*-clade and suffered from low taxon sampling (Hanyuda *et al.* 2002, Yoshii *et al.* 2004). These studies showed that

Cladophora conchopheria and *C. horii* form a sister-clade to the rest of the *Aegagropila*-clade, that *Wittrockiella lyallii* and *W. amphibia* (as *W. paradoxa* Wille) group together, that *C. kosterae* is allied to a species of *Basicladia*, and that *Aegagropila* and *Pithophora* have a close relationship. The relation of *Arnoldiella conchophila* and *C. okamurae* (as *Chaetomorpha okamurae*) to the other taxa was not resolved. The aerophytic unicellular alga *Spongiochrysis hawaiiensis* was recovered on a basal polytomy with *C. conchopheria* (Rindi *et al.* 2006).

In order to gain a better understanding of the evolution of morphology and specialized habitat preferences in the *Aegagropila*-clade, and to re-assess the taxonomy of the group, the phylogenetic relationships need to be resolved. This study extends the previous phylogenies by increasing taxon sampling and by combining SSU and LSU sequence data, which has been shown to lead to better resolved phylogenies in the Cladophorales (Leliaert *et al.* 2007a). The validity of pyrenoid ultrastructure as a diagnostic character for the lineage was tested with a wide range of taxa.

Materials and Methods

Taxon sampling and morphological analysis

Thirty specimens from the *Aegagropila*-clade were sampled in various habitats from a broad geographical range (Tables 2 & 4). The type species of the following of the currently recognized genera in the *Aegagropila*-clade have been included in the phylogenetic analyses: *Aegagropila* (*A. linnaei*), *Arnoldiella* (*A. conchophila*), *Cladophorella* (*C. calcicola*), and *Wittrockiella* (*W. amphibia*). Wittrock (1877) did not designate a type species for *Pithophora*, the sole genus included in Pithophoraceae at its inception, and we have found no prior lectotypication. We here select *P. kewensis* Wittrock as the type species (isotypes in BM (incl. K) & L, we choose the specimen from L (no. 938112 639) as the lectotype) for the genus *Pithophora* and the family Pithophoraceae (=*Aegagropila*-clade, see discussion), because it is the species that first attracted his attention, as Wittrock (1877) himself acknowledged in the introduction to his monograph, and it is the most thoroughly described and illustrated of the eight that make up his account of the genus. Furthermore, the type specimen of *P. kewensis* is fertile and displays the typical terminal and intercalary akinetes of the genus *Pithophora*, unlike the types of several other *Pithophora* species. *Basicladia crassa* Hoffmann & Tilden is the type species of the genus (Hoffmann & Tilden 1930), but no sequence data are available for this species. However, *B. crassa* is morphologically similar to *B. chelonum*, with intermediate forms frequently encountered (Proctor 1958), and both species are often found growing together. The recognition of *B. chelonum* and *B. crassa* as distinct species has been questioned (Proctor 1958, Garbary *et al.* 2007). It is therefore likely that *B. crassa* is closely related to *B. chelonum* and the other species of *Basicladia* included in this study. We excluded *Spongiochrysis hawaiiensis* (which has been proposed to be a member of the *Aegagropila*-clade by Rindi *et al.* 2006) from our analyses due to data conflict. We obtained partial LSU and partial SSU rDNA sequences of living material of *S. hawaiiensis* from the type locality. Our SSU sequence is identical to the ones published by Rindi *et al.* (2006) (GenBank accession nos. DQ077805, -806), and groups on a long, separate branch with the marine species *Cladophora horii* and *C. conchopheria* in our phylogenetic trees. However, our LSU sequence groups with members of the Trentepohliales in phylogenetic trees. For confirmation we re-extracted,

amplified and sequenced the partial LSU rDNA three times, always with the same result. In conclusion, the placement of *S. hawaiiensis* in the Cladophorales could not unequivocally be established, and further study of this organism is clearly necessary. We also did not include the published sequences of the *Basicladia* cultures UTEX LB810 and LB811 (University of Texas Culture Collection of Algae; GenBank accession nos. AB078726, -727; published in Yoshii *et al.* 2004), since our own SSU rDNA sequences of the same cultures differed by 10-20 bp. *Cladophora clavuligera* Grunow and *C. sterrocladia* Skuja were also included based on preliminary sequence data that indicated an affiliation with the *Aegagropila*-clade.

Fresh algal material was preserved in silica gel for DNA extraction, and vouchers were prepared from the same sample as herbarium sheets or preserved in a 5% formalin solution and deposited in L (herbarium abbreviations follow Holmgren *et al.* 1990). Specimens, either fresh, formalin-preserved or reconstituted from herbarium material, were examined with a Olympus SZX10 stereo-microscope and a Olympus BH2 light microscope (Olympus Optical Co. GmbH, Hamburg, Germany), and images were taken with a connected digital camera (ColorView Illu, Olympus Soft Imaging Systems, Münster, Germany). Polypyramidal pyrenoids had been proposed to be a diagnostic character for the *Aegagropila*-clade (Hanyuda *et al.* 2002), so we examined the ultrastructure of the pyrenoids of a range of members of this lineage by TEM. Morphological and ultrastructural investigations were performed on specimens also included in the molecular study (Table 2) or that had identical nucleotide sequences (Tables 2 & 4). For TEM, living cells from cultured material were fixed with 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), rinsed in buffer, postfixed with 1% OsO_4 in buffer, stained by dehydration in a gradual series of ethanol containing 1% uranylacetate, and finally embedded in Epon. Sections were cut with a diamond knife on a LKB-3 ultratome, mounted on grids and post-stained with 3% uranyl acetate and lead citrate. Preparations were viewed and photographed in a JEAL 1010 electron microscope operated at 60 kV.

Molecular phylogenetic analyses

The specimens used in the phylogenetic analyses are listed in Table 2 (members of the *Aegagropila*-clade) and Appendix S5 (other Cladophorales and outgroups, see Supplementary Materials). A total of 43 specimens were analysed (22 specimens of the *Aegagropila*-clade, eight taxa of the *Cladophora*-clade, ten taxa of the *Siphonocladus*-clade, three outgroup taxa). Molecular phylogenetic analyses were based on nuclear-encoded small subunit (SSU) and partial large subunit (LSU) rDNA sequences. DNA extraction, PCR amplification and sequencing were performed as in Boedeker & Immers (2009), modified for the SSU as follows: the complete SSU rRNA gene was amplified using the primer pairs SR1-SS11H and SSU897-18SC2 (Leliaert *et al.* 2007a). Obtained sequences have been deposited in GenBank (see Table 2 for accession numbers). Taxa for which new sequences were generated are listed in Table 2 (plus *Trentepohlia* sp. in Appendix S5). If multiple identical sequences were available for a species, only two sequences from specimens from different locations were included in the analysis (see Table 2). Sequences were aligned using MUSCLE (Edgar 2004) and subsequently edited by eye in Se-Al v2.0a11 (Rambaut 2007). Two short variable regions of the LSU alignment (33-38 bp in total, depending on outgroups) were ambiguous and excluded from all analyses. Evolutionary models of nucleotide substitution were determined by the Akaike Information Criterion for all

Table 2. Specimens of members of the *Aegagropila*-clade used in this study with collection data (voucher information, indicated in bold). n.i. = no information.

species	voucher[a], culture	no.
Aegagropila linnaei Kützing	L0793580	B54
Aegagropila linnaei	L0793577	C01
Aegagropila linnaei	L0793543	N36
Cladophora sp. 'Tateyama'		
Cladophora clavuligera Grunow	L0793298, L0793299	L70
Cladophora sterrocladia Skuja	L0793287	G91
Pithophora oedogonia (Montagne) Wittrock	L0793288	K01
Pithophora cf. *roettleri* (Roth) Wittrock[b]	L0793289, ACOI997	K93
Wittrockiella amphibia (Collins) Boedeker & Hansen	/ , CCMP1674	N60
Wittrockiella amphibia	L0793284	N73
Wittrockiella salina Chapman	L0793300, L0793301	B92
Wittrockiella lyallii (Harvey) van den Hoek, Ducker & Womersley	WELT A023866/n.i.	LSU: H67
Wittrockiella lyallii	SGO No. 158361	N61
Cladophorella calcicola Fritsch	L0793292, ACOI471	K92
Basicladia kosterae (C. Hoek) Garbary	L0793302, L0793303	J56
Basicladia kosterae	L0793294, CCAP505.6, UTEX LB1485	K06
Basicladia kosterae	L0793295, CCAP505.11	K09
Basicladia kosterae	L0793293	J79
Basicladia cf. *chelonum*[c]	L0793296, UTEX LB811	K98
Basicladia ramulosa Ducker	L0793304, L0793305	J85
Arnoldiella conchophila Miller		
Basicladia okamurae (S. Ueda) Garbary[d]	L0793306, FACHB795	L84
Basicladia okamurae	L0793310-312	J78
Cladophora horii van den Hoek & Chihara	HEC10983/n.i.	LSU: F53
Cladophora horii	L0793316-318	D78
Cladophora conchopheria Sakai	L0793297, UTEX LB2870/n.i.	LSU:K99
Cladophora conchopheria	L0793313, L0793314	N71
Spongiochrysis hawaiiensis Rindi *et al.*[e]	L0793315, GALW015489	LSU: G89

[a] Index Herbariorum: Holmgren *et al.,* 1990

[b] obtained as *P. oedogonia* (Montagne) Wittrock

[c] obtained as *Basicladia* sp.

[d] obtained as *Basicladia chelonum* (Collins) Hoffmann & Tilden

[e] not included in the phylogenetic trees due to data conflict

*not included in the phylogenetic trees, identical sequence to conspecific specimens above

location, collector, date of collection) and GenBank accession numbers (sequences generated in this study are

location	collectors	GenBank accession nos.	
		LSU	SSU
Lake Myvatn, Iceland	Á. Einarsson	EU655697	**FR719925**
cf. Lake Svityaz, Ukraine	(online aquarium shop)	EU655698	**FR719926**
Loch Watten, Caithness, Scotland	C. Scanlan	*	
Tateyama, Toyama, Japan	I. Wakana	/	AB062711
tropical aquarium	A. Immers	**FR719939**	**FR719927**
botanical garden, Leiden, Netherlands	C. Boedeker	**FR719940**	**FR719928**
botanical garden, Leiden, Netherlands	C. Boedeker	**FR719941**	**FR719929**
rice field, Montemor-o-Velho, Portugal	A. F. Frias	**FR719942**	**FR719930**
Lopez Island, Washington, USA	C. O'Kelly	GU384874	AB078732
Yaquina Bay, Oregon, USA	G.I. Hansen	GU384873	GU384872
Jawbone Reserve, Victoria, Australia	J. West	**FR719943**	**FR719931**
Bradshaw/Milford Sound, Fiordland, New Zealand	S. Heesch/T. Hanyuda	FN252712	AB062717
Dring Island, Aysén, Chile	M.E. Ramírez, D.M. John	GU198503	GU198502
Lagoa de Óbidos, Portugal	O. Lourenço	**FR719944**	**FR719932**
Kager Plassen, Leiden, Netherlands	C. Boedeker	**FR719945**	**FR719933**
botanical garden, Paris, France	C. van den Hoek	**FR719946**	AB078730
unknown	unknown	*	
canal, Meije/Bodegraven, Netherlands	C. Boedeker	*	
Missouri, USA	V. Proctor	**FR719947**	**FR719934**
Annie creek, Kimberley region, Australia	N. FitzSimmons	**FR719948**	**FR719935**
Lake Panke, Japan	S. Arai	/	AB062712
Wuhan, China	M. Chen	**FR719949**	**FR719936**
canal, Meije/Bodegraven, Netherlands	C. Boedeker	**FR719950**	**FR719937**
KwaZulu-Natal, South Africa/Ishigaki, Okinawa, Japan	E. Coppejans/S. Arai	AJ544728	AB078731
Trafalgar, KwaZulu Natal, South Africa	C. Boedeker	*	
Shimoda/Shirasaki, Ishikawa. Japan	C. v. d. Hoek/T. Hanyuda	**FR719951**	AB062705
Oishi, Sumoto, Awaji-shima, Japan	S. G. A. Draisma	*	
Waimanolo, O'ahu, Hawaiian Islands	A. Sherwood	not included	not included

alignments in PAUP* 4.0b10 /Modeltest v3.7 (Posada & Crandall 1998, Swofford 2002), or in PAUP/MrModeltest 2 v2.3 (Nylander 2004) for the subsequent use of MrBayes (see below). Uncorrected pairwise distances for the members of the *Aegagropila*-clade were calculated in PAUP.

First, the LSU (37 sequences plus *Okellya curvata* as outgroup) and SSU (39 sequences plus *Okellya curvata* as outgroup) datasets were analysed separately.
The I_{SS} statistic, a measure of substitution saturation in molecular phylogenetic datasets, was calculated with DAMBE (Xia & Xie 2001) for the SSU and partial LSU data separately. Congruence between the two genes was tested by conducting the incongruence length difference (ILD) test implemented in PAUP (Farris *et al.* 1995) under parsimony with 100 replicates, and indicated that the SSU and the partial LSU rDNA data were not significantly heterogeneous (P=0.57). The two genes were then combined into concatenated alignments, which were subsequently used for all analyses.

Three different concatenated LSU-SSU alignments were created (named 'test', 'outgroup' and 'ingroup', see Table 3). Initial phylogenetic analyses of the 'test' dataset were performed to evaluate the effect of removal of taxa with partial sequence data, and the effect of model selection on tree topology and branch support (Appendix S6). The 'outgroup' alignment contained two distant outgroups (*Ulva fasciata* and *Trentepohlia* sp.), *Okellya curvata*, and all ingroup sequences except two with missing LSU data (40 sequences in total). This alignment was created to establish whether the *Cladophora*-clade, the *Siphonocladus*-clade and the *Aegagropila*-clade represent monophyletic groups, and to infer the position of the root of the ingroup (=Cladophorales excluding Okellyaceae). Because distant outgroups can influence the inferred relationships of the ingroup taxa (Bergsten 2005), the 'ingroup' alignment was assembled which consisted of all 39 ingroup sequences. In addition to rooting with the three outgroup-taxa, the position of the root was also determined for the 'ingroup' dataset by midpoint rooting of the BI and ML trees and by molecular clock rooting (Appendix S7).

The datasets were analysed with maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). MP analyses were performed with PAUP and consisted of a full heuristic search with 1000 random sequence addition replicates and Tree Bisection Reconnection (TBR) branch swapping. Gaps were treated as missing data. Branches with zero length were collapsed. Robustness of the MP trees was tested by non-parametric bootstrapping (Felsenstein 1985) with 1000 pseudoreplicates. ML analyses were performed using PHYML v3.0 (Guindon & Gascuel 2003), starting with five random neighbour-joining trees and using SPR & NNI tree topology search and 1000 non-parametric bootstrap replicates. BI was performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Analyses consisted of two independent, simultaneous runs of one cold and three incrementally heated chains, and 3×10^6 generations with sampling every 100 generations. Posterior probabilities were calculated using a Metropolis-coupled Markov chain Monte Carlo approach. The average standard deviation of the spilt frequencies of the two parallel runs approached zero in all analyses (0.0005-0.0065, depending on alignment), indicating that the tree samples became increasingly similar and that a stationary distribution was reached. The log-files of the runs were checked with Tracer v1.4.1 (Rambaut & Drummond 2007) and a burn-in sample of 4000 trees was removed before calculating the majority rule consensus trees in MrBayes.

Table 4. Voucher and collection information of eight *Pithophora* specimens with identical LSU or SSU sequences. 'ID' indicates our own re-identification result, differing from the original species designation of the culture collections; Ø represents diameter; n.d. = no data.

specimen designation (GenBank accession numbers in brackets)	**voucher**	**no.**	**location**	**collector**	**identical sequences**		**morphological information**
					LSU	**SSU**	
Pithophora oedogonia (Mont.) Wittrock (LSU: FR719941, SSU: FR719929)	L0793288	K01	hothouse, botanical garden, Leiden, Netherlands	C. Boedeker	**x**	**x**	Main axis 40-75µm Ø; branches 38-50µm Ø, opposite or solitary; terminal akinetes 70-100µm Ø, pointy; intercalary akinetes 75-110µm Ø, cask-shaped.
Pithophora oedogonia (**ID**: *P.* cf. *roettleri*) (LSU: FR719942, SSU: FR719929)	L0793289, ACOI997[a]	K93	rice field, Montemor-o-Velho, Portugal	A.F. Frias	**x**	**x**	Main axis 60-100µm Ø; many short branches, 60-70-(110)µm Ø, sometimes opposite, mainly solitary; terminal akinetes ca. 100µm Ø, pointy; intercalary akinetes 100µm Ø, cask-shaped; many helicoid cells.
Pithophora roettleri (Roth) Wittrock	L0793290, CCALA408[a]	K96	thermal spa, Piestany, Slovakia	F. Hindák	**x**	n.d.	Main axis 50-65µm Ø; long branches, 50-60 µm Ø, solitary, widely spaced; terminal akinetes 70-100µm Ø, cylindrical-pointy; intercalary akinetes 125-140µm Ø, cask-shaped/ trapezoid, in chains.
Pithophora sp. (**ID**: *P.* cf. *polymorpha)*	L0793291, UTEX LB787[a]	K97	Brooklyn, Indiana, USA	C.J. O'Kelly	**x**[b]	n.d.	Main axis 38-70µm Ø, irregular outline; many short branches, 30-40µm Ø, opposite or solitary; no terminal akinetes; intercalary akinetes 125µm Ø, rare.
Pithophora sp. 'Kosrae'	missing	G99	Kosrae, Micronesia	G. Zuccarello	**x**	n.d.	sterile material (no akinetes)
Pithophora sp. 'Kamigori' (AB062713)	n.d.		Kamigori, Japan	S. Arai	n.d.	**x**	n.d.
Pithophora sp. 'Sano' (AB066646)	n.d.		Sano, Japan	S. Arai	n.d.	**x**	n.d.
Pithophora sp. 'Singu' (AB066647)	n.d.		Singu, Japan	S. Arai	n.d.	**x**	n.d.

[a]culture collection number
[b]this LSU sequence differs in three positions from the others

Table 3. Specification of datasets, summary of models and model parameters obtained.

Alignment name	LSU	SSU
Genes	partial LSU	SSU
Taxa	38	40
Ingroup taxa	37	39
Outgroup taxa	1 (*Okellya curvata*)	1 (*Okellya curvata*)
Alignment length/analysed length	643/610	1690/1690
Variable sites/parsimony informative sites	248/212	285/198
Model estimated[a]	GTR+I+G	GTR+I+G
Estimated base frequencies (A/C/G/T)	0.21/0.25/0.32/0.22	0.26/0.22/0.27/0.25
Estimated substitution frequencies (AC/AG/AT/CG/CT/GT)[a]	0.75/3.18/1.43/0.90/6.14/1	1.36/3.04/2.63/0.87/7.30/1
Among-site variation I/G[b]	0.34/0.58	0.67/0.46

[a]estimated by Akaike Information Criterion (AIC)

[b]proportion of invariable sites (I) and gamma distribution shape parameter (G) as estimated in PAUP/MrModeltest

Results

Datasets and alignments

Details of the different alignments including number of in- and outgroup taxa, alignment length and number of variable sites, as well as estimated parameters of nucleotide substitution are given in Table 3. For all datasets, a general time reversible model of evolution with a proportion of invariable sites and gamma shape (GTR+I+G) was determined. The SSU sequences are nearly three times as long as the partial LSU sequences but they contain comparable amounts of variable and parsimony-informative sites. Additional identical LSU sequences that were excluded from the phylogenetic analysis are listed in Tables 2 and 4. No significant saturation was detected in either the SSU or the partial LSU data, based on the I_{SS} statistic (Xia & Xie 2001).

'test'	'outgroup'	'ingroup'
partial LSU & SSU	partial LSU & SSU	partial LSU & SSU
40	40	39
39	37	39
1 (*Okellya curvata*)	3 (*Ulva fastigia*, *Trentepohlia* sp., *Okellya curvata*)	none
2333/2300	2358/2336	2333/2300
533/410	738/541	484/406
GTR+I+G	GTR+I+G	GTR+I+G
0.24/0.23/0.29/0.24	0.24/0.23/0.29/0.24	0.24/0.23/0.29/0.24
1.08/3.17/1.94/1.04/6.47/1	1.15/3.17/1.83/0.97/6.42/1	1.07/3.34/2.05/1.07/6.92/1
0.58/0.46	0.42/0.37	0.65/0.63

In the trees resulting from separate BI analyses of the SSU and the partial LSU datasets, the relationships among and within genera were largely unresolved (not shown). Topological differences between the SSU and the LSU tree were not supported. The combined SSU-LSU analyses were found to perform better in terms of resolution and support values than the separate SSU/LSU analyses. Analysing the 'test' alignment with a simple model of nucleotide substitution such as HKY (and employing phylogenetic methods of inference not utilising evolutionary models such as MP) resulted in slightly different tree topologies and support values compared to using the complex GTR+I+G model (Appendix S6). Excluding the three taxa with missing sequence data had no effect on tree topology or support values.

Outgroup tree

Three ingroup clades were recovered, representing the *Cladophora*-clade, the *Siphonocladus*-clade and the *Aegagropila*-clade (Fig. 3), which together with the monotypic

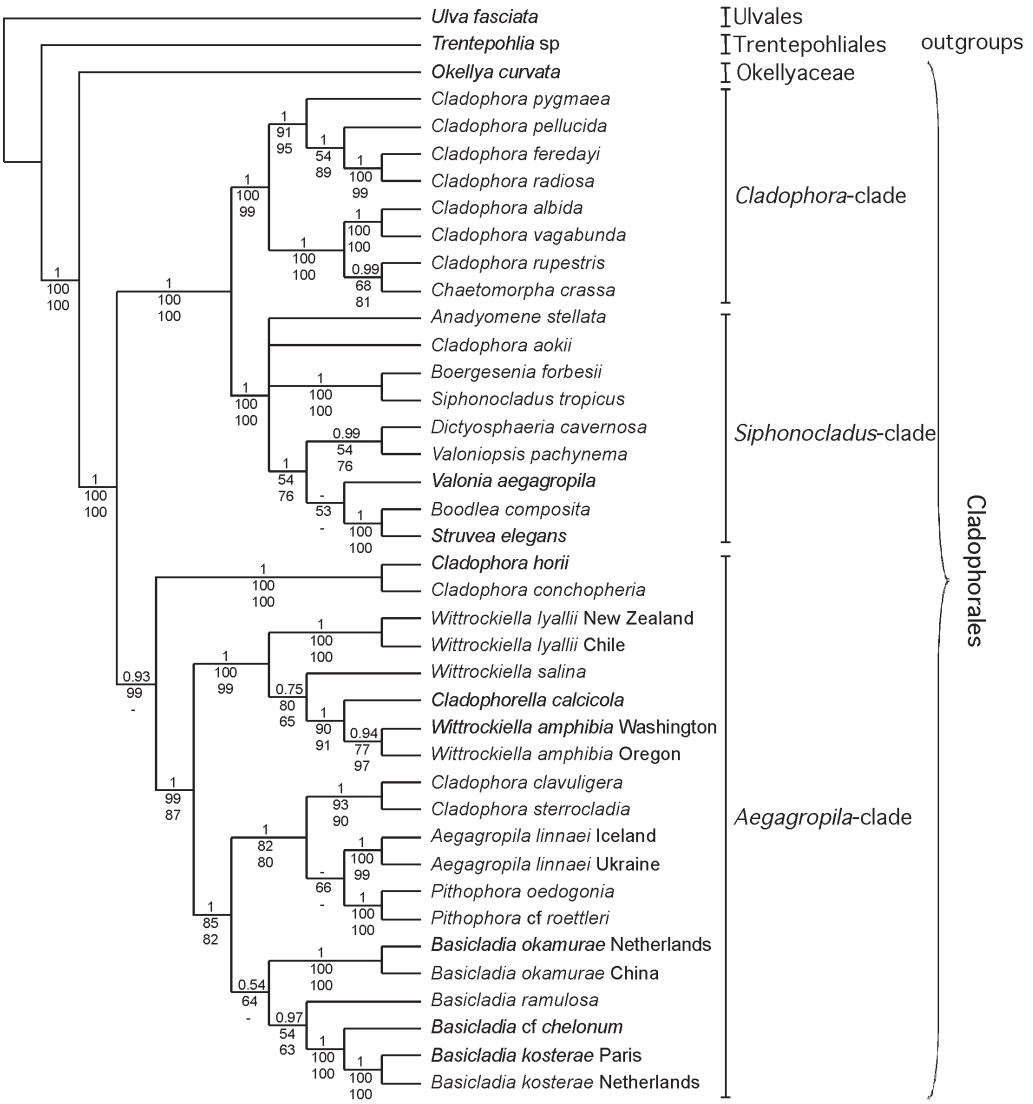

Figure 3. Maximum parsimony (MP) bootstrap consensus tree inferred from concatenated partial large and complete small subunit rDNA sequences with gaps treated as missing data showing the four groups within Cladophorales, rooted with two distant ulvophytes ('outgroup' dataset). Bayesian inference posterior probabilities are indicated above branches, MP and maximum likelihood bootstrap values (1000 replicates) are shown below branches.

Okellyaceae represent all currently known lineages of the Cladophorales. MP, ML and BI yielded similar tree topologies, but differed in levels of support on many branches. The *Aegagropila*-clade received high support in the MP analysis, but only moderate support in the BI analysis (posterior probability 0.93), and no support in the ML analysis. The root of the ingroup (Cladophorales excluding Okellyaceae) was placed on the branch separating the *Aegagropila*-clade from the sister groups *Siphonocladus*-clade and *Cladophora*-clade. The *Aegagropila*-clade is divided into four subclades. The earliest diverging clade consists

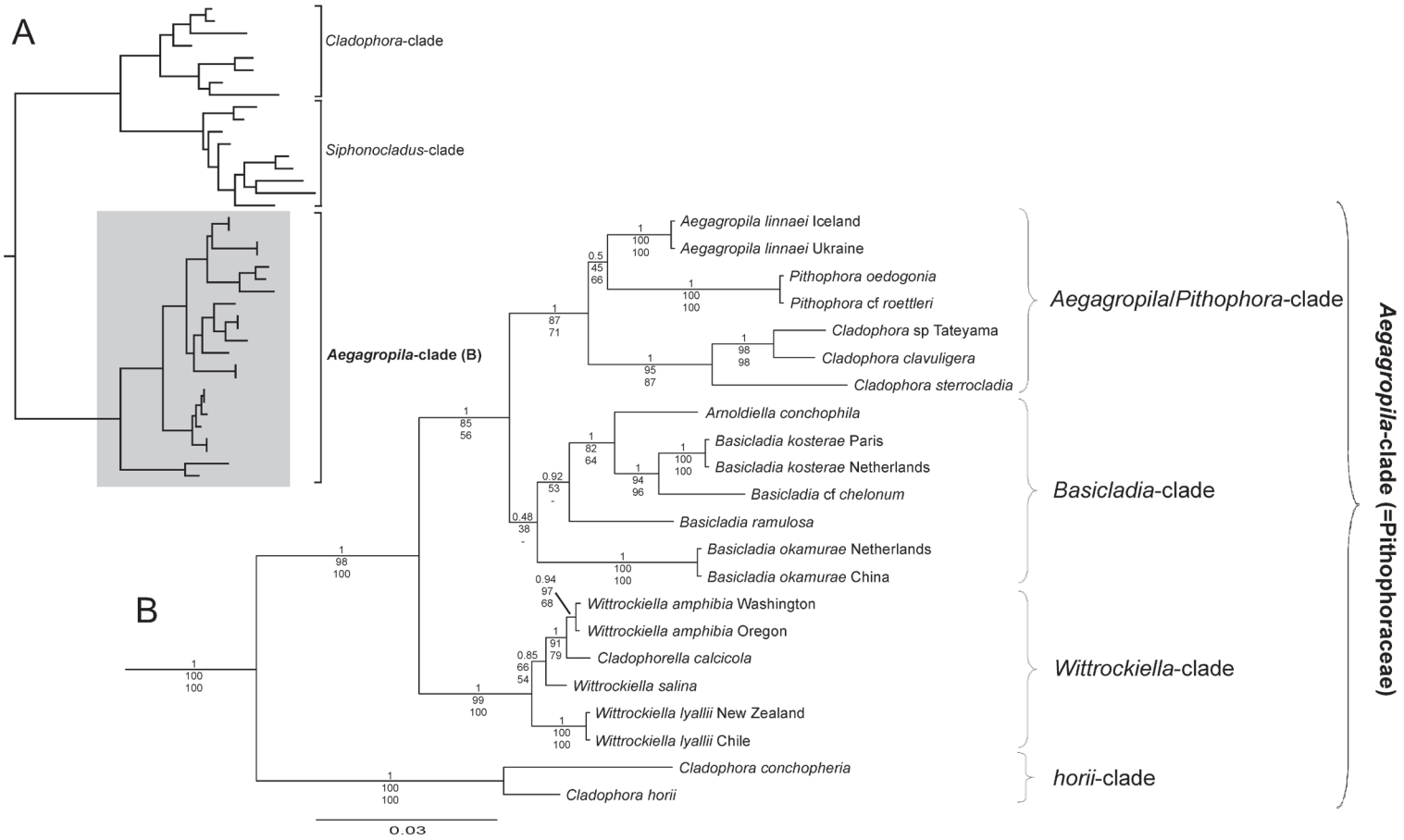

Figure 4. A: Bayesian inference (BI) tree inferred from concatenated partial large and complete small subunit rDNA sequences with gaps treated as missing data showing the three main clades of the Cladophorales ('ingroup' dataset). **B**: Close-up of the *Aegagropila*-clade highlighted in grey in **A**, with genus names indicated on the right. Bayesian inference posterior probabilities are indicated above branches, MP and maximum likelihood bootstrap values (1000 replicates) are shown below branches.

of *C. horii* and *C. conchopheria* ('*horii*-clade'), the next clade is composed of all *Wittrockiella* species and *Cladophorella calcicola* ('*Wittrockiella*-clade'), and a clade consisting of all *Basicladia* species ('*Basicladia*-clade') is sister to a clade composed of *Aegagropila linnaei*, *Pithophora* and two poorly known *Cladophora* species ('*Aegagropila*/*Pithophora*-clade'). The *Wittrockiella*-clade received high support, while support values for the *Aegagropila*/*Pithophora*-clade were moderate to high. The *Basicladia*-clade was only weakly supported in the MP analysis, and received no support in the BI and ML analyses.

Ingroup tree

The trees constrained with a strict and a relaxed molecular clock and midpoint rooting performed on BI and ML trees of the 'ingroup' dataset (Appendix S7) showed the same root position as revealed by outgroup rooting (Fig. 3). An overview of the relationships between the three main ingroup clades and branch length information is shown in Fig. 4A. The *Aegagropila*-clade is enlarged in Fig. 4B. This BI majority-rule phylogram shows the relationships, support values of the three phylogenetic inference methods used and the branch lengths. The *Aegagropila*-clade was recovered as a monophyletic group with high support in all three analyses. The same four main clades as shown in Fig. 3 were found, with the *Aegagropila*/*Pithophora*-clade further divided into three subclades with long branches.

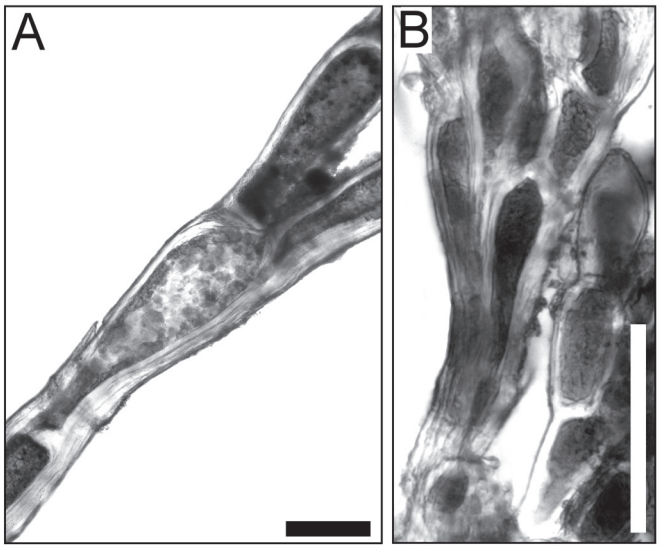

Figure 5.
Stem-like basal branches two members of the *horii*-clade (=*Pseudocladophora* gen. nov.) formed by descending intra- and extracuticular rhizoids that have fused with the cell walls of cells below, leading to a polysiphonous base. **A**: *Cladophora horii* (D78). **B**: *Cladophora conchopheria* (N71). Scalebars=100 µm.

Cladophora horii and *C. conchopheria* form a clade with high support that is sister to the rest of the *Aegagropila*-clade. Additional specimens of both taxa had identical LSU rDNA sequences (Table 2). While the two species differ markedly in habit (*C. horii* forming much larger thalli than *C. conchopheria* (Figs. 2 A, B), they share the characteristic stem-like basal branches that are formed by descending intra- and extracuticular rhizoids that fuse with the cell walls of cells below, leading to a polysiphonous base and very thick, stacked cell walls (Fig. 5).

The *Wittrockiella*-clade is highly supported in all analyses. This clade contains all currently recognized species of *Wittrockiella* plus *Cladophorella calcicola*. *Wittrockiella lyallii* (Fig. 2C) is sister to the rest and is represented by two identical sequences from

populations in Chile and New Zealand. The grouping of *W. salina* (Fig. 2D) with *C. calcicola* (Fig. 2E) and *W. amphibia* (Fig. 2F) received only moderate to low support. The sequences of *W. amphibia* from two locations in the northwestern Pacific are identical. The *Wittrockiella*-clade is characterized by short branches in comparison to all other clades in the phylogeny. The members of this clade share a heterotrichous growth form, with creeping main axes that produce relatively short upright filaments (Figs. 2C - F). Adventitious rhizoids can be produced in all cells along of the stolonoid axes (Fig. 2C), and can develop from any part of the cells.

The *Basicladia*-clade consists of four *Basicladia* species plus *Arnoldiella conchophila*. The clade received low or no support in the three analyses (Fig. 3), with *B. okamurae* being frequently recovered on a basal polytomy on the branch connecting the *Basicladia*-clade and the *Aegagropila*/*Pithophora*-clade (Figs. 3 & 4). However, this clade received high support in BI analyses under a HKY model of nucleotide evolution (Appendix Figs. S6B & D) and in the LSU-only analysis (not shown), and received moderate support in the ML analysis of the 'test'-dataset (Appendix Fig. S7D, bootstrap support=74). The placement of *B. ramulosa* Ducker with the remaining taxa of this clade was only weakly supported, while the grouping of *A. conchophila* with *B. chelonum* and *B. kosterae* was well supported. The four LSU rDNA sequences of *B. kosterae* from the Netherlands and France showed no intra-individual variation (Table 2). The LSU sequences of *B. okamurae* from the Netherlands and China were also identical. The stunted *Arnoldiella conchophila* is nested within *Basicladia*, between the northern Australian endemic *B. ramulosa* and *B. chelonum*-*B. kosterae*. All species in this clade share a heterotrichous growh form (e.g., Fig. 2H). Branched or unbranched upright shoots (Fig. 2G, I) with a characteristic long basal cell (Fig.2J) arise from an extensive prostrate, rhizome-like stratum consisting of coalescent, branched filaments. The upright filaments typically increase in diameter from the base to the apex.

The *Aegagropila*/*Pithophora*-clade is comprised of three subclades which represent the monotypic *Aegagropila* (*A. linnaei*), the genus *Pithophora*, and a subclade consisting of three Asian *Cladophora* species. The clade as a whole is highly supported in Bayesian analyses, but received only moderate bootstrap support in ML and MP analyses. The sister relationship of *Aegagropila* and *Pithophora* is only weakly supported in ML analyses, and unsupported in other inference methods, resulting in a basal polytomy of the three subclades. The sister relationship of *Aegagropila* and *Pithophora* received high support when employing the simpler HKY model of nucleotide evolution (Appendix Fig. S6). Among the three *Cladophora* species, *C. sterrocladia* is sister to both *C. clavuligera* and an undescribed species from Japan (*C.* sp. 'Tateyama'), and these relationships were highly supported. The two specimens of *A. linnaei* have identical sequences, and sequence variation of maximal 1 bp within this species in partial LSU rDNA sequences had been shown before with specimens from a wide range of locations (Boedeker & Immers 2009). All sequenced *Pithophora* specimens, sampled form different localities and including various morphotypes (Table 4), have identical LSU sequences, except for specimen K97 (*P.* cf. *polymorpha*), which has three point mutations.

The species in the *Aegagropila*/*Pithophora*-clade are morphologically distinct from the *Wittrockiella* and *Basicladia* clades by the absence of a prostrate system, *A. linnaei* and the *Cladophora* species have primary coralloid holdfasts, the genus *Pithophora* is only known unattached. Members of the *Aegagropila*/*Pithophora*-clade are characterized by secondary rhizoids, branches being inserted subterminally, and delayed cross-wall

formation in newly produced branches. The secondary rhizoids are frequently very long and seem to play an important additional role in attaching the thallus to the substrate. There is a tendency, particularly in apical parts, for opposite (Figs. 2N - P) or secund branching (Fig. 2M). Both *Pithophora* and *Aegagropila* are characterized by frequent inversion of polarity. A unique feature of the genus *Pithophora* are characteristic terminal or intercalary akinetes which occur either solitary (Fig. 2K), in pairs, or in short chains (Fig. 2L). Sterile plants without akinetes are morphologically similar to the other members of this clade (Fig. 2M). *Aegagropila linnaei*, *C. clavuligera* and *C. sterrocladia* are morphologically similar, and are the only known members of the Cladophorales that can have branches inserted serially (Fig. 2O). *Cladophora sterrocladia* (Fig. 2N) and *C. clavuligera* (Fig. 2O) can be distinguished from *A. linnaei* (Fig. 2P) by several upright shoots arising from the same base, the more sparsely branched basal parts of the main axes composed of regular cylindrical cells (Fig. 2N), while the basal parts of thalli of *A. linnaei* consist of irregular shaped cells with thick walls that can have branches inserted in any position of the mother cell. Furthermore, the arrangement of laterals in the upper parts of *C. clavuligera* is dominantly opposite (sometimes opposite branches in series on one cell), and frequently verticillate in *C. sterrocladia*, with up to 5 laterals per cell.

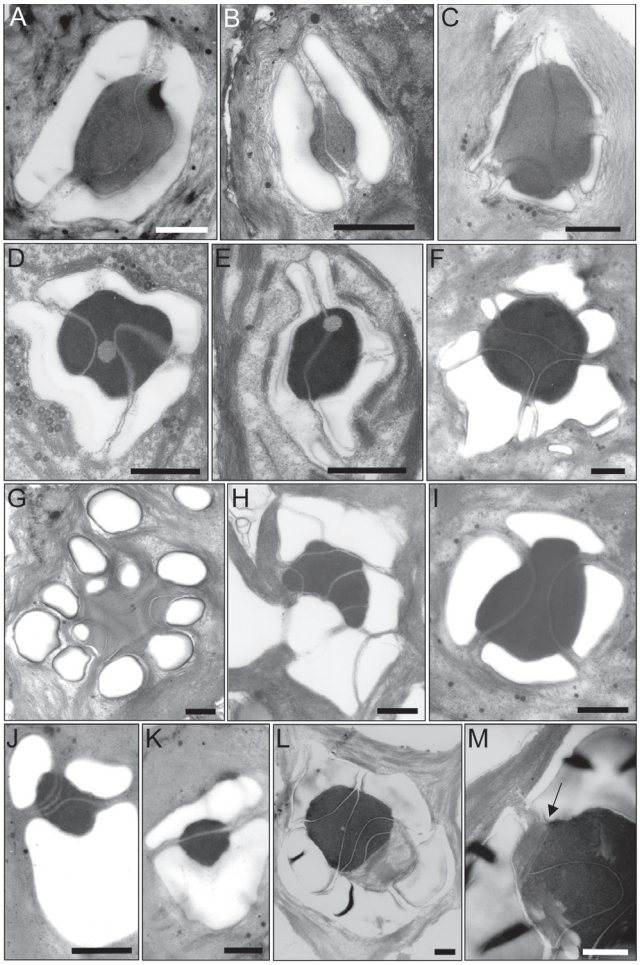

Figure 6.
Transmission electron microscope images of the pyrenoid ultrastructure in members of the *Aegagropila*-clade. Representative examples of all sectioned pyrenoids per species were chosen.
A, **B**: *Wittrockiella salina* (B92), bilenticular pyrenoids. **C**: *Cladophorella calcicola* (K92), polypyramidal pyrenoid. **D**, **E**: *Wittrockiella amphibia* (N60), intraspecific variation in pyrenoid type. **D**: polypyramidal pyrenoid. **E**: bilenticular pyrenoid. All pyrenoids of this species showed a characterstic light spot inside the pyrenoids. **F**: *Basicladia kosterae* (K09), polypyramidal pyrenoid. **G**: *Basicladia okamurae* (L84), polypyramidal pyrenoid with extremely segregated surrounding starch sheath. **H**: *Pithophora* cf. *roettleri* (K93), polypyramidal pyrenoid. **I**: *Pithophora* cf. *polymorpha* (K97), polypyramidal pyrenoid. **J**, **K**: *Pithophora roettleri* (K96), intraspecific variation in pyrenoid type. **J**: polypyramidal pyrenoid. **K**: bilenticular pyrenoid. **L**, **M**: *Aegagropila linnaei* (N36), polypyramidal pyrenoid. **M**: Close-up of a thylakoid membrane stack associated with the pyrenoid (arrow). All pyrenoids of this species showed this association. All scalebars=1 µm.

Pyrenoid ultrastructure

Pyrenoid ultrastructure was examined by TEM with respect to the number of thylakoid membranes transversing the pyrenoid (bilenticular *vs.* polypyramidal structure) and the arrangement of starch plates. In the *Wittrockiella*-clade, both polypyramidal and bilenticular pyrenoids were observed. All pyrenoids of *W. salina* (specimen B92, Figs. 6A & B, n=17) were bilenticular, with the surrounding starch layer divided into two halves. The majority of pyrenoids in *Cladophorella calcicola* (specimen K92, Fig. 6C, n=21) were polypyramidal with the starch layer divided into several pieces, but 33% of the pyrenoids had a bilenticular structure. Similarly, about half of the pyrenoids in *W. amphibia* was found to be polypyramidal (57%), the other 43% were bilenticular (specimen N60, Figs. 6D & E, n=14). All pyrenoids in *W. amphibia* displayed a small round spot of a different density than the surroundings. All pyrenoids of members of the *Basicladia*-clade were polypyramidal (n=64), with no intra-individual or intraspecific variation in the pyrenoid structure. The surrounding starch layer was highly fragmented (Fig. 6F), with the most extreme form of segregation observed in *B. okamurae* (Fig. 6G). Pyrenoids of the genus *Pithophora* showed variation in their ultrastructure, both intra-individually and between specimens. In specimen K93 (*P.* cf. *roettleri* Wittrock, Fig. 6H, n=31), all pyrenoids were polypyramidal with the surrounding starch layer divided into irregular pieces. In specimen K97 (*P.* cf. *polymorpha* Wittrock), 16% of the pyrenoids were bilenticular and 84% were polypyramidal (Fig. 6I, n=19). Specimen K96 (*P. roettleri*) about two thirds of the studied pyrenoids showed a polypyramidal structure (Fig. 6J, n=11) and about one third of the pyrenoids was bilenticular (36%, Fig. 6K). The starch plates in this specimen were very large (Figs. 6J & K). All pyrenoids in *A. linnaei* were polypyramidal (specimen N36, Fig. 6L, n=9). A unique feature found in all pyrenoids of this species was the association of a stack of thylakoid membranes with the pyrenoid, inside the surrounding starch layer (Fig. 6M).

Discussion

In the present study we aimed at establishing the phylogenetic relationships within the *Aegagropila*-clade with more confidence than previous treatments (Hanyuda *et al.* 2002, Yoshii *et al.* 2004) by increasing both the number of taxa and the number of characters (combining LSU and SSU sequence data). Earlier phylogenies of the *Aegagropila*-clade were based on SSU rRNA gene sequences only, a gene that has been shown in other phylogenetic studies of the Cladophorales to not resolve relationships well. By adding partial sequences of the more variable LSU rRNA gene, the number of informative characters was doubled (Table 3). Another potential benefit of combining genes is the emergence of relationships that are not recovered from individual partition trees (Gontcharov *et al.* 2004). The advantages of combined analyses have been demonstrated in numerous phylogenetic studies (e.g., Murray *et al.* 2005, Feau *et al.* 2006, Leliaert *et al.* 2007a). The relationships inferred in our phylogenetic analyses are in overall agreement with the previously published trees of the *Aegagropila*-clade (Hanuyda *et al.* 2002, Yoshii *et al.* 2004), and are generally well supported. In addition, we show that *Wittrockiella salina* and *Cladophorella calcicola* are part of the *Wittrockiella*-clade, and that all *Basicladia* species plus *Arnoldiella conchophila* are closely related and likely form a clade. We characterize a new subclade closely related to *Aegagropila* and *Pithophora* that contains tropical species

with a *Cladophora*-type morphology. For a discussion of slightly different tree topologies and support values recovered when employing simple models of nucleotide substitution, see Appendix S6.

Apomorhies of the Aegagropila*-clade*

Hanyuda *et al.* (2002) suggested that the *Aegagropila*-clade was characterized by a number of unique/derived biochemical and ultrastructural features, including the presence of the carotenoid pigment loraxanthin, the presence of chitin in the cell walls and polypyramidal pyrenoids. In contrast, the other two main lineages of the Cladophorales (the *Cladophora*-clade. and the *Siphonocladus*-clade) are assumed to be characterised by uniformly bilenticular pyrenoids (van den Hoek *et al.* 1995). Polypyramidal pyrenoids have been identified in all members of the *Aegagropila*-clade investigated so far, namely: *Cladophora horii* (van den Hoek & Chihara 2000); *C. conchopheria*, *Wittrockiella lyallii*, *Arnoldiella conchophila*, *B. okamurae*, *Pithophora mooreana* Collins and *Aegagropila linnaei* (Matsuyama *et al.* 1998; Hanyuda *et al.* 2002); as well as in *Basicladia chelonum* (Mrozinska *et al.* 2009). Polypyramid pyrenoids have, however, also been found in *Cladophora catenata* Kützing (Matsuyama *et al.* 1998), a species of the *Siphonocladus*-clade (Hanyuda *et al.* 2002), and *Rhizoclonium tortuosum* (Dillwyn) Kützing (Miyaji 1999), for which no molecular data are available at present but which is assumed to be a member of the *Cladophora*-clade. Furthermore, polypyramid pyrenoids are known for *Dictyospheria cavernosa* Forsskål (Børgesen) (Hori & Ueda 1975), a member of the *Siphonocladus*-clade. In the present study, polypyramidal pyrenoids have been confirmed for *Basicladia okamurae*, *B. kosterae*, one isolate of *Pithophora* (*P.* cf. *roettleri*, K93) and *Aegagropila linnaei*. Both polypyramidal and bilenticular pyrenoids were found in *Wittrockiella amphibia*, *Cladophorella calcicola*, *Pithophora* cf. *polymorpha* and *P. roettleri*. In *W. salina*, only bilenticular pyrenoids were observed, as had been previously mentioned by van den Hoek *et al.* (1984). Thus, the pyrenoid ultrastructure does not seem to be a stable diagnostic character to separate the *Aegagropila*-clade from the rest of the Cladophorales.

The other two suggested characters are also problematic. Loroxanthin, found in all members of the *Aegagropila*-clade studied to date, is also present in some members of the *Cladophora*-clade (Fawley 1991, Yoshii *et al.* 2004) and several orders of green algae (Fawley 1991). *Blastophysa rhizopus*, the closest known relative of the Cladophorales (Cocquyt *et al.* 2010), has siphonoxanthin (O'Kelly 1982), a character that otherwise appears to be derived within Cladophorales (Yoshii *et al.* 2004). Since lutein, loroxanthin, and siphonoxanthin are thought to be successive products in a biosynthetic series (Egeland *et al.* 1997, Yoshii *et al.* 2004), the actual xanthophylls produced may be less informative for phylogenetic purposes than the enzymes responsible for synthesizing them, and especially the genetic factors affecting their expression. Chitin is only known to be present in the cell walls of *Pithophora* species (Pearlmutter and Lembi 1978, 1980), for other members of the *Aegagropila*-clade data are lacking. There are, however, reports of the presence of chitin in the cell walls of *Cladophora glomerata* (Wurdack 1923) and *C. vagabunda* (Jónsson 1962, as *C. expansa*). Both species are members of the *Cladophora*-clade, rendering the presence of chitin invalid as a diagnostic character.

While polypyramidal pyrenoids and the presence of loroxanthin might represent apomorphies of the *Aegagropila*-clade, they do not represent diagnostic characters.

Thus, the *Aegagropila*-clade is currently only defined by molecular data. However, this multinucleate lineage can be characterized by occurring in brackish or freshwater habitats; a tendency for dominant asexual reproduction; extensive development of secondary rhizoids; a tendency for heterotrichous organization: from polysiphonous holdfast clusters (*horii*-clade), to a clear division into a prostrate and an upright system (*Wittrockiella*- and *Basicladia*-clade), to loss of the prostrate system coupled with ease of inversion of polarity or being unattached (*Aegagropila* & *Pithophora*); and subterminal insertion of laterals combined with delayed cell wall formation.

The *Aegagropila*-clade has not received a formal taxonomic rank, but considering its robustness in molecular phylogenetic analyses (Hanyuda *et al.* 2002, Yoshii *et al.* 2004, Rindi *et al.* 2006, present study), the designation of a family name is warranted. This lineage includes type species of three families: Pithophoraceae Wittrock 1877, Wittrockiellaceae Wille 1909 and Arnoldiellaceae Fritsch 1935. *Pithophora* and *Wittrockiella* are the sole genera within their families, while Arnoldiellaceae includes *Arnoldiella*, *Basicladia*, and the monotypic *Cladostroma* Skuja. The name **Pithophoraceae** has priority, and its use for the *Aegagropila*-clade is recommended here. In its new sense, the family includes the genera *Aegagropila*, *Aegagropilopsis*, *Arnoldiella* (including *Basicladia*), *Pithophora*, *Pseudocladophora* and *Wittrockiella*. Although the monotypic genus *Spongiochrysis* has been characterized as a member of the *Aegagropila*-clade (Rindi *et al.* 2006), we tentatively refrain from including it into this lineage because of data conflict between SSU and LSU rDNA data (unpublished data), resulting in an ambiguous phylogenetic position.

Genera of the Pithophoraceae (*=Aegagropila-*clade)

horii-clade

Cladophora conchopheria and *C. horii* are the only marine species in the Pithophoraceae, and form the earliest diverging clade. *Cladophora conchopheria* grows exclusively on shells of the marine snail *Lunella coronota,* occurring in South Korea and Japan (Sakai 1964, Matsuyama *et al.* 1999, van den Hoek & Chihara 2000). *Cladophora horii* has been found in shallow subtidal waters and intertidal rockpools, sometimes as an epiphyte on *C. prolifera*, along the east coast of South Africa (Leliaert & Coppejans 2003) and Okinawa, Japan (van den Hoek & Chihara 2000). The robust, broom-like tufts of *C. horii* and the minute turfs of *C. conchopheria* share a polysiphonous holdfast formed by fusion of descending rhizoids with the walls of basal cells. This character was not regarded as synapomorphic in a morphological treatment, in which *C. horii* was placed in the *Cladophora* section *Rugulosae* while *C. conchopheria* was placed in the section *Glomeratae* (van den Hoek & Chihara 2000). Other members of the section *Rugulosae* have extensive secondary rhizoidal development, often with annular constrictions, but the rhizoids do not fuse with the walls of other cells. Coalescent basal stipes are also known from other members of the section *Glomeratae* such as *C. albida* (Nees) Kützing or *C. opaca* Sakai (van den Hoek & Chihara 2000), but these differ in their mode of formation and are not characterized by very thick, layered cell wall wedges (Fig. 5).

Both *C. conchopheria* and *C. horii* are densely branched and show a typical *Cladophora*-like architecture, and the new genus *Pseudocladophora* is proposed here to accommodate the two species. We select *C. conchopheria* as the type species of the new genus.

***Pseudocladophora* Boedeker gen. nov.**
Type species: *Pseudocladophora conchopheria* (Sakai) Boedeker comb. nov. (*Cladophora conchopheria* Sakai 1964: 48).

Latin diagnosis
Algae marinae thallis rigidis erectis filamentis uniseriatis compositis divisionibus acropetalis intercalaribusque. Filamenta sparse ramosa in thalli partibus inferioribus, distaliter dense ramosa fere cellulis omnibus laterales uno vel duos rare tres septis obliquis ad ardue inclinatis e ramorum basi. Rami angulos acutos inclinati. Ramorum ordinatio parum acropetala ad opposita vel irregularia. Thalli substratum affixi hapeteronibus rhizoidealibus stipitibus numerosis e basi vulgari radiatis. Cellulae in thalli partibus mediis et inferioribus rhizoidea secundaria descendentia formantes saepe intracuticularia cellulis inferioribus. Rhizoidea adventitia parietibus cellulariis cellularum inferiorum connatescentia structuri caulibus similibus basin polysiphonam formantia. Cellularum parietes crassi partibus apicalibus 1.5-3-(5) µm*, basalibus* 5-15-(30) µm *crassis.*

Description
Marine algae with stiff, erect thalli composed of uniseriate filaments growing by acropetal and intercalary cell divisions. Cells are multinucleate with parietal net of polypyramidal chloroplasts. Filaments sparsely branched in lower parts of the thallus, densely branched in distal parts with almost every cell cutting of one or two (rarely three) laterals by oblique to steeply inclined cross walls at the base of branches. Branches inclined at acute angles. Branching patterns slightly acropetal to opposite to irregular. Thalli attached to the substratum by rhizoidal holdfasts with many stipes radiating from a common base. Cells in the middle and lower parts of the thallus producing descending secondary rhizoids, frequently intracuticular in cells below. The adventitious rhizoids become fused with the cell walls of the lower cells, producing stem-like structures that form a polysiphonous base. Cells cylindrical, apical cells rounded. Cell walls thick, in apical parts 1.5-3-(5) µm, in basal parts 5-15-(30) µm.

Members
Pseudocladophora conchopheria (Sakai) Boedeker comb. nov.
Holotype: Nagahama near Maizuru, Kyoto Prefecture, Japan, collector I. Umezaki, May 1949, SAP (SAP 029140), on shell of the marine gastropod *Lunella coronata* Gmelin.
Basionym: *Cladophora conchopheria* Sakai 1964: 48.

Pseudocladophora horii (van den Hoek & Chihara) Boedeker comb. nov.
Holotype: Sesoko Island, Okinawa, Japan, collectors S. Kamura, C. van den Hoek & T. Hori, April 1990, TNS (TNS-AL-46793).
Basionym: *Cladophora horii* van den Hoek & Chihara 2000: 68.

Wittrockiella-clade

Wittrockiella grows in brackish-water and estuarine environments, and currently contains three species (*W. amphibia*, *W. lyallii* and *W. salina* (including two varieties), which are primarily separated by their cell dimensions. The three species form a highly supported clade which also includes *Cladophorella calcicola*. *Cladophorella calcicola* is

most closely related to *W. amphibia*, a relationship that was already proposed based on morphological similarities (van den Hoek *et al*. 1984). *Cladophorella calcicola* is a warm-temperate to tropical semi-terrestrially species reported from moist limestone, bricks and mud in China, Bangladesh, and tropical hothouses in Europe (Fritsch 1944, Islam 1964, Cribb 1965, Ettl & Gärtner 1995, Liu 1999). The sample used in this study was found in an estuarine lagoon in Portugal (Table 2). Since all species of *Wittrockiella* occur in brackish environments (Wille 1909, Polderman 1976, South 1981, van den Hoek *et al*. 1984, Nelson *et al*. 2002), it seems likely that the freshwater/semi-terrestrial species *C. calcicola* evolved from a brackish ancestor (see Fig. 4). No molecular data are available yet for the other described *Cladophorella* species (*C. frischii* Islam, *C. sunderbanensis* Islam, *C. netzhualpilii* Galicia-García & Novelo).

Wittrockiella (1909) has priority over *Cladophorella* (1944), thus the new combination *Wittrockiella calcicola* (Fritsch) Boedeker is proposed (see below). For typification of *W. salina, W. amphibia* and *W. lyallii* see van den Hoek *et al*. (1984), Boedeker & Hansen (2010) and Boedeker *et al*. (2010c), respectively. We here select an epitype for *W. paradoxa*, the type species of the genus, to serve as a complement to the original drawings that represent the holotype.

***Wittrockiella* Wille**
Type species: *Wittrockiella amphibia* (Collins) Boedeker & Hansen (*Wittrockiella paradoxa* Wille 1909: 220-221).
Generitype: original specimen (collected in 1907 by N. Wille, Lyngør, Norway) not traceable, thus the original drawings represent the holotype material (Wille 1909: Tables XI-XIV).
Epitype: Lyngør, southeastern Norway, collector B. Lynge, January 1909, det. N. Wille, O (six iso-epitypes).

Wittrockiella calcicola (Fritsch) Boedeker comb. nov.
Holotype: original specimen (collected by F.E. Fritsch, tropical hothouse, Cambridge Botanical Garden, Britain, 1944, on moist limestone) lost or destroyed (used to be in BM), thus the original drawings represent the holotype material (Fritsch 1944: Figs. 1A-I, 2A-G, 3A-C, 4A-G).
Epitype: Lagoa de Óbidos, Portugal, collector O. Lourenço (sample no. K92; ACOI culture collection 471), 1989, L (L0793292).
Basionym: *Cladophorella calcicola* Fritsch 1944: 157-171.

Basicladia-clade

Seven species of *Basicladia* have been described, a genus that has a reputation to be restricted to freshwater turtles as the only possible substrate (e.g., Edgren *et al*. 1953, Normandin & Taft 1959). DNA sequences from three species could not be obtained for this study, namely *B. crassa*, *B. sinensis* and *B. vivipara*. The genus *Basicladia* was erected to accommodate *B. chelonum* Hoffmann & Tilden and *B. crassa* Hoffmann & Tilden (Hoffmann & Tilden 1930). These two species differ only in their cell dimensions and intermediate forms exist. Both species frequently occur in the same habitat, or even on the same turtle, and it was proposed that they could represent merely different growth forms of

a single plastic species, such as a sun and a shade form (Proctor 1958, Garbary *et al*. 2007). *Basicladia sinensis* (Gardner) G.M. Smith is only known from one specimen collected from a Chinese freshwater turtle that was imported into the USA (Gardner 1936), and its habit and cell dimensions are actually in the range of *B. crassa*. The species *B. vivipara* Normandin & Taft is only known from the freshwater gastropod *Viviparus malleatus* Reeve (Normandin & Taft 1959), and is morphologically very similar to *B. chelonum*. Thus, the actual number of species might not have been correctly assessed yet, and molecular data of these taxa would be very interesting. The grouping of *Arnoldiella conchophila* with *B. chelonum* and *B. kosterae* was well supported in our phylogenetic trees. It shares both the heterotrichous growth form of *Basicladia*, in which a dense basal stratum of branching filaments is united into a continuous layer, and a strong preference for epizoophytic freshwater habitats (Miller 1928, Kargupta 1994, Keshri & Hazra 2009).

Previous phylogenies had recovered *B. okamurae* on a basal polytomy on the branch connecting either *Basicladia* sp. or *Arnoldiella conchophila* and the *Aegagropila*/*Pithophora*-clade (Hanyuda *et al*. 2002, Yoshii *et al*. 2004). Our analyses yielded similar results (Figs. 3 & 4, Appendix S6 & S7), thus monophyly of *Basicladia* (including *A. conchophila*) could not be demonstrated with certainty. However, there is strong morphological evidence for keeping *B. okamurae* in the same genus as the other *Basicladia* species such as the formation of a dense basal stratum of branching filaments that unite into a continuous layer, and the very large basal cells of the upright filaments. Since *A. conchophila* is nested within *Basicladia* and has nomenclatorial priority over *Basicladia*, we propose the transfer of all *Basicladia* species used in this study to the emended genus *Arnoldiella*.

***Arnoldiella* V. Miller emend. Boedeker**
Type species: *Arnoldiella conchophila* V. Miller 1928: 20-21.

Description
Thallus differentiated into a prostrate layer consisting of coalescing filaments and a compact system of rigid upright filaments. Cells of the prostrate layer with one to few nuclei, cells of the erect filaments multinucleate. Erect filaments can be densely branched, rarely branched or entirely unbranched. If branched, branches more numerous in apical parts of the thallus. If unbranched, occasional branching can occur directly at the base of the upright filaments. Primary, secondary and tertiary branching can be present in erect filaments. Branches inserted subterminally or cut off by an almost horizontal cross wall resulting in a pseudichotomy. Cells gradually becoming shorter and wider from base to apex. Apical cells rounded or pointed. Thick cell walls, at least in basal parts. Terminal zoosporangia, sometimes formed in chains.

Members
Arnoldiella chelonum (Collins) Boedeker comb. nov.
Holotype: Walnut Lake, Michigan, USA, collector T.L. Hankinson, on turtle carapaces, NY (00887601).
Basionym: *Chaetomorpha chelonum* Collins 1907: 198-200.
Synonym: *Basicladia chelonum* (Collins) Hoffmann & Tilden 1930: 382-383.

Arnoldiella conchophila V. Miller
Holotype:, original specimen (collected by V. Miller, Lake Pereslavl, Vladimir district, Russia, 1921, on shell of freshwater bivalve) untraceable, thus the original drawings represent the holotype material (Miller 1928: Figs. 2-20).

Arnoldiella kosterae (van den Hoek) Boedeker comb. nov.
Holotype: Jardin des Plantes, Paris, France, collector C. van den Hoek, 25 April 1961, L (L 0054830).
Basionym: *Cladophora kosterae* van den Hoek 1963: 37-38.
Synonym: *Basicladia kosterae* (van den Hoek) Garbary 2010: 39.

Arnoldiella okamurae (S. Ueda) Boedeker comb. nov.
Holotype: Shirahama, Tokyo, Japan, collector S. Ueda, Tokyo University of Marine Science and Technology.
Basionym: *Chaetomorpha okamurae* S. Ueda 1932: 23-24.
Synonyms: *Cladophora okamurae* (S. Ueda) van den Hoek 1963: 39.
Basicladia okamurae (S. Ueda) Garbary 2010: 39.

Arnoldiella ramulosa (Ducker) Boedeker comb. nov.
Holotype: Stratford, Victoria, Australia, collector S.C. Ducker, 11 December 1956, on carapace of turtle, MEL. Isotypes in BM & MELU.
Basionym: *Basicladia ramulosa* Ducker 1958: 165-166.

Aegagropila/*Pithophora*-clade

This clade is characterized by a reduced rhizoidal system and the erect system has become the sole thallus. This is most extreme in *Pithophora*, which only occurs unattached. Also *Aegagropila* is commonly found unattached, and both genera display frequent inversion of polarity. Secondary rhizoids are formed by all members of this clade, but no prostrate system is formed.

1. *Aegagropila*
Aegagropila linnaei is currently the only species of the genus. More than 90 synonyms exist for *A. linnaei* (holotype in L, van den Hoek 1963), stemming from over-interpretation of plastic morphological characters. This species produces different growth forms, including attached filaments, free-floating mats and 'lake balls'. Little genetic variation was found among samples from the entire geographic range, indicating that *A. linnaei* indeed represents a single species (Boedeker *et al.* 2010b).

2. *Pithophora*
Species of *Pithophora* are widespread in the (sub)tropics (Wittrock 1877, Möbius 1895, Fritsch 1907a, Bourrelly 1966), but also widely distributed in the temperate regions of the eastern USA (John 2003). More than 35 taxa of *Pithophora* have been described (Index Nominum Algarum, http://ucjeps.berkeley.edu/INA.html), including a large number of varieties. The extent of phenotypic plasticity in the few morphological characters that had led to an inflation in the number of described species (Ernst 1908, Mothes 1930, also Fott 1971). Identification at the species level became basically impossible due to overlap of

character states (Möbius 1895, van Oye 1922). Conflicting with the number of described taxa based on subtle morphological differences, it was shown that akinete formation and germination is controlled by a wide range of environmental conditions (Ernst 1908, Agrawal 1986, Stevens & Neilson 1987), that the size of akinetes is age-dependent (Brand 1904), that akinete and branch formation are the same reversible process (Mothes 1930), and that helicoid formation is inducible as a wounding response (Mothes 1930). Pankow & Täuscher (1980) concluded that species level identifications are not feasible due to the amount of redundant species descriptions and recognized only two species, synonymizing all taxa with either *P. oedogonia* (Montagne) Kützing or with *P. roettleri* (Roth) Wittrock. The only morphological character separating these two species is the shape of intercalary akinetes. However, the distinction between isosporous and heterosporous is not clear at all (see Ernst 1908, van den Hoek 1959, Prescott 1951, Pankow & Täuscher 1980, Skinner & Entwisle 2004).

The lack of genetic variation in the LSU and SSU rDNA sequences (all SSU sequences identical, max. 3 bp differences in the LSU sequence of one out of five samples) of samples of different origin and morphologies (Table 4), hints to the existence of just one widespread, polymorphic species. The frequent formation of desiccation-resistant akinetes in *Pithophora* implies a high long-distance dispersal capacity. Based on morphological and molecular data we thus regard all described *Pithophora* taxa to be conspecific, rendering the genus monotypic. The name *P. roettleri* (Roth) Wittrock has priority (basionym: *Ceramium roettleri* Roth 1806, type in L), and is proposed to be used as the single species name. No type species had been selected for *Pithophora* to date, the sole genus included in Pithophoraceae at its inception. We here select *P. kewensis* Wittrock as the lectotype for the genus *Pithophora* and the family Pithophoraceae (see Materials and Methods), which is assumed to reside with the sequenced *Pithophora* specimens in the *Aegagropila/ Pithophora* clade.

The sister relationship with the temperate species *A. linnaei* invites for interesting speculation. Both genera are assumed to be asexual (Möbius 1895, Brand 1902, Ernst 1908, Heering 1921, Mothes 1930, Fritsch 1935, van den Hoek 1963, Soejima *et al.* 2009) and polyploid (chromosome counts in *Pithophora*: Geitler 1936, Verma 1979; own unpublished data of C-values for *A. linnaei*), factors that could play a role in the lack of speciation within the genera, the low intraspecific genetic variation and the extensive morphological plasticity.

***Pithophora* Wittrock**

Type species: *Pithophora roettleri* (Roth) Wittrock (*Pithophora kewensis* Wittrock 1877: 52-55).

Lectogeneritype: *Pithophora kewensis* Wittrock, tropical aquarium ('Waterlily-house'), Kew Gardens, Britain, collector V.B. Wittrock, August 1872, L (no. 938112 639). Isotypes in BM, L, UPS & S.

Pithophora roettleri (Roth) Wittrock

Holotype: Tranquebar, eastern India, collector Klein, January 1799, L (no. 93825 38). Isotype in UPS.

Basionym: *Ceramium roettleri* Roth 1806: 123.

Synonyms: all described species of *Pithophora* (including all intraspecific taxa) *P. aequalis* Wittrock, *P. affinis* Nordstedt, *P. chinensis* Skworzow, *P. clavifera* Schmidle, *P. cleveana*

Wittrock, *P. kewensis* Wittrock, *P. macrospora* Brand, *P. microspora* Wittrock, *P. mooreana* Collins, *P. oedogonia* (Montagne) Wittrock, *P. pachyderma* Schmidle, *P. pragensis* Sula, *P. polymorpha* Wittrock, *P. radians* W. & W.S. West, *P. reinecki* Schmidle, *P. sumatrana* (Martens) Wittrock, *P. varia* Wille, *P. variabilis* Schmidle, *P. zelleri* (Martens) Wittrock.

3. *Cladophora*-subclade

Three Asian *Cladophora* species are united in this subclade. One of them is still wanting a formal description, even though it has been included in a number of studies under several designations: in Nagai (1988) as *C. sauteri* forma *sauteri*, in Kanda (1991) as *C. sauteri*, in Hanyuda *et al.* (2002) and Yoshii *et al.* (2004) as *Cladophora* sp. 'Tateyama', and in Wakana *et al.* (2001b) as *Aegagropila* sp. nov. ('Tateyama-Marimo'). This species is morphologically very similar to *A. linnaei*, and in addition to epilithic growth forms it occurs also as free-floating tufts (Wakana *et al.* 2001b). Our phylogeny shows a sister relationship with *C. clavuligera*, a poorly-known species that has been reported from shells of a freshwater gastropod from Sri Lanka (Grunow 1868), and from brackish and freshwater gastropods (*Pila globosa* Swainson) and bivalves as well as from wood and stones in India (Verma 1981, Krishnamurthy 2000), but some of these identifications seem doubtful. Morphologically, *C. clavuligera* is very close to *C. yuennanensis* Skuja from China and *C. beneckei* Möbius from Java (see also Table 5). Our identification of *C. clavuligera* must be viewed as tentative too, since the material was collected in a tropical aquarium and is thus of unknown geographic origin. The third member of this subclade is *C. sterrocladia*, sister to both *C. clavuligera* and *Cladophora* sp. 'Tateyama'. It has been described from the shell of a freshwater snail (*Paludina*) from Myanmar (Skuja 1949). In a number of studies, this (sub)tropical species has been confused with the temperate species *A. linnaei* (e.g., Prasad & Misra 1992, Gardavský 1993, Liu 1999, Islam & Irfanullah 2005). As in the genus *Arnoldiella*, there seems to be a tendency in this clade for epizoophytism, especially to colonise freshwater gastropods. In comparison to *Arnoldiella*, the rhizoidal system in the *C. clavuligera/sterrocladia*-subclade is reduced, but long secondary rhizoids are formed.

So far, this subclade is restricted to Asia. Tropical Asian freshwater *Cladophora* species with a morphology similar to *C. clavuligera* and *C. sterrocladia* are for example *C. basicladioides* Jao, C. *beneckei*, *C. codiola* Zeller, *C. exigua* Zeller, *C. glomerata* var. *nana* Wang, *C. shensiensis* Jao, and *C. yuennanensis* Skuja. *Cladophora dusenii* Brand from Cameroon and *C. parvula* Möbius from Australia are probably also closely related to this group of species. However, the freshwater algal floras of both Africa and South America are less well known than of Asia, and the actual number of taxa and their distributions are most likely underestimated. Additional taxon sampling is clearly required, also in Asia (see Table 5).

The species of this subclade need to be transferred from *Cladophora* to a new genus. We decide against the possibility to merge *Aegagropila*, *Pithophora* and the three *Cladophora* species into *Aegagropila*, and erect a new genus to accommodate the three *Cladophora* species. The sequence divergence between the four main clades within the *Aegagropila*-clade is in the range 4.2-6.6%. Maximum intrageneric sequence divergence in *Wittrockiella*, *Pseudocladophora* and *Arnoldiella* emend. is 1.3%, 2.6% and 3.2-3.5%, respectively. In comparison, the sequence divergence between the three lineages in the *Aegagropila*/*Pithophora*-clade is 2.8-5%, and the recognition of three separate genera does not seem to be exaggerated. We propose the name *Aegagropilopsis* gen. nov., based on the morphological similarity to the genus *Aegagropila*, and select *C. sterrocladia* as the

type species.

***Aegagropilopsis* Boedeker gen. nov.**
Type species: *Aegagropilopsis sterrocladia* (Skuja) Boedeker comb. nov. (*Cladophora sterrocladia* Skuja 1949: 94-95).

Latin diagnosis
*Algae aquae dulcis interdum nonnihil salsugineae caespitibus minutis minus quam 1.5 cm altis filamentis erectis unserialibus hapterone coralloideo rhizoideis secundariis longis. Thalli dense ramosi saepe partibus inferioribus sparse ramosis. Rami praecipue oppositi interdum pectinati insertione subterminali interdum seriali angulis acutis septis tarde formantibus ardue inclinatis. Cellulae usque ad cinque laterales saepe verticillatas typice parum ad nodos constrictas partibus apicalibus longis eramosis. Rhizoidea adventitia e thalli omnibus partibus facta. Thalli praecipue epizoophtici ad cochleas aquae dulcis (sub) tropicis (*Pila*, *Paludina*) sed quoque substratis immobilis affixi inventus*.

Description
Freshwater algae, sometimes penetrating into slightly brackish waters, forming minute tufts or turfs less than 1.5 cm tall, consisting of erect uniseriate filaments, attached by a coralloid holdfast and long secondary rhizoids. Sometimes several shoots arising from the same holdfast, basal cells are short. Thalli densely branched, with lower parts of the thallus frequently sparsely branched. Branches mainly opposite, sometimes pectinate. Insertion of branches subterminally, sometimes serially, at acute angles, with delayed cross wall formation, cross walls steeply inclined. Up to five laterals per cells, frequently forming whirls. Cells typically slightly constricted at nodes. Apical parts long and unbranched. Adventitious rhizoids formed in all parts of the thallus. Cells in main axis 3-6 times as long as broad, cells in branches can be up to 1.5 mm long. Cell shape cylindrical to irregular. Apical cells 20-50 µm in diameter, branches 20-60 µm, basal parts up to 130 µm. Cell walls relatively thin, up to 6 µm in basal parts, 1 µm in apical cells. Zoospore formation by transformation of terminal cells into slightly swollen zooidingia. Thalli mainly epizoophytic on (sub)tropical freshwater snails (*Pila*, *Paludina*), but also found attached to stationary substrates.

Members
Aegagropilopsis sterrocladia (Skuja) Boedeker comb. nov.
Holotype: original specimen (collected by H. Skuja, Burma/Myanmar, on shell of freshwater gastropod) lost (used to be in RIG), thus the original drawings represent the holotype material (Skuja 1949: Plate XXXVII).
Epitype: pond in tropical hothouse, Hortus Botanicus Leiden, The Netherlands, collector C. Boedeker (sample G91), 26 April 2006, attached on mangrove pneumatophores (submerged), L (L0793287).
Basionym: *Cladophora sterrocladia* Skuja 1949: 94-95.

Aegagropilopsis clavuligera (Grunow) Boedeker comb. nov.
Holotype: Ceylon/Sri Lanka (Expedition Novara), collector G. von Frauenfeld, W (2010/2274), on shell of freshwater gastropod.
Basionym: *Cladophora clavuligera* Grunow 1868: 40.

Specialised niches and competition

Almost all species in the Pithophoraceae occur in habitats that can be characterized by fluctuating environmental conditions and reduced competition. Such conditions are found either in brackish environments (*Wittrockiella*, and to some extent *Aegagropila linnaei*) or on the surface of mobile host animals (*Pseudocladophora conchopheria*, *Arnoldiella* and to some extent *Aegagropilopsis*). The environment types and host animals of the members of the Pithophoraceae are indicated in Fig. 7. *Pseudocladophora conchopheria* occurs exclusively on the shells of the intertidal gastropod *Lunella coronota* along the coasts of South Korea and central and southern Japan (Sakai 1964, Matsuyama *et al*. 1999, van den Hoek & Chihara 2000), facing frequent desiccation stress and changes in other abiotic parameters. No other algae have been found to colonise those shells. Members of the genus *Arnoldiella* are most frequently encountered as epizoophytes on aquatic animals, especially on freshwater molluscs and turtles, habitats that have a drastically reduced algal diversity compared to permanent and stationary substrates (e.g. Edgren *et al*. 1953, Belusz & Reed 1968). Due to the basking and burrowing behaviour of many freshwater turtle species, they represent one of the most challenging habitats for freshwater algae. The species of *Arnoldiella* (as *Basicladia*) have a reputation to be restricted to freshwater turtles as the only possible substrate (e.g., Edgren *et al*. 1953, Normandin & Taft 1959). The Australian endemic *A. ramulosa* is actually the only *Arnoldiella* species that is exclusively known from turtles (Ducker 1958, Skinner *et al*. 2008). In North America, *A. chelonum* and *B. crassa* are the most common algae found on the carapaces of a wide range of turtle species (Proctor 1958, Belusz & Reed 1968, Garbary *et al*. 2007). The species *B. vivipara* Normandin & Taft is only known from the freshwater gastropod *Viviparus malleatus* Reeve (Normandin & Taft 1959). *Arnoldiella kosterae* has only rarely been encountered on turtles (Belusz & Reed 1968, also Ernst & Norris 1978 (as *B. crassa*)), but has been found on freshwater bivalves and dead wood (samples J56 and J79, respectively), and on stones and concrete (van den Hoek 1963). Only *A. okamurae*, the earliest diverging member of *Arnoldiella*, has actually never been encountered on turtles, and is the only *Arnoldiella/Basicladia* species that so far has never been encountered on an animal host of any kind (but observations are few). Thus, the tendency for epizoophytism might represent a more derived state and the evolution of the heterotrichous habit, also present in *A. okamurae*, might have been a prerequisite to colonise animal hosts (see also discussion on heterotrichy below). However, we could not show well-supported grouping of *A. okamurae* with the other *Arnoldiella/Basicladia* taxa in our phylogenetic analyses, thus the possibility exists that *A. okamurae* represents a separate unbranched, non-epizoophytic lineage.

It has been demonstrated for *A. chelonum* and *B. crassa* that other substrates can also be colonized in culture (Proctor 1958). These findings indicate competition effects, since these taxa are predominantly encountered on turtles in nature and not on other substrates. While some mutualistic relationships between the turtles and the algae have been considered (Edgren *et al*. 1953, Neill & Allen 1954), it seems more likely that competitive exclusion is at work, and that *Arnoldiella/Basicladia* species found their niche as epizoophytic freshwater algae. Besides *Arnoldiella/Basicladia* and *Dermatophyton radians* Peter, probably a closely related species, very few other macroalgae are found on turtle carapaces (e.g., Edgren *et al*. 1953, Belusz & Reed 1968, Garbary *et al*. 2007). *Dermatophyton radians* is generally considered to consist of merely a prostrate crust, but it was shown in culture that an erect system simlar to *Arnoldiella* spp. can develop (Potter

1888, Feldmann 1936). *Arnoldiella/Basicladia* species are most frequently encountered on the shells and carapaces of freshwater animals, probably because they are well adapted to those habitats by their heterotrichous organization and because competition in those habitats is drastically reduced compared to other solid substrates.

Similarly, brackish environments are generally characterized by low species numbers, and macroalgal diversity declines with decreasing salinity (Munda 1978, Nielsen *et al*. 1995, Middelboe *et al*. 1997). As both marine and freshwater species are at a disadvantage and species diversity is at a minimum (Remane 1934, Den Hartog 1969), competition can be less intense in brackish environments. The species of *Wittrockiella* occur in habitats characterised by strongly reduced biotic interactions. *Wittrockiella calcicola* mainly occurs semi-terrestrially on moist stone (Fritsch 1944, Islam 1964) or on mud (Table 2), while the other members of the genus are found in estuarine or intertidal habitats that are characterized by high fluctuations of environmental parameters and by frequently reduced salinities due to freshwater seepage. Adaptations of *Wittrockiella* to habitats with fluctuating salinities include the formation of a thick mucilage cover in *W. amphibia* (Wille 1909), an endophytic lifestyle in *W. amphibia* (Polderman 1976), the presence of haematochrome/oil droplets in *W. amphibia* and *W. salina* (Wille 1909, van den Hoek *et al*. 1984, respectively), and a cushion-like growth habit to preserve moisture during exposure to air in *W. salina* and *W. lyallii*. Another example for competition possibly shaping the ecological niche is *Aegagropila linnaei*, a species that occurs in both freshwater and brackish environments. *Aegagropila linnaei* is only found in the northern parts of the brackish Baltic Sea where salinity levels are below 6 psu (Boedeker et al 2010b). Northwards of the transition zone between the Bothnian Sea and the Bay of Bothnia where the salinity drops from 5 to 3.5 psu, *A. linnaei* even becomes the dominant macroalga on hard substrates down to a depth of 10 m (Nielsen *et al*., 1995; Bergström & Bergström, 1999). However, *A. linnaei* can survive several years under fully marine salinities in culture (Boedeker, unpublished data). Since *A. linnaei* disperses via fragmentation, the absence from higher salinities cannot be explained by lower salinity tolerances of spores or gametes. Accordingly, the physiological niche of *A. linnaei* is much broader than its realized niche, and increased biotic interactions might cause the absence of this species in the Baltic Sea in areas with higher salinities than 6 psu. This distribution suggests that *A. linnaei* is a poor competitor and can only establish in areas with low biotic interactions. Similar to *A. linnaei*, it has been shown in culture that the closely related genus *Pithophora* can cope with salinities of up to 20 psu (Mothes 1930), but has only been encountered in brackish environments on very few occasions, possibly washed in from rivers or floodings. *Pithophora* is primarily found in nutrient-rich, stagnant water bodies in the (sub)tropics. These environments are characterized by a less diverse algal flora than similar habitats in the temperate zones due to low oxygen levels in the water (e.g. Fritsch 1907a & b). It appears that the more derived members of the Pithophoraceae have retained a high osmoacclimation potential (the desiccation potential of *Arnoldiella* emend. is physiologically comparable), that has not been lost when the marine or brackish ancestors colonized less saline or even pure freshwater environments (Fig. 7).

Heterotrichy as a morphological adaptation to harsh conditions

In addition to the physiological features discussed above, the heterotrichous growth form of *Wittrockiella* and *Arnoldiella* emend. also represents an adaptation to harsh conditions.

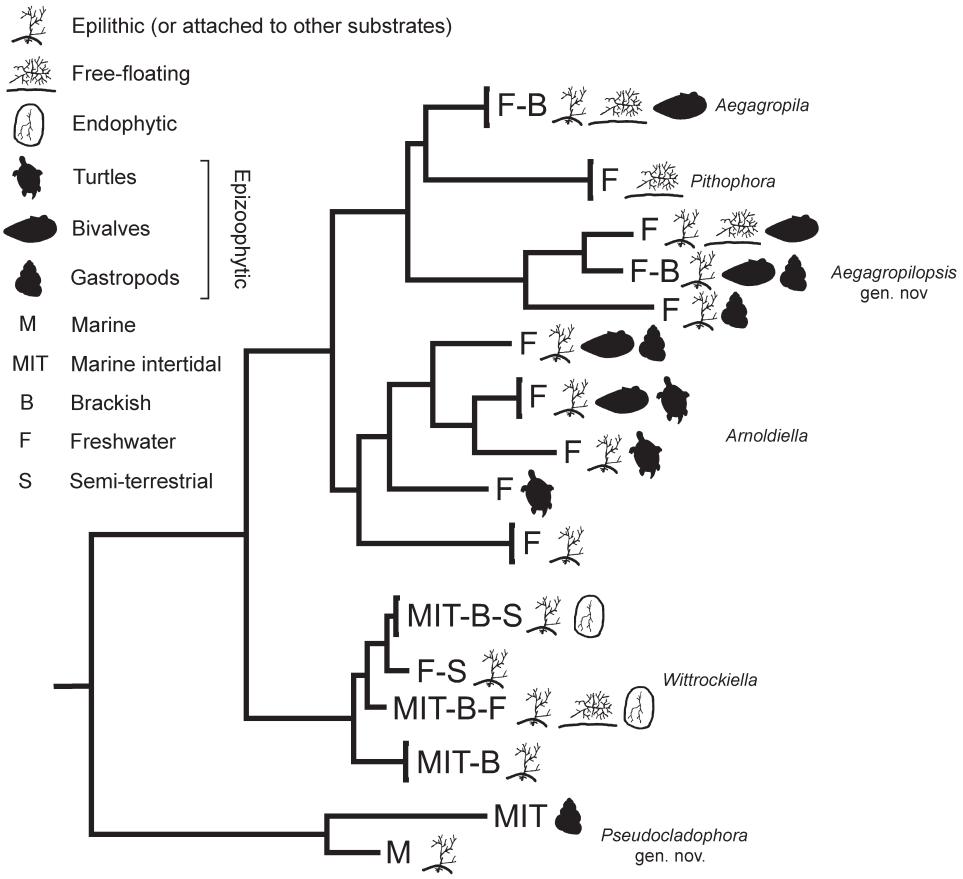

Figure 7. Phylogenetic tree of the *Aegagropila*-lineage (same as Fig. 4B), with letters indicating environment types in which the taxa occur, and stylised symbols indicating known host animals of epizoophytic taxa and growth forms.

Heterotrichy was defined by Fritsch (1935) as a prostrate and an erect system forming the (algal) thallus.

A heterotrichous growth form is generally considered of very significant adaptice importance, and is widespread in frequently disturbed habitats such as the marine intertidal zone (Fritsch 1935, Littler & Kauker 1984). The heterotrichous habit of the aerophytic green algal order Trentepohliales can maintain itself successfully in the extreme terrestrial environment (Fritsch 1945). Fritsch (1944) regarded the heterotrichous habit of *Wittrockiella calcicola* as a direct adaptation to growth on semi-terrestrial rock surfaces. The prostrate system is generally more sheltered from desiccation and abrasion (Seapy & Littler 1982, Littler & Littler 1983, Littler & Kauker 1984). Sand abrasion could be one factor that prevents other algal taxa to establish themselves on freshwater bilvalve shells or the carapaces of burrowing turtles (e.g. Miller 1928, Ducker 1958). Of all species in the Pithophoraceae, *A. conchophila* has the most reduced erect system, which might be either an adaptation to the movements of the host bivalves (*Anodonta* sp. and *Unio* sp.) in and

out of the sediment, or an artefact created by the constant abrasion of the developing erect system due to those movements.

Furthermore, the development of a prostrate system allows for retention of space during disturbances and fast recovery from disturbances. It has been shown that *A. ramulosa* perennates as the prostrate system during the hibernation of the host turtle on parts of the carapace that is covered with mud (Ducker 1958). The rhizoids of *Pseudocladophora conchopheria* penetrate the shell of the host gastropod (Matsuyama *et al*. 1999), possibly allowing for regrowth after the loss of the erect filaments. A common feature for heterotrichous species or life-stages is the tendency to reproduce vegetatively (Fritsch 1953, Perrone & Felicini 1988). Prostrate systems typically grow by apical cell divisions and spread outwards, so that a stolon-like growth pattern can develop and subsequently result in fragmentation and vegetative propagation. The frequent secondary rhizoids observed in the Pithophoraceae probably facilitate attachment after fragmentation events. Freshwater representatives of predominantly marine groups often have truncated life histories (Raven 1999). This and the heterotrichous lifestyle in disturbed habitats might explain why sexual reproduction has been observed in so few instances and so few species in the Pithophoraceae.

Our phylogeny indicates that a primary holdfast, present in the earliest diverging genus *Pseudocladophora*, has been lost in *Wittrockiella* and *Arnoldiella* and secondarily gained in *Aegagropila* and *Aegagropilopsis*. Members of the *Aegagropila*/*Pithophora*-clade are commonly found in quiet waters with less fluctuating environmental conditions, which might have led to the loss of the prostrate system. *Aegagropila linnaei* is even frequently encountered as unattached growth forms, and *Pithophora* is exclusively unattached (Fig. 7). In this context, the Chinese freshwater species *Cladophora rhizobrachialis* Jao is interesting as it displays an intermediate morphology between a prostrate and an erect growth form (Jao 1944). The thallus consists of a main upright filament that is accompanied by several elongate branches arising from nearly every cell of the basal portion, and these branches produce long rhizoids from the basal parts of most cells.

In addition to the colonization of highly specialised niches and the restriction to habitats with reduced competition, the Pithophoraceae represent a fascinating example of a transition from marine (*Okellya curvata* and most other Cladophorales, *Pseudocladophora*) to marine-brackish (*Wittrockiella*) to freshwater organisms (rest of the Pithophoraceae), with some of the most derived taxa penetrating secondarily into brackish habitats again (*Aegagropila linnaei*, *Aegagropilopsis clavuligera*) (Fig. 7). The Cladophorales are part of the essentially marine, species-rich BCD-clade (Bryopsidales-Cladophorales-Dasycladales; Cocquyt *et al*. 2010), and marine-freshwater transitions have only occurred in the Cladophorales: once in the Pithophoraceae, and once in the *Cladophora*-clade (*Cladophora glomerata*-complex and *Rhizoclonium riparium*-complex). One of the earliest diverging members of the Pithophoraceae, *Pseudocladophora horii*, occurs in marine intertidal environments that do not appear to represent a specialised niche or to be characterized by particularly low biotic interactions. However, the physiological capacity for osmoacclimatization is apparently present at the base of the tree and seems to have been retained by all taxa of the Pithophoraceae. The sister species of *P. horii*, *P. conchopheria*, is restricted to a highly specialised niche that seems to be almost competition-free. Thus, the ancestor of the rest of the Pithophoraceae might have evolved a heterotrichous habit

and have thrived in estuarine or intertidal environments and subsequently diversified into a brackish lineage (*Wittrockiella*) and a mainly epizoophytic freshwater lineage. Since selection generally operates on suites of traits, changes in morphology are often accompanied by changes in physiology (Pfennig *et al*. 2010). Phenotypic plasticity, very pronounced in the Cladophorales, can facilitate adaptive responses and colonization of novel environments (Pfennig *et al*. 2010), while at the same time phenotypic diversification is promoted by ecological opportunity (Yoder *et al*. 2010).

In many groups of organisms the boundary between marine and freshwater environments is crossed infrequently or not at all, and marine and freshwater taxa are normally not closely related (Round & Sims 1981, see also Logares *et al*. 2007 for additional references). In general, marine-freshwater transitions are rare events in algae. Among the algae, the marine-freshwater interface acts as an effective barrier for most groups of diatoms (Mann 1996), dinoflagellates (Logares *et al*. 2007), and transitions from one realm into the other are rare among Phaeophyceae (e.g., Wehr 2003), Bangiales (e.g., Nelson et al. 2006), trebouxiophytes (Henley *et al*. 2004), cryptophytes (Shalchian-Tabrizi *et al*. 2008) and goniomonads (von der Heyden *et al*. 2004). Differences in the biogeophysical conditions between marine and freshwaters are thought to constitute the blockade for cross-colonization, even though physiological adaptations that facilitate these transitions remain largely unknown. The marine-freshwater boundary represents a selective rather than a physical barrier. Obstacles to evolutionary migration across the salinity spectrum can be due to difficulties in access and dispersal, lack of physiological adaptability, or inability to complete the life cycle (Mann 1996). These do not appear to strongly apply to the brackish and freshwater Pithophoraceae, as their ancestor most likely has been physiologically pre-adapted to intertidal conditions and possibly to an epizoophytic lifestyle. Freshwater taxa of mainly marine groups often have truncated life histories (Raven 1999). Possibly the ability of the Pithophoraceae to reproduce vegetatively enabled them to persist in the novel environments despite disruption of the life cycle. One additional factor that can play a role in making the marine-freshwater interface difficult to cross could be competitive exclusion by adapted residents. Colonizing specialised niches with reduced competition might have enabled the Pithophoraceae to circumvent the barrier and successfully establish lineages in brackish and freshwater environments.

The Pithophoraceae is most likely very old, however, fossils and precisely dated phylogenies are missing. The split of *Cladophora*-clade and the *Siphonocladus*-clade can be deduced at around 200 mya (Cocquyt *et al*. 2010), considering that the siphonous orders are about 600 my old (Verbruggen et al 2009). Based on those estimates, the age of the *Aegagropila-clade* is at least 200 my old. Changes in sea levels during the last 250 my flooded large continental areas (Haq *et al*. 1987), which could have promoted the early invasion of estuarine and continental waters by the Pithophoraceae. Similar scenarios have been proposed for the evolution of freshwater lineages in the diatoms (Sims *et al*. 2006) and the dinoflagellates (Logares *et al*. 2007). The evolution and timing of habitat preferences invites for interesting speculation with regards to the beginning diversification of mollusks starting around 500 mya (Levin 1999) and of turtles starting around 200 mya (Gaffney *et al*. 1987). Early turtles were marsh dwellers, the highly aquatic and terrestrial forms are secondarily derived (Edgren *et al*. 1953), so maybe the common ancestor of the brackish genus *Wittrockiella* and of *Arnoldiella* evolved around that time in brackish

estuaries and saltmarshes.

Taxon sampling and diversity of the Pithophoraceae

The whole group must be regarded as undersampled, partly due to their unobtrusive habit, smallness in size, misidentification (e.g. as *Cladophora* spp.), and their occurrence in unusual habitats not regularly targeted in algal surveys. A general problem in inferring local diversity or species distributions is the detectability of the species in question, which leads to the reconstruction of apparent rather than real diversity or distributional range (Kéry *et al*. 2010). While basically nothing is known about the occurrence of members of the Pithophoraceae in Africa and South America, it seems likely that also in Europe interesting discoveries could be made with regard to the systematic position of many algal species of unknown affiliation (see Table 5). Based on the current sampling, the diversity of the Pithophoraceae appears to be highest in Asia (about 75% of the known taxa), and particularly high in Japan (about 50% of the known taxa). Several enigmatic species have not been sampled for molecular analyses but are assumed to shed light on issues such as thallus evolution (e.g., *Chaetonella goetzei* Schmidle, *Cladophora basicladioides* Jao, *Cladophora cornuta* Brand, *Cladophora rhizobrachialis*, *Cladostroma setschwanense* Skuja), niche evolution (*Basicladia vivipara*, *Cladogonium ogishimae* Hirose & Akiyama – epizoophytic on freshwater shrimps), (historical) biogeography (e.g. the Australian *Cladophora parvula*; or the taxa from Lake Baikal, see below). A group of morphologically closely related species and potential members of the genus *Aegagropila* (and of *Arnoldiella*) are some endemic cladophoralean species from ancient Lake Baikal, Russia (see Bourrelly 1966, Izhboldina 2007). *Aegagropila linnaei* or its ancestor is assumed to have dispersed throughout the Palaearctic (or the Holarctic) from Central or East Asia (Boedeker *et al*. 2010b). A similar scenario has also been proposed for several freshwater animals found as glacial relicts in Fennoscandian lakes and the brackish parts of the Baltic Sea as well as in some scattered Siberian locations, with ancestors in Lake Baikal (Segerstråle, 1962). Molecular data for the morphological relatives of *A. linnaei* from Lake Baikal would strongly add to our understanding of the biogeographic patterns and age of the fascinating Pithophoraceae.

Table 5. Inquirendae: list of genera and species that are potential members of the Pithophoraceae (= *Aegagropila*-clade) based on morphology and habitat, or based on a small subunit rDNA sequences in the case of *Spongiochrysis hawaiiensis*.

'Aegagropila' repens var. *antarctica* Gain, 1912

Bolbocoelon jolyi Yamaguishi-Tomita 1970

Chaetocladiella Meyer & Skabichevsky, 1968 (3 species)
- *C. microscopica* (Meyer) Meyer & Skabichevsky, 1968
- *C. pumila* (Meyer) Meyer & Skabichevsky, 1968
- *C. litoralis* (Skabichevsky) Meyer & Skabichevsky, 1968

Chaetomorpha baicalensis Meyer, 1922
Chaetomorpha curta (Skabichevsky) Skabichevsky, 1969
Chaetomorpha moniliformis Skabichevsky, 1936
Chaetomorpha solitaria Skabichevsky, 1931

Chaetonella Schmidle, 1901 (monotypic)
C. goetzei Schmidle, 1901

Cladogonium Hirose & Akiyama, 1971 (monotypic)
C. ogishimae Hirose & Akiyama, 1971

Cladophora aegagropiloidea Hoek & Womersley, 1984
Cladophora alpina Brand, 1899
Cladophora basicladioides Jao, 1947
Cladophora beneckei Möbius, 1893
Cladophora codiola Zeller, 1873
Cladophora compacta (Meyer) Skabichevsky, 1976
Cladophora contorta Zeller, 1873
Cladophora cornuta Brand, 1895
Cladophora dusenii Brand, 1902
Cladophora exigua Zeller, 1873
Cladophora floccosa Meyer, 1927 - 2 varieties
Cladophora globulus (Meyer) Skabichevsky, 1976
Cladophora glomerata var. *nana* Wang, 1935
Cladophora humida Brand, 1913
Cladophora intertexta Collins 1901
Cladophora koktschetavensis Sviridenko, 1995
Cladophora kozhowii Zagorenko & Izhboldina, 1977
Cladophora kursanovii (Meyer) Skabichevsky, 1976
Cladophora kusnetzowii Meyer, 1930
Cladophora mamillata Leliaert 2005
Cladophora meyerii (Meyer) Skabichevsky, 1976 – 2 varieties
Cladophora pachyderma (Kjellman) Brand, 1909
Cladophora parvula Möbius, 1895
Cladophora pithophoroides Phinney 1945
Cladophora pulvinata (Meyer) Skabichevsky, 1976
Cladophora rhizobrachialis Jao, 1944
Cladophora shensiensis Jao, 1948
Cladophora yuennanensis Skuja, 1937

Cladophorella fritschii Islam, 1964
Cladophorella netzhualpilii C. Galicia-García & E. Novelo, 2000
Cladophorella sundarbanensis Islam, 1964

Cladostroma Skuja, 1937 (monotypic)
C. setschwanense Skuja, 1937

Dermatophyton Peter, 1886 (monotypic)
D. radians Peter, 1886

Gemmiphora Skabichevsky, 1931 (monotypic)
G. compacta Skabichevsky, 1931

Rhizoclonium lapponicum Brand, 1913

Spongiochrysis Rindi, López-Bautista, Sherwood & Guiry, 2006 (monotypic)
S. hawaiiensis Rindi *et al.* 2006

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