CHAPTER 4: RESULTS

4.1 MORPHOLOGICAL STUDIES

All *Caulerpa* specimens used in this study were collected from Pulau Redang, Terengganu, Malaysia. The collected specimens were identified as:

i.   *Caulerpa serrulata var serrulata* (Cser6_1)

ii.   *Caulerpa serrulata var serrulata* (Cser6_2)

iii.   *Caulerpa serrulata var borynana* (Cser6_3)

The *Caulerpa serrulata* var. *serrulata* and *Caulerpa serrulata* var. *borynana* were found submerged in the sand bottom of the coral reefs areas. Both of the varieties appeared as dull green, consisting erect branches up to 8 cm tall. The branched stolons, rhizoid bore branches on both direction: upwardly and downwardly. The branches are commonly naked cylindrical at the lower portion and became flattened towards the tip, dichotomously branched, bearing teeth on both margins. The differences among the two varieties were *Caulerpa serrulata* var. *serrulata's* branches spiral upward and the entire strap-shaped blades were strongly twisted into spirals (Figure 4.1) while *C. serrulata* var. *boryana'* branches are linear and serrated on their edges (Figure 4.2).

Figure 4.1: Herbarium specimen of *Caulerpa serrulata* var. *serrulata*

(a: branches, b: stolon)

Figure 4.2: Herbarium specimen of *Caulerpa. serrulata* var. *boryana*

(a: branches, b: stolon, c: rhizoids)

4.2 MOLECULAR ANALYSE

4.2.1 PCR Amplification

Based on previous experience, it was found that the combination of 10X buffer (with $MgCl_2$) together with dNTP (dATP, dTTP, dCTP, dGTP) and *Taq* polymerase (1.5U) from FINNZYMES (Finland) produced the best amplification. Hence, this combination was used in this study and was found to be successful in amplifying the selected fragment of genes.

The primers employed for amplification were *tuf*A Forward and *tuf*A Reverse primers (De Senerpont Domis *et al.*, 2003). The optimum annealing temperature was found to be 52°C.

4.2.2 Determination of the Yield and Quality of the Selected Region of Fragments

Products of the PCR amplifications were checked by running 25 µl on 1.0 % (w v^{-1}) agarose gel (Figure 4.3). The amplified products were purified using QIAquick Gel Extraction Kit (Qiagen, Germany) and eluted to a final volume of 50 µl. The size of the amplified fragments was estimated to be about 852 bp.
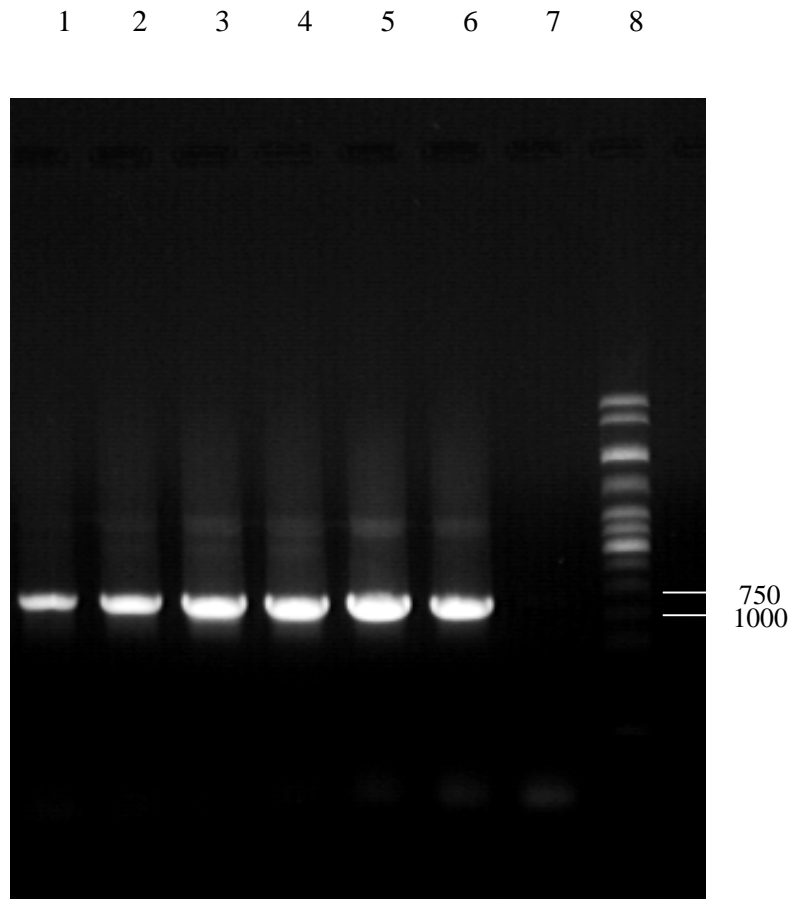
Figure 4.3: PCR amplified products on 1.0 % (w v^{-1}) agarose gel.

From left to right: Lane 1, *Caulerpa serrulata var serrulata* (Cser6_1), replicate 1; Lane 2, *Caulerpa serrulata var serrulata* (Cser6_1), replicate 2; Lane 3, *Caulerpa serrulata var serrulata* (Cser6_2), replicate 1; Lane 4, *Caulerpa serrulata var serrulata* (Cser6_2), replicate 2 ; Lane 5, *Caulerpa serrulata var borynana* (Cser6_3), replicate 1; Lane 6, *Caulerpa serrulata var borynana* (Cser6_3), replicate 2; Lane 7, negative control; Lane 8, 1kb plus DNA ladder.

4.3 PHYLOGENETIC ANALYSES

4.3.1. Maximum Parsimony Tree for *tuf*A gene

The Maximum Parsimony (MP) analysis resulted eight most parsimonious trees and only once is chosen as shown in Figure 4.4 of 431 steps with Consistency Index (CI) of 0.7865 and Retention Index (RI) of 0.9187. To determine phylogenetic relationships among *Caulerpella* and *Caulerpa* species and to root the tree properly, a *tuf*A sequence of *Caulerpella ambigua* was included in the alignment and was used as an outgroup in the MP analysis.

Two major clades, both with full bootstrap support, can be observed: one of them being the *C. flexilis* clade which was basal to the other major clade which includes all remaining ingroup sequences. This major clade can be divided into 2 main clades, namely Clade A with moderate bootstrap value (99%) and fully supported Clade B.

Within Clade A, four taxa, each with strong bootstrap support, can clearly be seen as monophyletic. They are *C. serrulata* (96%), *C. sertularioides* (100%), *C. mexicana* (100%) and *C. taxifolia* (100%). There was one individual of *C. scalpelliformis* was polyphyletic and was grouped as the sister taxa to all the other taxa while the other individual was grouped together with *C. brachypus*; this grouping, however, appeared to be unsupported.

The species of interest in the study were grouped in Clade A1. The two varieties of *Caulerpa serrulata,* namely, *Caulerpa serrulata* var. *serrulata* and *C. serrulata* var. *boryana* were grouped in a monophyletic clade with a strong bootstrap support of 96%. There is no clear separation between the two varieties.
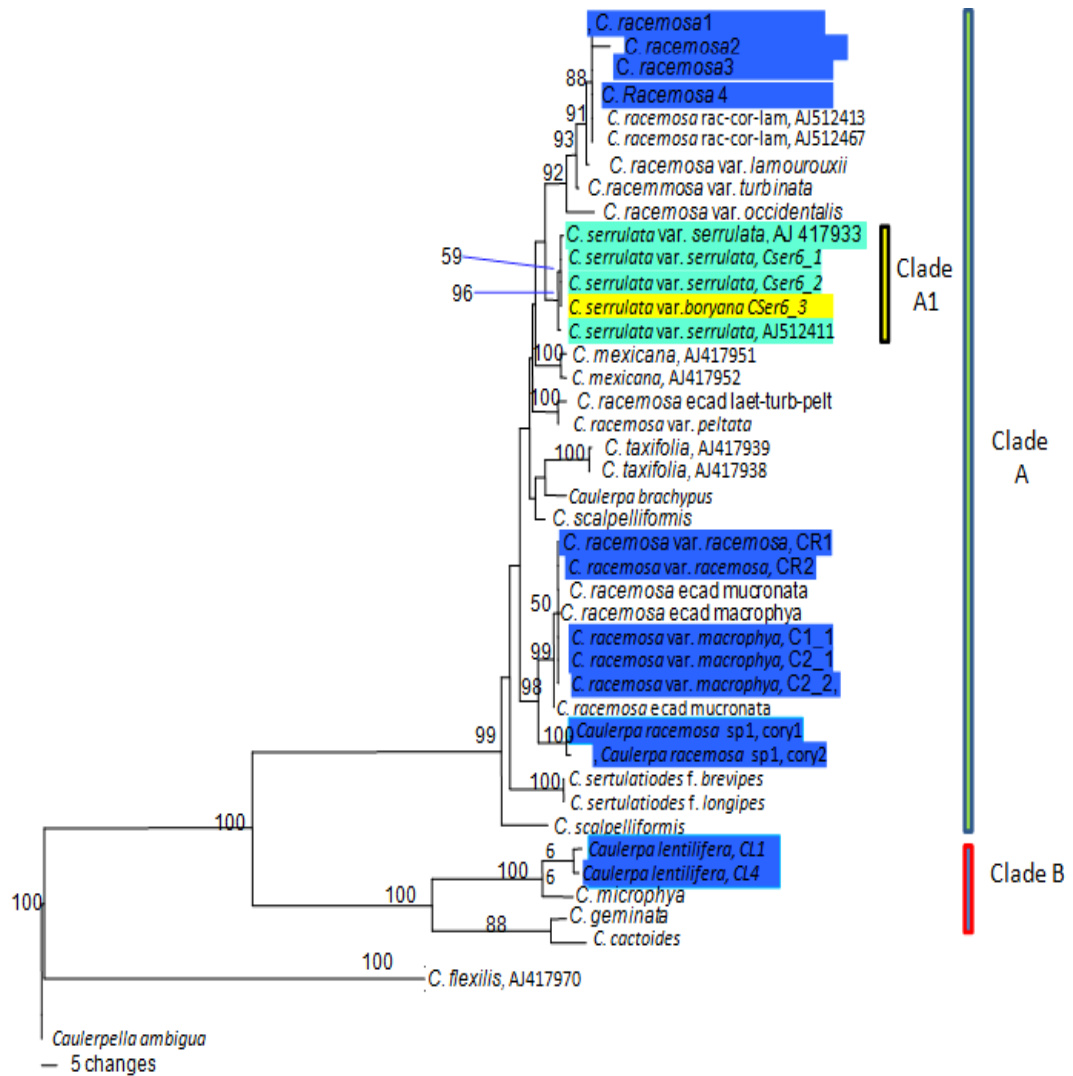
Figure 4.4: Phylogenetic tree of *Caulerpa* species based on partial *tuf*A sequences constructed using the most parsimonious method. The scale bar indicates number of nucleotide substitutions. Number at nodes indicates percentage support from 1000 replicates.

4.3.2 Bayesian Inference Tree for *tuf*A gene

The Bayesian Inference (BI) analysis resulted phylogenetic tree as shown in Figure 4.5. The phylogenetic tree showed similar topology with MP tree except variation in the bootstrap supports of some of the major clades.

Two major clades, both with full bootstrap support, can be observed: one of them being the *C. flexilis* clade which was basal to the other major clade which includes all remaining ingroup sequences. This major clade can be divided into 2 main clades, namely Clade A with moderate bootstrap value (98%) and fully supported Clade B.

The species of interest in the study were grouped in Clade A1. The two varieties of *Caulerpa serrulata,* namely, *Caulerpa serrulata* var. *serrulata* and *C. serrulata* var. *boryana* were grouped in a monophyletic clade with a full bootstrap support. There is no clear separation between the two varieties.
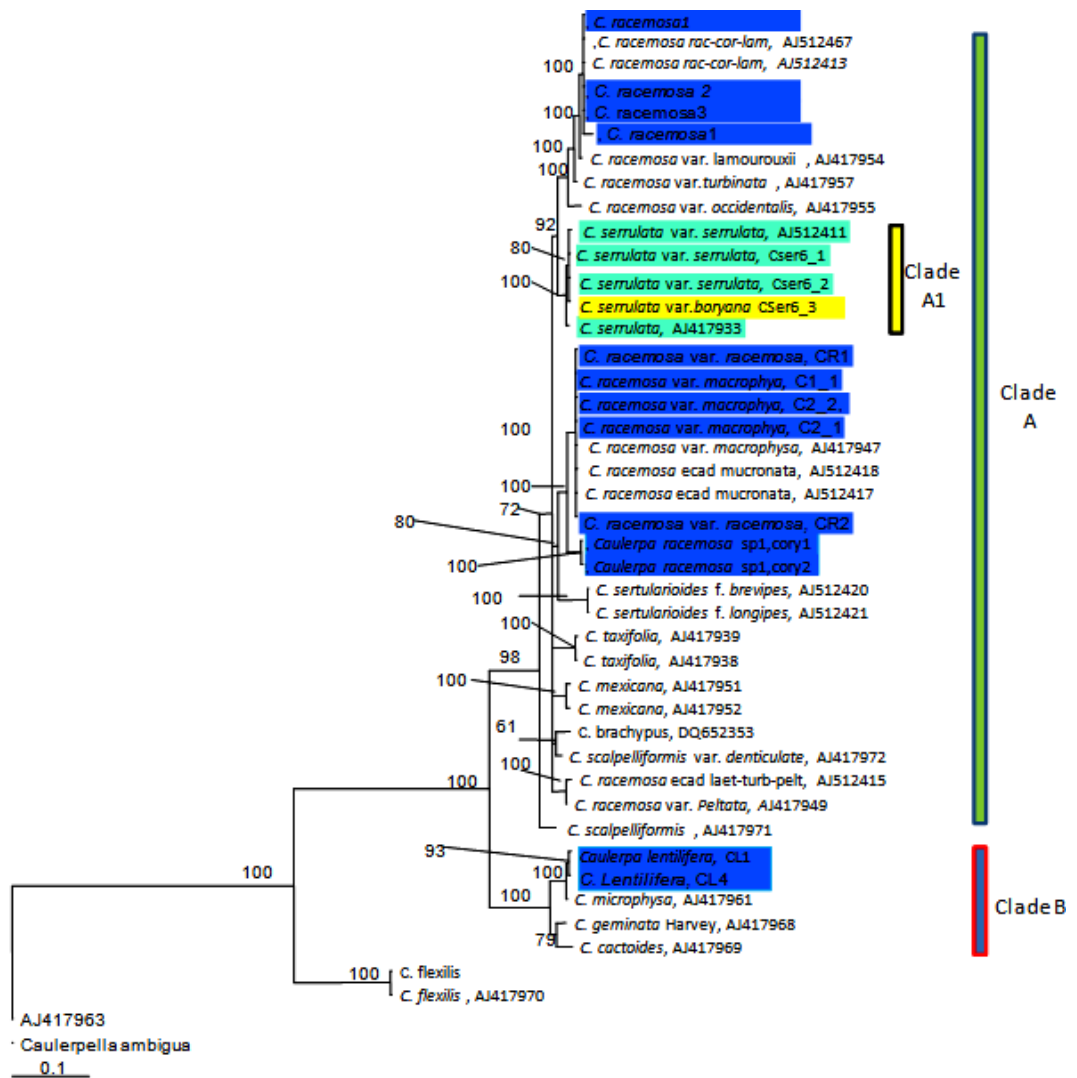
Figure 4.5: Phylogenetic tree of *Caulerpa* species based on partial *tuf*A sequences constructed using the Bayesian Inference method. The scale bar indicates number of nucleotide substitutions. Number at nodes indicates percentage support from 2,000, 000 generations.