

## MOLECULAR PHYLOGENY AND TAXONOMY OF THE *AEGAGROPILA* CLADE (CLADOPHORALES, ULVOPHYCEAE), INCLUDING THE DESCRIPTION OF *AEGAGROPILOPSIS* GEN. NOV. AND *PSEUDOCLADOPHORA* GEN. NOV.<sup>1</sup>

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The *Aegagropila* clade represents a unique group of cladophorean green algae occurring mainly in brackish and freshwater environments. The clade is sister to the species-rich and 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 a taxonomic reassessment. One lineage consists of two marine '*Cladophora*' species, for which the new genus *Pseudocladophora* and the new family Pseudocladophoraceae are proposed. For the other lineages, the family name Pithophoraceae is reinstated. Within the Pithophoraceae, the earliest diverging lineage includes *Wittrockiella* and *Cladophorella calcicola*, occurring mainly in brackish and sub-aerial habitats. The two other lineages are restricted to freshwater. One of them shows a strong tendency for epizoisism, and consists of *Basicladia* species and *Arnoldiella conchophila*. The other lineage includes *Aegagropila*, *Pithophora* and a small number of tropical '*Cladophora*' species. The latter are transferred to the new genus *Aegagropilopsis*. Previously, polypyramidal pyrenoids had been suggested to be apomorphic for this clade, but we report the finding of both polypyramidal and bilenticular pyrenoids in members of the Pithophoraceae, and thus show that this character has no diagnostic value.

**Key index words:** *Arnoldiella*; *Basicladia*; *Cladophorella*; freshwater; molecular phylogenetics; Pithophoraceae; Pseudocladophoraceae; ribosomal DNA; systematics; *Wittrockiella*

**Abbreviations:** bp, base pairs; BI, Bayesian inference; ML, maximum likelihood; MP, maximum parsimony; s.s., sensu stricto

The Cladophorales is a species-rich order of ulvophycean green algae with a siphonocladous organization that is widespread in marine and freshwater environments from tropical to polar regions. The Cladophorales have evolved in three main clades (Fig. 1, Hanyuda et al. 2002, Leliaert et al. 2003, Yoshii et al. 2004, Leliaert et al. 2012). The *Siphonocladus* clade (=Siphonocladales s.s.) is morphologically diverse and mainly distributed in marine tropical waters (Leliaert et al. 2007). Members of the *Cladophora* clade (=Cladophorales s.s. or Cladophoraceae sensu Wille) are morphologically simpler, consisting of branched or unbranched filaments, and have a distribution that extends into cold temperate and polar waters. The *Aegagropila* clade is sister to the *Cladophora* and *Siphonocladus* clades, and has not yet received a formal taxonomic rank, despite robustness in previous phylogenetic studies (Hanyuda et al. 2002, Yoshii et al. 2004). A fourth lineage, Okellyaceae, is sister to the three main clades (Fig. 1) and only includes *Okellya curvata*, an unbranched, microfilamentous species occurring in temperate marine subtidal habitats (Leliaert et al. 2009a).

The *Aegagropila* clade encompasses six species-poor or monotypic genera and a small number of freshwater *Cladophora* species (Hanyuda et al. 2002, Yoshii et al. 2004, Rindi et al. 2006). Hanyuda et al. (2002) suggested that the presence of loraxanthin, chitin and polypyramidal pyrenoids are diagnostic for the *Aegagropila* clade. Although molecular evidence clearly indicates the polyphyletic nature of the genus *Cladophora*, only few nomenclatural changes

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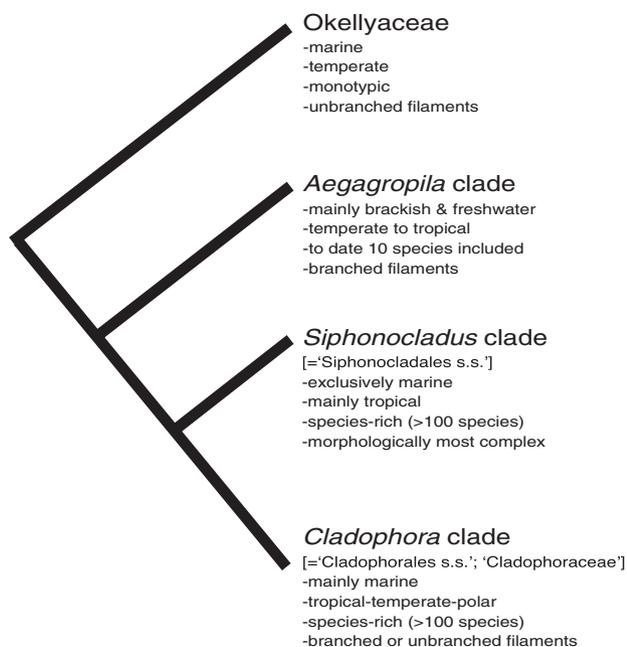


FIG. 1. Schematic phylogenetic tree of the Cladophorales with gross information on habitat, species numbers, and morphology for the four main lineages.

have been proposed, namely the recent transfer of *C. kosteræ* C. Hoek and *C. okamuræ* (Ueda) C. Hoek to the genus *Basycladia* (Garbary 2010) and the transfer of *C. amphibia* Collins to *Wittrockiella* (Boedeker and Hansen 2010). Species of *Cladophora* are (still) distributed in the three main clades of the Cladophorales (Fig. 1). The lectotype species of *Cladophora* is *C. oligoclona* (Setchell and Gardner 1920, van den Hoek 1963), a synonym of *C. rivularis*, which is a member of the *Cladophora* clade (Leliaert and Boedeker 2007). Thus, taxonomic changes of *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.

The *Aegagropila* clade is interesting from both an evolutionary and ecological perspective. It constitutes a mainly freshwater/brackish lineage within the predominantly marine Cladophorales. Several members occur in highly specialized niches such as on the carapaces of freshwater turtles (some members of *Basycladia*), on freshwater snails and bivalves (monotypic *Arnoldiella* and several *Basycladia* species), as epi- or endophytes of salt marsh plants and mangrove pneumatophores (some members of *Wittrockiella*), or on marine intertidal snails (*Cladophora conchophoria* Sakai). Recently, aerophytic unicells occurring on tree bark have been described and included in this lineage based on DNA sequence data (*Spongiochrysis hawaiiensis*, Rindi et al. 2006).

The majority of the species in the *Aegagropila* clade occurs in freshwater environments. The genus *Wittrockiella* grows in brackish habitats, and *Cladophora horii* C. Hoek (Fig. 2A) and *C. conchophoria* (Fig. 2B) are the only marine species. *Wittrockiella* currently encompasses three species, *W. lyallii* (Harv.) C. Hoek, Ducker et Womersley (Fig. 2C), *W. salina* V.J. Chapm. and *W. amphibia* (Collins) Boedeker et G.I. Hansen (Fig. 2F). In *Wittrockiella*, creeping prostrate axes give rise to upright, unbranched or sparsely branched filaments. A close relationship between *W. salina* and the (sub)tropical, subaerial or freshwater species *Cladophorella calcicola* F.E. Fritsch (Fig. 2E) has been suggested by van den Hoek et al. (1984) based on overall morphological similarity. The genus *Basycladia* consists of seven species and is characterized by branched or unbranched upright filaments (Fig. 2, G, I) arising from an extensive prostrate, rhizome-like stratum consisting of coalescent, branched filaments (Fig. 2H), with a characteristic long basal cell (Fig. 2J). *Basycladia* has a reputation for occurring on freshwater turtles, but culture experiments have shown that other substrates can be colonized as well (Proctor 1958), and the recently included species *B. okamuræ* (Fig. 2, G, H) and *B. kosteræ* (Fig. 2, G, I) are actually only rarely encountered on turtles. The diminutive and poorly known *Arnoldiella conchophila* V.V. Mill. has been found on shells of freshwater bivalves (Miller 1928), freshwater gastropods (Kargupta 1994, Keshri and Hazra 2009) and a range of other substrates (Cox Downing 1970). *Pithophora* Wittr. is easily recognized by the characteristic akinetes (Fig. 2, K, L) and includes more than 35 taxa, but this number is likely inflated due to plastic morphological characters (Ernst 1908, Mothes 1930, Fott 1971, Pankow and Täuscher 1980). This mainly (sub)tropical freshwater genus only occurs unattached in relatively stagnant, nutrient-rich waters and can form extensive floating masses that can be local nuisances (Entwisle and Price 1992, Lembi 2003). The freshwater species *Aegagropila linnaei* Kütz. (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 have been identified (Boedeker et al. 2010a). *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, Niyama 1989, Boedeker et al. 2010b).

Molecular phylogenetic studies based on SSU rDNA sequence data provided the first insights into the relationships within the *Aegagropila* clade (Hanyuda et al. 2002, Yoshii et al. 2004, Rindi et al. 2006). These studies showed that *Cladophora conchophoria* and *C. horii* form a sister clade to the rest of the *Aegagropila* clade. The relationships within that clade were only partly resolved. Surprisingly, the

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 <i>Cladophora</i> species that are members of the <i>Aegagropila</i> clade			reference for placement in <i>Aegagropila</i> clade
<i>Cladophora</i> species	<i>Cladophora</i> section/subgenus	current name	
<i>Cladophora horii</i> C. Hoek et Chihara 2000	section <i>Rugulosa</i> <sup>a</sup>	<i>Pseudocladophora horii</i> (C. Hoek et Chihara) Boedeker, comb. nov. (this study)	Leliaert et al. 2003, Yoshii et al. 2004
<i>Cladophora conchophoria</i> Sakai 1964	section <i>Glomeratae</i> <sup>a</sup> / section <i>Opacae</i> <sup>b</sup>	<i>Pseudocladophora conchophoria</i> (Sakai) Boedeker, comb. nov. (this study)	Hanyuda et al. 2002
<i>Cladophora hosterae</i> C. Hoek 1963	section <i>Basicaldi</i> <sup>c</sup>	<i>Arnoldiella hosterae</i> (C. Hoek) Boedeker, comb. nov. (this study)	Yoshii et al. 2004
<i>Cladophora okamurae</i> (Ueda) C. Hoek 1963	section <i>Basicaldi</i> <sup>c</sup>	<i>Basicaldia okamurae</i> (Ueda) Carbarby 2010	Hanyuda et al. 2002 (as <i>Chaetomorpha okamurae</i> ) Hanyuda et al. 2002
<i>Cladophora aegagropila</i> (L.) Rabenh. 1868	section <i>Aegagropila</i> <sup>c</sup> / subgenus <i>Aegagropila</i> <sup>b</sup>	<i>Aegagropila linnaei</i> Kütz. 1843	Hanyuda et al. 2002, Yoshii et al. 2004
<i>Cladophora</i> sp. ('Tateyama-Marimo')	/	undescribed species	this study
<i>Cladophora clavuligera</i> Grunow 1868	/	<i>Aegagropilopsis clavuligera</i> (Grunow) Boedeker, comb. nov. (this study)	this study
<i>Cladophora sterocladia</i> Skuja 1949	/	<i>Aegagropilopsis sterocladia</i> (Skuja) Boedeker, comb. nov. (this study)	Hanyuda et al. 2002
<i>Cladophora byallii</i> Harv.	/	<i>Witrockiella byallii</i> (Harvey) C. Hoek, Duckert et Womersley 1984	Yoshii et al. 2004 (as <i>W. paradoxa</i> )
<i>Cladophora amphibia</i> Collins 1907	/	<i>Witrockiella amphibia</i> (Collins) Boedeker et G.I. Hansen 2010	Hanyuda et al. 2002 (for the genus <i>Pithophora</i> )
<i>Cladophora oedogonia</i> (Mont.) Mont. 1856	/	<i>Pithophora oedogonia</i> (Mont.) Witt. 1877 <sup>d</sup>	Hanyuda et al. 2002 (for the genus <i>Pithophora</i> )
<i>Cladophora roettleri</i> (Roth) Kütz. 1849	/	<i>Pithophora roettleri</i> (Roth) Witt. 1877 <sup>d</sup>	Hanyuda et al. 2002 (for the genus <i>Pithophora</i> )
Candidate members of the <i>Aegagropila</i> clade that were shown by molecular data to have different affiliations			
<i>Cladophora</i> species	<i>Cladophora</i> section/subgenus	systematic placement	Reference
<i>Cladophora catenata</i> (L.) Kütz. 1843	section <i>Aegagropila</i> <sup>c</sup> / subgenus <i>Aegagropila</i> <sup>b, f</sup>	<i>Siphonocladus</i> clade	Hanyuda et al. 2002, Leliaert et al. 2007
<i>Cladophora echinus</i> (Biasol.) Kütz. 1849	section <i>Aegagropila</i> <sup>c</sup>	<i>Cladophora</i> clade	Leliaert et al. 2009b
<i>Cladophora patentiramea</i> (Mont.) Kütz. <sup>g</sup>	subgenus <i>Aegagropila</i> <sup>b</sup>	<i>Siphonocladus</i> clade <sup>g</sup>	Hanyuda et al. 2002, Leliaert et al. 2007
<i>Cladophora sibogae</i> Reinbold	subgenus <i>Aegagropila</i> <sup>b</sup>	<i>Siphonocladus</i> clade	Leliaert et al. 2003, Leliaert et al. 2007
<i>Cladophora socialis</i> Kütz.	subgenus <i>Aegagropila</i> <sup>b</sup>	<i>Siphonocladus</i> clade	Hanyuda et al. 2002, Leliaert et al. 2007
<i>Cladophora batterisii</i> C. Hoek 1963	section <i>Ruprestres</i> <sup>c</sup>	<i>Cladophora</i> clade	Leliaert et al. 2009b
<i>Cladophora pygmaea</i> Reinke 1888	section <i>Chamaethamnion</i> <sup>c</sup>	<i>Cladophora</i> clade	Leliaert et al. 2009b
'classic' freshwater <i>Cladophora</i> species <sup>h</sup>	sections <i>Glomeratae</i> & <i>Cladophora</i> <sup>c</sup>	<i>Cladophora</i> clade	Marks and Cummings 1996, Hanyuda et al. 2002

<sup>a</sup>van den Hoek and Chihara 2000.

<sup>b</sup>Sakai 1964.

<sup>c</sup>van den Hoek 1963.

<sup>d</sup>Pankow and Tauscher (1980) reduced *Pithophora* to two species, however disagreement over the number of species exists. Originally, Wittrock (1877) had also transferred *Cladophora acrosperma* Kütz., *C. sumatrana* G. Martens and *C. zelleri* G. Martens to *Pithophora* in addition to the two species listed here.

<sup>e</sup>van den Hoek 1982.

<sup>f</sup>As *C. fuliginosa* Kütz.

<sup>g</sup>Morphologically indistinguishable from *C. coelothrix* Kütz. (see van den Hoek and Chihara 2000).

<sup>h</sup>*C. fracta* (O.F. Müller ex Vahl) Kütz., *C. globulina* (Kütz.) Kütz., *C. glomerata* (L.) Kütz., *C. rivularis* (L.) C. Hoek.



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

aerophytic unicellular alga *Spongiochrysis hawaiiensis* was recovered as a member of the *Aegagropila* clade, although the exact phylogenetic position remained equivocal (Rindi et al. 2006).

This study aimed to gain insight into the evolutionary relationships in the *Aegagropila* clade and to reassess the taxonomy of the group. To this end, we extended the previous phylogenies by increasing taxon sampling and by combining SSU and LSU sequences into a partitioned dataset, which has been shown to lead to better resolved phylogenies in the Cladophorales and other groups (Murray et al. 2005, Feau et al. 2006, Leliaert et al. 2007). The validity of pyrenoid ultrastructure as a diagnostic character for the lineage was tested by examining a wide range of taxa.

#### MATERIALS AND METHODS

*Taxon sampling and morphological analysis.* Forty-two specimens from the *Aegagropila* clade were sampled in various habitats from a broad geographical range (Appendix S1, see Supplementary Materials). The type species of *Aegagropila*

(*A. linnaei*), *Arnoldiella* (*A. conchophila*), *Cladophorella* (*C. calcicola*), and *Wittrockiella* (*W. amphibia*) have been included in the phylogenetic analyses. Wittrock (1877) did not designate a type species for *Pithophora*, the sole genus included in the Pithophoraceae at its inception, and we have found no prior lectotypification. We here select *P. kewensis* Wittr. as the type species (isolecotypes in BM (incl. K) and L, we choose the specimen from L (no. 938112 639) as the lectotype) for the genus *Pithophora*, 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. The type species of *Basicladia* is *B. crassa* W.E. Hoffm. et Tilden (Hoffmann and Tilden 1930), but no sequence data are available for this species. However, *B. crassa* is morphologically very similar to *B. chelonum* (Collins) W.E. Hoffm. et Tilden, 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 *Basycladia* 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 showed a close affinity with *Cladophora horii* and *C. conchophoria*. However, our LSU sequence indicated a close relationship with the Trentepohliales. 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 *Basycladia* 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 SSU rDNA sequences of the same cultures differed by 10–20 bp. *Cladophora clavuligera* Grunow and *C. sterrocladia* Skuja were also included based on our 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, preserved in formalin or reconstituted from herbarium material, were examined with an Olympus SZX10 stereomicroscope and an 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). The pyrenoid ultrastructure of a several species was examined by TEM, following the method described in Nitschke et al. (2010). Morphological and ultrastructural investigations were performed on specimens for which sequence data were available (Appendix S1).

**Molecular phylogenetic analyses.** The specimens used in the phylogenetic analyses are listed in Appendix S1 (members of the *Aegagropila* clade) and Appendix S2 (see supplementary materials; other Cladophorales and outgroups). A total of 63 specimens were analyzed, including 42 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, and both genes were combined in a partitioned alignment. DNA extraction, PCR amplification, and sequencing were performed as in Boedeker and 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. 2007). Obtained sequences have been deposited in GenBank (Appendix S1, Appendix S2). Identical sequences (see Appendix S1) were excluded from the analysis. Sequences were aligned using MUSCLE (Edgar 2004) and subsequently edited by eye in Se-Al v2.0a11 (Rambaut 2007). Two short hypervariable regions of the LSU alignment (32 bp in total) were ambiguous and excluded from all analyses. Evolutionary models of nucleotide substitution were determined by the Akaike Information Criterion for the LSU and SSU alignments in PAUP/MrModeltest2 v2.3 (Swofford 2002, Nylander 2004) and in Treefinder (Jobb 2011). Uncorrected pairwise distances for the members of the *Aegagropila* clade were calculated in PAUP. The  $I_{SS}$  statistic, a measure of substitution saturation in molecular phylogenetic datasets, was calculated with DAMBE (Xia and Xie 2001).

Initially, the LSU (36 sequences) and SSU (38 sequences) datasets were analyzed 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 the results indicated that the SSU and the partial LSU rDNA data were not significantly heterogeneous ( $P = 0.57$ ). The two genes were then combined into a concatenated alignment with two additional outgroups, which was subsequently used for all analyses.

The concatenated alignment contained two outgroups (*Ulva fasciata* and *Trentepohlia* sp.) and 36 cladophoralean sequences in total. This alignment was created to establish whether the *Aegagropila* clade represents a monophyletic group, to infer the position of the root of the *Aegagropila* clade, and to gain insight into the phylogenetic relationships within this clade. The dataset was analyzed with maximum likelihood (ML) and Bayesian inference (BI) methodologies. Because the model parameters (rate matrix, gamma shape, and proportion of invariant sites) differ strongly between LSU and SSU alignments (Table 2), the dataset was partitioned into LSU and SSU, applying unlinked models to each partition.

ML analyses were performed using TreeFinder (Jobb 2011) with 1000 nonparametric bootstrap replicates (Felsenstein 1985). BI was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Analyses consisted of two independent, simultaneous runs of one cold and three incrementally heated chains, and  $3 \times 10^6$  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 split 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 and Drummond 2007) and a burnin sample of 4,000 trees was removed before calculating the majority rule consensus trees in MrBayes.

To evaluate alternative topologies regarding the monophyly of the *Aegagropila* clade, two constrained trees were generated. In the first constrained tree *Cladophora horii* and *C. conchophoria* (= *horii* clade), and the *Cladophora* and *Siphonocladus* clades formed a monophyletic group, in the second constrained tree, the *horii* clade was sister to a monophyletic group consisting of the *Cladophora* and *Siphonocladus* clades and the rest of the *Aegagropila* clade. These two topologies were compared with the result of our phylogenetic analysis by performing approximately unbiased (AU) tests (Shimodaira 2002) and Shimodaira–Hasegawa (SH; Shimodaira and Hasegawa 1999) tests. For this, site-specific likelihoods for the unconstrained and the two constrained trees were calculated in PAML (Yang 2007), and subsequently used as inputs in CONSEL (Shimodaira and Hasegawa 2001). All bioinformatic analyses except those involving PAUP and CONSEL were carried out on the freely available Bioportal (<http://www.bioportal.uio.no>).

## RESULTS

**Datasets and alignments.** Details of the separate and concatenated 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 2. No significant saturation was detected in either the SSU or the partial LSU data, based on the  $I_{SS}$  statistic (Xia and Xie 2001).

**Phylogenetic analyses.** BI analyses of the separate SSU and LSU datasets yielded poorly resolved trees (not shown). Topological differences between the

TABLE 2. Specification of datasets, summary of models and model parameters obtained.

Alignment	LSU	SSU	LSU-SSU
Taxa	36 34	38 36	38 36
Cladophoralean taxa			
Outgroup taxa	2 ( <i>Ulva fasciata</i> , <i>Trentepohlia</i> sp.)	2 ( <i>Ulva fasciata</i> , <i>Trentepohlia</i> sp.)	2 ( <i>Ulva fasciata</i> , <i>Trentepohlia</i> sp.)
Alignment length/analyzed length	656/624	1708/1708	2363/2331
Variable sites/parsimony informative sites	286/242	456/303	746/543
Model estimated <sup>a</sup>	GTR + I + G	GTR + I + G	GTR + I + G
Estimated base frequencies (A/C/G/T)	0.20/0.26/0.33/0.21	0.25/0.22/0.28/0.25	0.24/0.23/0.29/0.24 <sup>c</sup>
Estimated substitution frequencies (AC/AG/AT/CG/CT/GT) <sup>a</sup>	0.85/2.95/1.54/0.80/5.98/1	1.27/2.90/2.11/0.77/6.90/1	1.15/3.17/1.83/0.97/6.42/1 <sup>c</sup>
Among-site variation I/G <sup>b</sup>	0.27/0.52	0.51/0.43	0.42/0.37 <sup>c</sup>

<sup>a</sup>Estimated by Akaike Information Criterion (AIC).

<sup>b</sup>Proportion of invariable sites (I) and gamma distribution shape parameter (G) as estimated in PAUP/MrModeltest.

<sup>c</sup>The parameters estimated for the concatenated LSU-SSU dataset were not used in the phylogenetic analysis, instead the individual parameters estimated for the LSU and SSU datasets were used in a partitioned analysis of the LSU-SSU alignment.

two trees were not supported. The combined SSU-LSU analyses were found to perform better in terms of resolution than the separate SSU and LSU analyses. Analysis of the concatenated, partitioned alignment recovered four main lineages within the Cladophorales: the *Cladophora* clade, *Siphonocladus* clade, the *Aegagropila* clade and the Okellyaceae (Fig. 3A; log likelihood: -12969.811). The root of the Cladophorales was placed on the branch separating the Okellyaceae from the rest of the Cladophorales (Fig. 3A). The *Aegagropila* clade only received moderate support (BS: 79, PP: 0.93) (Fig. 3B). *Cladophora horii* and *C. conchophoria* (*horii* clade) represent a highly supported lineage (Fig. 3B). The SH and AU tests showed that the two alternative topologies with the *horii* clade either as sister to the *Cladophora-Siphonocladus* clade (log likelihood: -12972.127), or as sister to the *Cladophora-Siphonocladus* clade and the rest of the *Aegagropila* clade (log likelihood: -12973.753), could not be rejected (all *P*-values highly insignificant).

The remainder of the *Aegagropila* clade was recovered as a well-supported monophyletic group. Within this group, the *Wittrockiella* clade was well supported and consisted of all *Wittrockiella* species and *Cladophorella calcicola*. *Wittrockiella byallii* (Fig. 2C) was sister to the rest and is represented by identical sequences from populations in Chile and New Zealand. The grouping of the currently undescribed *Wittrockiella* sp. (Fig. 2D) with *C. calcicola* (Fig. 2E) and *W. amphibia* (Fig. 2F) received only moderate support. The sequences of *W. amphibia* from two locations in the north-western Pacific were identical. Branches within the *Wittrockiella* clade were short compared with the other clades. Two species of *Basicladia* (*B. okamurae* and *B. ramulosa*) did not group with the other *Basicladia* taxa, and the relationships with the clade consisting of *B. kosteriae*, *B. chelonum* and *Arnoldiella conchophila*, and the *Aegagropila/Pithophora* clade was not resolved. The four LSU sequences of *B. kosteriae* from the Netherlands and France showed no interindividual variation (Appendix S1). The LSU sequences of *B. okamurae* from the Netherlands and China were also identical. A well-supported *Aegagropila/Pithophora* clade is composed of *Aegagropila linnaei*, the genus *Pithophora* and three Asian *Cladophora* species. The relationships among the three lineages within this clade were not resolved. Among the three *Cladophora* species, *C. sterrocladia* was sister to both *C. clavuligera* and an undescribed species from Japan (*C.* sp. 'Tateyama'), and these relationships were highly supported. Within *A. linnaei*, LSU rDNA sequence variation of maximal 1 bp has been shown among specimens from a wide range of locations (Boedeker and Immers 2009). Various morphotypes of *Pithophora* form different localities (Appendix S1) had identical LSU sequences, except for specimen K97 (*P.* cf. *polymorpha* Wittrock), which had three point mutations.

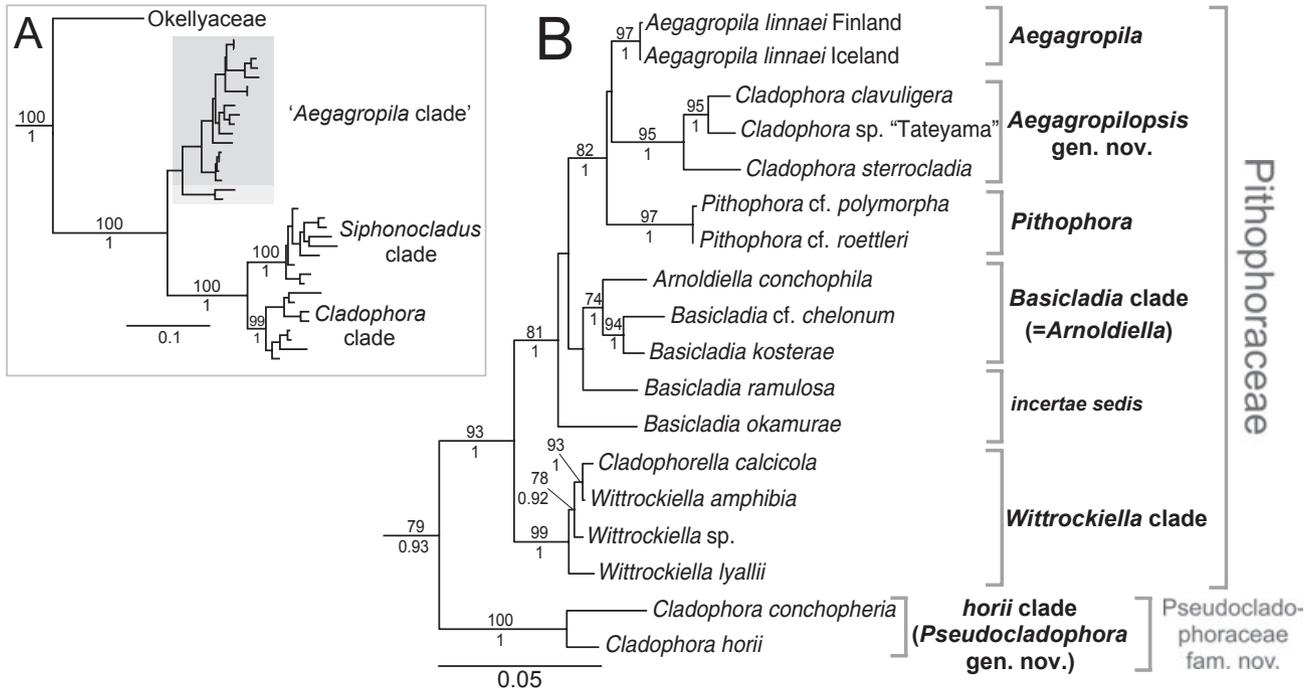


FIG. 3. (A) Maximum likelihood (ML) phylogram of the Cladophorales inferred from rDNA small subunit (SSU) and partial large subunit (LSU) sequences. ML bootstrap values (1000 replicates) are indicated above the branches, posterior probabilities (PP) from Bayesian inference (BI) are indicated below. The tree was rooted with two species from different ulvophyte families (*Trentepohlia* sp. and *Ulva fasciata*), which were removed from the phylogram for better visualization; (B) Close-up of the “Aegagropila clade” highlighted in grey in (A), with genus and family names indicated on the right.

**Morphological observations.** *Cladophora conchopheria* and *C. horii* share characteristic stem-like basal branches that are formed by descending intra- and extracuticular rhizoids that fuse with the cell walls of the cells below, leading to a polysiphonous base and very thick, stacked cell walls (Fig. 4).

The species in the *Aegagropila*/*Pithophora* clade are morphologically distinct from *Wittrockiella* and *Basicladia* by the absence of a prostrate system. *Aegagropila linnaei* and the *Cladophora* species have coralloid holdfasts, while 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 branches. The secondary rhizoids are frequently very long. There is a tendency, particularly in apical parts, for opposite (Fig. 2, N–P) or second branching (Fig. 2M). Both *Pithophora* and *Aegagropila* are characterized by frequent inversion of polarity. A unique feature of the genus *Pithophora* is the characteristic terminal or intercalary akinetes, which occur either solitary (Fig. 2K), in pairs, or in short chains (Fig. 2L). 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*

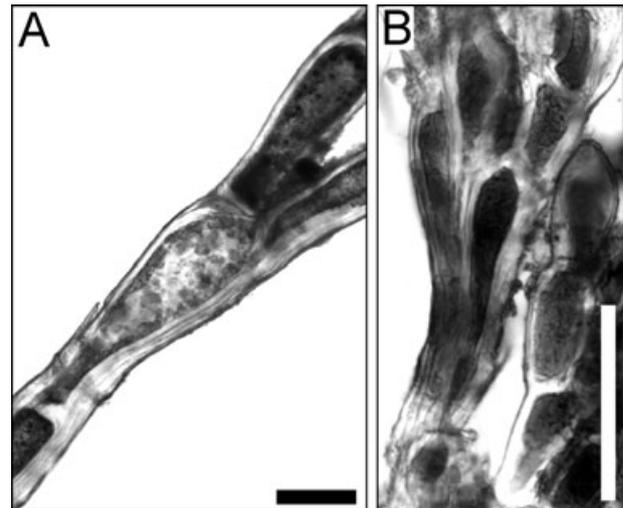


FIG. 4. Stem-like basal branches of the two members of the *horii* clade (= *Pseudocladophora* gen. nov.) formed by descending intra- and extracuticular rhizoids that have fused with the cell walls of cells below, leading to a polysiphonous base: (A) *Cladophora horii* (D78); (B) *Cladophora conchopheria* (N71). Scale bars = 100 μm.

(Fig. 2P) by several upright filaments arising from the same base and the more sparsely branched basal parts of the main axes composed of regular cylindrical cells (Fig. 2N). The basal parts of *A. linnaei* consist of irregularly shaped, thick-walled cells with 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 with up to five laterals per cell in *C. sterocladia*.

**Pyrenoid ultrastructure.** Pyrenoid ultrastructure was examined by TEM with respect to the number of thylakoid membranes transversing the pyrenoid (bilenticular versus polypyramidal structure) and the arrangement of starch plates. In the *Wittrockiella* clade, both polypyramidal and bilenticular pyrenoids were observed. All pyrenoids of *Wittrockiella* sp. (specimen B92; Fig. 5, A, B;  $n = 17$ ) were bilenticular, with the surrounding starch layer divided into two halves. The majority of pyrenoids in *Cladophorella calcicola* (specimen K92; Fig. 5C;  $n = 21$ ) 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* were found to be polypyramidal (57%), the other 43% were bilenticular (specimen N60; Fig. 5, D, E;  $n = 14$ ). All pyrenoids in *W. amphibia* displayed a small round spot of a different density than the surroundings. All pyrenoids of *Basicladia* species were polypyramidal ( $n = 64$ ), with no intraindividual or intraspecific variation in the pyrenoid structure. The surrounding starch layer was highly fragmented (Fig. 5F), with the most extreme form of segregation observed in *B. okamurae* (Fig. 5G). Pyrenoids of the genus *Pithophora* showed variation in their ultrastructure, both intraindividually and between specimens. In specimen K93 (*P. cf. roettleri* (Roth) Wittr.; Fig. 5H;  $n = 31$ ), all pyrenoids were polypyramidal with the surrounding starch layer divided into irregular pieces. In specimen K97 (*P. cf. polymorpha*), 16% of the pyrenoids were bilenticular and 84% were polypyramidal (Fig. 5I;  $n = 19$ ). Specimen K96 (*P. roettleri*) about two-thirds of the studied pyrenoids showed a polypyramidal structure (Fig. 5J;  $n = 11$ ) and about one-third of the pyrenoids were bilenticular (36%, Fig. 5K). The starch plates in this specimen were very large (Fig. 5, J, K). All pyrenoids in *A. linnaei* were polypyramidal (specimen N36; Fig. 5L;  $n = 9$ ). 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. 5, L, M).

#### DISCUSSION

The relationships inferred in this study are in overall agreement with previous phylogenies of the *Aegagropila* clade (Hanyuda et al. 2002, Yoshii et al. 2004). The main difference is that the *Aegagropila* clade was not strongly supported. Instead, two distinct lineages were recovered with high support: a first lineage containing the brackish and freshwater taxa and a second lineage including the two marine species (*C. horii* and *C. conchophoria*). We recognize the *horii* clade as a separate family, since the SH and

AU tests showed that this clade is not necessarily most closely related to remainder of the *Aegagropila* clade, and since it is also morphologically and ecologically distinct from the brackish and freshwater lineage. In addition, we show the phylogenetic positions of several species not included in earlier treatments (including *Cladophorella calcicola*, *Wittrockiella* sp., *Arnoldiella conchophila*, *Basicladia chelonum* and *B. ramulosa*), and we characterize a new subclade closely related to *Aegagropila* and *Pithophora* that contains (sub)tropical species with a *Cladophora*-type morphology. On the basis of our phylogenetic results, we propose a number of taxonomic changes.

**Apomorphies of the *Aegagropila* clade.** A number of unique biochemical and ultrastructural features have been suggested for the *Aegagropila* clade as a whole, including the presence of the carotenoid pigment loroxanthin, the presence of chitin in the cell walls and polypyramidal pyrenoids (Hanyuda et al. 2002). The other two main lineages of the Cladophorales (the *Cladophora* clade and the *Siphonocladus* clade) were assumed to be characterized by bilenticular pyrenoids (van den Hoek et al. 1995). Polypyramidal pyrenoids had been identified in all members of the *Aegagropila* clade investigated so far, namely: *Cladophora horii* (van den Hoek and Chihara 2000); *C. conchophoria*, *Wittrockiella lyallii*, *Arnoldiella conchophila*, *B. okamurae*, *Pithophora mooreana* Collins and *Aegagropila linnaei* (Matsuyama et al. 1998; Hanyuda et al. 2002); as well as *Basicladia chelonum* (Mrozinska et al. 2009). Polypyramidal pyrenoids have, however, also been found in *Cladophora catenata* (Matsuyama et al. 1998) and *Dictyosphaeria cavernosa* (Hori and Ueda 1975), two members of the *Siphonocladus* clade, and *Rhizoclonium tortuosum* (Miyaji 1999), for which no molecular data are available at present, but which is assumed to be a member of the *Cladophora* clade based on morphological features. In the present study, polypyramidal pyrenoids have been confirmed for *Basicladia okamurae*, *B. kosteriae*, 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 *Wittrockiella* sp., only bilenticular pyrenoids were observed, as had been previously observed by van den Hoek et al. (1984) for *W. salina*. 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 other 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).

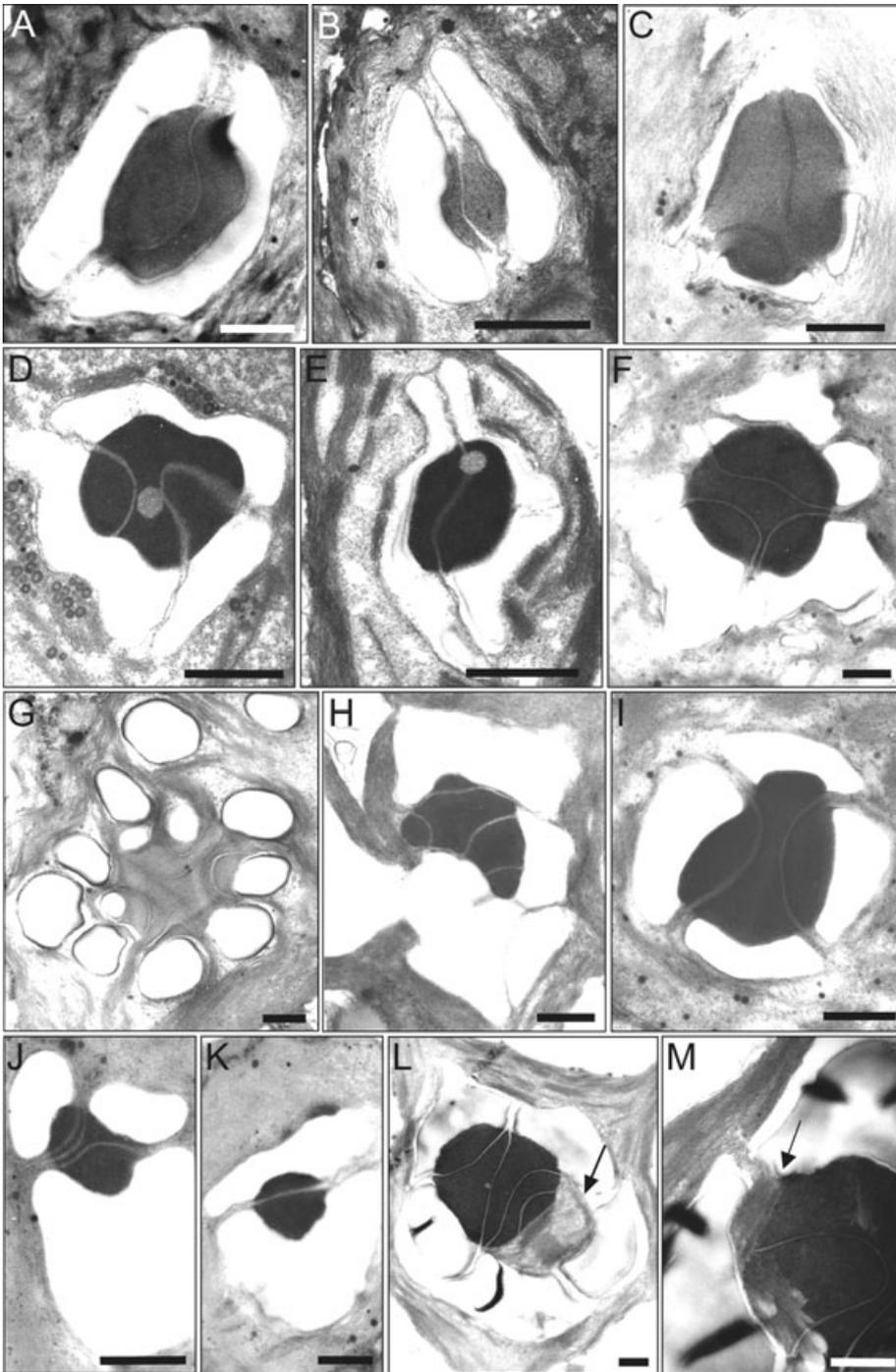


FIG. 5. Transmission electron microscope images of the pyrenoid ultrastructure in members of the *Aegagropila* clade, with representative examples of all sectioned pyrenoids per species chosen: (A) and (B) *Wittrockiella* sp. (B92), bilenticular pyrenoids; (C) *Cladophorella calcicola* (K92), polypyramidal pyrenoid; (D) and (E) *Wittrockiella amphibia* (N60), intraspecific variation in pyrenoid type [(D) polypyramidal pyrenoid, (E) bilenticular pyrenoid; all pyrenoids of this species showed a characteristic 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) and (K) *Pithophora roettleri* (K96), intraspecific variation in pyrenoid type [(J) polypyramidal pyrenoid, (K) bilenticular pyrenoid]; (L) and (M) *Aegagropila linnaei* (N36), polypyramidal pyrenoids [note thylakoid membrane stacks associated with the pyrenoid (arrows), all pyrenoids of this species showed this association]. Scale bars = 1  $\mu$ m.

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 phylogenetically informative 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.

*Two families: Pithophoraceae and Pseudocladophoraceae.* Because neither polypyramidal pyrenoids nor the presence of loroxanthin or chitin represents diagnostic characters for the *Aegagropila* clade, and because monophyly of the clade is not strongly supported (also shown by the SH and AU tests), we

opt to recognize the two distinct lineages within this clade as discrete groups at the family level.

One lineage (Pithophoraceae) can be characterized by occurring in brackish or freshwater habitats; development of secondary rhizoids in all parts of the thallus; a tendency for heterotrichous organization (from a clear division into a prostrate (= horizontal) and an upright system (*Wittrockiella*, *Arnoldiella*, and *Basicladia*), to loss of the prostrate system coupled with ease of inversion of polarity or being unattached (*Aegagropila* and *Pithophora*)); and subterminal insertion of laterals combined with delayed cross wall formation. However, it should be noted that none of these characters by themselves are unique but also occur in some members of the *Cladophora* and *Siphonocladus* clades. We propose the designation of a family for the monophyletic brackish and freshwater clade. 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*. The name **Pithophoraceae** has priority, and its use for this lineage is recommended here. In its new sense, the family includes the genera *Aegagropila*, *Aegagropilopsis* gen. nov., *Arnoldiella* (including *Basicladia chelonum* and *B. kosteriae*), several *Basicladia* species with uncertain phylogenetic affinities, *Pithophora* and *Wittrockiella* (Fig. 3B). 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 in this lineage because of data conflict between SSU and LSU rDNA data (unpublished data), resulting in an ambiguous phylogenetic position. Molecular data are lacking for a number of small genera that likely belong to the Pithophoraceae (e.g., *Chaetocladia*, *Chaetonella*, *Cladogonium*, *Cladostroma*, *Dermatophyton* and *Gemmiphora*; see Appendix S3).

The other lineage includes the marine species *Cladophora conchophoria* and *C. horii* and is characterized by polysiphonous holdfast clusters. We transfer the two species to the new genus *Pseudocladophora*, for which we erect the new family **Pseudocladophoraceae** (see below for description and taxonomic details). *Cladophora conchophoria* grows exclusively on shells of the marine snail *Lunella coronata*, occurring in South Korea and Japan (Sakai 1964, Matsuyama et al. 1999, van den Hoek and Chihara 2000). *Cladophora horii* has been found in shallow subtidal waters and intertidal rock pools, sometimes as an epiphyte on *C. prolifera* (Roth) Kützing, in Okinawa, Japan (van den Hoek and Chihara 2000) and along the east coast of South Africa (Leliaert and Coppejans 2003). While the two species differ markedly in habit (*C. horii* forming much larger and more robust, broom-like thalli), 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. 4). This character was not regarded as synapomorphic in a morphological treatment, in which *C. horii* was placed in the *Cladophora* section *Rugulosae* while *C. conchophoria* was placed in the section *Glomeratae* (van den Hoek and 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* or *C. opaca* (van den Hoek and Chihara 2000), but these differ in their mode of formation and are not characterized by very thick, layered cell wall wedges (Fig. 4). Both *C. conchophoria* and *C. horii* are densely branched and show a typical *Cladophora*-like architecture.

#### *Clades and genera of the Pithophoraceae.*

*Wittrockiella*: *Wittrockiella* grows in stable brackish water and fluctuating estuarine environments, and currently contains three species, *W. amphibia*, *W. byallii*, and *W. salina* (including two varieties), which can be primarily distinguished by their cell dimensions. The three species of *Wittrockiella* included in the phylogeny (including the undescribed *Wittrockiella* sp.) form a highly supported clade, which also includes *Cladophorella calcicola*. The latter 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 subaerial species reported from moist limestone, bricks and mud in China, Bangladesh, and tropical hothouses in Europe (Fritsch 1944, Islam 1964, Cribb 1965, Etl and Gärtner 1995, Liu 1999). The sample used in this study was found in an estuarine lagoon in Portugal (Appendix S1). The members of this clade share a heterotrichous growth form, with creeping main axes that produce relatively short upright filaments (Fig. 2, C–F). Adventitious rhizoids can be produced in all cells along the stolonoid axes (Fig. 2C) and can develop from any part of the cells. 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/subaerial species *C. calcicola* evolved from a brackish ancestor. Since our analyses showed the type species of *Cladophorella*, *C. calcicola*, to be nested within *Wittrockiella*, the genus *Cladophorella* cannot be maintained anymore. No molecular data are available for the other three described *Cladophorella* species (*C. fritschii* A.K. Islam, *C. sundarbanensis* A.K. Islam, and *C. netzhualpilii* Galicia et Novelo), but the morphology implies close relationships or conspecificity with *C. calcicola*. *Wittrockiella* (Wille 1909) has priority over *Cladophorella* (Fritsch 1944), thus new combinations for all *Cladophorella* species are proposed here (see below).

*Arnoldiella* (*Basycladia* clade), *Basycladia okamurae* and *B. ramulosa*. *Arnoldiella conchophila* grouped together with two *Basycladia* species with moderate (ML) to high (BI) support. The grouping of *Arnoldiella conchophila* with species of *Basycladia* is corroborated by morphological characteristics such as a shared heterotrichous growth form, in which a dense basal stratum of branching filaments is united into a continuous layer, and a strong preference for epizoic freshwater habitats (Miller 1928, Kargupta 1994, Keshri and Hazra 2009).

The northern Australian endemic *B. ramulosa* and the widespread *B. okamurae* were recovered on long branches and did not form a clade with *Arnoldiella* and the other *Basycladia* species. *Basycladia ramulosa* was included in a phylogenetic analysis for the first time, while the relationship of *B. okamurae* with *Basycladia* sp. or *A. conchophila* and the *Aegagropila/Pithophora* clade was also unresolved in previous studies (Hanyuda et al. 2002, Yoshii et al. 2004). *B. ramulosa* is densely branched in the upper part of the thallus (Ducker 1958), while *B. okamurae* consists of entirely unbranched upright filaments. Both species might represent separate lineages, but, for the time being, we refrain from transferring them to new genera. Additional markers and increased taxon sampling will hopefully establish the phylogenetic position of these species, allowing for appropriate nomenclatural changes.

Seven species of *Basycladia* have been described. DNA sequences from three species could not be obtained for this study, namely *B. crassa*, *B. sinensis* and *B. vivipara*. The genus *Basycladia* was erected to accommodate the type species *B. crassa* and *B. chelonum* (Hoffmann and Tilden 1930). These two species differ only in their cell dimensions and intermediate forms have been reported (e.g., Proctor 1958). Both species frequently occur in the same habitat, or even on the same turtle, and it has been proposed that they represent merely different growth forms of a single plastic species, such as a sun and a shade form (Proctor 1958, Garbary et al. 2007). *Basycladia sinensis* 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 in the range of *B. crassa*. *Basycladia vivipara* is only known from the freshwater gastropod *Viviparus malleatus* (Normandin and Taft 1959), and is morphologically very similar to *B. chelonum*. Thus, based on morphology *B. crassa*, *B. chelonum*, *B. sinensis* and *B. vivipara* might actually represent a single species, or can be assumed to be at least closely related. Since *Arnoldiella conchophila* formed a supported clade with *B. chelonum* and *B. kosteriae* and has nomenclatural priority over *Basycladia*, we propose the transfer of all described *Basycladia* species to the emended genus *Arnoldiella*, except *B. okamurae* and *B. ramulosa*, which remain as *incertae sedis* for the time being.

*Aegagropila*, *Pithophora* and *Aegagropilopsis*. This clade includes *Aegagropila*, *Pithophora* and three Asian *Cladophora* species, which are all characterized by a reduced rhizoidal system (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, i.e., inversion of the cell pole from which laterals arise (from distal to proximal). Secondary rhizoids are formed by all members of this clade, but no prostrate system is formed.

*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 overinterpretation 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. 2010a).

*Pithophora* is widespread in the (sub)tropics (Wittrock 1877, Möbius 1895, Fritsch 1907, Bourrelly 1966) and temperate regions of the eastern USA (John 2003). More than 40 taxa of *Pithophora* have been described (Index Nominum Algarum, <http://ucjeps.berkeley.edu/INA.html>), including a large number of forms and varieties. The extent of phenotypic plasticity in the few morphological characters has led to 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). Culture studies have shown that akinete formation and germination is controlled by a wide range of environmental conditions (Ernst 1908, Agrawal 1986, Stevens and Neilson 1987), that the size of akinetes is dependent on age (Brand 1904), that akinete and branch formation are the same reversible process (Mothes 1930), and that helioid formation is inducible as a wounding response (Mothes 1930). Pankow and 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*. The only morphological character separating these two species is the shape of intercalary akinetes: *P. oedogonia* being isosporous (i.e., all akinetes throughout the thallus having the same shape) and *P. roettleri* being heterosporous (i.e., akinetes with different shapes within an individual). However, the distinction between isosporous and heterosporous is not always clear (see Ernst 1908, Prescott 1951, van den Hoek 1959, Pankow and Täuscher 1980, Skinner and Entwisle 2004).

The little genetic variation among *Pithophora* samples of different origin and morphologies hints at

the existence of a single widespread, plastic species. The frequent formation of desiccation-resistant akinetes in *Pithophora* implies high dispersal potential. On the basis of 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, and is proposed to be used as the single species name. No type species has 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).

The close relationship between *Pithophora* and *Aegagropila* invites 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 account for the lack of speciation within the genera, the low intra-specific genetic variation and the extensive morphological plasticity.

Three Asian *Cladophora* species are united in a subclade. One of them is still lacking a formal description, even though it has been included in a number of studies under several designations: in Nagai (1988) as *C. sauteri* f. *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. (2001) as *Aegagropila* sp. nov. ('Tateyama-Marimo'). This species is morphologically similar to *A. linnaei*, and in addition to epilithic growth forms, it occurs as free-floating tufts (Wakana et al. 2001). 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*) 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* from China and *C. beneckeii* from Java. 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*, which 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 and Misra 1992, Gardavský 1993, Liu 1999, Islam and Irfanullah 2005). As in the genus *Arnoldiella*, there seems to be a tendency in this clade for epizoisism, especially to colonize freshwater gastropods. In comparison with *Arnoldiella*, the rhizoidal system in the *C. clavuligera/sterrocladia* subclade is reduced, but long secondary rhizoids are formed.

So far, this subclade is believed to be restricted to Asia. Tropical Asian freshwater *Cladophora* species similar to *C. clavuligera* and *C. sterrocladia* include *C. basicladioides*, *C. beneckeii*, *C. codiolo*, *C. exigua*, *C. glomerata* var. *nana*, *C. shensiensis* and *C. yuennanensis*. *Cladophora dusenii* from Cameroon, *C. amplectens* from Angola and *C. parvula* from Australia are probably also closely related to this group. 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 Appendix S3 in supplementary materials).

The species of this subclade need to be transferred from *Cladophora* to a new genus. We are against the possibility of merging *Aegagropila*, *Pithophora* and the three *Cladophora* species into *Aegagropila* based on the large sequence divergence between the three subclades (2.7–5%), which exceeds the maximum intrageneric sequence divergence in the other genera of Pithophoraceae (1.3–1.7%), and because of the unique morphological features of *Pithophora*. We propose the new genus name *Aegagropilopsis* on the basis of morphological similarity with the genus *Aegagropila*, and select *C. sterrocladia* as the type species.

*Taxon sampling and diversity of the Pithophoraceae.* The diversity of the Pithophoraceae is likely underestimated as a result of the unobtrusive habit of most taxa, the diminutive size of many representatives, misidentifications (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 ranges (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 interesting discoveries could also be made in Europe with regard to the systematic position of many algal species of unknown affiliation (see Appendix S3). 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).

The phylogenetic position of several enigmatic species would be very illuminating with regard to issues such as thallus evolution (e.g., *Chaetonella goetzei*, *Cladophora basicladioides*, *Cladophora cornuta*, *Cladophora rhizobranchialis*, *Cladostroma setschwanense*), niche evolution (*Basicladia vivipara*; *Cladogonium ogishimae* – epizoic on freshwater shrimps), (historical) biogeography (e.g., the Australian *Cladophora parvula*; or the taxa from Lake Baikal). The cladophoralean algae from ancient Lake Baikal, Russia, seem particularly interesting for our understanding of the evolution of the Pithophoraceae. The endemic

baikalian genus *Chaetocradiella* might represent non-epizoic species of *Arnoldiella* (Bourrelly 1966). Furthermore, a group of morphologically similar species and potential members of the genus *Aegagropila* (see Izhboldina 2007) appear to have diversified sympatrically in Lake Baikal. *Aegagropila linnaei* or its ancestor is assumed to have dispersed throughout the Palaearctic (or the Holarctic) from Central or East Asia (Boedeker et al. 2010a). 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 Pithophoraceae. In conclusion, additional taxon sampling and molecular analyses hold promise for new evolutionary insights.

The diagnoses of the new family Pseudocladophoraceae and the new genera *Pseudocladophora* and *Aegagropilopsis*, the emended description of *Arnoldiella*, as well as typification details and nomenclatorial changes are as follows:

**Pseudocladophoraceae** Boedeker et Leliaert, **fam. nov.**

*Algae marinae thallis rigidis erectis filamentis uniseriatis compositis divisionibus acropetalis intercalariibusque. 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 inclinatis. 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 descendencia 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)  $\mu\text{m}$ , basalibus 5–15 (–30)  $\mu\text{m}$  crassis. Distinguitur a *Cladophora* differentiis in sequentiis molecularibus.*

Marine algae with stiff, erect thalli composed of uniseriate filaments growing by acropetal and intercalary cell divisions. Cells are multinucleate with a 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, opposite or 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)  $\mu\text{m}$ , in basal parts 5–15(–30)  $\mu\text{m}$ . Distinct from *Cladophora* in molecular sequences.

*Type genus: Pseudocladophora* Boedeker et Leliaert, **gen. nov.**

*Cum characteribus familia.* Characters as for family.

*Type species: Pseudocladophora conchopheria* (Sakai) Boedeker et Leliaert, **comb. nov.**

*Basionym: Cladophora conchopheria* Sakai 1964, *Sci. Pap. Inst. Algol. Res. Fac. Sci. Hokkaido Imp. Univ.* 5: 48; fig. 22; Plate XVI, fig. 2.

*Holotype:* Nagahama near Maizuru, Kyoto Prefecture, Japan, collector I. Umezaki, May 1949, SAP 029140, on shell of the marine gastropod *Lunella coronata* Gmelin.

*Additional species:*

*Pseudocladophora horii* (C. Hoek et Chihara) Boedeker et Leliaert, **comb. nov.**

*Basionym: Cladophora horii* C. Hoek et Chihara 2000, *Nat. Sci. Mus. Monographs* 19: 67–68; fig. 28.

*Holotype:* Sesoko Island, Okinawa, Japan, collectors S. Kamura, C. van den Hoek and T. Hori, April 1990, TNS AL-46793.

**Pithophoraceae**

**Wittrockiella** Wille

*Type species: Wittrockiella amphibia* (Collins) Boedeker et G.I. Hansen (*Wittrockiella paradoxa* Wille 1909: 220–221; Tables XI–XIV).

*Basionym: Cladophora amphibia* Collins 1907: 200.

*Holotype:* original specimen of *W. paradoxa* (collected in 1907 by N. Wille, Lyngør, Norway) not traceable, thus the original drawings represent the holotype material (Wille 1909, *Nytt Mag. Naturvidensk.* 47: Tables XI–XIV).

*Epitype:* Lyngør, south-eastern Norway, collector B. Lyngø, January 1909, det. N. Wille, O (six iso-epitypes), designated here.

For typification of *W. salina*, *W. amphibia* and *W. lyallii* see van den Hoek et al. (1984), Boedeker and Hansen (2010) and Boedeker et al. (2010c), respectively.

*Synonym: Cladophorella* F.E. Fritsch 1944, *Ann. Bot.* 8: 157–170.

*Additional species:*

**Wittrockiella calcicola** (F.E. Fritsch) Boedeker, **comb. nov.**

*Basionym: Cladophorella calcicola* F.E. Fritsch 1944, *Ann. Bot.* 8: 157–171, figs. 1, A–I; 2, A–G; 3, A–C; and 4, A–G.

*Holotype:* original specimen (collected by F.E. Fritsch, tropical hothouse, Cambridge Botanical Garden, Britain, 1944, on moist limestone) lost or destroyed (previously BM), thus the original drawings represent the holotype material (Fritsch

1944, *Ann. Bot.* 8: figs. 1, A–I; 2, A–G; 3, A–C; and 4, A–G).

*Epitype*: Lagoa de Óbidos, Portugal, collector O. Lourenço (sample no. K92; ACOI culture collection 471), 1989, L (L079292), designated here.

***Wittrockiella fritschii*** (A.K. Islam) Boedeker, **comb. nov.**

*Basionym*: *Cladophorella fritschii* A.K. Islam 1964, *Rev. Algol.* 7: 282, 286; Plates I–III; Plate 4, figs. 1–6.

*Holotype*: no information.

***Wittrockiella netzahualpillii*** (Galicia et Novelo) Boedeker, **comb. nov.**

*Basionym*: *Cladophorella netzahualpillii* Galicia et Novelo 2000, *Phycologia*: 288–289; figs 1–46.

*Holotype*: Brackish water extraction wells, Lake Texcoco Management, Mexico, FCME TEX1 (figs. 1–30).

***Wittrockiella sundarbanensis*** (A.K. Islam) Boedeker, **comb. nov.**

*Basionym*: *Cladophorella sundarbanensis* A.K. Islam 1964, *Rev. Algol.* 7: 286, 289; Plate VII.

*Holotype*: no information.

***Arnoldiella*** V.V. Mill. **emend.** Boedeker

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 or sparsely branched. Branches more numerous in apical parts of the thallus. 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 pseudodichotomy. 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.

*Type species*: ***Arnoldiella conchophila*** V.V. Mill. 1928: 20–21; figs 2–20.

*Holotype*: original specimen (collected by V. V. Miller, Lake Pereslavl, Vladimir district, Russia, 1921, on shell of freshwater bivalve) untraceable, thus the original drawings represent the holotype material (Miller 1928, *Planta* 6: figs. 2–20).

*Synonym*: *Basicladia* W.E. Hoffm. et Tilden, 1930, *Bot. Gaz.* 89: 380, 381.

*Additional species*:

***Arnoldiella chelonum*** (Collins) Boedeker, **comb. nov.**

*Basionym*: *Chaetomorpha chelonum* Collins 1907, *Rhodora* 9: 198–200.

*Holotype*: Walnut Lake, Michigan, USA, collector T.L. Hankinson, on turtle carapaces, NY 00887601.

*Synonym*: *Basicladia chelonum* (Collins) W.E. Hoffm. et Tilden 1930: 382–383; fig. 1.

***Arnoldiella crassa*** (W.E. Hoffm. et Tilden) Boedeker, **comb. nov.**

*Basionym*: *Basicladia crassa* W.E. Hoffm. et Tilden 1930, *Bot. Gaz.* 89: 382; figs. 2–22.

*Holotype*: lost, thus the original drawings represent the holotype material (Hoffmann and Tilden 1930, *Bot. Gaz.* 89: figs. 2–20).

***Arnoldiella kosteræ*** (C. Hoek) Boedeker, **comb. nov.**

*Basionym*: *Cladophora kosteræ* C. Hoek 1963, *Revision of the European species of Cladophora*, Brill E.J., Leiden, Netherlands: 37–39; Plate II, figs. 22–27; Plate III, figs. 28–36; Plate IV, figs. 37–40.

*Holotype*: Jardin des Plantes, Paris, France, collector C. van den Hoek, 25 April 1961, L 0054830.

*Synonym*: *Basicladia kosteræ* (C. Hoek) Garbary 2010: 39.

***Arnoldiella sinensis*** (N.L. Gardner) Boedeker, **comb. nov.**

*Basionym*: *Chaetomorpha sinensis* N.L. Gardner 1936, *Madroño* 4: 28–32; Plate VII.

*Holotype*: Kiangsu, China, 1936, on carapace of turtle, UC 543979.

*Synonym*: *Basicladia sinensis* (N.L. Gardner) G.M. Sm. 1950: 218.

***Arnoldiella vivipara*** (Normandin et Taft) Boedeker, **comb. nov.**

*Basionym*: *Basicladia vivipara* Normandin et Taft 1959, *Ohio J. Sci.* 59: 60; figs. 1–5.

*Holotype*: Ohio State University, Botany and Zoology Department, Columbus, Ohio, USA (Normandin and Taft 1959, not checked).

***Pithophora*** Wittr.

*Type species*: *Pithophora roettleri* (Roth) Wittr. (*Pithophora kewensis* Wittrock 1877, *Nova Acta Reg. Soc. Sci. Upsaliensis* 3 (19): 52; Plate I, fig. 8; Plate II, figs. 1–12; Plate III; Plate IV, figs 2–11; Plate V, figs. 9–10).

*Holotype*: *Pithophora kewensis* Wittr., tropical aquarium ('Waterlily house'), Kew Gardens, Britain, collector V. B. Wittrock, August 1872, L 938112 639, designated here. Isotypes in BM, L, UPS and S.

*A single recognized species*:

***Pithophora roettleri*** (Roth) Wittr.

*Basionym*: *Ceramium roettleri* Roth 1806, *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur*, Io. Fr. Gledischiano, Lipsiae [=Leipzig], Germany: 123–124.

*Holotype*: Tranquebar, eastern India, collector Klein, January 1799, L 93825 38. Isotype in UPS.

*Synonyms*: *P. aequalis* Wittr. 1877: 50; Plate I, figs. 4–7; *P. affinis* Nordst. 1878: 19; *P. chinensis* Skvortsov 1946: 8; Plate 13, figs. 4–7; *P. clavifera* Schmidle

1897: 304; figs. A, 1–6; *P. cleveana* Wittr. 1877: 58; Plate II, figs. 13–15; Plate IV, figs. 12–18; Plate V, figs. 1–8; *P. heterospora* M. Howe et Herv. 1924: 351; figs. 1–14 (in Howe 1924); *P. kewensis* Wittr. 1877: 52; Plate I, fig. 8; Plate II, figs. 1–12; Plate III; Plate IV, figs. 2–11; Plate V, figs. 9–10; *P. macrospora* F. Brand 1904: 175; Plate V, figs. 2–4; *P. microspora* Wittr. 1884: 132 (in Henriques 1884); *P. mooreana* Collins 1912: 97; Plate I, fig. 9; *P. oedogonia* (Mont.) Wittr. 1877: 55; *P. pachyderma* Schmidle 1900: 17; *P. pragensis* Šula 1930: 24; figs. 1–2; *P. polymorpha* Wittr. 1877: 62; Plate I, figs. 13–17; Plate IV, fig. 19; *P. radians* W. et G.S. West 1897: 36; *P. reineckeae* Schmidle 1897 (in Reinecke 1897): 264; fig. 7; *P. sumatrana* (G. Martens) Wittr. 1877: 48; *P. tropica* Woodhead et Tweed 1958: 181; fig. 93; *P. varia* Wille 1902: no. 983 (nomen, in Collins et al. 1902); *P. variabilis* Schmidle 1901: 344; Plate XII, fig. 2; *P. zelleri* (G. Martens) Wittr. 1877: 64.

***Aegagropilopsis* Boedeker, gen. nov.**

*Algae aquae dulcis (sub)tropicis interdum nonnihil salsugineae caespitibus minutis minus quam 1.5 cm altis filamentis erectis uniseriatis 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 epizootici ad cochleas aquae dulcis (Pila, Paludina) sed quoque substratis immobilis affixi inventus. Distinguitur a *Aegagropila* differentiis in sequentiis molecularibus.*

Tropical to subtropical 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 filaments 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 zooidangia. Thalli mainly epizootic on freshwater snails (*Pila*, *Paludina*), but also found attached to stationary

substrates. Distinct from *Aegagropila* in molecular sequences.

*Type species: Aegagropilopsis sterrocladia* (Skuja) Boedeker, **comb. nov.**

*Basionym: Cladophora sterrocladia* Skuja 1949, *Nova Acta Reg. Soc. Sci. Upsaliensis* 4(14): 94–95; Plate XXXVII).

*Holotype:* original specimen (collected by H. Skuja, Burma/Myanmar, on shell of freshwater gastropod) lost (previously in RIG), thus the original drawings represent the holotype material (Skuja 1949, *Nova Acta Reg. Soc. Sci. Upsaliensis* 4(14): 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 079287, designated here.

*Additional species:*

***Aegagropilopsis clavuligera* (Grunow) Boedeker, comb. nov.**

*Basionym: Cladophora clavuligera* Grunow 1868, *Reise Seiner Majestät Fregatte Novara um die Erde. Botanischer Theil*, K. K. Hof und Staatsdruckerei, Vienna, Austria: 40.

*Holotype:* Ceylon/Sri Lanka (Expedition Novara), collector G. von Frauenfeld, W 2010/2274, on shell of freshwater gastropod.

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Agrawal, S. C. 1986. Effects of different factors on the akinete germination of *Pithophora oedogonia*. *J. Basic Microbiol.* 26:195–9.

Boedeker, C. & Immers, A. 2009. No more lake balls (*Aegagropila linnaei* Kützing, Cladophorophyceae, Chlorophyta) in The Netherlands? *Aquat. Ecol.* 43:891–902.

Boedeker, C. & Hansen, G. I. 2010. Nuclear rDNA sequences of *Wittrockiella amphibia* (Collins) comb. nov. (Cladophorales, Chlorophyta) and morphological characterisation of the mat-like growth form. *Bot. Mar.* 53:351–6.

- Boedeker, C., Eggert, A., Immers, A. & Wakana, I. 2010a. Biogeography of *Aegagropila linnaei* (Cladophorophyceae, Chlorophyta): a widespread freshwater alga with low effective dispersal potential shows a glacial imprint in its distribution. *J. Biogeogr.* 37:1491–1503.
- Boedeker, C., Eggert, A., Immers, A. & Smets, E. 2010b. Global decline of and threats to *Aegagropila linnaei*, with special reference to the lake ball habit. *BioScience* 60:187–98.
- Boedeker, C., Ramírez, M. E. & Nelson, W. A. 2010c. *Cladophoropsis brachyartrus* from southern South America is a synonym of *Wittrockiella lyallii* (Cladophorophyceae, Chlorophyta), previously regarded as an endemic from New Zealand. *Phycologia* 49:525–36.
- Bourrelly, P. 1966. *Les Algues D'eau Douce. I Les Algues Vertes*. Éditions N. Boubée & Cie, Paris, France, 511 pp.
- Brand, F. 1902. Die *Cladophora*-Aegagropilen des Süßwassers. *Hedwigia* 41:34–71.
- Brand, F. 1904. Über die Anheftung der Cladophoraceen und über verschiedene polynesische Formen dieser Familie. *Beih. Bot. Centralblatt* 18:165–93.
- Cocquyt, E., Verbruggen, H., Leliaert, F. & De Clerck, O. 2010. Evolution and cytological diversification of the green seaweeds (Ulvothyceae). *Mol. Biol. Evol.* 27:2052–61.
- Collins, F. S. 1907. Some new green algae. *Rhodora* 9:198–200.
- Collins, F. S. 1912. The green algae of North America, supplementary paper. *Tufts Coll. Stud.* 3:69–109.
- Collins, F. S., Holden, I. & Setchell, W. A. 1902. *Phyc. Bor.-Amer. A collection of dried specimens of the algae of North America*. Fasc. XX, nos 951–1000. Malden, Massachusetts.
- Cox Downing, R. 1970. Shoreline algae of Western Lake Erie. *Ohio J. Sci.* 70:257–76.
- Cribb, A. B. 1965. An ecological and taxonomic account of the algae of a semi-marine cavern, Paradise Cave, Queensland. *Papers Dept. Bot. Univ. Queensland* 4:259–82.
- Ducker, S. C. 1958. A new species of *Basycladia* on Australian freshwater turtles. *Hydrobiologia* 10:157–74.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32:1792–7.
- Egeland, E. S., Guillard, R. R. L. & Liaaen-Jensen, S. 1997. Additional carotenoid prototype representatives and a general chemosystematic evaluation of carotenoids in Prasinophyceae (Chlorophyta). *Phytochemistry* 44:1087–97.
- Entwistle, T. J. & Price, I. R. 1992. New record of two potential weed species of freshwater macroalgae from Queensland. *Papers Dept. Bot. Univ. Queensland* 102:57–63.
- Ernst, A. 1908. Beitrag zur Morphologie und Physiologie von *Pithophora*. *Ann. Jard. Bot. Buitenzorg* 22:18–29.
- Ettl, H. & Gärtner, G. 1995. *Syllabus der Boden-, Luft- und Flechtalgen*. Gustav Fischer, Stuttgart, Germany, 721 pp.
- Farris, J. S., Källersjö, M., Kluge, A. G. & Bult, C. 1995. Constructing a significance test for incongruence. *Syst. Biol.* 44:570–2.
- Fawley, M. W. 1991. Note: disjunct distribution of the Xanthophyll Loroxanthin in the green algae (Chlorophyta). *J. Phycol.* 27:544–8.
- Feau, N., Hamelin, R. C. & Bernier, L. 2006. Attributes and congruence of three molecular data sets: inferring phylogenies among *Septoria*-related species from woody perennial plants. *Mol. Phylog. Evol.* 40:808–29.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–91.
- Fott, B. 1971. *Algenkunde*. 2nd ed. Gustav Fischer, Stuttgart, Germany, 581 pp.
- Fritsch, F. E. 1907. The subaerial and freshwater algae of the tropics. *Proc. Roy. Soc., Ser. B.* 79:235–75.
- Fritsch, F. E. 1935. The structure and reproduction of the algae. Volume I. Introduction, Chlorophyceae, Xanthophyceae, Chrysophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae, Chloromonineae, Euglenineae, Colourless Flagellata. Vol. I. Cambridge University Press, Cambridge, UK, 791 pp.
- Fritsch, F. E. 1944. *Cladophorella calcicola* nov. gen. et sp., a terrestrial member of the Cladophorales. *Ann. Bot.* 8:157–71.
- Galicía-García, C. & Novelo, E. 2000. *Cladophorella netzahualpillii* sp. nov. (Cladophorales, Ulvothyceae), a species reproducing by spores. *Phycologia* 39:288–95.
- Garbary, D. J. 2010. Taxonomy of *Basycladia* (Cladophorales, Chlorophyta) with two new combinations. *Novon* 20:38–40.
- Garbary, D. J., Bourque, G., Herman, T. B. & McNeil, J. A. 2007. Epizoid algae from freshwater turtles in Nova Scotia. *J. Freshw. Ecol.* 22:677–85.
- Gardavský, A. 1993. *Rhizoclonium fractiflexum* sp. nova, a new member of Cladophorales (Chlorophyta) described from freshwater aquaria. *Archiv für Protistenkunde* 143:125–36.
- Gardner, N. L. 1936. A new species of *Chaetomorpha* from China. *Madroño* 4:28–32.
- Geitler, L. 1936. Vergleichende Untersuchungen über den feineren Kern und Chromosomenbau der Cladophoraceen. *Planta* 25:530–78.
- Grunow, A. 1868. Erster Band. Algen. In Fenzl, E. [Ed.] *Reise Seiner Majestät Fregatte Novara um die Erde. Botanischer Theil*. K. K. Hof und Staatsdruckerei, Vienna, Austria, pp. 1–104.
- Hanyuda, T., Wakana, I., Arai, S., Miyaji, K., Watano, Y. & Ueda, K. 2002. Phylogenetic relationships within Cladophorales (Ulvothyceae, Chlorophyta) inferred from 18S rRNA gene sequences, with special reference to *Aegagropila linnaei*. *J. Phycol.* 38:564–71.
- Heering, W. 1921. Siphonales. In Pascher, A. [Ed.] *Süßwasserflora Deutschlands, Österreichs und der Schweiz*. Vol. 7. Gustav Fischer, Jena, Germany, pp. 69–99.
- Henriques, J. 1884. Contribuição para o estudo da flora d'algamas possessões portuguesas. *Bol. Soc. Broter.* 3:129–54.
- Hoffmann, W. E. & Tilden, J. E. 1930. *Basycladia*, a new genus of Cladophoraceae. *Bot. Gaz.* 89:374–84.
- Holmgren, P. K., Holmgren, N. H. & Barnett, L. C. 1990. *Index Herbariorum, Part I: The herbaria of the world*. New York Botanical Garden, New York, USA, 693 pp.
- Hori, T. & Ueda, R. 1975. The fine structure of algal chloroplasts and algal phylogeny. In Tokida, J. & Hirose, H. [Eds.] *Advance of Phycology in Japan*. Junk, The Hague, Netherlands, pp. 11–42.
- Howe, M. A. 1924. Notes on algae of Bermuda and the Bahamas. *Bull. Torrey Bot. Club* 51:351–9.
- Islam, A. K. M. N. 1964. The genus *Cladophorella* newly found in East Pakistan. *Rev. Algol.* 7:275–89.
- Islam, A. K. M. N. & Irfanullah, H. M. 2005. Hydrobiological studies within the tea gardens at Srimangal, Bangladesh. III. Chlorophyceae (excluding desmids). *Bangl. J. Plant Taxon.* 12:19–37.
- Izhboldina, L. A. 2007. *Guide and key to benthonic and periphyton algae of lake Baikal (meio- and macrophytes) with short notes of their ecology*. Nauka-Center, Novosibirsk, Russia, 248 pp.
- Jobb, G. 2011. *TreeFinder*. Available at: <http://www.treefinder.de> (last accessed 06 June 2011).
- John, D. M. 2003. Filamentous and plantlike green algae. In Wehr, J. D. & Sheath, R. G. [Eds.] *Freshwater algae of North America*. Academic Press, San Diego, USA, pp. 311–52.
- Jónsson, S. 1962. Recherches sur des Cladophoracées marines. Structure, reproduction, cycles comparés, conséquences systématiques. *Ann. Sci. Nat. Bot. Biol.* 12:27–265.
- Kanda, F. 1991. The observation of specimens of *Cladophora sauteri* from Toyama, Japan. *Jpn. J. Phycol.* 39:27–30.
- Kargupta, A. N. 1994. Monotypic genus *Arnoldiella* (Cladophorales : Chlorophyceae) - a new record from Indian subcontinent. *J. Econ. Tax. Bot.* 18:439–441.
- Kéry, M., Gerdner, B. & Monnerat, C. 2010. Predicting species distributions from checklist data using site-occupancy models. *J. Biogeogr.* 37:1851–62.
- Keshri, J. P. & Hazra, A. G. 2009. Observations on the rare alga *Arnoldiella conchophila* V. Miller (Ulvothyceae: Cladophorales) from India. *Crypt. Algol.* 30:271–4.
- Krishnamurthy, V. 2000. *Algae of India and neighbouring countries I. Chlorophycota*. Science Publishers, Inc., Enfield, USA, 210 pp.
- Kurogi, M. E. 1980. Lake Ball 'Marimo' in Lake Akan. *Jap. J. Phycol.* 28:168–9.
- Leliaert, F. & Boedeker, C. 2007. Cladophorales. In Brodie, J., Maggs, C. A. & John, D. [Eds.] *Green seaweeds of Britain and*

- Ireland. Natural History Museum Publications, London, UK, pp. 131–183.
- Leliaert, F. & Coppejans, E. 2003. The marine species of *Cladophora* (Chlorophyta) from the South African east coast. *Nova Hedwigia* 76:45–82.
- Leliaert, F., Rousseau, F., de Reviere, B. & Coppejans, E. 2003. Phylogeny of the Cladophorophyceae (Chlorophyta) inferred from partial LSU rRNA gene sequences: is the recognition of a separate order Siphonocladales justified? *Eur. J. Phycol.* 38:233–46.
- Leliaert, F., De Clerck, O., Verbruggen, H., Boedeker, C. & Coppejans, E. 2007. Molecular phylogeny of the Siphonocladales (Chlorophyta: Cladophorophyceae). *Mol. Phylogenet. Evol.* 44:1237–56.
- Leliaert, F., Rueness, J., Boedeker, C., Maggs, C. A., Cocquyt, E., Verbruggen, H. & De Clerck, O. 2009a. Systematics of the marine microfilamentous green algae *Uronema curvatum* and *Urospora microscopica* (Chlorophyta). *Eur. J. Phycol.* 44:487–96.
- Leliaert, F., Boedeker, C., Peña, V., Bunker, F., Verbruggen, H. & De Clerck, O. 2009b. *Cladophora rhodolithicola* sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European maerl beds. *Eur. J. Phycol.* 44:155–69.
- Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F. & De Clerck, O. 2012. Phylogeny and molecular evolution of the green algae. *Crit. Rev. Plant Sci.* 31:1–46.
- Lembi, C. A. 2003. Control of nuisance algae. In Wehr, J. D. & Sheath, R. G. [Eds.] *Freshwater algae of North America*. Academic Press, San Diego, USA, pp. 805–34.
- Liu, G. 1999. *Study on the systematics of freshwater Cladophoraceae from China*. Master's Thesis, Institute of Hydrobiology, Chinese Academy Sciences, Wuhan, 117 pp.
- Marks, J. C. & Cummings, M. P. 1996. DNA sequence variation in the ribosomal internal transcribed spacer region of freshwater *Cladophora* species. *J. Phycol.* 32:1035–42.
- Matsuyama, K., Matsuoka, T., Miyaji, K., Tanaka, J. & Aruga, Y. 1998. Ultrastructure of the pyrenoid in the family Cladophoraceae (Cladophorales, Chlorophyta). *J. Jpn. Bot.* 73:279–86.
- Matsuyama, K., Aruga, Y. & Tanaka, J. 1999. Ecological and morphological studies of *Cladophora conchopheria* Sakai (Ulvophyceae, Cladophoraceae). *J. Jpn. Bot.* 74:136–41.
- Miller, V. 1928. *Arnoldiella*, eine neue Cladophoraceaeengattung. *Planta* 6:1–21.
- Miyaji, K. 1999. A new type of pyrenoid in the genus *Rhizoclonium* (Cladophorales, Chlorophyta). *Phycologia* 38:267–276.
- Möbius, M. 1895. Beitrag zur Kenntnis der Algengattung *Pithophora*. *Ber. Deut. Bot. Ges.* 13:356–61.
- Moeser, K. 1930. Morphologische und physiologische Studien an der Cladophoracee *Pithophora*. *Ber. Deut. Bot. Ges.* 48:110–21.
- Mrozinska, T., Czerwik-Marcinkowska, J. & Webb-Janich, M. 2009. *Basicladia chelonum* (Collins) W.E. Hoffmann et Tilden (Chlorophyta, Cladophorophyceae) from Cuba (Caribbean): new observation of the ultrastructure of its vegetative cells. *Acta Soc. Bot. Pol.* 78:63–7.
- Murray, S., Jorgensen, M. F., Ho, S. Y. W., Patterson, D. J. & Jermini, L. S. 2005. Improving the analysis of dinoflagellate phylogeny based on rDNA. *Protist* 156:269–86.
- Nagai, M. 1988. "Tateyama-Marimo" in Tateyama Town, Toyama Prefecture. *Iden* 42:101–5.
- Nelson, W. A., Villouta, E., Neill, K., Williams, G. C., Adams, N. M. & Slivsgaard, R. 2002. Marine macroalgae of Fiordland. *Tuhinga* 13:117–52.
- Niiyama, Y. 1989. Morphology and classification of *Cladophora aegagropila* (L.) Rabenhorst in Japanese lakes. *Phycologia* 28:70–6.
- Nitschke, U., Boedeker, C., Karsten, U., Hepperle, D. & Eggert, A. 2010. Does the lack of mannitol accumulation in an isolate of *Rhodella maculata* (Rhodellophyceae, Rhodophyta) from the brackish Baltic Sea indicate a stressed population at the distribution limit? *European Journal of Phycology* 45:436–49.
- Nordstedt, C. F. O. 1878. De algis aquae dulcis et de Characeis ex insulis Sandvicensibus a Sv. Bergren 1875 reportatis. *Minneskr. Fys. Sällsk. Lund* 7:1–24.
- Normandin, R. F. & Taft, C. E. 1959. A new species of *Basicladia* from the snail *Viviparus malleatus* Reeve. *Ohio J. Sci.* 59:58–62.
- Nylander, J. A. A. 2004. *MrModeltest2 v2.3*. Available at: <http://www.abc.se/~nylander/> (last accessed 24 August 2010).
- O'Kelly, C. J. 1982. Chloroplast pigments in selected marine Chaetophoraceae and Chaetosiphonaceae (Chlorophyta): the occurrence and significance of siphonaxanthin. *Bot. Mar.* 25:133–7.
- Pankow, H. & Täuscher, L. 1980. Über eine *Pithophora*-Art aus den Gewächshäusern des Botanischen Gartens in Rostock. *Nova Hedwigia* 33:465–474.
- Pearlmutter, N. L. & Lembi, C. A. 1978. Localization of chitin in algal and fungal cell walls by light and electron microscopy. *J. Histochem. Cytochem.* 26:782–791.
- Pearlmutter, N. L. & Lembi, C. A. 1980. Structure and composition of *Pithophora oedogonia* (Chlorophyta) cell walls. *J. Phycol.* 16:602–16.
- Polderman, P. J. G. 1976. *Wittrockiella paradoxa* Wille (Cladophoraceae) in N.W. European saltmarshes. *Hydrobiol. Bull.* 10:98–103.
- Prasad, B. N. & Misra, P. K. 1992. *Fresh Water Algal Flora of Andaman and Nicobar Islands*. Vol. II. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 284 pp.
- Prescott, G. W. 1951. Algae of the western Great Lakes area, exclusive of desmids and diatoms. *Cranbrook Inst. Sci. Bull.* 31:1–946.
- Proctor, V. W. 1958. The growth of *Basicladia* on turtles. *Ecology* 39:634–45.
- Rambaut, A. 2007. *Se-AL (multiple sequence alignment program), v2.0a11*. Available at: <http://tree.bio.ed.ac.uk/software/seal> (last accessed 24 August 2010).
- Rambaut, A. & Drummond, A. J. 2007. *Tracer v1.4*. Available at: <http://tree.bio.ed.ac.uk/software/tracer> (last accessed 24 August 2010).
- Reinecke, P. 1897. Die Flora der Samoa-Inseln. *Bot. Jahrb.* 23:237–368.
- Rindi, F., Lopez-Bautista, J. M., Sherwood, A. R. & Guiry, M. D. 2006. Morphology and phylogenetic position of *Spongiochrysis hawaiiensis* gen. et sp. nov., the first known terrestrial member of the order Cladophorales (Ulvophyceae, Chlorophyta). *Int. J. Syst. Evol. Microbiol.* 56:913–922.
- Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Roth, A. G. 1806. *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur*. Io. Fr. Gledischiano, Lipsiae [=Leipzig], Germany, 1–350 pp.
- Sakai, Y. 1964. The species of *Cladophora* from Japan and its vicinity. *Sci. Pap. Inst. Algal. Res. Fac. Sci. Hokkaido Imp. Univ.* 5:1–104.
- Schmidle, W. 1897. Epiphyllae Algen nebst einer *Pithophora* und *Dasya* aus Neu-Guinea. *Flora* 83:304–26.
- Schmidle, W. 1900. Algologische Notizen. XIV. Einige neue von Professor Dr. Hansgirg in Vorderindien gesammelte Süßwasser-algen. *Allg. Bot. Zeitschr. Syst., Florist., Pflanzengeogr.* 6:17–9 33–5, 53–5, 77–9.
- Schmidle, W. 1901. Einige Algen, welche Prof. Dr. Volkens auf den Carolinen gesammelt hat. *Hedwigia* 40:343–9.
- Seegerstråle, S. G. 1962. The immigration and prehistory of the glacial relicts of Eurasia and North America. A survey and discussion of modern views. *Int. Rev. Ges. Hydrobiol.* 47:1–25.
- Setchell, W. A. & Gardner, N. L. 1920. The marine algae of the Pacific coast of North America. Part II. Chlorophyceae. *University of California Publications in Botany* 8:139–374.
- Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51:492–508.
- Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16:1114–16.
- Shimodaira, H. & Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17:1246–47.
- Skinner, S. & Entwistle, T. J. 2004. Non-marine algae of Australia: 6. Cladophoraceae (Chlorophyta). *Teloepa* 10:731–48.
- Skuja, H. 1949. Zur Süßwasser-algenflora Burmas. *Nova Acta Reg. Soc. Sci. Upsaliensis* 4:1–188.

- Skvortsov, B. V. 1946. Species novae et minus cognitae Algarum, Flagellatarum et Phycomicetarum Asiae, Africae, Americae et Japoniae nec non Ceylon anno 1931-1945, descripto et illustrato per tab. 1-18. *Proc. Harbin Soc. Nat. Hist. & Ethnogr.* 2:1-34.
- Šula, J. 1930. *Pithophora pragensis*, eine neue *Pithophora* aus Prager Aquarien. *Preslia*. 9:22-5.
- Smith, G. M. 1950. *The fresh-water algae of the United States*. McGraw-Hill, New York, Toronto & London, 719 pp.
- Soejima, A., Yamazaki, N., Nishino, T. & Wakana, I. 2009. Genetic variation and structure of the endangered freshwater benthic alga Marimo, *Aegagropila linnaei* (Ulvophyceae) in Japanese lakes. *Aquat. Ecol.* 43:359-70.
- South, G. R. 1981. *Wittrockiella paradoxa* Wille (Chlorophyta, Cladophoraceae), new for the Pacific coast of North America. *Syesis* 14:93-5.
- Stevens, R. J. & Neilson, M. A. 1987. Environmental control of *Pithophora oedogonia* (Chlorophyceae) akinete germination. *J. Phycol.* 16:424-7.
- Swofford, D. L. 2002. PAUP\*. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.
- van den Hoek, C. 1959. Caribbean fresh and brackish water Chlorophyta. *Blumea*. 9:590-625.
- van den Hoek, C. 1982. A taxonomic revision of the American species of *Cladophora* (Chlorophyceae) in the North Atlantic Ocean and their geographic distribution. North Holland Publishing Company, Amsterdam, Netherlands, 236 pp.
- van den Hoek, C. 1963. *Revision of the European species of Cladophora*. Brill E.J., Leiden, Netherlands, 248 pp.
- van den Hoek, C. & Chihara, M. 2000. A taxonomic revision of the marine species of *Cladophora* (Chlorophyta) along the coasts of Japan and the Russian Far-east. *Nat. Sci. Mus. Monographs* 19:1-242.
- van den Hoek, C., Duckert, S. C. & Womersley, H. B. S. 1984. *Wittrockiella salina* Chapman (Cladophorales, Chlorophyceae), a mat and ball forming alga. *Phycologia* 23:39-46.
- van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995. *Algae. An Introduction to Phycology*. Cambridge University Press, Cambridge, UK, 623 pp.
- van Oye, P. 1922. Kurzer Beitrag zur Kenntnis von *Pithophora sumatrana* (Mart.) Wittr. *Hedwigia* 63:43-7.
- Verma, B. N. 1979. Cytological studies in four species of *Pithophora* Wittrock. *Cytologia* 44:29-38.
- Verma, B. N. 1981. New records of *Cladophora* Kuetz from Bihar. *Phykos* 20:44-8.
- Wakana, I., Arai, S., Sano, O. & Honoko, H. 2001. Current situation and living environment of freshwater alga "Tateyama-marimo" (*Aegagropila* sp. nov.) in the Saryfutsu-Hamatonbetsu lake group, Hokkaido, Japan. *J. Plant Res.* 114(Suppl):1-59.
- West, W. & West, G. S. 1897. Welwitsch's African freshwater algae. *J. Bot. (London)* 35:1-7 33-42, 77-89, 113-22, 172-83, 235-43, 264-72, 297-304, 365-9.
- Wille, N. 1909. Algologische Notizen XV. Über *Wittrockiella* nov. gen. *Nytt Mag. Naturvidensk* 47:209-25.
- Wittrock, V. B. 1877. On the development and systematic arrangement of the Pithophoraceae, a new order of algae. *Nova Acta Reg. Soc. Sci. Upsaliensis* 3:1-80.
- Woodhead, N. & Tweed, R. D. 1958. Freshwater algae of Sierra Leone. *Hydrobiol.* 12:181-225.
- Wurdack, M. E. 1923. The chemical nature of the walls of certain algae. *Ohio J. Sci.* 23:181-91.
- Yang, Z. 2007. PAML 4: a program package for phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24:1586-91.
- Yoshii, Y., Hanyuda, T., Wakana, I., Miyaji, K., Arai, S., Ueda, K. & Inouye, I. 2004. Carotenoid compositions of *Cladophora* balls (*Aegagropila linnaei*) and some members of the Cladophorales (Ulvophyceae, Chlorophyta): their taxonomic and evolutionary implication. *J. Phycol.* 40:1170-7.
- Xia, X. & Xie, Z. 2001. DAMBE: data analysis in molecular biology and evolution. *J. Hered.* 92:371-3.

### Supplementary Material

The following supplementary material is available for this article:

**Appendix S1.** Specimens of members of the *Aegagropila* clade used in this study with collection data (voucher information, location, collector, date of collection) and GenBank accession numbers (sequences generated in this study are indicated in bold). n.i. = no information.

**Appendix S2.** List of outgroup taxa and their respective GenBank accession numbers for the LSU and SSU rDNA sequences (those generated in this study are indicated in bold).

**Appendix S3.** Inquirendae: potential members of the Pithophoraceae (= *Aegagropila* clade)

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