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Cladophora goensis sp. nov. (Cladophorales, Ulvophyceae) –a bloom forming marine algae from Goa, India

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A new species of green seaweed, *Cladophora goensis* sp. nov. (Cladophorales, Ulvophyceae), had been discovered in our last field trip to Vasco-da-Gamma, Goa, India. This species formed algal bloom of moderate intensity in the Bay of Mormugao, on the west coast of India. Our observations suggest combination of a number of morphological characteristics of this alga distinct from previously described members of this genus, including parietal chloroplast surrounding central hollow and bilenticular pyrenoids. Molecular sequence data at Nuclear ribosomal DNA Internal Transcribed Spacer-1 and 2 (ITS1 and ITS2) regions along with intervening 5.8S rRNA indicated Kimura-2-Parameter (T3P) pair-wise distance of 1.77 x 10^{-1} between this species and the nearest phylogenetic accession of *Cladophora glomerata*. In our phylogenetic reconstructions using Bayesian Inference and Maximum Likelihood, this species was not part of any monophyletic clades comprising any of the previously described species of this genus at the locus studied, thereby ascertaining conformity with phylogenetic species concept. With this discovery, a new phylogenetically primitive morphological synapomorphy of "pseudodichotomous profuse branching" has been revealed for cladophoralean algae, and we argue that this is the single most important morphological characteristic of this bloom-forming seaweed.

[Keywords: Green tide; ITS1; systematics, phylogenetics; seaweed]

Genus *Cladophora* (Kützing) consists of some of the ubiquitous bloom-forming green algae with cosmopolitan in distribution. This macroalgal genus is renowned for the adaptation to environments of various salinity and wave-exposure regimes, with representative species of this genus described from marine, brackish and freshwater habitats. Massive ephemeral blooms of this genus have been described from marine, as well as freshwater, habitats throughout the world [1-3] and, therefore, algae of this genus is considered to play a major role in marine, estuarine and limnetic ecosystem dynamics.

Algal thalli of the genus *Cladophora* consist of moderately branched uniseriate filaments with coenocytic cells having a typical banded pattern under low magnification. Dominant form of life history in this genus is isomorphic diplohaplontic alternation and exhibit considerable morphological plasticity in relation with environmental factors [4]. This genus is known for lack of clear-cut species delineating morphological characters. Species delineation within this genus have been traditionally based upon combinations of various microscopic characteristics, such as insertion of branches in the main axis, cell shape and wall thickness, shape of the apical cell tip-if tapered, apical/intercalary growth, arrangement of chloroplasts and so on. There are 188 morphospecies of this genus currently accepted taxonomically; almost all of them described based solely on microscopic characters and, therefore, species-level identification remains very cumbersome for the field phycologists [5]. A number of recent molecular phylogenetic studies have revealed polyphyly of this genus owing to the unaccountability of morphological synapomorphies, with members of this genus clustered within at least two orders; Cladophorales and Siphonocladales [6, 7]. For taxonomically reliable and reproducible description of cladophoralian species, molecular data and its phylogenetic reconstruction have recently been integrated in a European species [8].

Previously reported species of genus *Cladophora* in Indian subcontinent include *Cladophora callicoma* [9], *Cladophora uberrima* [10] and *Cladophora crispata* [11]. At least three other species had also been reported, viz., *Cladophora prolifera* [12], *Cladophora fasicularis* [13] and *Cladophora glomerata* [14], albeit taxonomy was not the point of focus in those reports, and no detailed information on species' identification was mentioned. *Cladophora* from Indian Subcontinent has never been subjected to molecular assessment. In addition, algal bloom caused by *Cladophora* have never been reported from India.

In our 2012 field-trip to the west coast of India, a particular Cladophoralian algae was observed causing bloom of moderate intensity at Mormugao Bay, Vasco-da-Gamma, Goa. This bloom was found to be occurring all along the bay coastline, from the northern tip of Headland Sada till North-East of Vasco fishing harbor, with intervening Mormugao Harbor area. The algae was seen to be profusely growing on mooring lines, buoys, hulls of wooden dinghies and intertidal substrata, including natural rocks and concrete breakwaters.

A bloom specimen was collected from mid-point of the Vasco fishing harbor (15.402639N, 73.814673E), which was observed to be growing attached to the mooring line made of coir. A map of the bloom area along with sampling location is accessible at http://bit.ly/GoaBloom. Algal specimen was transported to the laboratory in the ice box (c 4°C) and processed immediately. Samples did not freeze at any point of time and therefore possibility of the freeze-fracture of chloroplasts can be ruled out. Morphological features were recorded using an upright microscope (BX53, Olympus, Japan) and photographs were taken using an attached digital camera (E450, Olympus, Japan). Public domain software ImageJ (http://rsbweb.nih.gov/ij/) was used for scale calibration and size measurements.

10 g wet weight of thalli was used for DNA extraction and sequencing. Unabridged protocols used for DNA extraction, amplification and sequencing used in the present study are as per [15]. In summary, total genomic DNA was extracted from dried specimens using HiPurA™ Algal Genomic Extraction Kit (HiMedia Laboratories, India). A region consisting of ITS1-5.8S-ITS2 was amplified from the extracted DNA using ITS1-forward primer and ITS4-reverse primer[16] and subsequently subjected to bidirectional Sanger sequencing (Applied Biosystems 3730*xl* Genetic Analyzer, Foster City, CA, USA). Sequences were then assembled and additional sequences of related taxa were procured from Genbank, with numbers of these accessions listed in Table S1. Phylogenetic analyses using distance matrix, Maximum Likelihood (ML) and Bayesian Inference (BI) methods were conducted with Kimura-2-Parameter (K2P) model of nucleotide substitution, gamma distribution and complete deletion of gaps and missing data in dataset, in force. Step-by-step protocols for sequence analysis used in the present study are accessible under LabArchives Protocols http://dx.doi.org/10.6070/H4Q23X56). All of our phylogenetic data, including alignment in FASTA format, Results of ModelTest, trees in Newick format and DNA sequence assemblies with original electropherograms are freely available at LabArchives (http://dx.doi.org/10.6070/H4FJ2DQ4).

Thallus was erect filamentous, light green in color with size ranging between 3 cm-6 cm (Average 4.34±1cm; n=50) (Fig.1-6). Under low magnification, irregular branching pattern of uniseriate filaments was evident. Cells produced mostly dichotomous branching, but third, fourth and fifth order branching were also occasionally observed. Cells were mostly cylindrical, with slightly narrowed at posterior ends. Cell diameter of basal, main axial and apical cells ranged between 65-165 µm, 59-150 µm and 45-141 µm, respectively. Apical cells were tapering with obtuse tips. Each cell had 100-130 distinct pyrenoids within parietal network of the chloroplast, most having a central hollow. In our understanding, these morphological features are not shared with any of the previously described species of *Cladophora* and, therefore, we conclude that our isolate is a new species. Comparison of taxonomic characteristics between this isolate and *Cladophora sericea*, a closely related bloom-forming species, and *Cladophora glomerata*, phylogenetically closely related species revealed in the present study, is presented in Table 1.

A genomic (nuclear ribosomal) DNA region consisting of partial 18S gene, complete ITS1-5.8S-ITS2 cistron, and partial 28S gene, with a sequence length of 858 base pairs was

obtained from contiguous assembly of 4 single reads (two each for ITS1 and ITS2 regions, with flanking genes) from either direction. Sequence assembly was verified manually for ambiguities and labelled accordingly. After full annotation, this was deposited in Genbank.

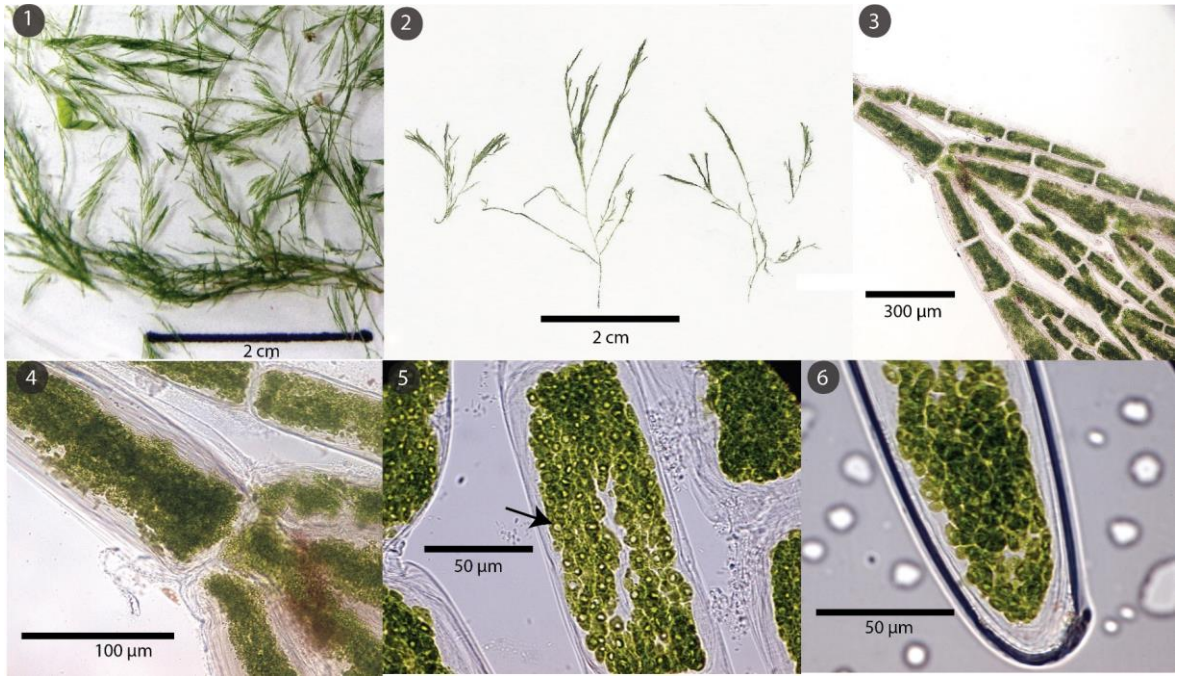

Fig. 1-6. Holotype of *Cladophora goensis* from Vasco-da-Gamma, Goa, India (F. Bast, 19th May, 2012). 1) Morphology of collected specimen. 2) Pressed herbarium voucher 3) Irregular branching pattern under low magnification 4) Detail of the branch showing pseudodichotomy 5) Ovular pyrenoids (arrow) with parietal organization of chloroplast and 6) Apical cells with round tips.

Table 1. Distinguishing characters of *Cladophora goensis* from *Cladophora sericea* and *Cladophora glomerata.*

S. No	Features	*Cladophora goensis*	*Cladophora sericea*[17]	*Cladophora glomerata*[18]
1	Habit	Light green, moderately tufted	Light to grass green, densely tufted, up to 20 cm high	Dark green, densely tufted, 1 meter or more high
2	Cell diameter (µm)	Apical: 45-141 µm, Main axis cells: 59-150 µm, Basal cells: 65-165 µm	Apical: 15-70, Matured: 35-55	Apical: 19-91 µm, Main axis cells: up to 150 µm.
3	Branching system	Irregular profuse branching of uniseriate filaments forming tufts. Branches are inserted apically on the cell without apical meristem (pseudodichotomy). Creeping growth/intercalary cell division absent.	Terminal branch systems acropetal to irregular; main axis pseudodichotomously branched; growth mainly by intercalary cell division	Pseudodichotomous profuce branching with terminal bunch of arcuate branches. Growth mainly by intercalary cell division

4	Other Characters	Apical cells tapering with an obtuse tip; Chloroplasts form parietal layer, mostly with central hollow, and consists of a network of numerous ovular bilenticularpyrenoids.	Apical cells tapering with an obtuse tip.	Branches are attached obliquely, rarely laterally.
5	Ecology	Common in sheltered marine habitats with stable salinity, not found in low saline locations	Common in intertidal rockpools, able to penetrate in water with low salinity	Common in freshwater to marine habitats

Distance matrix (Table S1) revealed that the accession *Cladophora glomerata* AB665566 had lowest K2P distance (1.77×10^{-1}) with our isolate and, therefore, these two isolates are evolutionarily closely related. There were 18 unique pairs of taxa in our matrix that had pairwise distance less than 1.77×10^{-1} and all of these taxa are currently recognized as distinct species of the genus *Cladophora*. Phylogenetic analyses using ML and BI resulted in well-resolved phylograms with similar topology (Fig 7). *Cladophora* isolate from Goa did not form any monophyletic clade together with any of the available cladophoralian species in the phylogenetic reconstruction, therefore, ascertaining conformity with phylogenetic species concept. Our isolate formed a basal position of a well-supported clade in both ML and BI phylograms (Clade A in Fig. 7) comprising of *C. stimpsonii*, *C. gomerata*, *C. gracilis*, *C. vagabunda*, *C. fascicularis* and *C. laetevirens*. Basal position is suggestive of the phylogenetically primitive position of this species in this clade. This phylogenetic clade is circumscribed by the morphological synapomorphy of "pseudodichotomous profuse branching" (PPB)- branchlets being inserted on the apex without apical meristem profusely- a trait shared exclusively by all the members of this clade. As clearly seen in our phylograms, this character state divides two clades of the *Cladophora* first time in the evolution and, therefore, occupies a paramount position in the systematics of cladophoralean algae. In our opinion, this is the single most important morphological feature for the genus *Cladophora*.

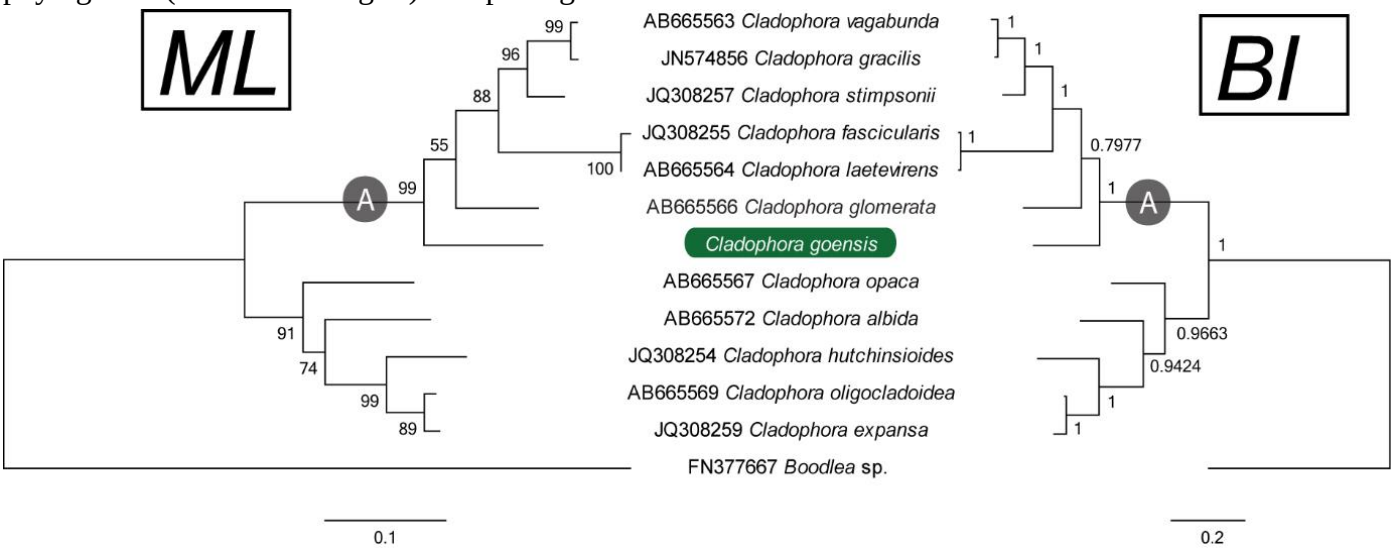

Fig. 7. Phylogeny reconstruction of *Cladophora goensis* using Maximum Likelihood (ML) on the left and Bayesian Inference (BI) on the right. Numbers near nodes represent bootstrap proportions with 500 replicates in ML and Bayesian Posterior Probabilities in BI. Both trees are rooted with *Boodlea* sp. (Siphonocladales) as outgroup. Scale bars on bottom is in the units of average nucleotide substitutions per site.

In summary, results from our morphological observations, as well as from three of our phylogenetic analyses were congruent in diagnosing the isolate from Goa, India as a distinct species. Therefore, we propose formal recognition of a new species of bloom-forming *Cladophora*.

***Cladophora goensis* Bast sp. nov.**

Diagnosis: Plants light green, soft textured with size between 3 cm-6 cm. Irregular profuse branching of uniseriate filaments forming moderate tufts. Branches are inserted apically on the cell without apical meristem (pseudodichotomy). Creeping growth and intercalary rhizoids are absent. Mostly dichotomous branching, but occasionally third, fourth and fifth-order branching. Cells are cylindrical with slightly tapered at posterior ends. Cell diameters range between 45-165 µm. Apical cells have round tips. Cell walls are thicker near base (c 50 µm, range 30-50 µm, Fig 3-4) and thinner towards apex (c 5 µm, range 2-5 µm, Fig 5-6). Chloroplasts form parietal layer, mostly with central hollow, and consists of a network of numerous (c 100-120 per cell) ovular pyrenoids. Pyrenoids are mostly bilenticular, resembling human eyes.

Habitat: Partially submerged splash zones on the hulls of ships, mooring lines, floating buoys and intertidal rocks.

Holotype: Collected from coir mooring line at mid-point of the Vasco fishing harbor, Vasco-da-Gamma, Goa, India (15.402639N, 73.814673E). Deposited at Central National Herbarium, Botanical Survey of India, Calcutta (*Index Herbariorum* code: CAL) under voucher # CAL-CUPVOUCHER-CG-2013-1. DNA sequences of nrDNA ITS1-5.8S-ITS2 complete region of the holotype deposited at Genbank under accession # KF318887.

Isotype: Deposited at Herbarium, Central University of Punjab under voucher No.: CUPVOUCHER-CG-2013-1. Frozen voucher maintained at Centre for Biosciences, Central University of Punjab under voucher No.: CUPFVOUCHER-CG-2013-1.

Etymology: Specific epithet refers Indian state of Goa where the alga is first described.

There are three implications of the discovery of this species. First is the revelation of the ancient morphological synapomorphy of PPB within *Cladophora*; this phylogenetic clade is ought to be analyzed by other means, including unrelated genetic loci, life history, ontogeny, ultrastructure and biochemistry, to confirm our finding. Second is that the type locality of this species, Mormugao port area, is one of the busiest shipping harbor in India and therefore possibility of an introduction of this species from elsewhere cannot be ruled out. Cladophoralean algal blooms have not yet been reported as invasive, especially by global shipping route, and, therefore such a conclusion could have deep ramifications in policy making concerning the environmental containment of factors like ballast water. Third, this is the first report of the cladophoralean algal bloom in India, either gone undetected for centuries by chance, or it is being a recent phenomenon. In either case, more research is warranted to test hypotheses regarding the origin of the new species described in this report and its possible impacts. On the other hand, this is the first molecular assessment of cladophoralean algae from India, highlighting imminent necessity to catalogue this important plant lineage.

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References

1. Higgins, S.N., Pennuto, C.M., Howell, E.T., Lewis, T.W., & Makarewicz, J.C., Urban influences on *Cladophora* blooms in Lake Ontario. *Journal of Great Lakes Research*. **38**(2012) pp. 116-123.

2. Smith, J.E., Runcie, J.W., & Smith, C.M., Characterization of a large-scale ephemeral bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui, Hawai'i. *Marine Ecology Progress Series*. **302**(2005) pp. 77-91.
3. Bach, S.D. & Josselyn, M.N., Mass blooms of the alga *Cladophora* in Bermuda. *Marine Pollution Bulletin*. **9**(1978) pp. 34-37.
4. Hoek, C., *A taxonomic revision of the American species of Cladophora (Chlorophyceae) in the North Atlantic Ocean and their geographic distribution*. Vol. 550. (Amsterdam: North-Holland) 1982.
5. Guiry, M. & Guiry, G., *AlgaeBase. World-wide electronic publication, National University of Ireland, Galway*).2013.
6. Bakker, F.T., Olsen, J.L., & Stam, W.T., Evolution of nuclear rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). *Journal of Molecular Evolution*. **40**(1995) pp. 640-651.
7. Hanyuda, T., Wakana, I., Arai, S., Miyaji, K., Watano, Y., & Ueda, K., Phylogenetic relationships within Cladophorales (Ulvophyceae, Chlorophyta) inferred from 18s rRNA gene sequences, with special reference to *Aegagropila linnaei. Journal of Phycology*. **38**(2002) pp. 564-571.
8. Leliaert, F., Boedeker, C., Peña, V., Bunker, F., Verbruggen, H., & De Clerck, O., *Cladophora rhodolithicola* sp. nov.(Cladophorales, Chlorophyta), a diminutive species from European maerl beds. *European Journal of Phycology*. **44**(2009) pp. 155-169.
9. Shyam, R., On the life cycle, cytology and taxonomy of *Cladophora callicoma* from India. *Am J Bot*, (1980) pp. 619-624.
10. Sinha, J. & Ahmed, S., A Study on the Cytology and Life-history of *Cladophora uberrima* Lambert. from Ranchi, India. *Cytologia*. **38**(1973) pp. 99-105.
11. Dixit, S., The chlorophyceae of the Bombay presidency, India—I. *Proceedings: Plant Sciences*. **5**(1937) pp. 16-25.
12. Ely, R., Supriya, T., & Naik, C., Antimicrobial activity of marine organisms collected off the coast of South East India. *Journal of experimental marine biology and ecology*. **309**(2004) pp. 121-127.
13. Kumar, J.N., Oommen, C., & Kumar, R.N., Biosorption of heavy metals from aqueous solution by green marine macroalgae from Okha Port, Gulf of Kutch, India. *American-Eurasian J. Agric. & Environ. Sci*. **6**(2009) pp. 317-323.
14. Yuvaraj, N., Kanmani, P., Satishkumar, R., Paari, K., Pattukumar, V., & Arul, V., Extraction, purification and partial characterization of *Cladophora glomerata* against multidrug resistant human pathogen *Acinetobacter baumannii* and fish pathogens. *World J Fish Mar Sci*. **3**(2011) pp. 51-57.
15. Bast, F., Sequence Similarity Search, Multiple Sequence Alignment, Model Selection, Distance Matrix and Phylogeny Reconstruction. *Nature Protocol Exchange*, (2013).
16. White, T.J., Bruns, T., Lee, S., & Taylor, J., *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. PCR protocols: a guide to methods and applications. Vol. 18. (Waltham, Massachusetts: Academic Press) 1990. pp. 315-322.
17. Leliaert, F. & Coppejans, E., The marine species of Cladophora (Chlorophyta) from the South African east coast. *Nova Hedwigia*. **76**(2003) pp. 1-2.
18. Kützing, F.T., *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange: Mit 80 farbig gedruckten Tafeln*: Brockhaus) 1843.

Table S1. Evolutionary distance between pairs of taxa calculated using Kimura-2-Parameter model of molecular evolution with Gamma Distribution. Columns containing gaps and missing data in the dataset were completely deleted prior to the matrix construction.

	AB665566	JQ308257	AB665563	JN574856	JQ308255	AB665564	AB665567	JQ308254	AB665569	JQ308259	AB665572	FN377667
AB665566 *Cladophora glomerata*												
JQ308257 *Cladophora stimpsonii*	0.1548											
AB665563 *Cladophora vagabunda*	0.1426	0.0693										
JN574856 *Cladophora gracilis*	0.1509	0.0647	0.0117									
JQ308255 *Cladophora fascicularis*	0.2008	0.1491	0.1691	0.1575								
AB665564 *Cladophora laetevirens*	0.1887	0.1494	0.1578	0.1633	0.0078							
AB665567 *Cladophora opaca*	0.3446	0.3223	0.3387	0.3511	0.3678	0.3544						
JQ308254 *Cladophora hutchinsioides*	0.3651	0.3388	0.3634	0.3634	0.3759	0.3800	0.1996					
AB665569 *Cladophora oligocladoidea*	0.3547	0.3270	0.3453	0.3626	0.3764	0.3626	0.1773	0.1015				
JQ308259 *Cladophora expansa*	0.3666	0.3324	0.3618	0.3618	0.3755	0.3796	0.1872	0.0962	0.0217			
AB665572 *Cladophora albida*	0.3242	0.3455	0.3376	0.3547	0.3478	0.3349	0.1850	0.1779	0.1609	0.1691		
FN377667 *Boodlea* sp. (outgroup)	0.6975	0.7034	0.6942	0.7034	0.7810	0.7636	0.6285	0.6473	0.6180	0.6635	0.5862	
Cladophora goensis sp. nov.	0.1770	0.1865	0.1950	0.2075	0.2271	0.2143	0.3235	0.3813	0.3469	0.3539	0.3305	0.8426