CHAPTER V

MORPHOMETRIC STUDY ON SHELL MORPHOLOGY AND PHYLOGENETIC ANALYSIS OF THAI *CYCLOPHORUS*

5.1 Result and discussion on Morphometric analysis

Specimens were studied on the shell morphology i.e. shape, size and color pattern. Each shell specimen was measured for shell height (SH), spire height (SP), major diameter (MA), minor diameter (MI), aperture height (AH) and aperture width (AW) by a vernier caliper (Fig. 3.1). The five ratios such as SH/SW, SP/SW, AH/SW, AW/SW and MI/SW were compared using ANOVA Duncan's multiple range test and discriminant analysis.

Duncan's multiple comparisons of means showed that the differences for 15 taxa showed a significant difference ($p \leq 0.05$) in the shell dimension among populations at the species and sub-species level (Table 5.1, Appendix II). It was also used to separate significant difference among means.

Canonical Discriminat Analysis (CDA) provides a means of summarizing a large amount of morphometric information. It does this by reducing a multidimensional data set so that it can be presented in two dimensions. In the case of CDA, groups, in this case individuals within species are selected prior to analysis. The method proceeds by reducing morphometric multidimensional space by capturing the maximum information between groups and minimizing information within groups. The value of the method, applied in the way, is that it depicts summary of overall morphological resemblance between species. Thus for example (Fig. 5.1), *C. semisalcatus* can be seen to exhibit an overall distinctness from *C. cantori*: Similarly, *C. malayanus* is equally distinct from *C. fulguratus* ssp.1 and from *C. saturnus saturnus*. However, *C. saturnus saturnus* and *C. fulguratus* ssp.1

occupy a similar multidimensional space. It is important that ratios of measurements are used to minimize the effects of size, which would otherwise totally dominate the results leading to a clustering of species based on size alone.

Shells of gastropods contain a rich source of taxonomic information that can be used to interpret evolutionary relationship among taxa (Goodfriend, 1983; Chiu *et al.*, 2002). Shell morphological characters are used as primary guidelines for species identification in general handbooks and the taxonomic literature.

Table 5.1 Results of Duncan's multiple range test, showing the mean values. Superscript number means group number and the number unique group was demonstrated in different colour.

	Species	SH/SW	SP/SW	AH/SW	AW/SW	MI/SW
1.	*C. volvulus*	$.8737^{7}$	$.5891^{7}$	$.5794^{4}$	$.5587^{4,5}$	$.7986^{8,9}$
2.	*C. aurantiacus*	$.7516^{3}$	$.5212^{1}$	$.5648^{2,3,4}$	$.5407^{3}$	$.7479^{2}$
3.	*C. semisulcatus*	$.7249^{2}$	$.5170^{1}$	$.5141^{1}$	$.4915^{1}$	$.7869^{6,7}$
4.	*C. speciosus*	$.8224^{4}$	$.5531^{4}$	$.5777^{4}$	$.5572^{4}$	$.7757^{5,6}$
5.	*C. cantori*	$.8568^{6}$	$.5701^{5}$	$.6776^{6}$	$.6197^{9}$	$.7413^{2,3}$
6.	*C. fulguratus fulguratus*	$.8849^{7}$	$.5679^{5}$	$.5856^{4,5}$	$.5697^{6}$	$.7839^{6,7,8}$
7.	*C. fulguratus* ssp.1	$.8755^{7}$	$.5923^{7}$	$.5706^{3,4}$	$.5439^{3}$	$.8063^{9}$
8.	*C. malayanus*	$.7057^{1}$	$.5177^{1}$	$.5482^{2,3}$	$.5301^{2}$	$.7669^{4,5}$
9.	*C. saturnus saturnus*	$.7543^{3}$	$.5310^{2}$	$.5444^{2}$	$.5441^{3}$	$.7273^{1}$
10.	*C. saturnus* ssp.1	$.8135^{4}$	$.5416^{3}$	$.5677^{2,3,4}$	$.5565^{4}$	$.7553^{3,4}$
11.	*C. courbeti*	$.8056^{4}$	$.5792^{6}$	$.5816^{4}$	$.5539^{4}$	$.7654^{4,5}$
12.	*C. subfloridus*	$.8455^{5}$	$.5689^{5}$	$.5909^{4,5}$	$.5817^{7}$	$.7763^{5,6}$
13.	*C. diplochilus*	$.8349^{5,6}$	$.5691^{5}$	$.6073^{5}$	$.5925^{8}$	$.7364^{1,2}$
14.	*C. orthostylus*	$.6973^{1}$	$.5755^{5,6}$	$.5187^{1}$	$.5273^{2}$	$.7949^{7,8,9}$
15.	*Cyclophorus* sp.	$.7646^{3}$	$.5563^{4}$	$.5788^{4}$	$.5674^{5,6}$	$.7553^{3,4}$

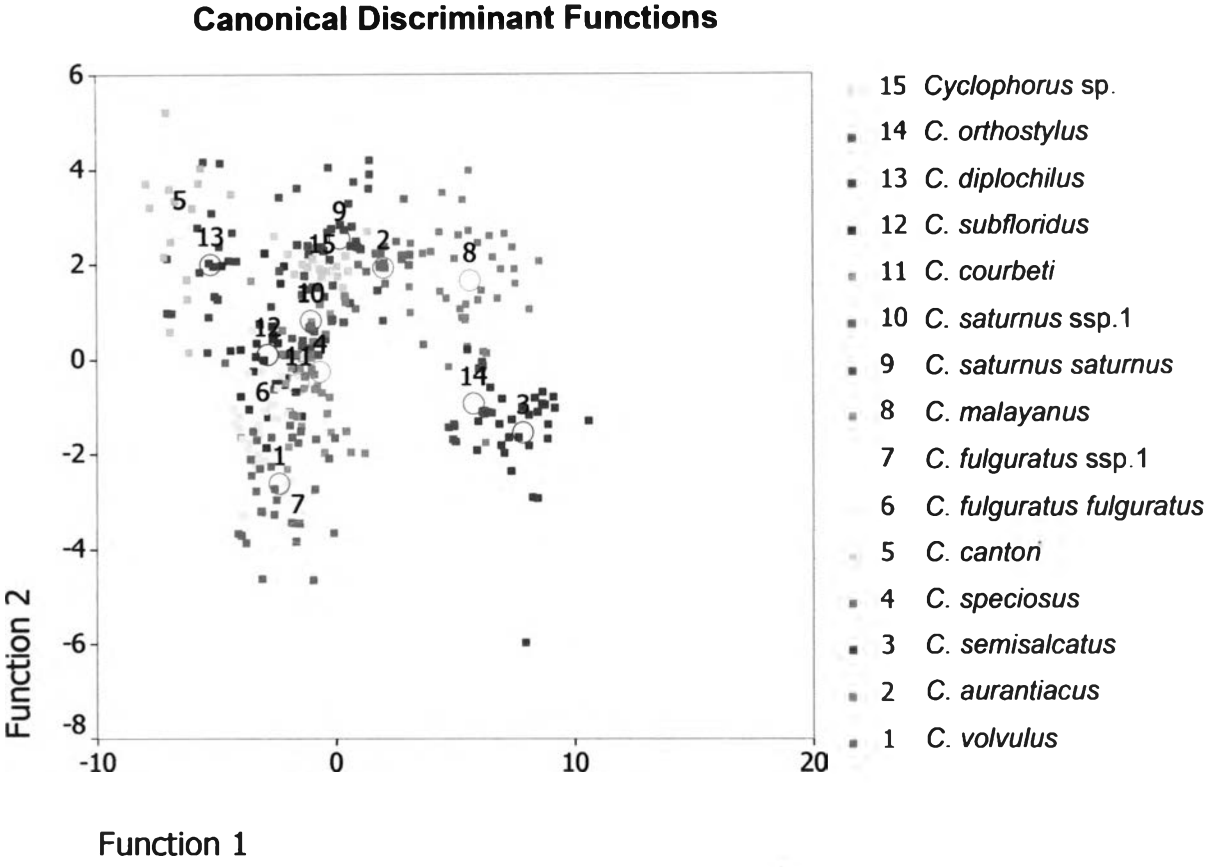

Figure 5.1 Canonical Discriminant Analysis. Plots are shown for individuals in each of the species level groups. To minimize the influence of the overall size of particular species in dominating discrimination, the plot shows a summary of variation in shell characters based on shell ratios (SH/SW, SP/SW, AH/SW, AW/SW, and MI/SW) rather than raw data. Open circles indicate the mean spatial location of characters within each species group.

5.2 Result and discussion of phylogenetic analysis

Twenty two characters were coded, including those from the shell characters (1-15), habitat type (1), radula and reproductive characters (17-22) (Table 3.1). *Leptopoma perlucidum* was used as an outgroup, because of its family member and the related shell morphology and soft parts anatomy. Table 3.2 gives the distributions of these character states among taxa. The cladistic analysis using Branch and Bound was resulted in 4 equally parsimonious trees. The strict consensus tree (Fig. 4.2) has 47 steps, consistency index (CI) of 0.55 and retention index (RI) of 0.55.

The dendrogram revealed the monophyletic of ingroup taxa, which supported by 57 bootstrap values. All five large shell species *C. aurantiacus*, *C. malayanus*, *C. saturnus saturnus*, *C. saturnus* ssp.1, *C. speciosus* and *Cyclophorus* sp. formed a monophyletic group with 55 bootstrap values.

The phylogenetic analysis using shell morphology only may appear more homoplasy and increase the risk that the similarity of the characters observed is not the result of the common ancestry but rather of parallel or convergent evolution (Schander and Sundberg, 2001). In this study, the high level of homoplasy and polytomies were observed in cyclophorid taxa. Molecular analysis needed for *Cyclophorus* phylogeny. However, the present study on karyotype analysis confirmed species identification and presented species relationship similar to morphological phylogeny for example *C. volvulus* has karyotypic formular of 14m which isolated from other species. The morphological phylogeny is also present species isolation. *C. aurantiacus* and *C. malayanus* showed the same karyotype relationship of 7m + 7sm and also appeared sister relationship in morphological phylogeny.

This will be at least the very important step before going further to the complete phylogenetic analysis of *Cyclophorus*.

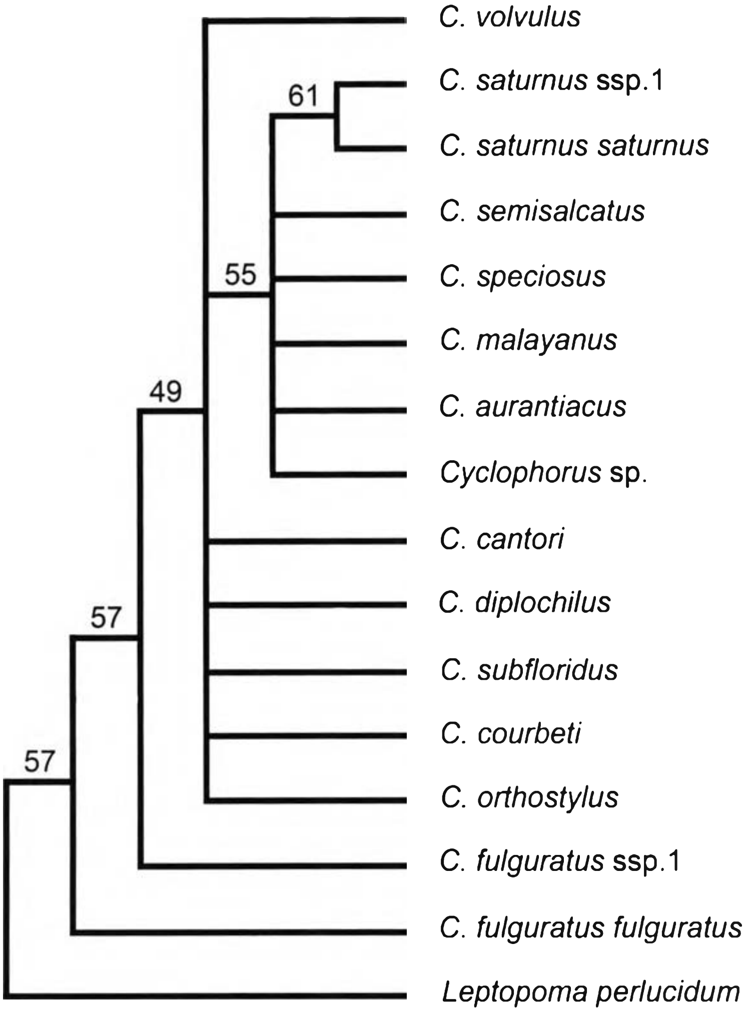

Figure 5.2 Phylogenetic tree of *Cyclophorus* with *Leptopoma perlucidum* as outgroup based on shell, radula and reproductive characters. The numbers above branch indicate the bootstrap probability from 1000 replicates.