The Aegagropila clade represents a unique group of cladophoralean green algae occurring mainly in brackish and freshwater environments. The clade is sister to the species-rich and primarily marine Cladophora and Siphonoclados 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 Cladophorrella calcicola, occurring mainly in brackish and subaerial habitats. The two other lineages are restricted to freshwater. One of them shows a strong tendency for epizoism, 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; Cladophorrella; 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 siphonoclados 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). 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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have been proposed, namely the recent transfer of *C. kosterae* C. Hoek and *C. okamurae* (Ueda) C. Hoek to the genus *Basicladia* (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 *Siphonoclados* 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 *Basicladia*), on freshwater snails and bivalves (monotypic *Arnoldiella* and several *Basicladia* 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, aephytrophic unicells occurring on tree bark have been described and included in this lineage based on DNA sequence data (*Spongiochloris hawaiensis*, 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. byallii* (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 *Basicladia* 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). *Basicladia* 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. okamurae* (Fig. 2, G, H) and *B. kosterae* (Fig. 2, G, I) are actually only rarely encountered on turtles. The diminutive and poorly known *Arnoldiella conchophoria* V.V. Mill. has been found on shells of freshwater bivalves (Miller 1928), freshwater gastropods (Karpugia 1994, Keshri and Hazra 2009) and a range of other substrates (Cox Downing 1970). *Pithophora* Wittr. is easily recognized by the characteristic aki-netes (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, Niiyama 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.

<table>
<thead>
<tr>
<th>Former Cladophora species that are members of the Aegagropila clade</th>
<th>Cladophora section/subgenus</th>
<th>current name</th>
<th>reference for placement in Aegagropila clade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladophora horii C. Hoek et Chihara 2000</td>
<td>section Rugulosae (^a)</td>
<td>(Pseudocladophora) horii (C. Hoek et Chihara) Boedeker, comb. nov. (this study)</td>
<td>Leliaert et al. 2003, Yoshii et al. 2004</td>
</tr>
<tr>
<td>Cladophora conchopheria Sakai 1964</td>
<td>section Obscurae (^b)</td>
<td>(Pseudocladophora) conchopheria (Sakai) Boedeker, comb. nov. (this study)</td>
<td>Hanyuda et al. 2002</td>
</tr>
<tr>
<td>Cladophora kosterae C. Hoek 1963</td>
<td>section Basidalia (^c)</td>
<td>Aneurocallista kosterae (C. Hoek) Boedeker, comb. nov. (this study)</td>
<td>Yoshii et al. 2004</td>
</tr>
<tr>
<td>Cladophora okamurae (Ueda) C. Hoek 1963</td>
<td>section Basidalia (^c)</td>
<td>(Basicladia) okamurae (Ueda) Garbary 2010</td>
<td>Hanyuda et al. 2002 (as (Chaetomorpha) okamurae)</td>
</tr>
<tr>
<td>Cladophora aegagropila (L.) Rabenh. 1868</td>
<td>section Aegagropilae / subgenus Aegagropila (^b)</td>
<td>Aegagropila linnaei Kütz. 1843</td>
<td>this study</td>
</tr>
<tr>
<td>Cladophora catenata (L.) Kütz. 1843</td>
<td>/</td>
<td>(Siphonochlorella) clade</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora echinus (Bisal.) Kütz. 1849</td>
<td>section Aegagropilae</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora patens (Mont.) Kütz. (^8)</td>
<td>subgenus Aegagropilae (^b)</td>
<td>Cladophora clade</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora sphaerica Reinbold</td>
<td>subgenus Aegagropilae (^b)</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora soriata Kütz.</td>
<td>/</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora sibogae Reinbold</td>
<td>section Rupestre (^c)</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora socialis (Roth) Kütz.</td>
<td>/</td>
<td>`classic' freshwater Cladophora species (^b)</td>
<td>Marks and Cummings 1996, Hanyuda et al. 2002</td>
</tr>
<tr>
<td>Cladophora socialis (Roth) Kütz.</td>
<td>/</td>
<td>Cladophora clade</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
</tbody>
</table>

Candidate members of the Aegagropila clade that were shown by molecular data to have different affilations

<table>
<thead>
<tr>
<th>Cladophora species</th>
<th>Cladophora section/subgenus</th>
<th>systematic placement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladophora catenata (L.) Kütz. 1843</td>
<td>section Aegagropilae / subgenus Aegagropila (^b, 4)</td>
<td>(Siphonochlorella) clade</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora echinus (Bisal.) Kütz. 1849</td>
<td>section Aegagropilae</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora patens (Mont.) Kütz. (^8)</td>
<td>subgenus Aegagropilae (^b)</td>
<td>(Siphonochlorella) clade (^g)</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora sphaerica Reinbold</td>
<td>subgenus Aegagropilae (^b)</td>
<td>(Siphonochlorella) clade (^g)</td>
<td>Leliaert et al. 2002, Leliaert et al. 2007</td>
</tr>
<tr>
<td>Cladophora soriata Kütz.</td>
<td>/</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora sibogae Reinbold</td>
<td>section Rupestre (^c)</td>
<td>Cladophora clade</td>
<td>Leliaert et al. 2009b</td>
</tr>
<tr>
<td>Cladophora socialis (Roth) Kütz.</td>
<td>/</td>
<td>`classic' freshwater Cladophora species (^b)</td>
<td>Marks and Cummings 1996, Hanyuda et al. 2002</td>
</tr>
<tr>
<td>Cladophora socialis (Roth) Kütz.</td>
<td>/</td>
<td>Cladophora clade</td>
<td>Hanyuda et al. 2002, Leliaert et al. 2007</td>
</tr>
</tbody>
</table>

\(^a\)van den Hoek and Chihara 2000.
\(^b\)Sakai 1964.
\(^c\)van den Hoek 1963.
\(^d\)Pankow and Täuscher (1980) reduced \(Pithophora\) to two species, however disagreement over the number of species exists. Originally, Wittrock (1877) had also transferred \(Cladophora\) acrosperma Kützing, \(C.\) sumatrana G. Martens and \(C.\) zelleri G. Martens to \(Pithophora\) in addition to the two species listed here.
\(^e\)van den Hoek 1982.
\(^f\)As \(C.\) fuliginosa Kütz.
\(^g\)Morphologically indistinguishable from \(C.\) coelothrix Kütz. (see van den Hoek and Chihara 2000).
\(^h\)\(C.\) fracta (O.F. Müller ex Vahl) Kütz., \(C.\) globulina (Kütz.) Kütz., \(C.\) glomerata (L.) Kütz., \(C.\) rivularis (L.) C. Hoek.
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 lectotypication. We here select *P. kewensis* Wittr. as the type species (isolectotypes 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 Basicladia included in this study. We excluded Spongiochrysis hawaiiensis (which has been proposed to be a member of the Aegagropila clade by Rindi et al. 2006) from our analyses due to data conflict. We obtained partial LSU and partial SSU RNA 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. conchopheria. However, our LSU sequence indicated a close relationship with the Trentephoblades. For confirmation, we re-extracted, amplified, and sequenced the partial LSU rDNA three times, always with the same result. In conclusion, the placement of S. hawaiiensis in the Cladophorales could not unequivocally be established, and further study of this organism is clearly necessary. We also did not include the published sequences of the Basicladia clade, and further study of this clade is clearly necessary. 

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 of the same cultures differed by 10–20 bp. Cladophora clavuligera Grunow and C. stenoclados 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 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).

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. conchopheria (= 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>
<thead>
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<th>Alignment</th>
<th>LSU</th>
<th>SSU</th>
<th>LSU–SSU</th>
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</thead>
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<tr>
<td>Taxa</td>
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<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Cladophoralean taxa</td>
<td>34</td>
<td>36</td>
<td>36</td>
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<tr>
<td>Outgroup taxa (Ulva fasciata, Trentepohlia sp.)</td>
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<td>2 (Ulva fasciata, Trentepohlia sp.)</td>
<td></td>
</tr>
<tr>
<td>Alignment length/analyzed length</td>
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<td>1708/1708</td>
<td>2363/2381</td>
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<td>456/303</td>
<td>746/543</td>
</tr>
<tr>
<td>informative sites</td>
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<td></td>
<td></td>
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<tr>
<td>Model estimated</td>
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<td>GTR + I + G</td>
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<tr>
<td>Estimated base frequencies (A/C/G/T)</td>
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<td>0.25/0.22/0.28/0.25</td>
<td>0.24/0.25/0.29/0.24</td>
</tr>
<tr>
<td>Estimated substitution frequencies (AC/AG/AT/CG/CT/GT)^a</td>
<td>0.85/2.95/1.54/0.80/5.98/1</td>
<td>1.27/2.90/2.11/0.77/6.90/1</td>
<td>1.15/3.17/1.83/0.97/6.42/1</td>
</tr>
<tr>
<td>Among-site variation I/G^b</td>
<td>0.27/0.52</td>
<td>0.51/0.43</td>
<td>0.42/0.37</td>
</tr>
</tbody>
</table>

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^a Estimated by Akaike Information Criterion (AIC).

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

^c 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.

---

...
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 secund 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. 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

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.

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.
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. stercoreladi*a.

**Pyrenoid ultrastructure.** Pyrenoid ultrastructure was examined by TEM with respect to the number of thylakoid membranes transversing the pyrenoid (bilateral versus polypyramidal structure) and the arrangement of starch plates. In the *Wittrockiella* clade, both polypyramidal and bilateral 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. *roetleri* (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; \( n = 19 \)). Specimens K96 (P. *roetleri*) 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 conchophoria*, *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 loraxanthin, 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 byallii*, *Arnoldiella conchophoria*, *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* (Miyagi 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. kosterae*, one isolate of *Pithophora* (P. cf. *roetleri*, K93) and *Aegagropila linnaei*. Both polypyramidal and bilenticular pyrenoids were found in *Wittrockiella amphibia*, *Cladophorella calcicola*, *Pithophora* cf. *polymorpha* and *P. roetleri*. 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 (Cocqyt et al. 2010), has siphonoxanthin (O’Kelly 1982), a character that otherwise appears to be derived within Cladophorales (Yoshii et al. 2004).
Since lutein, loroxanthin, and siphonoxanthin are thought to be successive products in a biosynthetic series (Egeland et al. 1997, Yoshii et al. 2004), the actual xanthophylls produced may be less 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 \textit{Pithophora} species (Pearlmutter and Lembi 1978, 1980); for other members of the \textit{Aegagropila} clade, data are lacking. There are, however, reports of the presence of chitin in the cell walls of \textit{Cladophora glomerata} (Wurduck 1923) and \textit{C. vagabunda} (Jönsson 1962, as \textit{C. expansa}). Both species are members of the \textit{Cladophora} clade, rendering the presence of chitin invalid as a diagnostic character.

\textbf{Two families: Pithophoraceae and Pseudocladophoraceae.} Because neither polypyramidal pyrenoids nor the presence of loroxanthin or chitin represents diagnostic characters for the \textit{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 Siphonocladales 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 Cladostrona. 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 cheloniunm 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., Chaetocladiulla, Chaetonella, Cladogonium, Cladostrona, Dermatophyton and Gemmiphora; see Appendix S3).

The other lineage includes the marine species Cladophora conchopheria 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 conchopheria grows exclusively on shells of the marine snail Lusella 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. conchopheria 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. conchopheria 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. lyallii, 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 subaeral species reported from moist limestone, bricks and mud in China, Bangladesh, and tropical hothouses in Europe (Fritsch 1944, Islam 1964, Cribb 1965, Ettl 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. fritschi A.K. Islam, C. sundarbanensis A.K. Islam, and C. netzhualpili 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 (Basicladia clade), Basicladia okamurae and B. ramulosa. Arnoldiella conchophila grouped together with two Basicladia species with moderate (ML) to high (BI) support. The grouping of Arnoldiella conchophila with species of Basicladia 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 Basicladia species. Basicladia ramulosa was included in a phylogenetic analysis for the first time, while the relationship of B. okamurae with Basicladia 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 Basicladia 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 Basicladia 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). Basicladia 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. Basicladia 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 Basicladia, we propose the transfer of all described Basicladia 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 linnæi is currently the only species of the genus. More than 90 synonyms exist for A. linnæi (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. linnæi 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 helicoid 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 aki-
etes 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. clavuligera) are likely to account for the lack of speciation within the genera, the low intraspecific 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. sauter*, 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-Mari-mo’). This species is morphologically similar to *A. linniae*, 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. beneckei* 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. sterrecladia*, which has been described from the shell of a freshwater snail (*Paludina*) from Myanmar (Skuj 1949). In a number of studies, this (sub)tropical species has been confused with the temperate species *A. linniae* (e.g., Prasad and Misra 1992, Gardavsky 1993, Liu 1999, Islam and Irfanullah 2005). As in the genus *Arnoldiella*, there seems to be a tendency in this clade for epizoic, especially to colonize freshwater gastropods. In comparison with *Arnoldiella*, the rhizoidal system in the *C. clavuligera/C. sterrecladia* 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. sterrecladia* include *C. basicladioides*, *C. beneckei*, *C. codiola*, *C. exigua*, *C. glorera* 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. sterrecladia* 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., *Chaetonephila* goetzii, *Cladophora basicladioides*, *Cladophora cornuta*, *Cladophora rhizobrachialis*, *Cladostroma sitchensense*), niche evolution (*Basicladia vivipara*, *Cladogonium ogishimai*—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 Chaetocladiella might represent non-epizoic species of Arnoldiella (Bourrelley 1966). Furthermore, a group of morphologically similar species and potential members of the genus Agagropila (see Ihzholdina 2007) appear to have diversified sympatrically in Lake Baikal. Agagropila linnaei or its ancestor is assumed to have dispersed throughout the Palaeartic (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 Agagropilopsis, the emended description of Arnoldiella, as well as typification details and nomenclatorial changes are as follows:

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


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 polypyrimal 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 descending secondary rhizoids, frequently intracuticular in cells below. The adventitious rhizoids become fused with the cell walls of the lower cells, producing stem-like structures that form a polysiphonous base. Cells cylindrical, apical cells rounded. Cell walls thick, in apical parts 1.5–3 (–5) μm, in basal parts 5–15 (–30) μm. 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.


_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.


**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 1999, _Nytt Mag. Naturv. Densk._ 47: Tables XI–XIV).

_Epitype:* Lyngør, south-eastern Norway, collector B. Lyng, 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._


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


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


**Wittrockiella fritschii** (A.K. Islam) Boedeker, comb. nov.
*Holotype* no information.

**Wittrockiella netzahualpillii** (Galicia et Novelo) Boedeker, comb. nov.
*Holotype* Brackish water extraction wells, Lake Texcoco Management, Mexico, FCME TEX1 (figs. 1–30).

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

*Additional species*
**Arnoldiella chelonum** (Collins) Boedeker, comb. nov.
*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.
*Holotype* lost, thus the original drawings represent the holotype material (Hoffmann and Tilden 1930, *Bot. Gaz.* 89: figs. 2–20).

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

**Arnoldiella sinensis** (N.L. Gardner) Boedeker, comb. nov.
*Basionym*: *Chaetomorpha sinensis* N.L. Gardner 1936, *Madrono* 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.
*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 kewenensis* Wittrock 1877, *Noua 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 kewenensis* 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.
*Holotype* Tranquebar, eastern India, collector Klein, January 1799, L 93825 38. Isotype in UPS.
*Synonym*: *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
Aegagropilopsis Boedeker, gen. nov.

Algae aquae dulcis (sub) trophiis interdum nonnihil salisugineae caespitibus minutis minus quam 1,5 cm altis filamentis erectis uniseriabilis haftoreone coralloide rhizoides secundarius longis. Thalli dense ramosi saepe partibus inferioribus sparse ramosi. Rami praecipe oppositi interdum pecinati insertione subterminali interdum seriali angulis acutis tarde formantibus ardue inclinatis. Cellulae usque ad cinque laterales saepe verticillatas typice parum ad nodos constrictas partibus apicalibus longis eramosis. Rhizoidea adventita et thalli omnibus partibus facta. Thalli praecipe epizooptici ad cochlea aquae dulcis (Pila, Paludina) sed quoque substratis immobiles affixi inventus. Distinguuitur Aegagropila differentis in sequentibus 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 whirs. 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 epizoic 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.


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).


Additional species:

Aegagropilopsis clavuligera (Grunow) Boedeker, comb. nov.


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

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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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