Asian Fisheries Science **28** (2015): 37-46

ISSN 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2015.28.1.004

Asian Fisheries Science
The Journal of the Asian Fisheries Society

Morphometric and Meristic Divergence of Two Hybrid Catfish: Backcross (F1 hybrid female x *Pangasianodon gigas* Chevey 1931 male) and Reciprocal Backcross (*P. gigas*, female x F1 hybrid male)

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Abstract

The parental F1 hybrids (*Pangaisianodon gigas*, PG Chevey 1931, male x *Pangasianodon hypophthalmus*, PH (Sauvage 1878), female) were used as brood-stock. The two new catfish hybrids, backcross, BC {F1 hybrid, female x PG, male} and reciprocal backcross, RCBC {F1 hybrid (male) x PG (female)} were successfully produced. Both the BC and the RCBC types showed fast growth; however, at the fingerling stage it is very difficult to distinguish between the two hybrids because of their very similar morphology. The aim of this study was to identify the characteristics of BC and RCBC types and to compare the results with two purebred catfish specimens (PG and PH) at a total length of 13-15 cm. using 14 morphometric and 4 meristic measurements, respectively. The parental hybrid catfish were produced from PG (male) x PH (female). The results revealed statistically significant differences ($p< 0.05$); the BC exhibited distinct variations in anal fin height, anal fin length, body depth (BD), body width (BW), and head length (HLSL) from the other fish. The RCBC showed the highest level of distance from snout to isthmus (DSI) and dorsal fin length (DFL). A multifactor analysis (MFA) was used to perform clustering after the principal component analysis (PCA) had been done from both the morphometric and meristic traits and indicated that the backcross was greater in terms of BD and BW parameters when compared with the other groups. The RCBC, however, was intermediate between the two purebreds. The combined morphometrics and meristics data demonstrated that the BC and the PH had the most divergent morphology. The original group cases correctly classified the results for the morphometric and meristic characteristics, and their combined data from all the results were 100%, 66.1% and 100%, respectively. The results indicated that the two catfish species and their hybrids were highly different from one another and all characteristics except the dorsal fin ray count could be utilized for the identification of the two pangasiid catfish species and the two hybrids at the fingerling stage.

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Introduction

Many hybrid catfish have been produced for aquaculture because these hybrids display results with increased growth rates and yields, as well as greater disease resistance than pure line species (Dunham and Masser 1998; Argue et al. 2003). Freshwater catfish in the family Pangasiidae, such as *Pangasianodon hypopthalmus* Sauvage, 1878 (PH), *Pangasius bocourti* Sauvage, 1880 and their hybrids (F1), are considered very important economically and there is a great commercial demand for their culture in Southeast Asia, particularly in Thailand and Vietnam (Hung et al. 2003). At present, we have succeeded in developing the brood stock of the parental F1 hybrids (*Pangaisianodon gigas* Chevey, 1931 (PG) male x PH female and have successfully produced two new fingerling types, backcross, BC (PG male x F1 hybrid female) and reciprocal backcross, RCBC (PG female x F1 hybrid male) hybrid catfish, in 2012 and 2013 (Figs. 1a and 1b), respectively. The fingerlings of the RCBC showed fast growth, which indicated that the hybrids have the potential to be utilized for commercial aquaculture in the future

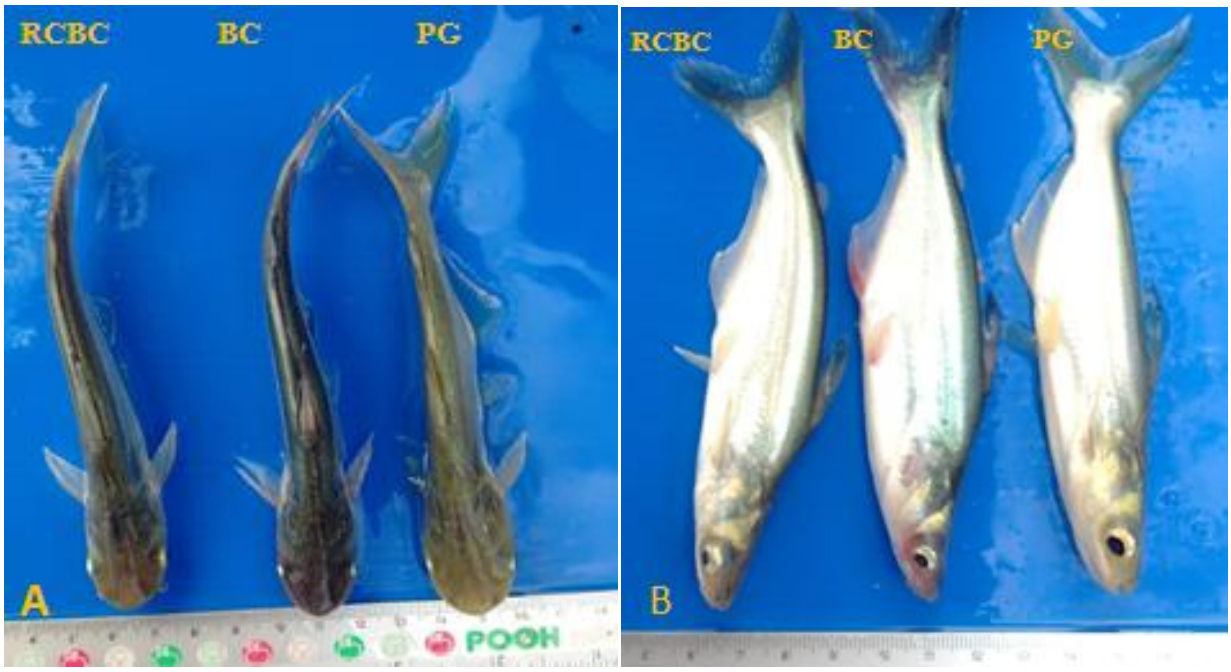

Fig. 1. Fingerlings of *P. gigas* (PG), backcross (BC) and reciprocal backcross (RCBC) a; top view and b; side view.

However, at the fingerling stage (total length of 10-15 cm) of the PG, PH, BC and the RCBC, identification was very difficult because they revealed very similar morphology. Hence, more studies are needed on the morphology as a primary source of information for the purposes of taxonomic and evolutionary studies. The numerous characteristics available for morphological studies are usually divided into two categories, morphometric and meristic. Morphometric measurements have been widely used to identify the differences between fish populations (Buj et al. 2008; Torres et al. 2010; Yakubu and Okunsebor 2011).

The aim of this study was to investigate the morphological characteristics of BC and RCBC to provide a quick identification tool in classifying them.

Materials and Methods

Morphometric and meristic characters

The four types of fingerlings were produced using the single-pair mating method. Body weight of the parents, PG, F1 hybrid catfish (produced from PG male x PH female) and PH were 15 kg, 3 kg and 2.5 kg, respectively, and the age of these parents were 10, 3 and 3 years, respectively. A total of 107 specimens consisting of 24 PG, 29 BC, 31 RCBC and 23 PH specimens were used. The hybrids were obtained from the Fisheries Technology and Aquatic Resource Faculty, Maejo University, Chiang Mai Province, Thailand. Four specimens of fingerling catfish (with an average total length and body weight of 15 cm and 18-22 g, respectively) were examined under the morphological study. The body length was measured using the graduated ruler. Fourteen morphometric measurements were made using the dial calipers according to the method of Pouyaud (1999). Prior to analysis, size effects from the dataset were eliminated as follows; anal fin height (AFH), anal fin length (AFL), dorsal fin length (DFL), body width (BW), body depth (BD), distance of snout to isthmus (DSI), maxillary barbel length (MBSL) and head length (HLSL) which were all adjusted in terms of percent of standard length (% SL); whereas pre dorsal length (PDL), pectoral fin length (PFL), head depth (HD) and head width (HW) were adjusted in terms of percentage of head length (% HL). Eye dimension (EDMB) was adjusted in terms of percentage of maxillary barbel length and percentage of head length (EDHL). Four meristic measurements, pectoral fin rays (PFR), dorsal fin rays (DFR), pelvic fin rays (PeFR) and anal fin rays (AFR) were counted for each fish.

Data analysis

There were no morphological differences between the sexes of all the fingerlings and it was difficult to identify their gender at the fingerling stage. For the morphometric study, size-adjusted data were standardized with data obtained from the morphometric and meristic characters which were subjected to ANOVA analysis. For cluster analysis, the multifactor analysis (MFA) was performed after the data had been analyzed by the principal component analysis (PCA) on morphometric, meristic and a combination of both using the R program v. i386 2.15.2 in the package of FactoMineR. The boxplots of each character of the four fish groups were created by R program. The percentage of correctly assigned fish was determined with an additional measurement of the differentiation among the samples. The number of cases correctly and incorrectly assigned to each group were based on discriminant analysis using SPSS for Window V. 17.

Results

Morphometric and meristic analysis

The results of the 14 morphometric measurements for PG, BC, RCBC and PH are shown in Fig. 2. The PG demonstrated the highest EDMB and the lowest DFL, PFL and MBSL. The BC showed the highest AFH, AFL, BD, BW and HLSL and the lowest PDL. The RCBC showed the highest PDL but other traits had intermediate values. PH showed highest values for many of the traits, DFL, EDSL, HD, HW, PFL and MBSL, but on the other hand, showed the lowest HLSL. A cluster analysis of 14 morphometric traits demonstrated clear morphological divergence between the PG, BC, RCBC and PH specimens. Principal component analysis (PCA) showed that the first two principal components had an Eigen-value of greater than 1 (5.404 and 3.494, respectively) and explained a combined 63.25% score of the total variation of the full data (Table 1). Principal component axis 1 (PC1 or Dim 1) and axis 2 (PC2 or Dim 2) were represented as 35.2% and 28.05% of the variance, respectively. This study, which is based on the morphometric analysis, indicated two main groups: 1) BC and 2) PG, RCBC and PH (Fig. 3a and 3b).

Analysis of variance showed fish group to have significant ($P < 0.05$) effect on meristic measurements except for DFR. The PG showed the highest PFR and PeFR values, while the RCBC had the highest AFR. On the other hand, BC showed intermediate values among all fish, while PH showed the lowest PFR and PeFR values (Table 2). Multifactor analysis of the four meristic characteristics showed the BC and PG to have overlapping characteristics but these two fish groups had clear distinction from the others (Fig. 4). Principal component axis 1 (PC1 or Dim 1) represented 33.89 %, while principal component axis 2 (PC2 or Dim 2) represented 22.84 % of the variance revealing a total of 56.73% variation for the full data.

When morphometric and meristic measurements were combined there was a clear separation of each of the fish groups (Fig. 5). Clustering was done for the two main groups: first) PG and the two hybrids, and second) only PH. Multiple factor analysis (MFA) was performed after principle component analysis (PCA), and it explained a combined 59.72 % score of the total variation of the full data. Principal component axis 1 represented 33.2% of the variance and principal component axis 2 represented 26.52 % of the variance.

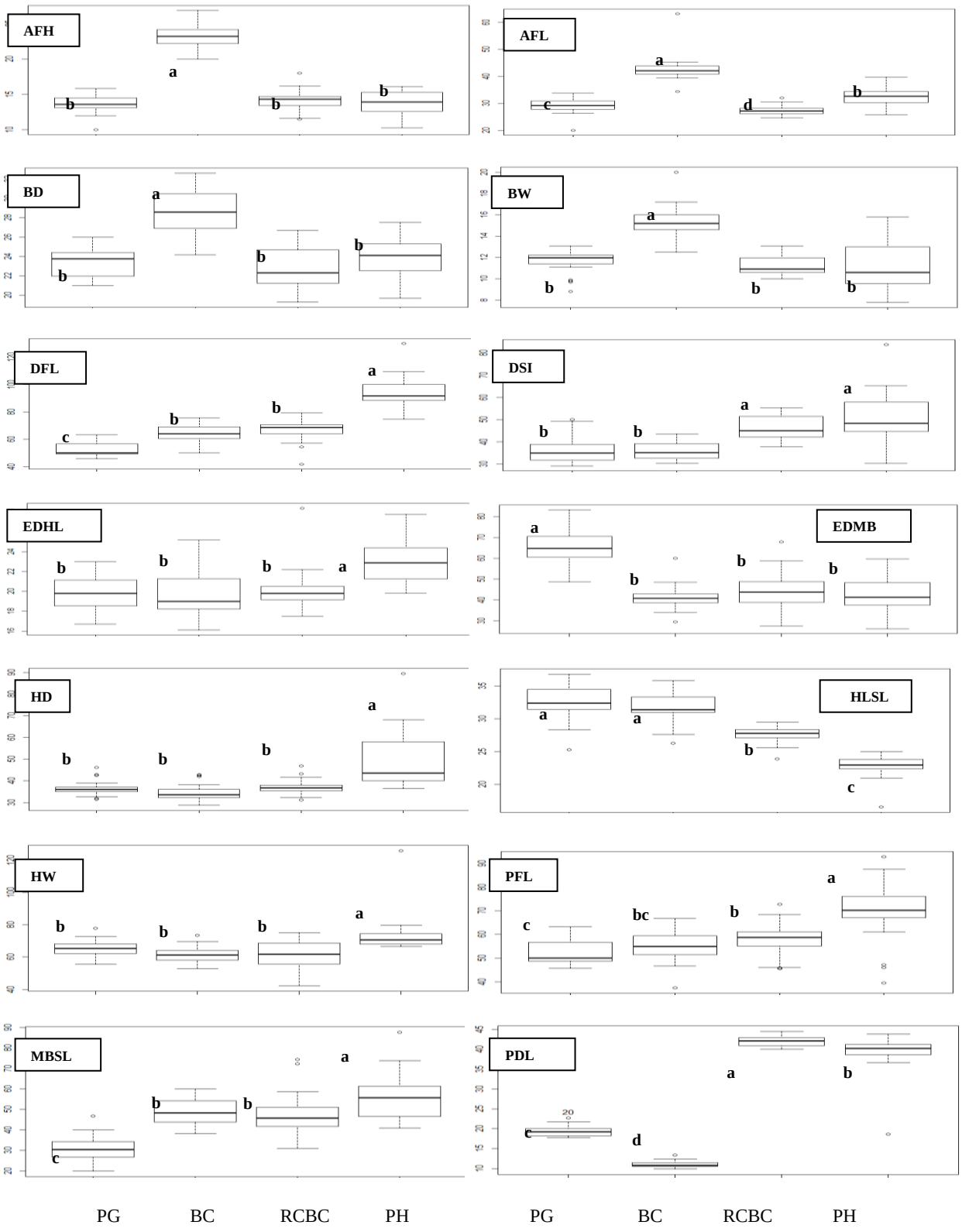

Fig. 2. Boxplot outputs of 14 morphometric measurements from R program for PG, BC, RCBC and PH. Groups assigned different letters show significant differences in their means at $p < 0.05$ (Duncan's new multiple range test).

Table 1 The loading scores of the first two principal components for the 14 morphometric characteristics.

Morphometric Characters	BW	DSI	HD	BD	AFL	HLSL	PFL	HW	DFL	EDHL	AFL	PDL	EDMB	MBSL	**Eigen value**
PC1	0.92	0.89	0.84	0.71	0.71	-0.41	-0.30	0.63	0.52		-0.56	0.55	0.51	-0.51	**5.40**
PC2	-0.23	0.23	0.27	-0.31	-0.53	-0.84	0.70	0.74	0.64	0.62	-0.12	-0.27	-0.28	0.56	**3.49**

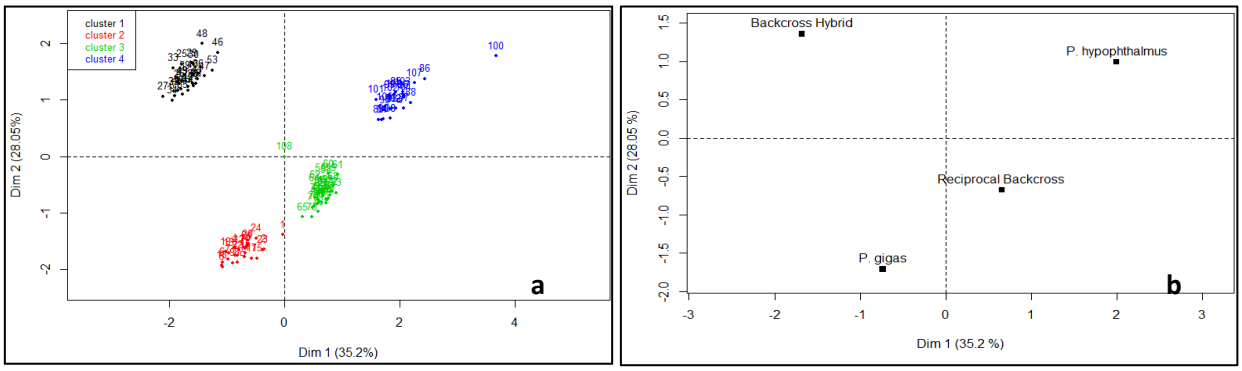

Fig.3. Clustering outputs by multifactor analysis (MFA) of *P. gigas*, BC, RCBC and *P. hypopthalmus* based on morphometric indices; (a) different colours represent the different fish groups, (b) ▪ represents each of the fish groups. Each group was clearly separated from the others.

Table 2. Meristic characteristics of two hybrids and two purebreds

Characters	PG (n=24)	BC (n=29)	RCBC (n=31)	PH (n=23)
	range			
	mean ± SE			
PFR	7-11	7-9	8-9	6-7
	8.9±0.2 ^{a}	8.3±0.1^{b}	8.4± 0.1^{b}	6.4±0.1^{c}
DFR	5-7	6	5-7	5-7
	6.0±0.1	6.0±0.0	6.0±0.5	6.0±0.4
PeFR	7- 9	7-8	8	6-7
	8.2±0.1^{a}	7.8±0.1^{b}	8.0±0.0^{ab}	7.0±0.1^{c}
AFR	27-30	27-30	29-32	27-30
	28.5±0.3^{b}	28.6±0.2^{b}	30.0±0.2^{a}	28.6± 0.2^{b}

- PFR (Pectoral fin rays), DFR (Dorsal fin rays), PeFL (Pelvic fin rays) and AFR (Anal fin rays)

-Values marked with different letters show significant differences in the meristic characteristics among the four groups ($P < 0.05$).

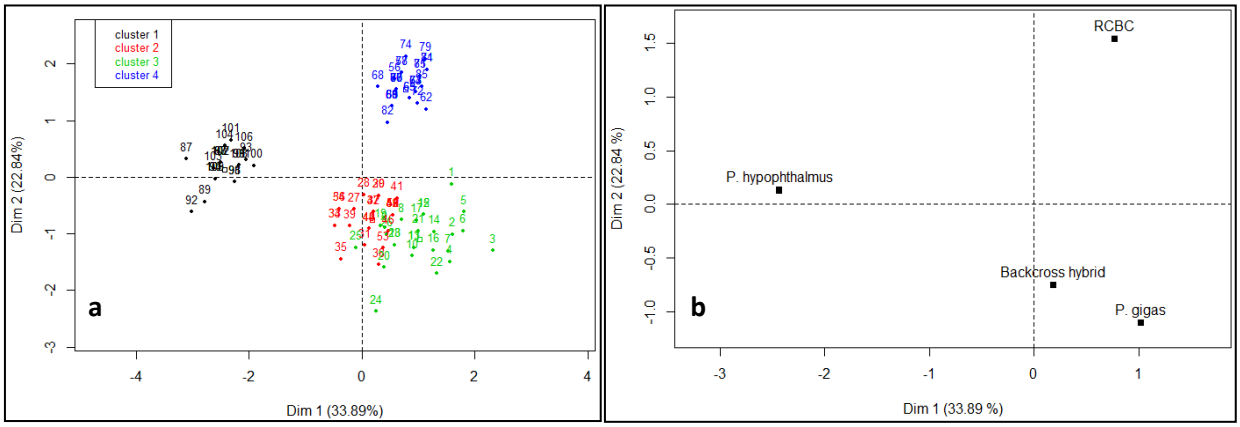

Fig. 4. Clustering outputs by multifactor analysis (MFA) of each group was based on meristic characteristics; (a) different colours represent the different fish groups, (b) ▪ represents each of the fish groups. backcross and PG overlapped, but PH and RCBC clearly diverged from each other.

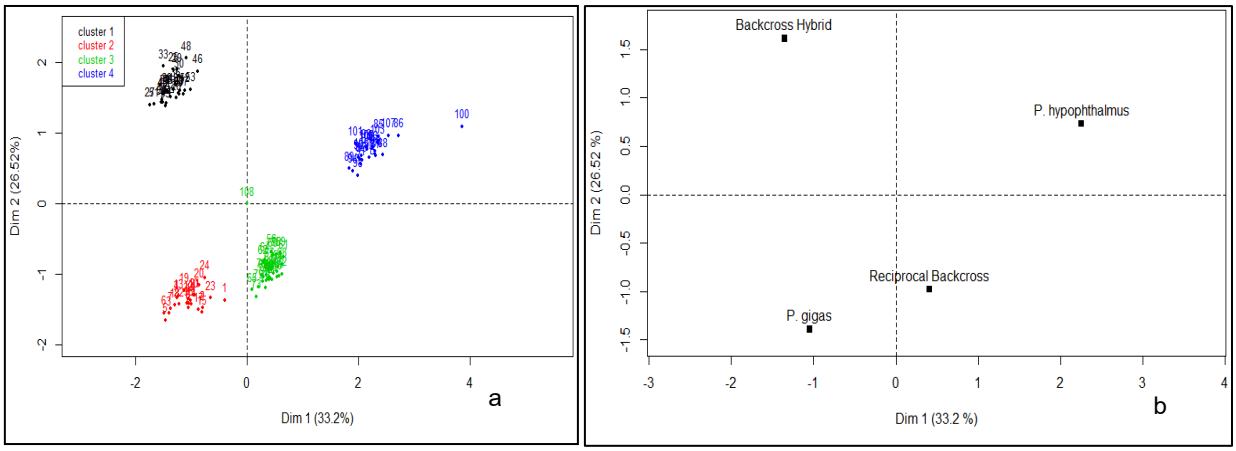

Fig. 5. Clustering outputs when morphometric and meristic characteristics were combined after multifactor analysis; (a) different colours represent the different fish groups, (b) ▪represents each of the fish groups.

The original four fish groups were correctly classified by the morphometric traits; discriminant function analysis gave 100% membership assignment for all groups. Membership assignments based on meristic traits were 54.2% for PG, and 48.3% for the BC, while RCBC and PH had 71.1% and 100% assignment, respectively (Table 3).

Table 3 Classification results of discriminant function analysis using morphometric, meristic and combined data of backcross hybrid, reciprocal backcross and the two purebred fish.

Characters	Discriminant function analysis		Predicted group membership				
			PG	BC	RCBC	PH	total
Morphometric characters	% Original count	PG	100				24
		BC		100			29
		RCBC			100		31
		PH				100	23
Meristic Characters	% Original count	PG	54.2				24
		BC		48.3			29
		RCBC			71.1		31
		PH				100	23
Morphometric and meristic combined data	% Original count	PG	100				24
		BC		100			29
		RCBC			100		31
		PH				100	23

- BC (backcross), RCBC (reciprocal backcross), PG (*Pangasianodon gigas*) and

PH (*Pangasianodon hypophthalmus*)

Discussion

Mophometric characteristics are commonly used to identify fish species (Anene 1999; Akyol and Kinacigil 2001). Turan et al. (2005) used these techniques to identify the morphology of *Clarias gariepinus* (Burchell, 1822) populations from six rivers in Turkey and the results showed that the observed differences were mainly from the head of the fish. All of the fish groups used in this study showed no differences in total length and body weight ($p > 0.05$). The average of the total length and the body weight were 15 cm and 18- 22 g, respectively. We found that only two morphometric traits (MBSL and DFL) could divide the two hybrids from PG and PH and these two hybrids could be separated from each other by seven morphometric characteristics (AFH, AFL, BD, BW, DSI, HLSL and PDL) at the fingerling stage, especially, the backcross hybrid which showed the highest AFH, BD, AFL and BW. According to Kirczuk and Domagala (2011) the hybrid and reciprocal hybrid salmon at age one-year old showed similar measurements to trout (*Salmo trutta* Linnaeus 1758) in terms of head length, minimum body depth and dorsal fin height. Moreover, both hybrids showed similar measurements to salmon at ages of 1 and 2 years in terms of the caudal fin and some characteristics exceeded the parental species while the meristic traits could not easily be used to identify hybrids. Gustiano (2004) published the reciprocal hybrid strains, *Pangasius djambal* Bleeker 1846 x PH, which have intermediate characteristics and the hybrid strain has a tendency to be closer to PH rather than *P. djambal*.

Meristic characteristics such as dorsal fin ray count, pectoral fin ray count and anal fin ray count were used to identify different fish species (Mekkawy and Mohammad 2011). Meristic results of this study clustered the fishes into three groups, 1) PH 2) reciprocal backcross and 3) PG and backcross hybrid. All meristic characteristics except DFR could be used to distinguish fish from each other. The hybrid species of *Salmo alpinus* Linnaeus 1758 x *Salmo fontinalis* Mitchill 1814 revealed a wide range of variations in meristic characteristics (Hammar et al. 1991). We suggest that the morphometric characteristics provide a more powerful method of identifying hybrids than the meristic characteristics because they could be used to more accurately identify the classified scores than through the use of the meristic analysis. Research on other fish has shown that meristic characteristics could not be used to reliably identify the anchovy species, *Engraulis encrasicolus* (Linnaeus 1758) from the north of Spain and the Gulf of Biscay (Junquera and Gaandaras 1993). A combination of morphometric and meristic characteristics has revealed a few differences in the total variations of the full data because the result of the total variations from meristic analysis is lower. The results indicate that the morphometric characteristics could be used for cluster analysis because it showed the highest level of the total variations in the fish groups investigated in our study.

Conclusion

The BC and RCBC appeared similar to the PG and PH species at the fingerling stage. However, analysis of 14 morphometric and 4 meristic characteristics revealed that the tools used in this study were suitable in identifying and differentiating the two hybrids and two purebred species. This was true with the exception of the dorsal fin rays (DFR), which could not be used to distinguish these fish species. Moreover, the morphometric characteristics could better identify the fingerlings of the two catfish species and two hybrids, BC and RCBC than the meristic characteristics. However, to improve this identification process, it is possible to integrate this method of characteristic assessment with the molecular identification system such as AFLP (Amplified Fragment Length Polymorphism) or DNA barcoding.

Acknowledgements

I would like to thank the National Thailand Research Council for financial support and the Plabuk Integration Knowledge Base, Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Thailand for the use of their facilities and for providing fish specimens. I would like to thank Miss. Nantaporn Sutthi, Miss. Sasinath Pinvattanachai, Miss. Supaporn Sattand and Mr. Teerapong Suksri for their assistance in data collection in this research study. I would like to thank Mr. Russell Kirk Hollis, Mr. Damrong Nimmanpisut and Ms. Surangkana Chuanpongpanich for their assistance in language adjustment and revision.

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Received: 03/09/2014; Accepted: 28/03/2015 (MS14-104)