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

Boedeker, C.

Citation

Boedeker, C. (2010, December 21). *Phylogenetic, taxonomic and biogeographical studies in the Pithophoraceae (Cladophorales, Chlorophyta)*. Retrieved from https://hdl.handle.net/1887/16263

Appendix S5. List of outgroup taxa and their respective GenBank accession numbers for the LSU and SSU rDNA sequences (sequences generated in this study are indcated in bold).

outgroup taxa	**GenBank accession nos.**	
	LSU	SSU
Trentepohlia sp.	**FR719952**	**FR719938**
Ulva fasciata Delile	AJ544726	AB425964
Okellya curvata (Printz) Leliaert & Rueness	FN257507	FN257508
Cladophora coelothrix Kützing	AM503443	AM498749
Anadyomene stellata (Wulfen) C. Agardh	AJ544746	AF510147
Boergesenia forbesii (Harvey) J. Feldmann	AJ544742	AF510164
Cladophora aokii Yamada	AM503434	AM498747
Siphonocladus tropicus (P. Crouan & H. Crouan) J. Agardh	AJ544744	AM498761
Boodlea composita (Harvey) Brand	AJ544731	AF510157
Struvea elegans Børgesen	AJ544737	AF510149
Dictyosphaeria cavernosa (Forsskål) Børgesen	AJ544745	AM498756
Valonia aegagropila C. Agardh	AJ544748	AM498762
Valoniopsis pachynema (G. Martens) Børgesen	AJ544741	AM498765
Cladophora pygmaea Reinke	FM205040	FM205051
Cladophora feredayi Harvey	FM205030	FM205049
Cladophora pellucida (Hudson) Kützing	FM205037	Z35314
Cladophora radiosa (Suhr) Kützing	FM205042	FM205052
Cladophora albida (Nees) Kützing	AM503433	Z35317
Cladophora vagabunda (Linnaeus) van den Hoek	AJ544760	AB062710
Cladophora rupestris (Linnaeus) Kützing	AJ544764	Z35319
Chaetomorpha crassa (C. Agardh) Kützing	AJ544767	AB062701

Appendix S6

Effects of model choice and missing data on tree topologies and support

The 'test' alignment was assembled to test for the effects of using a complex *vs.* a simple evolutionary model both with and without outgroup and consisted of 39 ingroup taxa (see Table 3 in Chapter 7). Since the phylogenetic position of the early-diverging clade consisting of *Cladophora horii* and *C. conchopheria* was not stable in preliminary BI and MP analyses performed with the 'test' alignment, the effects of using a complex (GTR+I+G, determined by Modeltest) *vs.* a simple (HKY) model of nucleotide substitution on the tree topology was evaluated in BI analyses, both with (Figs. S6 A & B) and without *Okellya curvata* as outgroup (Figs. S6 C&D). In both cases, the trees resulting from the analysis with the simple HKY model were better resolved and had higher support for several branches. Main differences in the topologies under the two different models were the placement of *Cladophora horii* and *C. conchopheria* (Figs. S6 A & B), the monophyly of the *Basicladia*-clade (Figs. S6 A & B, C & D), and the overall resolution within the *Aegagropila/Pithophora* clade. When an outgroup was used, the clade consisting of *C. horii* and *C. conchopheria* grouped without support with the *Siphonocladus*-clade and the *Cladophora*-clade under the GTR+I+G model (Fig. S6 A). Under the HKY model, this clade grouped with high support with the *Aegagropila*-clade (Fig. S6 B). Without ougroup, this clade grouped with high support with the *Aegagropila*-clade under both models (Figs. S6 C & D). The *Basicladia*-clade was only monophyletic with high support when a simple model (HKY) was used instead of a more complex model (GTR+I+G) that was estimated to have the best fit for the data, or in MP analyses. Under a GTR+I+G model *B. okamurae* was recovered on a polytomy. MP analysis is considerd as one of the least reliable methods, especially if sites are not evolving independently as in the rRNA genes, since no model of nucleotide evolution is employed (Schöniger & von Haeseler 1995). Similarly, only under the HKY model the relationships within the *Aegagropila/Pithophora* clade were resolved, while the three subclades formed a polytomy under a complex model. Both with and without using outgroup sequences, the trees resulting from the analysis with the simple HKY model were better resolved and had higher support for several branches. However, it is incorrect to regard better resolved or better supported trees as superior since there is no *a priori* knowledge of the true phylogeny and support does not necessarily correlate with accuracy (Gontcharov *et al*. 2004). Employing simple models of evolution for complex datasets can possibly lead to the recovery of wrong topologies (Posada & Crandall 2001). Only the trees obtained under the inferred complex model are shown in the main text. Possible explanations for the failure to unequivocally establish the phylogenetic placement of *B. okamurae* with high support could be a higher proportion of homoplasic characters in *B. okamurae*, long branch attraction (Graham *et al*. 2001) or insufficient taxon sampling.

The 'test' alignment was also used to test for the effects of missing sequence data (not shown). Taxa with missing LSU sequences (two taxa: *Cladophora* sp. 'Tateyama' and *Arnoldiella conchophila*) and with missing partial SSU sequences (one taxon: *Cladophora sterrocladia*) were excluded from this alignment, and we subsequently compared the resulting tree topologies from BI analyses (not shown). Excluding the three taxa with missing sequence data had no effect on the tree topology. The model choice (GTR+I+G *vs.* HKY) had the same effects on datasets without missing data as outlined above.

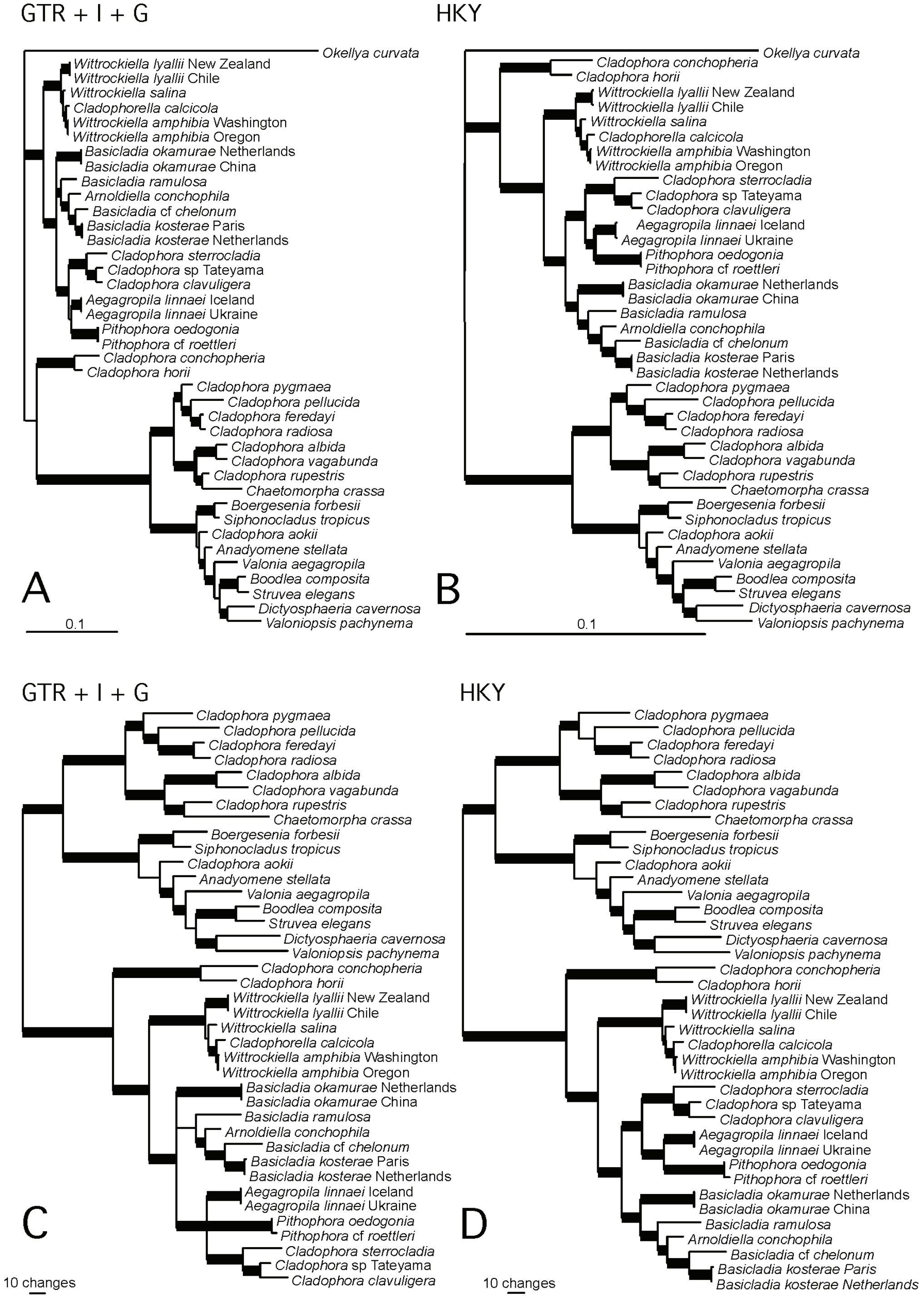

Fig. S6. Comparison of model performance (GTR+I+G *vs*. HKY) used in Bayesian Inference analysing the combined SSU_LSU dataset. **A**: LSU_SSU dataset with outgroup under a GTR+I+G model. **B**: SSU_LSU dataset with outgroup under a HKY model. **C**: SSU_LSU dataset without outgroup under a GTR+I+G model. **D**: SSU_LSU dataset without outgroup under a HKY model. Thick branches indicate posterior probabilities > 0.95.

Appendix S7

Molecular clock and midpoint rooting of the ingroup

In addition to rooting with the three outgroup-taxa, the position of the root was determined for the 'ingroup' dataset (see Table 3 in Chapter 7) by molecular clock rooting using BEAST v1.5.2 (Rambaut & Drummond 2007b) and by midpoint rooting of the BI and ML trees (Fig. S7). The molecular clock analysis automatically roots the tree along its oldest branch. The likelihood ratio tests of the LSU and SSU alignments separately and combined rejected rate constancy in all cases. However, molecular clock rooting is robust to moderate degrees of rate heterogeneity (Huelsenbeck *et al*. 2002), so it is still possible to accurately root trees with a (relaxed) molecular clock when local clock deviations exist. The dataset was analysed under both a relaxed (Drummond *et al*. 2006) and a strict molecular clock assumption n BEAST. For all analyses, two independent runs of 50 x 10^6 generations with a fixed mean substitution rate, default priors and a Yule model as the tree prior were performed at the BioHPC cluster (http://cbsuapps.tc.cornell.edu/beast.aspx), which were subsequently combined with LogCombiner (part of the BEAST package). A lognormal relaxed clock was utilized. Trees were sampled every 5000 generations, and a burnin of 1000 trees was removed before generating consensus trees with TreeAnnotator (part of the BEAST package). The log-files were checked with the program Tracer.

The trees constrained with a strict and a relaxed molecular clock (Figs. S7 A & B, respectively) both show the same root position as the outgroup rooting results (Fig. 3 in Chapter 7), separating the *Aegagropila*-clade as a monophyletic clade (including the *horii*-clade) with high support from the sister groups *Siphonocladus*-clade and *Cladophora*-clade. Additional midpoint rooting performed on BI and ML trees of the 'ingroup' dataset (Figs. S7 C & D, respectively) showed the same root position as revealed by outgroup rooting and molecular clock rooting.

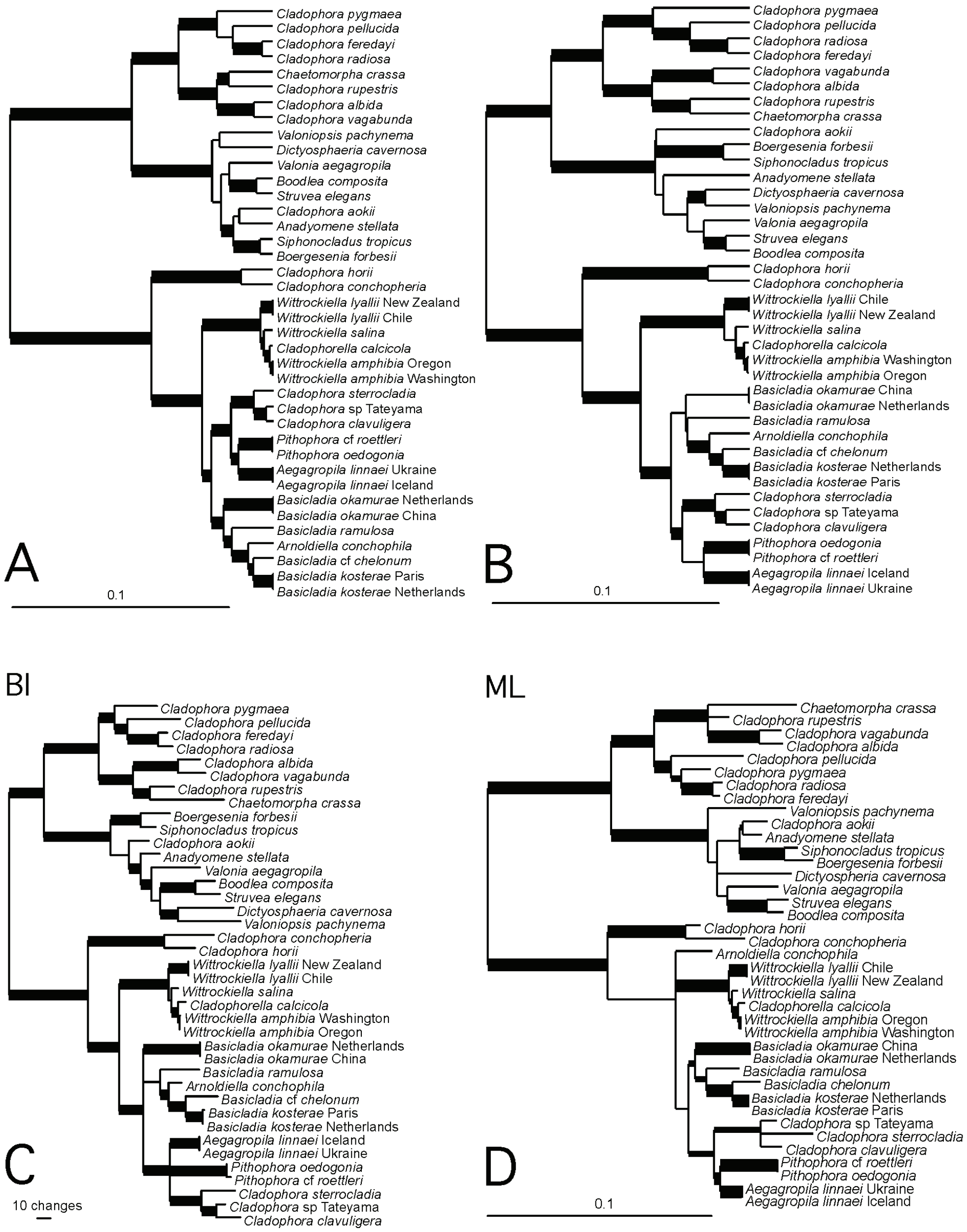

Fig S7: Ingroup_only analysis of the SSU_LSU dataset under different phylogenetic inference methods (all with GTR+I+G as substitution model). Molecular clock rooting was performed in BEAST under a strict (**A**) and a relaxed (**B**) molecular clock algorithm. The Bayesian tree (**C**) and the Maximum Likelihood tree (**D**) were rooted using midpoint. Taxa of the *Cladophora*-clade and the *Siphonocladus*-clade are included to evaluate root placement. Thick branches indicate posterior probabilities > 0.95 in **A**-**C**, while in **D** medium-thickness branches reflect bootstrap values over 70 and thick branches reflect bootstrap values over 90 (1000 replicates).

Appendix S8

List of all nomenclatorial and taxonomic changes proposed in this thesis

Nomenclatorial-taxonomic changes proposed in this thesis are underlined.

New descriptions, new combinations, new synonymizations

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

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

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

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

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

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

***Arnoldiella kosterae* (van den Hoek) Boedeker comb. nov.**
Holotype: Jardin des Plantes, Paris, France, collector C. van den Hoek, 25 April 1961, L (L 0054830).

Basionym: *Cladophora kosterae* van den Hoek 1963: 37-38.
Synonym: *Basicladia kosterae* (van den Hoek) Garbary 2010: 39.

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

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

Pithophora **Wittrock**
Type species: *Pithophora roettleri* (Roth) Wittrock (*Pithophora kewensis* Wittrock 1877: 52-55).
Lectogeneritype: *Pithophora kewensis* Wittrock, tropical aquarium (‘Waterlily-house’), Kew Gardens, Britain, collector V.B. Wittrock, August 1872, L (no. 938112 639). Isotypes in BM, L, UPS & S.

Pithophora roettleri **(Roth) Wittrock**
Holotype: Tranquebar, eastern India, collector Klein, January 1799, L (no. 93825 38). Isotype in UPS.
Basionym: *Ceramium roettleri* Roth 1806: 123.
Synonyms: all described species of *Pithophora* (including all intraspecific taxa) *P. aequalis* Wittrock, *P. affinis* Nordstedt, *P. chinensis* Skworzow, *P. clavifera* Schmidle, *P. cleveana* Wittrock, *P. kewensis* Wittrock, *P. macrospora* Brand, *P. microspora* Wittrock, *P. mooreana* Collins, *P. oedogonia* (Montagne) Wittrock, *P. pachyderma* Schmidle, *P. pragensis* Sula, *P. polymorpha* Wittrock, *P. radians* W. & W.S. West, *P. reinecki* Schmidle, *P. sumatrana* (Martens) Wittrock, *P. varia* Wille, *P. variabilis* Schmidle, *P. zelleri* (Martens) Wittrock.

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

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

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

***Wittrockiella* Wille**

Type species: *Wittrockiella amphibia* (Collins) Boedeker & Hansen (*Wittrockiella paradoxa* Wille 1909: 220-221).

Generitype: original specimen (collected in 1907 by N. Wille, Lyngør, Norway) untraceable, thus the original drawings represent the holotype material (Wille 1909: Plates XI-XIV).

Epitype: Lyngør, southeastern Norway, collector B. Lynge, January 1909, det. N. Wille, O (six iso-epitypes).

***Wittrockiella amphibia* (Collins) Boedeker et Hansen comb. nov.**

Isotypes: Alameda, San Francisco Bay, California, USA, Leg. W.J.V. Osterhout and N.L. Gardner, 26 September 1903, in L, voucher numbers L1284 and L2989, L1284 distributed as Exsiccatae Phycotheca Boreali-Americana (PB-A) No. 1284 (Collins *et al.* 1905). Additional isotypes distributed as No. 1284 of the PB-A to AHFH, BM, FH, PC, TRH, UC and WIS, among others. We select the specimen L1284 as the lectotype.

Basionym: *Cladophora amphibia* Collins 1907: 200.

Synonym: *Wittrockiella paradoxa* Wille 1909: 220-221.

***Wittrockiella calcicola* (Fritsch) Boedeker comb. nov.**

Holotype: original specimen (collected by F.E. Fritsch, tropical hothouse, Cambridge Botanical Garden, Britain, 1944, on moist limestone) lost or destroyed (used to be in BM), thus the original drawings represent the holotype material (Fritsch 1944: Figs. 1A-I, 2A-G, 3A-C, 4A-G).

Epitype: Lagoa de Óbidos, Portugal, collector O. Lourenço (sample no. K92; ACOI culture collection 471), 1989, L (L0793292).

Basionym: *Cladophorella calcicola* Fritsch 1944: 157-171.

***Wittrockiella lyallii* (Harvey) C. Hoek, Ducker & Womersley 1984: 45**

Holotype: Stewart Island, New Zealand, collector D. Lyall, TCD (isolectotypes in BM).

Basionym: *Cladophora lyallii* Harvey 1855: 262

Synonyms: *Siphonocladus brachyartrus* Svedelius 1900: 304, *Cladophoropsis brachyartra* (Svedelius) Wille 1910: 116, *Cladophoropsis lyallii* (Harvey) V.J. Chapman 1956: 471.

Doubtful, erroneous, and excluded names

Refused synonyms of *Aegagropila linnaei* Kützing (Chapter 4, Appendix S1)

Cladophora aegagropila var. *thermalis* Wolle 1885
    > description suggests different affinity (*Cladophora sp.*)
Cladophora sterrocladia Skuja 1949
    > synonymity had been proposed by Liu (1999)
Cladophora yuennanensis Skuja 1937
    > synonymity had been proposed by van den Hoek (1963)
Cladophora shensiensis Jao 1948
    > synonymity had been proposed by Liu (1999)
Cladophora glomerata var. *nana* Wang 1935
    > synonymity had been proposed by Liu (1999)
Cladophora parvula Möbius 1895

> synonymity had been considered by Skinner & Entwisle (2000)

Cladophora dusenii Brand 1902

> possible synonymity had been discussed by Brand (1902)