PHYLOGENETIC INFERENCE OF PRIACANTHIDAE FROM MORPHOMETRIC STUDIES

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Kwang-Tsao Shao and Weise Chang (1985) Phylogenetic inference of Priacanthidae from morphometric studies. Bull. Inst. Zool., Academia Sinica 24(2): 273-288. Phylogenetic relationship of eight different species of priacanthids around Taiwan were studied via morphometric measurements. These species are Pristigenys multifasciatus, Pg. niphonia, Cookeolus boops, Priacanthus cruentatus, P. tayenus, P. macracanthus, P. hamrur, and P. blochii. A total of twenty-three individuals (OTUs), two to seven individuals for each species, were analyzed simultaneously to evaluate various numerical taxonomic techniques in regard to the criterion that conspecific taxa should be clustered near each other. It is shown that the measurements of truss method tend to give better results compared to that of using tradition. Among phenetic methods, Bray-Curtis dissimilarity coefficient performs the best among total of seven resemblance coefficients under test, and UPGMA gives better phenograms than the other three clustering methods. For cladistic analysis, distance Wagner of using parsimony principle and Fitch-Margoliash method were applied to construct cladograms which in general agree with the geneological relationship of priacanthids inferred from various phenetic classifications. The phylogeny of Priacanthidae contains three convex groups which are exactly in corresponding to three different genera. Pg. multifasciatus and Pg. niphonia are grouped each other. Cookeolus boops is intermediate between both genera of Pristogenys and Priacanthus. Within the genus of Priacanthus, P. tayenus is the one which more related to C. boops than the other four congeneric species. P. brochii and P. harmur are sister group but its relationship with P. macracanthus and P. cruentatus is not clear. This paper also gives a demonstration of showing how to use morphometric data to infer the fish phylogeny.

The family Priacanthidae, commonly called big-eye, contains approximately three genera and eighteen nominal species of fishes distributed throughout the tropic and subtropics of the Atlantic, Indian, and Pacific Ocean (Nelson, 1984). In Taiwan, eight different species which belong to three genera had been recorded previously (Lee 1980; 1984). They are Pristigenys niphonia (Cuvier and Valenciennes), Pg. multifasciatus Yoshino and Iwas, Cookeolus boops (Synder), P. cru-

entatus (Lacepede), P. harmrur (Forskal), P. macracanthus Cuvier and Valenciennes, P. blochii Bleeker, and P. tayenus Richardson. For the phylogenetic relationships of this group of fish, Lee (1984) firstly proposed a phylogenetic relationship of these fishes by comparing the electropherograms of muscle myogens and indicated that the electrophoretic result is in agree with the results of the conventional systematic studies in terms of their external features including meristic data. To test the nonspecificity

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hypothesis by using different character suits of the same study organisms has attracted much attention in the numerical taxonomic field (Rohlf, 1965; Farris, 1971; Sneath and Sokal, 1973). Recently, this issue has even been extended to examine whether the classification methods used in phenetics or in cladistics have greater stability when sampling different classes of characters (Colless, 1980; Mickevich and Johnson, 1976; Rohlf et al., 1983a, b: Schuh and Farris, 1980). purpose of this paper is therefore to test the nonspecificity hypothesis by comparing the phylogenies of priacanthids based on the morphometric characters against the one proposed previously based on the electropherogams of myogens. Similar comparative studies by using fish as study material have been published elsewhere except that they used the allozymes as the internal characters (Smith and Koehn 1971; Mickevich and Johnson, 1976; Colless, 1980; Shaklee and Tamaru, 1981: Yoshiyama and Sassaman, 1983).

Numerical taxonomical methods has been increasingly used to study the phylogency of real organisms since last decade. However, there still are some methodological problems that require further investigations. Thus, the other purpose of this paper is to compare some different effectiveness of numerical taxonomical techniques by using the morphometric measurements of priacanthids as the testing data set. The techniques we examined in the studies includes: 1) truss measurement (Humphries et al., 1981; Strauss and Bookstein, 1982) versus the measurement based on the traditional way; 2) various phenetic methods, including different resemblance coefficients and different clustering methods; 3) phenetic versus cladistic methods. To judge the goodness-of-fit of the above different techniques on the phylogenetic studies, one often uses the cophenetic correlation coefficient for phenetic clustering methods, and the shortest tree length for the cladistic method (Sneath and Sokal, 1973). In the present paper, we proposed an alternative way by applying the

maximum consensus tree method, MCT (Shao, 1983). The validity of this application is based on the assumption that the contaxon individuals (OTUs) should be clustered near each other when the identification of each species is guaranteed. Consequently, the clustering methods which could more satisfy the above critrion would be considered to be the better.

MATERIAL AND METHODS

Data were obtained for 33 morphometric variables (Fig. 1) from twenty-three individuals belonging to eight different species in All data were measured from preserved specimens. Their body size range are Pg. niphonia (3 specimens, PgN1-PgN3, 71-248 mm); Pg. multifasciatus (2 specimens, PgM1 and PgM2, 270-272 mm); C. boops (2) specimens, CB1 and CB2, 342-365 mm); P. hamrur (3 specimens, PH1-PH3, 245-248 mm); P. macracanthus (7 specimens, PM1-PM7, 205-224 mm); P. blochii (2 specimens, PB1 and PB2, 283-285 mm); P. tayenus (2 specimens, PT1 and PT2, 145-298 mm); P. cruentatus (2 specimens, PC1 and PC2, 242-245 mm). Measurements were made to the nearest millimeter using Vernier calipers (+0.02 mm). Variables included: 1. standard body length: 2. head length; 3. snout length; 4. eye diameter; 5. caudal fin length; 6. pectoral fin length; 7. ventral fin length; 8. height of first dorsal spine; 9. height of the longest dorsal ray; 10. height of the longest anal fin ray; 11. premaxilla length; 12. body depth; 13. interorbital distance; 14. interprenasal distance; 15. dorsal fin base length; 16. anal fin base length; 17. length from the tip of upper jaw to the origin of dorsal; 18. distance between the origins of ventral and anal fin; 19. the tip of upper jaw to the origin of ventral fin; 20. height of caudal peduncle; and the subsequent thirteen characters CD, EF, HI, AI, BI, BH, BG, BF, CI, CG, CF, CE, DF which were defined based on the nine landmarks, A-I, marked on the outline of the fish (Fig.

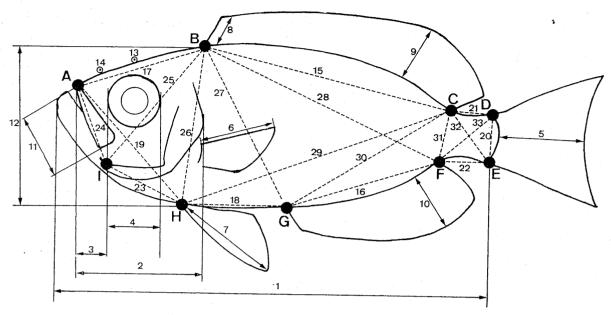


Fig. 1. [An outline drawing of *Priacanthus hamrur* showing the variables comprising the morphometric data. The definitions of each variable were described in the text. Alphabatical A-I are nine homologous landmarks used to define the variables of truss measurements.

1). For comparing the different effect between traditional and truss measuring methods on the phylogenetic results, the above 32 characters (exclude the standard body length) were arranged into two subsets of data. Each of the subsets contains ninteen characters: the second to the twentieth of the above variables were regarded as the traditional measurements, and the fifteenth to the last as the truss Among them, variables fifteenth to method. the twentieth were overlapped in both data All these 32 characters were subjected to the ratio correction based on the standard body length to normalizing the body size effect. After some preliminary studies, we did not choose other sophisticate normalizing methods, such as regression, PCA, or sheared PCA (Humphries, et al., 1981, Strauss and Bookstein, 1982) in the present study because of the following two reasons: 1), the size problem is not significant in our fish data after examing the projections of OTUs on the first three principal axes; 2), testing various resemblance coefficient could not be done if PCA is used first. Because only a single

distance matrix could be obtained by applying this ordination technique. For phenetic analysis, four clustering methods and seven resemblance coefficients in the CLUSTAR program package were used to compute the phenograms and their corresponding cophenetic correlation coefficient values. The minimum spanning tree, principal coordinate analysis, and the Kruskal's non-metric multidimensional scaling (MDSCAL) analysis subjected to the Euclidean distance matrix were computed by using the NTSYS package (Rohlf, et al., 1983). To do the cladistic analysis based on he continuous data, only mid-point rooting Wagner in the WAGNER78, written by F. J. Farris (1978), and Fitch-Margoliash methods in the PHYLIP package, among many currently available cladistic programs, could be used to construct morphometric unrooted trees. For using both cladistic programs, the Manhattan distance matrix computed from CLUSTAR (Romesburg and Marshall, 1984) were used as the input data. Theoretical comparison of the above two cladistic methods and the UPGMA has been studied detailly by

Swofford (1981).

For comparing the phylogenetic results of eight species of priacanthids easily, methods were used independently to obtain the tree with 8 OTUs only. The first method is to reconstruct a reduced 8 OTUs data matrix directly from the original data matrix of 23 OTUs. The value of each variable in the new matrix is the mean value of that variable of all conspecific OTUs. The resulting phenograms or cladograms constructed from this 8 OTUs data matrix will naturally be 8 OTUs. The second method is to apply the maximum consensus method to compare each testing tree of 23 OTUs against the standard tree with the same number of OTUs in which the criterion of conspecific taxa should be clustered together has been set (Fig. 3). Those ambiguous subsets, i. e. a conspecific cluster has other species sandwiched in, at higher level will be degenerated to be unresolved subsets and down to lower hierarchical level. But all of the rest unambiguous subsets in the tree will be retained as much as possible. Consequently, an 8 OTUs tree could be easily extracted from the MCT of 23 OTUs without any ambiguity. The algorithm for computing the maximum consensus tree and its associated consensus indices was furnished in Shao (1983).

RESULTS

From both of the CPCC and CIc values in Table 1, it is clearly shown that the truss measurement is superior than the traditional measurment except in five cases of CPCC and one case of CIc values. The mean CIc and CPCC values from all 19 different phenetic methods are 0.8596 and 0.8862 for the truss and 0.7719, and 0.8129 for the traditional respectively. Although the product moment correlation coefficient between all CIc values and all CPCC values is not so high (r=0.5097), the tendency of CIc and CPCC values consistently shown the relation of UPGMA> Complete linkage>Single linkage. The mean

CIc and CPCC values of different clustering methods averaging from various resemblance coefficients is given in Table 2(b). The results also revealed that the UPGMA is the best among four different phenetic clustering methods. The Ward's clustering method by using Ward's coefficient performs relatively poor. For cladistic methods, only CIc of Wagner method could be calculated since the CPCC can only be used for phenetic clustering methods. Nevertheless, both Wagner trees based on traditional and truss method gave the same CIc values of 0.9524 which is the highest if compared to other phenetic methods.

The same criterion of *CIc* used on the Fitch-margoliash networks is not appropriate since its plethora of available trees when shuffled the OTUs sequences in the input distance data matrix.

The same criterion for comparing the effect of different resemblance coefficients is furnished in Table 1 and Table 2(a). It is shown that the Bray-Curtis has the highest value among total of seven different resemblance coefficients investigated. The order is Bray-Curtis > Manhattan distance > Average Euclidean distance > Correlation > Ward's > Shape > Cosine coefficient with respect to high to low of the CIc and CPCC values. Due to the limiting space, we only choose the best phenograms, i.e. the UPGMA based on Bray-curtis coefficient similarity matrix according to the above criterion (Fig. 3), as the representatives among a great number of various phenograms. It is shown that almost all congeneric or conspecific taxa were clustered near each other. The only exception is the PH3 which is grouped with P. cruentatus rather than with PH1 and PH2. Figure 4 shows the summarized phylogenetic relationship of eight species after applied the maximum consensus tree method on the two phenograms in Fig. 3 and the standard tree in Fig. 2. The resulting phylogenetic relationship of both from traditional and truss method is quite similar. All congeneric species are grouped together to form three monophyletic

Colless consensus index* (CIc) of the maximum consensus tree and the cophentic correlation coefficient (CPCC) values of various phenograms computed from seven different resemblance coefficients in combination with four different clustering methods. Two character data matrices, one based on the traditional characters, and the other based on the truss were used separately as the input data. For detail explanation

see the text

Resemblance coefficients*	Clustering methods#	Traditional		Truss	
		CIc	CPCC	CIc	CPCC
Correlation	Single	0.7143	0.7200	0.8095	0.8564
	Complete	0.7619	0.8813	0.8095	0.8424
	UPGMA	0.8095	0.8830	0.9048	0.8644
Average Euclidean distance	Single	0.7143	0.6873	0.8095	0.9015
	Complete	0.8095	0.7876	0.7619	0.9226
	UPGMA	0.8571	0.8358	0.9048	0.9251
Cosine	Single	0.7143	0.7832	0.7619	0.7791
	Complete	0.7143	0.8221	0.8571	0.8366
	UPGMA	0.7619	0.8616	0.8571	0.8561
Shape difference	Single	0.6667	0.8106	0.8095	0.864
	Complete	0.7143	0.8741	0.9048	0.8677
	UPGMA	0.7143	0.8846	0.8571	0.9044
Bray-Curtis	Single	0.6191	0.7702	0.8571	0.9185
	Complete	0.9524	0.8575	0.9524	0.9291
	UPGMA	0.9048	0.8757	0.9048	0.9318
Manhattan distance	Single	0.6667	0.6951	0.8571	0.9120
	Complete	0.9524	0.8521	0.9524	0.9236
	UPGMA	0.8095	0.8616	0.9524	0.9269
Ward's	Ward's method	0.8095	0.7016	0.8095	0.8764

* The Colless consensus index is computed by counting the number of distinct subsets (excluding the set of all objects) and then divide it by t-2, where t is the number of objects in the study.

* The formulas of computing resemblance coefficients were furnished in the User's Manual for CLUSTAR/CLUSTID package (Romesburg and Marshall 1984) except the Manhattan distance coefficient which was added into the package by KTS. For further information about these

resemblance coefficients and clustering methods, please refer to some clustering textbooks (Sneath and Sokal 1973, Romesburg 1984).

TABLE 2

The overall effects of different resemblance coefficients (a), and clustering methods (b) on the *CIc* of MCT and CPCC values. The table values are the means averaged from the values in the previous table. The indices values of both traditional and truss methods were lumped together when calculated the means.

(a) (b) Resemblance Clustering CIc**CPCC** CIcCPCC coefficients methods Correlation 0.8016 0.8413 Single linkage 0.7500 0.8079 Average Euclidean distance 0.8095 0.8433 Complete linkage 0.8452 0.8664 Cosine 0.7778 0.8231 Shape difference 0.7778 0.8676 **UPGMA** 0.8532 0.8844 **Bray-Curtis** 0.8651 0.8805 Manhattan distance 0.8651 0.8619 Ward's methods 0.8095 0.7890 Ward's 0.8095 0.7890

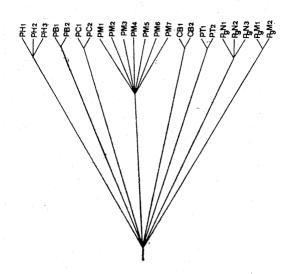


Fig. 2. A standard tree which only give the information that the conspecific taxa are grouped each other. This tree is used to compute the maximum consensus tree by comparing it with various phenograms or cladograms obtained in the studies.

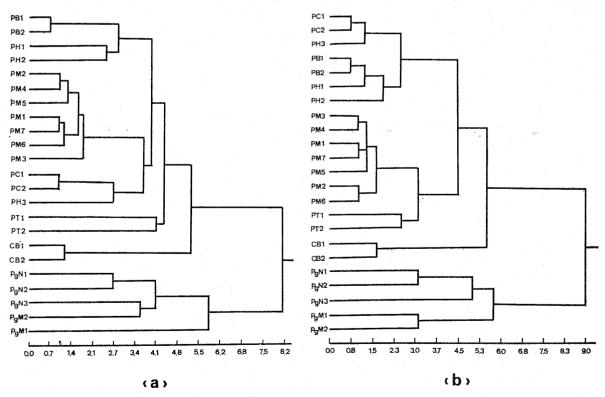


Fig. 3. Phenograms constructed from the UPGMA based on the Bray-Curtis dissimilarity matrix. The input data were (a) Traditional mesurements, and (b) truss measurements of the 23 OTUs.



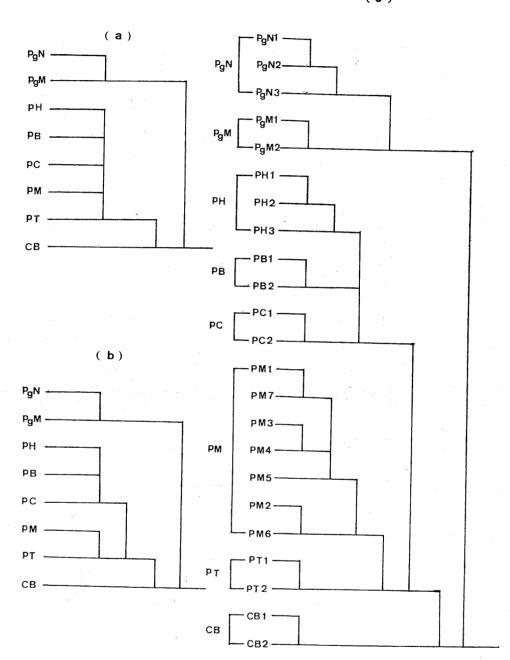


Fig. 4. The maximum consensus tree of the UPGMA phenograms of Bary-Curtis coefficient based on (a) traditional measurements, and (b) truss measurements of the 23 OTUs data matrix compared to the standard tree shown in Fig. 2. The 8 OTUs trees of (a) and (b) are extracted from original 23 MCT. For example, the tree b was redrawn from the consensus tree c. Note that the hierachical level is no longer existed after the consensus tree method is operated since only the branching pattern of tree are taken into consideration.

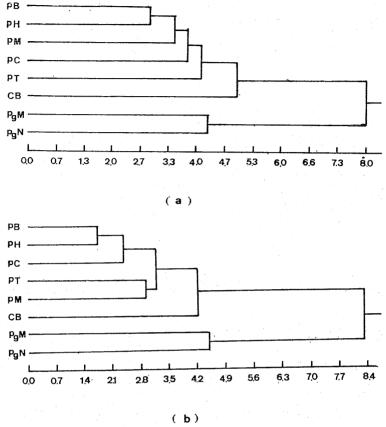


Fig. 5. Phenograms constructed from the Bray-Curtis similarity matrix by UPGMA. The input data matrices were (a) traditional measurement, and (b) truss measurements of eight species averaged from the original 23 individuals data set.

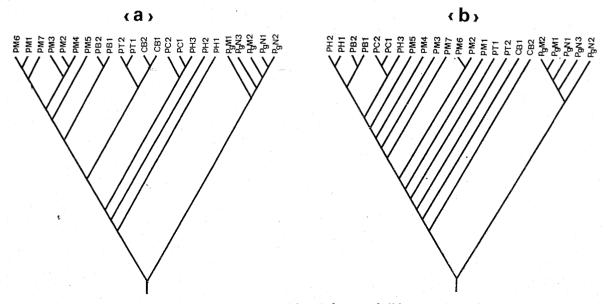


Fig. 6. Distance Wagner trees of (a) traditional data, and (b) truss data sets of 23 OTUs.

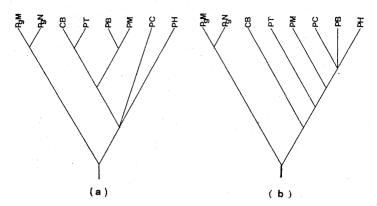
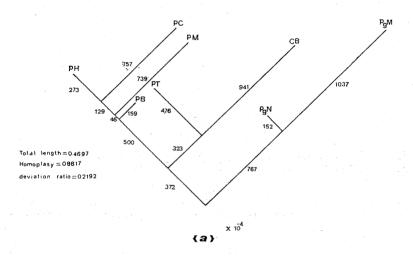


Fig. 7. The maximum consensus tree of distance Wagner trees versus the standard. Tree (a) was based on traditional measurements, (b) based on the truss. For other explanations see Fig. 4.



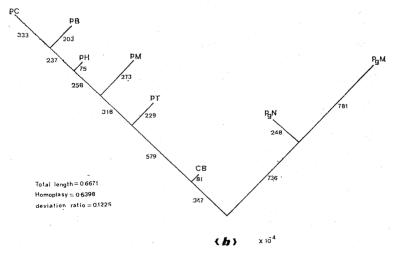


Fig. 8. Distance Wagner trees of (a) traditional measurements, and (b) truss measurements of eight OTUs average data matrices. Divergences are reflected in the relative lengths of the branches.

groups as represented in three different genera. The only difference is the *P. macracanthus* which is grouped with *P. tayneus* as a separated cluster in the truss but unresolved with other *Priacathus* species in the traditional tree. Nevertheless, these congeneric relationships could be supported by the phylogenies prepared by using reducing data matxix directly. Fig. 5 shows the two UPGMA phenograms based on the same Bray-Curtis similarity coefficient. From these two phenograms, species inter-relationship within *Pria*-

cathus could be recognized as the *P. blochii* and *P. hamrur* are sister group, which is next to *P. cruentatus* and then *P. macracanthus* and *P. tayenus*.

As to the phylogeny constructed by the cladistic methods, Fig. 6 is two Wagner cladograms of original 23 OTUs. Their two maximum consensus trees (MCTs) comparing to the standard tree are shown in Fig. 7. In Fig. 7, the phylogenetic relationship of the truss give more compatible relationships suggested by phenograms (Fig. 5b) than that of

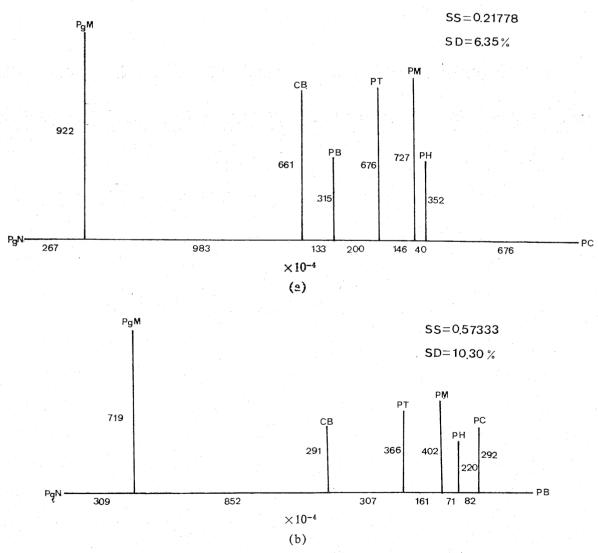
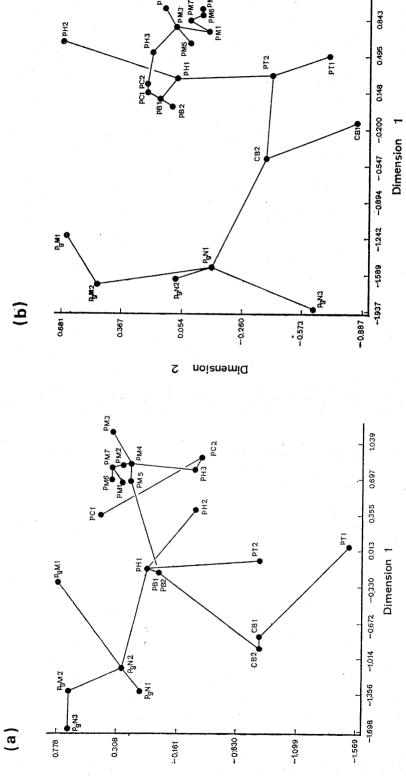


Fig. 9. Fitch-Margoliash networks for (a) traditional measurements and (b) truss measurements based on the eight OTUs data matrices. Divergences are reflected in the relative lengths (indicated by numbers) of the branches.



Dimension 2

Fig. 10. The projection of 23 individuals (OTUs) of eight different species on the two dimensional space by using Kruskal's nonmetric multi-dimensional scaling (MDSCAL) technique. The lines connected among OTUs is the result of minimum spanning tree (MST). Plot (a) was based on the traditional measurement data, and (b) on the truss.

traditional (Fig. 5a). The MCTs based on the traditional data set (Fig. 8a) is relatively poor since all Priacanthus species are not grouped monophyletically. Figure 8 contains two Wagner cladograms directly constructed from the reduced data sets of eight OTUs. The species inter-relationships are comparable to that of Fig. 7 but more resolved. length of each stem marked on the trees revealed that the path length between the terminal nodes of P. blochii and P. hamrur are 0.067 in (a) and 0.0515 in (b) which are shorter than 0.1030 of sister group of P. hamrur-P. cruentatus in (a) and 0.0536 of P. cruentatus-P. blochii respectively. This difference can be used to explain why the P. blochii and P. hamrur were clustered together in phenetic but not in cladistics. Similarly, the path lengths on the two Fitch-Margoliash networks (Fig. 9) could be used to interpret their phenetic relationships besides the information of their cladistics relations per se.

The diagrams produced by principal coordinate analysis and MDSCAL show similar species inter-relationships. Figure 10 represents the 2-dimensional views of the MDSCAL which showing three fairly well divisions of points, respectively the genus Pristigenys (upper, left-hand), the genus Cookeolus (lower, middle), and the genus Priacanthus (upper, right-hand). Within the genus Priacanthus, individuals of P. macracanthus, are tightly grouped as well as the individuals of P. blochii. The groups of P. hamrur and P. cruentatus are Among Priacanthus, P. relatively sparse. tyaneus still shows the closest relation with C. boops. The ordination result is quite reliable since the final stress of these plots are close to zero as 0.2710 for traditional data and 0.1049 for the truss data. The minimum spaning tree (MST) superimposed on the plots could be detect the possible distortions of reducing dimensions. From the result of MST it is also shown that the one based on the truss is superior to the one based on the traditional data since there is only one intersection in truss but more intersections in traditional'

DISCUSSION

From comparing all of the above phenograms, cladograms, and ordination plots, the phylogenetic relationship of pricanthids should be able to be inferred from their consistent portions. Three genera Priacanthus, Cookelous, and Pristigenys estiablished from conventional systematics can be supported by our morphometric phylogenetic results since all congeneric species were grouped together to form three monophyletic groups. Pg. multifasciats and Pg. niphonias form a sister group although their actual divergence is great. The genus Pristigenys could be the ancestor of priacanthids as the midpoint rooting of distance Wagner procedure had done automatically by choosing the root at the stem which has the longest path length among all possible pairs of OTUs. Other evidences also come from the comparisons of morphological or meristic characters. The Pristigenys is characteristically with the lowest meristic count including dorsal ray, anal ray, and later line scales comparing to that of the intermediates of Cookeolus and the highest counts of Priacanthus. The large hard scale with spinules of Pristigenys is another character in contrast to the small, smooth and soft scale of the other two genera. If the lower meristic count and the the harder scale is considered to be the primitive state, then the Pristigenys would be assigned to be the ancestor as well if the compatibility analysis was applied. Cookeolus should share the common ancestor with *Priacanthus* since the single species of C. boops is clustered with Priacathus on the same main branch. Its distance from the nearest species of Priacanthus, P. tyaenus, is much shorter than that from the species of Pristi-The interspecific relationships among Priacanthus is relatively difficult to determine. Phenetically the P. blochii and P. hamrur is in a sister group but may be not in cladistics. However, three species of P. blochii, P. hamrur, and P. cruentatus are suggested to be close to each other from both phenetic and cladistic

result if the truss data is used. If the traditional data was used, the interspecific relations within the genus *Priacanthus* is unclear. This may suggest that the measurement by traditional method can not reflect the shape information of the fishes as effectively as by the truss method. Nevertheless, the similar morphology among the species of *Priacanthus* may also imply that the degree of divergence within this genus is not so great and the time of their divergence might be recent. The above proposed phylogeny should be reliable since we have applied many different numerical taxomical methods as more as we can.

The meristic data summarized in Lee (1984) also encourages our proposed phylogeny. The modes of dorsal fin rays and anal fin rays of the eight species are Pg. niphonia (11, 10); Pg. multifasciatus (12, 11), C. boops (12, 13); P. tayneus (12, 13); P. macracanthus (13, 14); P. cruentatus (13, 14); P. hamrur (14, 15); and P. blochii (14, 15) respestively. A trend from lower to higher count indicates that the P. hamrur and P. blochii are more advance species than P. tayenus which is close to C. boops, and the two Pristigenys species should be more primitive. As to the congruence study between our morphometric phylogeny and the eletrophoretic one (Lee 1984), the result is moderate since there are some discrepancies of interspecific relationships within Priacanthus though the genus relationship is in common. Lee (1984) suggested that P. hamrur and P. macracanthus were clustered together, and P. blochii and P. cruentatus are in a sister group. This difference is not surprised since there were only a few data sets which show modest degree of congruence with respect to the effects of sampling of different suites of characters (Rohlf, 1983).

As to the evalution of different analytical methods based on our data set, the study results suggest the following points: (1) The truss method obviously does better job in reflecting the shape information than the traditional (Struss and Bookstein, 1982). (2)

Because of the consistency between CIc and CPCC values, it is suggested that the Colless' consesus index of maximum consensus tree could be applied as an alternative criterion to evaluate the goodness-of-fit of various numerical taxonomical techniques, such as different data coding, data transformation, resemblance coefficients, or clustering methods From our phenetic studies, we found that the Bray-Cutis similarity coefficient, and the UPGMA clustering methods perform the best among total of 19 various methods. (3) It seems that the cladistic method is superior to the phenetic method based on the CIc However, the conclusion is not warranted since we only used one single cladistic method—distance Wagner and only used one data set-priacathids in this studies. (4) The maximum consensus method can be applied to solve another common problem of using numerical taxonomical techniques. It is that the contaxon individuals (OTUs) often can be clustered near each other if multiple specimens are used simultaneously to represent one "taxon". Consequently, the final phylogenetic inference based on that taxon level is difficult. The procedures of using the maximum consensus tree method for resolving this problem and its resulting unambiguous tree has been demonstrated in the above section.

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由形態測定方法探討大眼鯛科魚類之親緣關係

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本文係以本省產八種大眼鯛科魚類爲材料,利用形態測定法從事其類緣關係之研究。這八種魚分別是 Pristigenys multifasciatus, Pg. niphonia, Cookeolus boops, Priacanthus tayesus, P. cruentatus, P. macracanthus, P. hamrur, 與 P. blochii,研究共使用二十三尾標本,每種魚由二至七尾不等同時進行數值分類分析。根據同種或同屬之個體是否被聚類在一起之標準下評估不同分析方法之優劣。其結果顯示桁架 (truss) 測定法要比傳統測定方法有效。 在相似性係數中以 Bray-Curtis 係數 ,聚類分析中以 UPGMA 最佳。在分枝分類 (cladistic) 方法中,由 Distance Wagner 及 Fitch-Margoliash 方法所求得之類緣關係與由表型分類 (phenetic) 方法所得者相近似。 綜而言之,大眼鯛的確依三個不同的屬而分成三個獨立的羣 , Pg. multifasciatus 與 Pg. niphonia 爲一羣 , C. boops 爲獨立之一支介於 Pristigeny 和 Priacanthus 兩屬之間。 在 Priacanthus 屬內 , P. tayenus 爲最近緣於 C. boops 者 , P. blochii 與 P. hamrur 應爲一姊妹羣 ,而他們與 P. macracanthus 及 P. cruentatus 間之關係則不明瞭。本研究過程中所使用之方法亦可提供日後利用形態測定來從事魚類之類緣關係研究時之參考。