Chapter 5

A CLADISTIC ANALYSIS: BASED ON MORPHOLOGICAL CHARACTERISTICS

CONTENTS

5.1 Introduction

The "phylogenetic systematics" or cladistics is of primary importance in modern phylogenetic studies (the term "cladistics" reflects the important role of cladogenesis and, therefore, reflects the essence of this method more precisely" (Bock, 1974), is used after this). The traditional phylogenetics study is the primary criterion of relationship in cladistics is homologous similarity. However, the foundation of cladistic methodology means an essential difference between two types of homology: common ancestral (symplesiomorphic) and standard evolutionarily advanced (synapomorphic). In the context of cladistics, only the second type of homology (synapomorphic) should be used to establish phylogenetic relationships (Hennig, 1950). Synapomorphies (evolutionarily advanced condition of characters uniform within a monophyletic (Hennig, 1950) group of organisms) are inherited by descendants from a common ancestor, which is acquired by them. Hence, they are evidence of confirmation. Although Hennig proposed several criteria for distinguishing between apomorphic (evolutionarily advanced) and plesiomorphic (evolutionarily primitive) characters, at present, due to the global distribution of computer data processing, only the principle of the outgroup is predominantly used for this. According to this principle, the only criterion for the recognition of an apomorphic condition is the fact that it is absent in the “outer” (more primitive, basal) taxa. In particular, the plumage is missing in all amniotes, except for some archosaurs, and, consequently, represents an undoubted apomorphy of birds (and some theropod dinosaurs).

The system of morphometric measurements called the truss network system (Strauss and Bookstein, 1982) has been widely used for population and taxonomic studies for the organism (Elliott et al., 1995; Çakmak and Alp, 2010; Rajakumaran et al., 2014). The conservation of body skeleton and definite body ratio facilitated using the truss network system as a taxonomical tool to differentiate physically similar species and compare traditional morphological methods; such a tool can be used as a clear, accurate, and precise descriptor (Fitzgerald et al., 2002). Study the morphological variation and differentiation between any prawn and shrimp population, a truss network has been used (Sun et al., 2014; Carvalho et al., 2019).

These morphometric measurements are used in fisheries biology to determine the dissimilarity or similarity of taxa (Reist, 1985) or relationships among various taxonomic groups (Turan, 2011). The analysis of phenotypic variations forms the basis for identifying stocks and estimating their population (Mojekwu and Anumudu, 2015). The use of multivariate techniques is thus receiving more attention in the stock assessment (Bektas and Belduz, 2009). These traditional methods continue to play essential roles in inventory identification despite molecular genetics today (Solomon et al., 2015).

Morphometric studies are considered one of the simplest, most commonly, and cost-effective used tools to identify the evolutionary relationship between species. Few published have addressed the phylogenetic relation of prawn and shrimp species; only a few have listed the cladistic analysis of this group. In 2006 Aktas et al. used the morphometric values to describe the taxonomical relationship between *Melicertus kerathurus*, *Metapenaus* *dobsoni,* and *Penaeus semisulcatus.* Thus, the morphometric characters have been successfully used for species discrimination and previous taxonomic works of Burkenroad (1934), Kubo (1949), Perez Farfante (1969), Tirmizi (1971), and Burukovsky (1972). Rajakumaran and his team studied the phylogenetic relationship among 13 Penaeidae shrimp based on morphometric analysis. The importance of this chapter was to evaluate the traditional hypothesis of marine prawn and shrimp species relationships based on the morphological characters. The objective is to reexamine the classical taxonomic

5.2 Material and Methods

5.2.1 Specimen Information

The morphometric analysis includes 152 specimens belonging to 39 valid species (Table 3.1). For the morphometric analysis, 4 families viz. are Callianassidae Alpheidae, Upogebiidae, and Spongicolidae were omitted because the morphological structure is different. Family Pandaloidea was also not considered because, in this species, only ovigerous females were reported during the present study. After identifying species belonging to suborder dendrobranchiate and pleocyemata shrimp, from each species of Penaeidae, three male samples were taken, and the female was omitted due to the size variation. In the case of caridean shrimps (in a few species), female specimens were also considered due to a smaller number of samples were reported.

5.2.2 Multivariate Analysis of Morphometric characters

The morphometric measurements were taken based on the truss network system shown in fig 5.1 (Aktas et al., 2006). In this system total of eighteen landmarks and forty variables were measured. The total length (TL) was also calculated from the tip of the rostrum to the posterior end of the telson, and forty-one variables (Table 5.1) were measured using a digital vernier caliper (accuracy ±0.1 mm). Small specimens were measured using a stereomicroscope equipped with a measuring tool. All the measurements were taken from thawed samples.

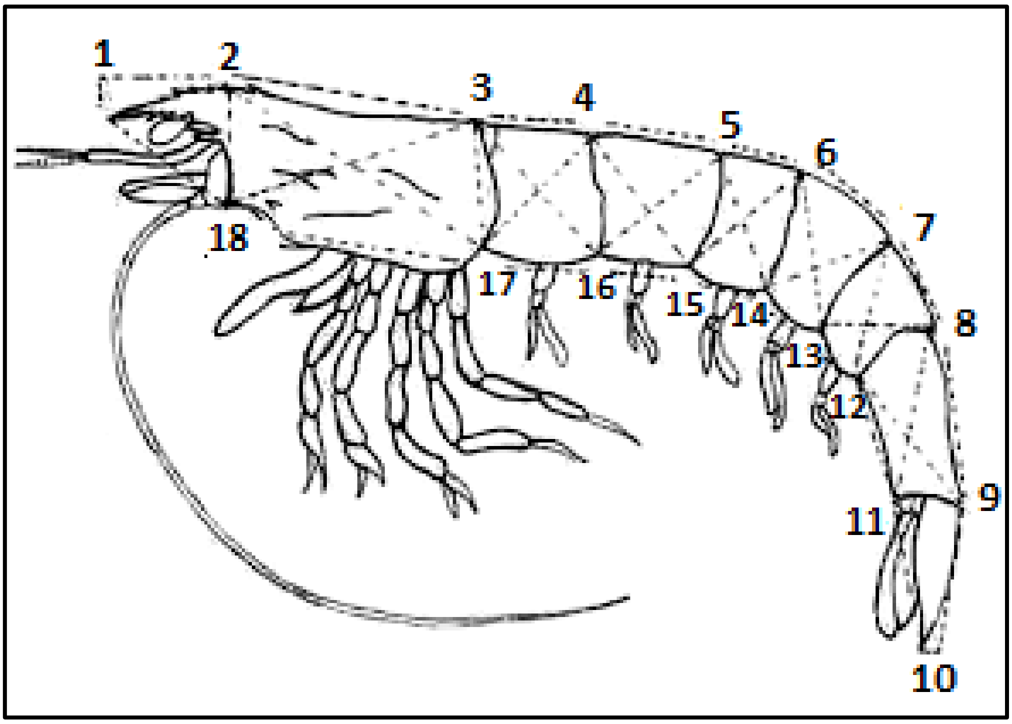

Figure 5.1 Truss network system used for morphometric analysis among prawn and shrimp species.

The average value was calculated. In this chapter, a significant correlation was observed between the average measurement of each variable and the total length. Therefore, it was necessary to remove the effect of size variation on species. To eliminate the variation resulting from each morphometric variable, they were standardized, according to Elliott et al. (1995).

$$\mathrm{Madj} = \mathrm{M}(\mathrm{Ls}/\mathrm{Lo})^{\mathrm{b}}$$

M is the original (primary) measurement; Madj is the size-adjusted measurement of each variable. Ls is the overall mean of the standard length of all samples, and Lo is the standard length of the model or sample. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all prawn and shrimp groups. The Ls/Lo values computed in the MS excel sheet and detecting the b parameter were carried out using the PAST (Paleontological Statistics

Software) package version 3.25. The cluster analysis was performed UPGMA multivariate method to evaluate the morphometric relationship among all species in each order. In this chapter, hierarchical clustering analysis was represented as a dendrogram, where a joint of the tree illustrated each step in the clustering formation. The single linkage method was used with the Euclidean distance (statistic tool that quantifies the extent to which species within-cluster are similar) with 1000 bootstrapped.

Table 5.1 List of the morphometric variables used for the study of the taxonomical relationship of the prawn and shrimp species

S. No.	No Variables*	Variable Name
1	var1-2	Rostrum length
2	var2-3	Carapace dorsal
3	var3-4	First Abdominal segment dorsal
4	var4-5	Second Abdominal segment dorsal
5	var5-6	Third Abdominal segment dorsal
6	var6-7	Fourth Abdominal segment dorsal
7	var7-8	Fifth Abdominal segment dorsal
8	var8-9	Sixth Abdominal segment dorsal
9	var9-10	Telson dorsal
10	var10-11	Telson ventral
11	var11-12	Sixth Abdominal segment ventral
12	vae12-13	Fifth Abdominal segment ventral
13	var13-14	Fourth Abdominal segment ventral
14	var14-15	Third Abdominal segment ventral
15	var15-16	Second Abdominal segment ventral
16	var16-17	First Abdominal segment ventral
17	var17-18	Carapace ventral
18	var1-18	Diagonal of anterior end carapace to rostrum end
19	var2-18	Perpendicular Carapace 1

20	var2-17	Diagonal of Carapace 1
21	var3-18	Diagonal of Carapace 2
22	var3-17	Perpendicular Carapace 2
23	var3-16	Diagonal of first Abdominal segment 1
24	var4-17	Diagonal of first Abdominal segment 2
25	var4-16	Perpendicular of first Abdominal segment
26	var4-15	Diagonal of second Abdominal segment 1
27	var5-16	Diagonal of second Abdominal segment 2
28	var5-15	Perpendicular of second Abdominal segment
29	var5-14	Diagonal of third Abdominal segment 1
30	var6-15	Diagonal of third Abdominal segment 2
31	var6-14	Perpendicular of third Abdominal segment
32	var6-13	Diagonal of fourth Abdominal segment 1
33	var7-14	Diagonal of fourth Abdominal segment 2
34	var7-13	Perpendicular of the fourth segment
35	var7-12	Diagonal of fifth Abdominal segment 1
36	var8-13	Diagonal of fifth Abdominal segment 2
37	var8-12	Perpendicular fifth Abdominal segment
38	var8-11	Diagonal of sixth Abdominal segment 1
39	var9-12	Diagonal of sixth Abdominal segment 2
40	var9-11	Perpendicular of fifth Abdominal segment
41	TL	Tip of the rostrum to the posterior margin of telson

5.3 Data analysis and Results

During the present study, detailed cladistic analyses of prawn and shrimp species were studied based on the 41 morphometric variables. For this study, a total of 39 species (28 Dendrobranchiata and 11 Pleocyemata) of prawns and shrimps were used, and its morphometric measurements values are given in Annexure 5.1 and 5.3. All these available morphometrical values exhibited a significant correlation with the size of prawn species. The size modification also was carried out to remove the effect of the size. Annexure 5.2 and 5.4 provide the modified value for all variables of all species of Dendrobranchiate and Pleocyemata.

The size of the species evaluated was virtually eliminated via these modified values. These size-modified morphometrical values described relationships among the species. The cladistic cluster analysis of Dendrobranchiata species based on the morphometric is shown in fig. 5.2. In this dendrogram, *T. curvirostris* (first clade) and *P. monodon* (last clade) formed a separated clade with a high bootstrap value. The species like *P. penicillatus, P. japonicus, P. merguiensis,* and *P. cancaliculatus* are close. Species belonging to the family solenoceridae are close to each other, and they are not arranged in a single clade. *M. dobsoni* and *P. latisulcatus* are placed in a single clade. Species like *P. indicus*, *P. merguiensis,* and *P. penicillatus* are morphologically close to each other. All the species belonging to the genus *Metapenaeus* came near.

The species like *M. moyebi* and *M. brevicornis* are morphometrical close and formed in a single clade with high value. Only a single species viz., *G. uncta* was reported from the genus *Ganjampenaeopsis.* This genus was separated from the genus *Parapenaeopsis* and was arranged in between. Species like *M. hardwickii* and *P. sculptilis* were morphometrically similar to a high value (99%). Another species, *P. stylifera* is morphological and morphometrical identical to both species. The *Metapenaeopsis* species *M. barbata* and *M. stridulans* and species of the genus *Parapenaeus* like *P. longipes,* and *P. fissuroides indicus* are arranged together with low value. Species like *S. crassicornis,* and *S. koelbeli* are placed nearby with low bootstrap values (27%). *M. kutchensis* formed a different clade, which is endemic in the Northwest coast of India.

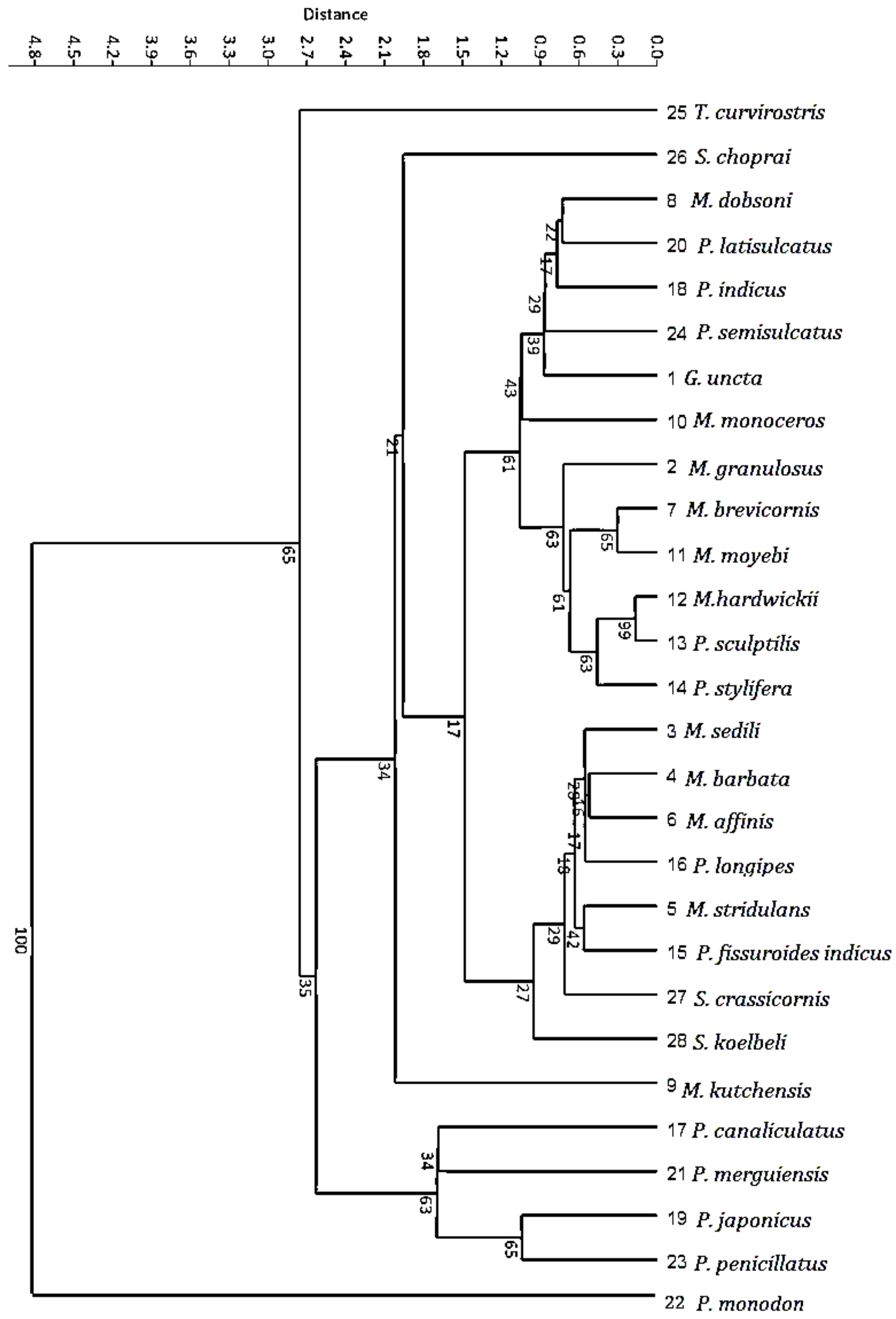

Figure 5.2 Taxonomical relationship among 28 species of Dendrobranchiata based on morphometrical characters.

The cladistic cluster analysis of the pleocyemata species found on the morphometric is shown in fig. 5.3. The species *N. tenipes* formed a separate clade (first clade) from other species with high bootstrap value. Species like *P. serrifer, P. pacificus*, *P. stylifers,* and *A. brevicarpalis* are close species *P. serrifer*, and *P. pacificus* was positively related to it 68% bootstrap value. *P.*

stylifers, and *E. ensirostris ensirostris* are formed a different clade with a sister taxon with a good bootstrap value. *Thor amboinensis* comprised a separate clade (fourth clade). Other species like *Saron marmoratus* and *Lysmata vittata* are arranged nearby with low bootstrap value. Another species like *L. anoplonyx,* formed a different clade.

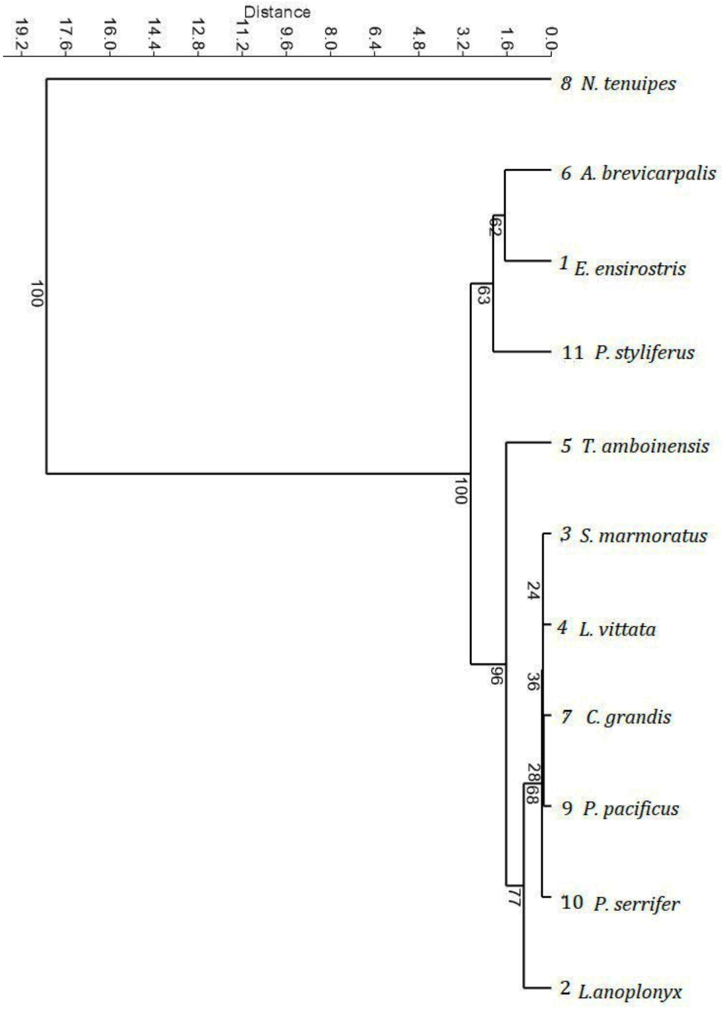

Figure 5.3 Taxonomical relationship among Pleocyemata species based on morphometrical characters.

5.4 Discussion

The taxonomical relationship is the pioneering exploration of life on earth, which lays the foundation for the phylogenetic tree of life (Wilson, 2004.) For the prawn taxonomical studies, morphometric traits have been used in discriminating many prawn and shrimp species (Aktas et al., 2006; Bissaro et al., 2013). In this study, the resulting trees of Dendrobranchiata species had low bootstrap support values for half of the branches and lineages (BS <50%; fig. 5.2). This might result from the small size of data matrices; however, moderately sized matrices. During the present study, we have not been able to identify a reason for these low values. Still, the lack of support may be due to the nature of the morphological cladistical analysis. In another resulting tree, Pleocyemata species had high bootstrap support value for most branches and lineages (BS>50%; fig. 5.3). A low support value to a low number of synapomorphies within the clade. Since other studies have analyzed and interpreted the evolutionary relations of their corresponding taxa despite low support values, the results and interpretations made here are valid.

In the present study, we tried to establish the morphometrical based taxonomical relationship among prawn and shrimp species by increasing the number of the trait using the truss network system. The resulting tree showed more statistical support of the taxonomical relationship of prawn and shrimp species belonging to suborder dendrobranchiate and pleocyemata. All the prawns and shrimps differ in various morphological characteristics that are the expression of genetic differences among them. Research on morphological differences among species could be used for taxonomic distinctions. Morphometry is a quantitative study of the pattern of covariance with shape (Bookstein, 1991). Many morphological attributes of natural form reflect the evolutionary process (Darwin, 1859; Simpson, 1944; Mayr, 1963).

In the morphometric analysis species like, *P. merguensis*, *P. japonicus*, and *P. penicillatus* are arranged in the same clade. It also supported the molecular phylogeny data studied by Kundu et al. in 2018 from Indian waters. Another species *P. indicus,* was too close to the *P. semisulcatus.* In this study *M. brevicornis* shared a sister clade with M. *hardwickii*, *P. sculptilis*, and *P. stylifera*. A similar kind of observation was reported by Rajakumaran et al. in 2014. They studied the morphometric phylogeny of 13 species of penaeidae shrimp from the East coast of India. Species of family solenoceridea formed a clade with *P. fissuroides indicus* that is supported by Voloch et al. (2005), who conducted the molecular study of prawns. In this study, we found that the family solenoceridea is closely related to the family Penaeidae.

In the case of suborder pleocyemata, the size of standardized morphometric values identified by the families Hippolytidae Lysmatidae, Thoridae, and Palaemonidae are taxonomically close by morphometric values. This study was also supported by molecular data (Aznar-Cormano et al., 2015). In 2014, De Grave et al. separated the family Hippolytidae into five families: Merguiidae, Bythocarididae, Thoridae, Hippolytidae s.s. and Lysmatidae. In 2015, Aznar-Cormano et al. attempted to resolve the taxonomy and evolutionary relationships among caridean shrimp. They said that additional data was needed to revise the taxonomy of some caridean families like Palaemonidae, Hippolytidae s.l., and Pasiphaeidae. However, such taxonomic revision needs additional taxonomic sampling to resolve the taxonomic relationship between these families.

These kinds of research can potentially help in aquaculture management, stock assessment, systematic, ecological, and evolutionary studies, and the conservation program for a population and lead to a better understanding of species ecology and behavioral traits. The morphological variability reflects both environmental and genetic influences.