

Phylogenetic analyses of *Cladophora vagabunda* (L.) C. Hoek (Cladophorales, Chlorophyta) from Brazil based on SSU rDNA sequences¹

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ABSTRACT – (Phylogenetic analyses of *Cladophora vagabunda* (L.) C. Hoek (Cladophorales, Chlorophyta) from Brazil based on SSU rDNA sequences). The complete SSU rDNA was sequenced for 10 individuals of *Cladophora vagabunda* collected along the coast of Brazil. For *C. rupestris* (L.) Kütz. a partial SSU rDNA sequence (1634 bp) was obtained. Phylogenetic trees indicate that *Cladophora* is paraphyletic, but the section *Glomeratae sensu lato* including *C. vagabunda* from Brazil, Japan and France, *C. albida* (Nees) Kütz., *C. sericea* (Hudson) Kütz., and *C. glomerata* (L.) Kütz. is monophyletic. Within this group *C. vagabunda* is paraphyletic. The sequence identity for the SSU rDNA varied from 98.9% to 100% for the Brazilian *C. vagabunda*, and from 98.3% to 99.7% comparing the Brazilian individuals to the ones from France and Japan. Sequence identity of the Brazilian *C. vagabunda* to *C. albida* and *C. sericea* vary from 98.0% to 98.6%. The SSU rDNA phylogeny support partially the morphological characteristics presented by Brazilian populations of *C. vagabunda*. On the other hand, *C. rupestris* from Brazil does not group with *C. rupestris* from France, both sequences presenting only 96.9% of identity. The inclusion of sequences of individuals from Brazil reinforces the need of taxonomical revision for the genus *Cladophora* and for the complex *C. vagabunda*.

Key words - Brazil, *Cladophora vagabunda*, Cladophorales, molecular phylogeny, SSU rDNA

RESUMO – (Análises filogenéticas de *Cladophora vagabunda* (L.) C. Hoek (Cladophorales, Chlorophyta) do Brasil, baseada nas seqüências SSU rDNA). A seqüência completa do SSU rDNA foi obtida para 10 indivíduos de *Cladophora vagabunda* coletadas ao longo da costa do Brasil. Uma seqüência parcial do SSU rDNA (1634 bp) foi obtida para *C. rupestris* (L.) Kütz. As árvores filogenéticas indicam que *Cladophora* é parafiletico, mas a seção *Glomeratae sensu lato* compreendendo *C. vagabunda* do Brasil, Japão e França, *C. albida* (Nees) Kütz., *C. sericea* (Hudson) Kütz. e *C. glomerata* (L.) Kütz., é monofilética. Dentro deste grupo, *C. vagabunda* é parafiletica. A identidade da seqüência para o SSU rDNA variou de 98,9% à 100% para *C. vagabunda* brasileira e de 98,3% a 99,7% quando comparados os indivíduos brasileiros aos da França e do Japão. A identidade da seqüência entre *C. vagabunda* brasileira e as duas outras espécies (*C. albida* e *C. sericea*) variou entre 98,0% e 98,6%. A filogenia do SSU rDNA suporta parcialmente as características morfológicas apresentadas pelas populações brasileiras de *C. vagabunda*. Por outro lado, *C. rupestris* do Brasil não se agrupou a *C. rupestris* da França, as duas seqüências apresentaram somente 96,9% de identidade. A inclusão de seqüências de indivíduos do Brasil reforçam a necessidade de uma revisão taxonômica para o gênero *Cladophora* e para o complexo *C. vagabunda*.

Palavras chave - Brasil, *Cladophora vagabunda*, Cladophorales, filogenia molecular, SSU rDNA

Introduction

The genus *Cladophora* Kütz. (Cladophorales, Chlorophyta) is composed by a heterogeneous group of species that shows a high morphological plasticity

influenced by thallus age and habitat. The genus is widespread in marine, freshwater, estuarine and brackish water environments, from temperate to tropical regions (Hoek 1963, 1982, Dodds & Gudder 1992, Hoek *et al.* 1995, Hoek & Chihara 2000, Leliaert *et al.* 2003). Taxonomical problems due to morphological plasticity and wide geographical distribution led to the use of molecular biology analyses to study the genus.

Bot *et al.* (1989a,b, 1990, 1991) estimated the genetic distance among individuals of *Cladophora* species with wide geographic disjunction, using the single strand complementary DNA-DNA hybridization. Unexpected wide genetic distances were observed between North Atlantic and Indo-Pacific individuals of *C. albida* (Nees) Kütz., corresponding to distances found among higher plants genera and mammals families. On the other hand, no divergence was found among *C. sericea* (Hudson)

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Kütz. amphiatlantic individuals, interpreted as result of a transatlantic dispersion after the last Pleistocene glaciations (Bot *et al.* 1989a,b, 1991). The moderated divergence observed between *C. laetevirens* (Dillwyn) Kütz. and *C. vagabunda* (L.) C. Hoek individuals from North Atlantic and Australia, respectively, was suggested to be a reflex of their eurythermic tolerances, which potentially allowed the dispersion through tropical regions (Bot *et al.* 1990).

Bakker *et al.* (1992) defined biogeographic groups of *C. albida* using the ribosomal genes (rDNA) internal transcribed spacers (ITS) sequences, observing that there was a 21% difference in the ITS sequences when the inter-oceanic individuals were compared. Bakker *et al.* (1994) studied the *Cladophora* complex based on the small subunit rRNA gene (SSU rDNA) to verify the evolutionary relationships among species traditionally attributed to Siphonocladales/Cladophorales Orders. They concluded that there is no basis for independent recognition of Cladophorales and Siphonocladales because the first is paraphyletic and the second is polyphyletic.

Bakker *et al.* (1995) studied the global phylogeography of the cosmopolitan *C. vagabunda* and concluded that this species represents an intermediary case between the old tropical species, that show a well-marked vicariance, and cold-temperate to boreal, more recently derived strains that do not present a well-marked vicariance.

Hoek & Chihara (2000) presented the taxonomic revision of the species of *Cladophora* from Japan to Russian Far-East, recording the occurrence of 32 taxa and a new approach to the distribution of the species inside the morphological sections was given according to phylogenetic relationships based on SSU rDNA sequences. Further, Hanyuda *et al.* (2002) observed the phylogenetic relationships in Cladophorales inferred from SSU rDNA sequences and concluded that *Cladophora* is polyphyletic because many species of this genus are more closely related to species belonging to other genera than are to other *Cladophora* species.

Recently, Leliaert *et al.* (2003) inferred the phylogenetic relationships in Cladophorophyceae based on the large subunit rRNA gene (LSU rDNA) sequences and showed that Siphonocladales forms a monophyletic group while Cladophorales remains paraphyletic.

On Brazilian coast, *Cladophora vagabunda* is widely distributed from Maranhão (North coast) to Rio Grande do Sul States (South coast), according to Oliveira Filho (1977) and Horta *et al.* (2001). So far, there has been no specific study regarding the phylogenetic

relationships among *C. vagabunda* and other allies in Brazil. Thus, the aim of this study is to verify the relationships among *C. vagabunda* individuals collected from different regions of the Brazilian coast inferred from SSU rDNA sequences.

Material and methods

Samples – Ten individuals of *Cladophora vagabunda* and one of *C. rupestris* (L.) Kütz. were collected from seven locations: three of them in the Northeastern region and the others in the Southeastern region of the Brazilian coast (table 1). The thalli were washed in seawater to remove the excess of sediment and undesirable organisms, briefly dried in absorbent paper, directly transferred to a recipient with silica gel and stored at room temperature.

DNA Extraction – Dehydrated thalli of *C. vagabunda* (about 40-50 mg) were macerated in liquid nitrogen with mortar and pestle, until a thin powder was obtained. Total genomic DNA was extracted using the “DNeasy Plant Mini Kit” (Qiagen, Santa Clarita, CA, USA), according to manufacturer’s instructions, adding one step of centrifugation before the extract addition to the first purification column. DNA samples were stored at -20 °C. A DNA aliquot (5 µL) was submitted to electrophoresis in 0.7% agarose gel (Sambrook *et al.* 1989) to verify quality and quantity of the DNA extracted.

Polymerase chain reaction amplification and sequencing – Primers 18S5’ and 18S3’ were used for the amplification and sequencing of the SSU rDNA (Sogin 1990, Bird *et al.* 1992, Oliveira & Ragan 1994, Milstein & Oliveira 2005). Polymerase chain reaction (PCR) amplification conditions for a total volume of 50 µL were 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.2 µM each primer, around 5 ng of genomic DNA and 1.25 U of *Taq* DNA polymerase (Invitrogen – Life Technologies, CA, USA). All PCR reactions were performed in a thermocycler “PTC – 150 Mini Cycler” (MJ Research Inc., Maryland) as follows: 94 °C for 5 min, 35 cycles of 94 °C for 30 sec, 55 °C for 1 min and 72 °C for 2 min and a final extension step at 72 °C for 7 min. *Taq* DNA polymerase was added to reactions only when the template was totally denatured (in the first step of 5 min at 94 °C) for a higher specificity of the product to be synthesized. At least three independent PCR reactions were pooled together for each fragment to be sequenced (Baldwin *et al.* 1995).

PCR products were purified using “MicroSpin™ S-300” columns (Amersham Pharmacia Biotech, Piscataway, NJ, USA), according to manufacturer’s specifications.

PCR products were directly sequenced using BigDye™ Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, NJ, USA) by standard methods, on an “ABI PRISM 3100 Genetic Analyzer” (Applied Biosystems). The sequences were assembled to obtain a consensus sequence, using the program Eyeball Sequence Editor (ESEE) version 3.2 (Cabot & Beckenbach 1989). When site ambiguity

Table 1: Collection information for *Cladophora vagabunda* individuals and *C. rupestris* from Brazil, the SSU rDNA gene size (bp = bases pairs), and GenBank accession number.

Locality	Collection date	Collector	Observation	Abbreviation (GenBank Accession n.)	SSU rDNA (bp)
<i>Cladophora vagabunda</i>					
Ponta do Cabo Branco Beach – João Pessoa (PB) (07°09'48" S-034°48'36" W)	7 Oct. 2002	<i>L. Gestinari & A. Kanagawa</i>	growing on rocks; intertidal zone	CB1 (FJ715640)	1783
				CB2 (FJ715641)	1781
Boa Viagem Beach – Recife (PE) (08°07'51,9" S-034°53'55,32" W)	9 Aug. 2002 21 Sep. 2002	<i>L. Gestinari & J. Torres</i>	growing on the fringe of sandstone reef; infralittoral	BV1 (FJ715642)	1781
				BV2 (FJ715643)	1783
Sereias Beach – Maceió (AL) (09° 33'53,3" S-035°38'43,9" W)	04 Oct. 2002	<i>L. Gestinari & E. Guedes</i>	growing on the fringe of sandstone reef; infralittoral	SER1 (FJ715644)	1782
				SER2 (FJ715645)	1781
Portocel Beach – Aracruz (ES) (19°53'18" S-040°05'30" W)	29 Jun. 2003	<i>C. Nassar</i>	growing on rocks; intertidal zone	POR (FJ715646)	1781
Prainha Beach – Arraial do Cabo (RJ) (22°57'30" S-042°01'30" W)	1 Aug. 2003	<i>L. Gestinari & J. Torres</i>	growing on rocks; intertidal zone	PRA (FJ715647)	1781
Conchas Beach – Cabo Frio (RJ) (22°53'00" S-042°01'30" W)	1 Aug. 2003	<i>L. Gestinari & J. Torres</i>	growing on rocks; intertidal zone	CON (FJ715648)	1781
Boa Viagem Island – Niterói (RJ) (23°54'36" S-043°07'48" W)	16 Jun. 2003	<i>L. Gestinari & J. Torres</i>	growing on rocks; intertidal zone	IBV (FJ715649)	1781
<i>Cladophora rupestris</i>					
Ponta do Cabo Branco Beach - João Pessoa (PB) (07°09'48" S-034°48'36" W)	7 Oct. 2002	<i>L. Gestinari & A. Kanagawa</i>	growing on rocks; intertidal zone	RUP (FJ715639)	1634 ¹

¹ partial SSU rDNA sequence.

was observed, a careful analysis was performed on the chromatograms to confirm the correct nucleotides on those sites. If the confirmation was not possible, new sequences were generated. Full gene sequence was obtained from both strands, except for sample RUP (table 1), for which only partial sequence was obtained (1634 bp).

Matrix – Consensus sequences obtained for the SSU rDNA from each individual analyzed were aligned using ClustalW in BioEdit program (Hall 1999) and were manually inspected. SSU rDNA sequences from other Cladophorales species were imported from GenBank (see figure 1 for accession numbers) and included in the alignments. The sequences from *C. horii* C. Hoek & Chihara and *C. conchopheria* Sakai were chosen as outgroups. Sequences corresponding to amplification primers 18S5' and 18S3' and variable regions, which could not be unambiguously aligned, were removed from the

alignments, yielding a final matrix of 29 taxa sequences with 1558 positions.

Phylogenetic trees construction – All phylogenetics analyses were performed with PAUP 4.0b8 (Swofford 2000). An appropriate evolution model was selected using Modeltest 3.06 (Posada & Crandall 1998). The selected model estimated from our data was Tamura & Nei (1993) with equal base frequencies. Base substitution model was determined as [A ↔ C, A ↔ T, C ↔ G, G ↔ T] = 1.0000, [A ↔ G] = 1.8236 and [C ↔ T] = 5.1336. The proportion of invariable sites considered was 0.7620 and the gamma distribution rate parameter was 0.5866 for rate heterogeneity on variable sites.

Trees were inferred using three different methods. For the distance method, a neighbor-joining (NJ) tree (Saitou & Nei 1987) was built with Tamura & Nei (1993) substitution model. A maximum parsimony (MP) tree was inferred by

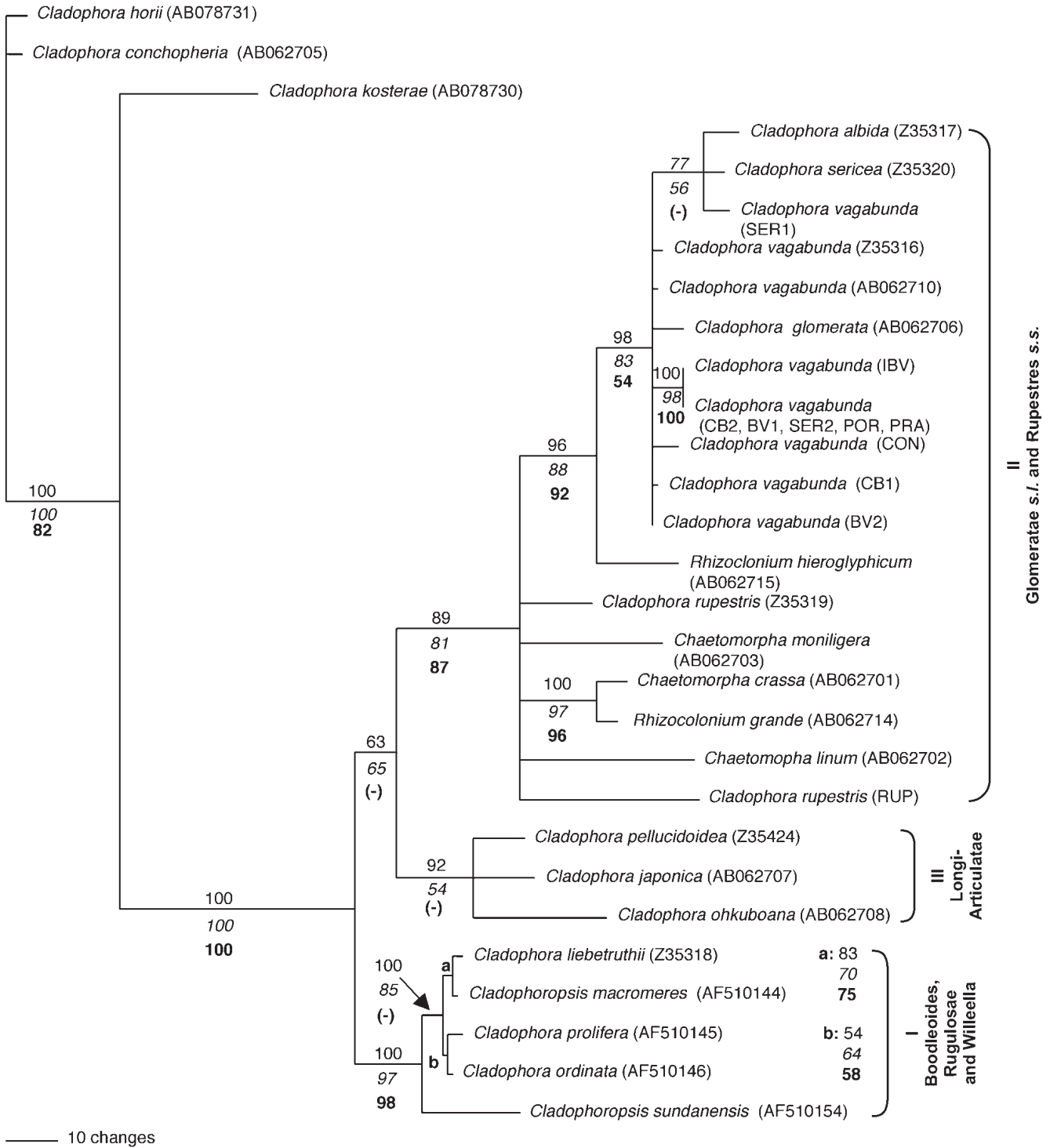


Figure 1. Maximum parsimony consensus tree based on SSU rDNA. Bootstrap values for the different analyses are plotted on the branches: neighbor joining (first line, normal typeface) and maximum parsimony (second line, italic typeface) were performed for 2000 replicates; maximum likelihood (third line, bold typeface) was performed for 100 replicates. An arrow or a letter (a, b) indicate these values where they do not fit. (-) indicates no support. Within brackets there is the GenBank accession number or abbreviation for the Brazilian collections (see table 1). I – III are the subgroup formed with the respective sections.

heuristic search, with starting trees obtained by stepwise addition, with random sequence addition (10 replicates) using the tree bisection-reconnection (TBR) branch-swapping algorithm. The number of parsimony-informative sites was 138. In both NJ and MP trees, gaps were treated as missing data and all sites were weighted equally. Bootstrap analyses (Felsenstein 1985) were performed with 2000 replicates for the methods described above.

Maximum likelihood (ML) analysis was performed with heuristic search using TBR algorithm, with starting trees obtained via stepwise addition as described for the MP tree. Other specifications were estimated with Modeltest as described above. Bootstrap re-sampling was done for 100 replicates due to computational limitations.

For all analyses, bootstrap values were considered low up to 70%, moderate from 71% to 90%, and high above 90%.

Results

The complete SSU rDNA was sequenced for 10 individuals identified as *Cladophora vagabunda* collected along the coast of Brazil. The size of the SSU rDNA varied from 1781 to 1783 bp (table 1). For *C. rupestris* only a partial SSU rDNA sequence of 1634 bp was obtained (the 3' end of the gene is missing). Sequence identity for the SSU rDNA varied from 98.9% to 100% for the Brazilian *C. vagabunda* individuals (table 2). Five individuals presented 100% identity for the SSU rDNA sequences (CB2; BV1; SER2; POR; and PRA). Comparing the Brazilian *C. vagabunda* individuals with the other SSU rDNA available in GenBank, the identities varied from 98.3% to 99.1% to *C. vagabunda* (Z35316) from France (Bakker *et al.* 1994), and from 99.1% to 99.7% to *C. vagabunda* (AB062710) from Japan (Hanyuda *et al.* 2002). The sequence identity of the Brazilian *C. vagabunda* individuals to *C. albida* (Z35316) varied from 98.0% to 98.4% and to *C. sericea* (Z34320) from 98.0% to 98.6%. On the other hand, *C. rupestris* from Brazil showed only 96.9% of identity to *C. rupestris* (Z35319) from France and from 96.5% to 96.8% of identity to the Brazilian *C. vagabunda* individuals.

Phylogenetic trees obtained from three different inference methods were very similar and only minor changes in topology were observed at branches with low bootstrap values. Therefore, only the maximum parsimony (MP) tree is presented in figure 1 with the bootstrap values of the other analyses plotted in the common branches.

Species of Cladophoraceae and Siphonocladaceae (Order Cladophorales *sensu* Wynne 2005) were included in the phylogenetic analyses. *Cladophora horii* (section *Rugulosae*) and *C. conchophoria* (section *Glomeratae*)

Table 2: Percentage of sequence identity among the SSU rDNA sequences in the different *Cladophora* specimens. (CRU = *C. rupestris* Z35319; CSE = *C. sericea* Z35320; CAL = *C. albida* Z35317; CVZ = *C. vagabunda* Z35316; CVA = *C. vagabunda* AB062710; CBI, CB2, BV1, BV2, SER1, SER2, POR, PRA, CON, IBV = Brazilian populations of *C. vagabunda*; RUP = Brazilian population of *C. rupestris*).

	CRU	CSE	CAL	CVZ	CVA	CBI	CB2	BV1	BV2	SER1	SER2	POR	PRA	CON	IBV	RUP
CRU	-															
CSE	97.1	-														
CAL	97.0	98.7	-													
CVZ	96.9	98.0	97.9	-												
CVA	97.0	98.1	98.3	99.1	-											
CBI	97.0	98.2	98.2	99.0	99.7	-										
CB2	97.2	98.0	98.0	98.7	99.5	99.3	-									
BV1	97.2	98.0	98.0	98.7	99.5	99.3	100	-								
BV2	97.1	98.2	98.2	99.1	99.7	99.9	99.4	99.4	-							
SER1	97.0	98.6	98.4	98.3	99.1	98.9	98.9	98.9	99.0	-						
SER2	97.2	98.0	98.0	98.7	99.5	99.3	100	100	99.4	98.9	-					
POR	97.2	98.0	98.0	98.7	99.5	99.3	100	100	99.4	98.9	100	-				
PRA	97.2	98.0	98.0	98.7	99.5	99.3	100	100	99.4	98.9	100	100	-			
CON	97.3	98.3	98.2	98.8	99.6	99.4	99.6	99.6	99.4	99.0	99.6	99.6	99.6	-		
IBV	97.2	98.0	98.0	98.8	99.4	99.4	99.9	99.9	99.4	98.9	99.9	99.9	99.9	99.5	-	
RUP	96.9	96.3	96.2	96.3	96.7	96.7	96.7	96.7	96.7	96.5	96.7	96.7	96.7	96.8	96.7	-

s.l.) were chosen as outgroup taxa. *Cladophora kosteræ* C. Hoek, a species that belongs to the section *Basicladia*, appeared basally rooting the remaining taxa, which grouped in all analyses with strong bootstrap support.

In all phylogenetic analyses two main groups were formed, presenting bootstrap support (figure 1): group I composed of *Cladophora* and *Cladophoropsis* species; and group II composed of *Cladophora*, *Chaetomorpha* and *Rhizoclonium* species. A third group, basal to group II, was formed in the MP tree, but with no support from the other inference methods, it is composed of three *Cladophora* species of the section *Longi-articulatae*: *C. pellucidoidea* C. Hoek, *C. japonica* Yamada and *C. ohkuboana* Holmes (group III).

Group I, with high bootstrap support, was formed by three different sections of *Cladophora* with very distinct morphologies: *Boodleoides* (*C. liebethuthii* Grunow), *Rugulosae* [*C. prolifera* (Roth) Kütz.] and *Willeella* [*C. ordinata* (Børgesen) C. Hoek], and by two species of the Siphonocladaceae family: *Cladophoropsis macromeres* W.R. Taylor and *C. sundanensis* Reinbold.

In all phylogenetic analyses group II presented moderate to high bootstrap support, including all species of the sections *Glomeratae s.l.* and *Rupestris s.s.* and also species of *Chaetomorpha* and *Rhizoclonium*. Within this group II, *Rhizoclonium hieroglyphicum* (C. Agardh) Kütz is rooting, a monophyletic group including *C. vagabunda*, *C. albida*, *C. sericea* and the freshwater taxa *C. glomerata* (L.) Kütz. This clade, with moderate to high bootstrap support, includes all the *C. vagabunda* individuals from Brazil, Japan and France. The Brazilian individual SER1 grouped together with *C. albida* (Roscoff, France) and *C. sericea* (Roscoff, France).

Both *C. rupestris* (L.) Kütz, from France (Z35319) and from Brazil are in a basal polytomy of group II which also includes a second *Rhizoclonium* species (*R. grande* Børgesen) that groups with high bootstrap support to *Chaetomorpha crassa* (C. Agardh) Kütz, as well as two species of *Chaetomorpha* [*C. moniligera* Kjellman and *C. linum* (Müller) Kütz.].

Discussion

In this paper we present the first molecular phylogenetic analyses, which include SSU rDNA sequences from ten individuals identified as *Cladophora vagabunda* and one as *C. rupestris* from Brazil. Based on the results presented in this paper and previous papers by other authors (Bakker *et al.* 1994, Hanyuda *et al.* 2002) the genus *Cladophora* is clearly paraphyletic, as it includes representatives from at least three other genera,

Cladophoropsis, *Rhizoclonium* and *Chaetomorpha*. Therefore, an extensive taxonomic review, using different types of data (*e.g.* morphology and molecular markers), is necessary to clarify and rearrange the taxonomy of those genera.

The section *Glomeratae sensu lato* (*C. vagabunda*, *C. albida*, *C. sericea*, and *C. glomerata*) appears as a monophyletic group in the phylogenetic analyses carried out in this study and also in the studies of Bakker *et al.* (1994) and Hanyuda *et al.* (2002). Thalli architecture of the members of the section *Glomeratae sensu lato* is relatively heterogeneous and varies from strict acropetal to irregular, being the growth by apical or intercalary cell divisions (Hoek & Chihara 2000).

The species *C. vagabunda* is paraphyletic as it includes other species of the section *Glomeratae sensu lato* (*C. albida*, *C. sericea*, and *C. glomerata*), corroborating previous results by Hanyuda *et al.* (2002), and therefore, the *C. vagabunda* complex needs to be taxonomically reviewed. Due to the relatively high amount of variation in the SSU rDNA in the *C. vagabunda* complex, but considering the monophyly of the complex with other closely related species, we suggest that a new genus is erected to accommodate the different species in this complex.

The morphological characteristics presented by the Brazilian individuals of *C. vagabunda* corroborate, partly, the phylogenetic relationships presented in this study (Gestinari 2004). Although, the individual SER1, collected from Sereias Beach (AL), presents similar morphological characteristics to the representatives of the other Brazilian individuals of *C. vagabunda*, it is grouped with *C. albida* and *C. sericea* from France. Probably, SER1 is a *C. dalmatica*, a species strictly related to *C. vagabunda*, not only morphologically, but also ecologically; the differences in *C. dalmatica* are the diameter of the apical cells (38 ± 12 μm diam.), terminal branches (47 ± 14 μm diam.) and main branch (102 ± 35 μm diam.), which are thinner than in *C. vagabunda* (70 ± 19 μm diam., 83 ± 22 μm diam. and 200 ± 29 μm diam., respectively) (Gestinari 2004, Hoek 1982, Hoek & Chihara 2000). *Cladophora dalmatica*, as *C. vagabunda*, belongs to the “amphiatlantic tropical-to-temperate distribution group”, occurring from the tropics on both sides of the Atlantic Ocean to about 45° N latitude along American Atlantic coasts and almost 57° N latitude along European Atlantic coasts, while *C. vagabunda* occurs from the tropics to about 50° N latitude along American Atlantic coasts and almost 60° N latitude along European shores (Hoek 1979).

The Brazilian populations of *C. vagabunda* (IBV) and the individuals that have identical SSU rDNA: CB2,

BV1, SER2, POR and PRA) collected from Rio de Janeiro (Southeast) to Paraíba (Northeast) States formed a branch with high bootstrap support in all analyses. This branch presented individuals with highly similar morphological characteristics, with more delicate thalli, bearing lesser branches and with cells dimensions slightly smaller than the representatives from the populations CON, CB1 and BV2 which presented more rigid thalli, a higher number of ramifications and similar cell size (Gestinari 2004). However, individuals CB2, BV1 and SER2 were collected from reef areas with oligotrophic waters, while individuals IBV, PRA and POR were collected on rocky shores exposed to the wave action and also under strong influence of nitrogenous compounds from natural or artificial origin. This indicates that individuals with identical SSU rDNA and similar morphologies can be found in quite different types of environments. The specimens of CB1 and BV2, which have divergent SSU rDNA sequences, proceeding from relatively close locations, with very similar environmental conditions (temperature, salinity), were both collected in the intertidal region from the sandstone reef, growing in places with moderate exposition to the waves action. The specimen CON was collected from rocky shores exposed to the wave action and under strong influence of nitrogenous compounds from natural origin.

Many authors have mentioned that *C. vagabunda* presents an extremely plastic morphology, highly influenced by environmental factors as well as the age of the thallus (Hoek 1963, 1982, Dodds & Gudder 1992, Hoek *et al.* 1995). The slight differences observed among these populations, probably, could be related to the morphological plasticity and environmental conditions of the different collection sites, since the morphological characteristics agree with those mentioned in the literature for this species (Hoek 1963, 1982, Lawson & John 1982, Hoek & Womersley 1984, Hoek & Chihara 2000).

According to Hoek & Chihara (2000), *C. rupestris* is the type species within the section *Rupestres s.s.* The Brazilian *C. rupestris* does not group with *C. rupestris* from France, both are in a polytomy including *Chaetomorpha* species. The proximity of *C. rupestris* with the genus *Chaetomorpha* has been reported in previous papers (Bakker *et al.* 1994, Hanyuda *et al.* 2002). *Cladophora rupestris* grouped with *Chaetomorpha* species in the ML analysis inferred from LSU rDNA carried out by Leliaert *et al.* (2003) indicating that, as suggested by Hoek (1982), *Chaetomorpha* could be considered as a reduced form and not as a primitive sister genus of *Cladophora*.

In this study, Section *Longi-articulatae* comprised by *C. pellucidoidea*, *C. japonica* and *C. ohkuboana*

formed a monophyletic group, not closely related to *C. prolifera*. Similar results were also observed in Bakker *et al.* (1994) SSU rDNA phylogeny, in which species of the section *Rugulosae*, such as *C. prolifera*, stand clearly apart from the section *Longi-articulatae*, corroborating the morphological differences between these two sections: branch insertion and structure and disposition of the rhizoids (Hoek 1982, Hoek & Chihara 2000).

The inclusion of sequences from Brazilian individuals of *Cladophora* reinforces the need of taxonomical revision for the genus and for the complex *C. vagabunda*. To have a better understanding of the monophyly and of the divergence among species and isolates of the *Cladophora vagabunda* complex it will be necessary to obtain sequences for other molecular markers and from a broader geographic sampling.

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