HAYATI Journal of Biosciences September 2013
Vol. 20 No. 3, p 138-143
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.20.3.138

SHORT COMMUNICATION

Morphometric Variations of Rasbora Group (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh Province, Indonesia, Based on Truss Character Analysis

ZAINAL ABIDIN MUCHLISIN

Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia

Received February 18, 2013/Accepted August 28, 2013

The genetic variation of Rasbora group in Lake Laut Tawar has been reported previously, however information on morphometric variations of this Genus was not available. Hence, the objective of the present study was to evaluate the morphometric characters of Rasbora group in Lake Laut Tawar, in order to contribute useful information on the biology of this important fish. The truss morphometric method was utilized in this study. A total of 45 samples of Depik, 42 samples of Eas and 44 samples of Relo were used in this evaluation. Eight homologous landmarks were determined along the outline of the fish, and based on these landmarks, 14 characters or linear measurements were recognized. Discriminant function analysis was employed to distinguish the fish sample. The results showed that the truss morphological characters could highlight the high differentiation between Relo and the other two groups, and the closeness of Eas and Depik. The morphometric data strongly indicated that Eas and Depik should be regarded as a same species, *Rasbora tawarensis*, and Relo may be considered as a cryptic species. This finding is in agreement to the genetic data which was published previously.

Keywords: Depik, Eas, Relo, Truss network and Discriminant Function Analysis

INTRODUCTION

The freshwater fish fauna of Aceh and Lake Laut Tawar has been previously described by Muchlisin and Siti-Azizah (2009); a total of eleven species of freshwater fish were recorded in the Lake Laut Tawar by the authors. However, the number of fishes in Lake Laut Tawar was most likely underestimated, because of limitation of sampling duration and variety of fishing gear. According to the local fishermen of Lake Laut Tawar, there are three taxa of *Rasbora* according to its size, i.e. the small one as Relo, medium one as Depik, and the largest one as Eas. However, clarification has been made by Muchlisin *et al*. (2012), by using further study on genetic variation of Rasbora group in this lake, that proposed Eas and Depik as a same species *Rasbora tawarensi*, while Relo was a different species.

Information on morphometric variation is needed to contribute importance information on the *Rasbora* group, in relation to provide comprehensive understanding on the biology of this genus. Quantitative morphology of fishes can be studied through morphometric and meristic techniques. These are the two main numerical techniques used in the process of scientific description of fishes (Barriga-Sosa *et al*. 2004; Pinheiro *et al*. 2005).

In general, morphometrics can be defined as a technique for describing body form. It is a widely used tool in the study of ichthyological systematics or taxonomy which looks at measurable component (i.e. measuring the length or distance between physical features or landmarks) of fish anatomy such as the size of body parts and fins and its ratio of body length. For about the past 50 years, the morphometrics method have been successfully discriminated numerous fish stocks throughout the world (Dwivedi & Dubey 2013). This technique is very useful for testing and graphically displays the differences in shape when combined with multivariate statistical procedures (Baur & Leuenberger 2011). Observation of fish shape, size, colour, and other general description are also conducted during such procedure.

In recent years the combination of morphometric and genetic data have been commonly used by researchers, for example in sea bass *Decentrarchus labrax* (Erguden & Turan 2005), the threespine stickleback, *Gasterosteus aculeatus* (Hermida *et al*.

Corresponding author. Phone: +62-651-7407659, Fax: +62-651-7554229, E-mail: muchlisinza@yahoo.com

2005), gouramy *Osphronemus gouramy* (Setijaningsih *et al*. 2007), vandace *Coregonus albula* (Kaupinis & Bukelskis 2010), tilapia (Espinosa-Lemus *et al*. 2009), Japanese threadfin bream *Nemipterus japonicus* (Lim 2008) and the endemic freshwater killifish, *Fundulus lima* (Reyes-Valdez *et al*. 2011) to investigate species and population variation. Information on genetic data of Rasbora group in Lake Laut Tawar has been reported by Muchlisin *et al*. (2012), however no morphometric data was available hitherto. Hence, the objectives of the present study was to evaluate the morphometric variations among three presumed *Rasbora* taxa (locally known as Eas, Depik and Relo) based on their truss morphometric character, in order to contribute important information on the *Rasbora* group in Lake Laut Tawar, Aceh Province, Indonesia.

MATERIALS AND METHODS

The landmark-based technique of geometric morphometric or known as Truss Network Morphometric was utilized in this study. The truss network poses no restriction on the directions of variation and localization of shape change, and are much more effective in capturing information about the shape of an organism (Jayasankar *et al*. 2004). This approach is considered a revolutionary tool in morphometrics since it overcomes inherent weakness of traditional characters sets which tend to be aligned to the same horizontal axes (Poulet *et al*. 2004).

Sampling and Morphometric Data Collection. The sampling was conducted in Lake Laut Tawar (04°362 433 N 096°552 253 E) which situated in Central Aceh, Aceh Province, Indonesia, during March 2009. The lake is located approximately 1200 m above sea level. The lake is an old volcanic caldera of circa 16 km in length, 5 km in width with an estimated depth of 80 m and surrounded by mountains reaching over 2000 meters. At least 25 short tributaries discharge into this lake, the main outflow being Peusangan River. The watershed is covered by forests, which are increasingly affected by deforestation, and agricultural activities. The lake is a water source and fishing ground for the fishermen of the Gayonese people (Muchlisin *et al*. 2010).

Fish sample was captured by using gillnets (mesh size 1.0 cm, 2 m depth and 30 m length). The gill nets were set up for eleven hours (18.00 PM to 05.00 AM). Collected fishes were counted and cleaned. The live fishes were anesthetized in MS.2222, then after, all fishes were preserved in 10% formalin in 3 kg sized plastic bags. These bags were tagged by catching location, date and name of fish at the sampling sites. The investigated species were selected while other species were set aside for future studies. The fish samples were then transported to the laboratory for further evaluation.

A total of 45 samples of Depik, 42 samples of Eas and 44 samples of Relo were used in this evaluation. Eight homologous landmarks were determined along the outline of the fish and based on these landmarks, 14 characters or linear measurements were recognized (Figure 1). According to Kocovsky *et al*. (2009) the number of fish samples should be at least 3.5 times the number of landmark positions (as conducted in this study). Measurements were conducted to the nearest 0.01 mm by using a digital caliper. The description of each character is presented in Table 1. The truss network data were transformed using the formula proposed by Palma and Andrade (2002) as follows:

$$M_{trans} = \text{Log } M - \beta (\text{Log TL} - \text{Log TL}_{mean})$$

Where, M: the original measurement, M_{trans}: transformed measurement, TL: total length, â: within group slope regression of the Log M against Log TL, TL_{mean}: overall mean of total length.

Univariate Data Analysis. The data of truss network morphometric were analyzed by one-way Analysis of Variance (ANOVA) to test differences among species for each character, and then followed by a Duncan multiple range tests to investigate sources of variation if data have a significance differences.

Multivariate Data Analysis. Discriminant Function Analysis (DFA) was utilized in this study. The eigenvalues, cumulative percentage, percentage of total variance, and canonical correlation were generated in this analysis. Functions are considered useful for explaining the data if the eigenvalues are higher than 1. A structure matrix was also performed and the largest absolute correlation between each character and any discriminant function utilized to explain the data. The Mahalanobis squared distance was used in a stepwise method to calculate overlap

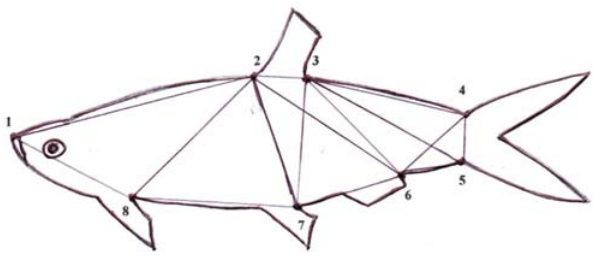

Figure 1. Illustration of left side of specimen showing the truss morphometric characters of Rasbora.

between each group. The group separation was shown in a scatterplot of function 1 versus function 2.

RESULTS

Univariate Analysis. The one-way ANOVA test showed that all truss network characters were significantly different at 95% of confidence level among the three groups. The Duncan's multi range test showed that all characters were significantly different among three presumed taxa ($P < 0.05$) and the Relo characters were significantly lower than Depik and Eas (Table 2). These results indicated that all transformed values were able to discriminate at least one of the group from the others.

Multivariate Aanalysis. The pooled within group correlation matrix of DFA is shown in Table 3. Pairwise correlations were low (below 0.5) except between K2 and I2. Therefore all of the characters were retained for the rest of the analysis. Two functions were generated by DFA both having eigenvalues higher than 1 (Table 4). Function 1 had an eigenvalue of 283.81 explaining 99.60% of the total variance, while Function 2 had an eigenvalue of 1.14 explaining only 0.40% of the total variance accounting for cumulative percentage of 100%.

Function 1 was highly loaded by characters A1, C1, and M2. Function 2 was highly loaded by characters H1, I2, and K2. Character J2 loaded highly in both functions. The characters which contributed to Function 1, were correlated to head and caudal regions as similarly observed in traditional character; while the characters which contributed to Function 2, were strongly correlated to median region of the body, but this accounted for only at small amount of group variation. Figure 2 showed that Function 1 successfully discriminated the individuals into three separate groups, where the Eas group was closely related to the Depik group as in traditional morphometric.

Pairwise Differences Between Groups Based on Mahalanobis F-Statistics. All pairwise Mahanalobis F-statistic analyzed for presumed

Table 1. Description of truss network characters used in the study

Character codes	Landmarks	Description of characters
A1	1-2	Anterior tip of snout to the origin of dorsal fin base
B1	2-3	Origin of dorsal fin to the end of dorsal fin base
C1	3-4	End of dorsal fin base to origin of caudal fin
D1	4-5	Upper to lower of caudal fin origin
E1	5-6	Origin of lower of caudal fin to end of the anal fin base
F1	6-7	Origin of anal fin to origin of pelvic fin
G1	7-8	Origin of pelvic fin to origin of pectoral fin
H1	8-1	Origin of pectoral fin to the end of snout tip
I2	2-7	Origin of dorsal fin to origin of pelvic fin
J2	2-6	Origin of dorsal fin to origin of anal fin
K2	3-7	End of dorsal fin base to origin of pelvic fin
L2	3-6	End of dorsal fin base to origin of anal fin
M2	3-5	End of dorsal fin base to lower caudal fin origin
N2	4-6	Origin of the upper caudal fin to end of the anal fin base

Table 2. The mean transformed values of truss network characters according to the presumed taxa

Characters code	Presumed taxa		
	Eas (sample size 35)	Depik (sample size 50)	Relo (ssmple size 45)
A1	1.67 ± 01^{c}	1.56 ± 01^{b}	1.38 ± 01^{a}
B1	0.94 ± 04^{c}	0.87 ± 04^{b}	0.70 ± 04^{a}
C1	1.52 ± 02^{c}	1.40 ± 03^{b}	1.22 ± 02^{a}
D1	0.96 ± 03^{c}	0.86 ± 02^{b}	0.73 ± 02^{a}
E1	1.25 ± 03^{c}	1.15 ± 04^{b}	0.92 ± 04^{a}
F1	1.40 ± 04^{c}	1.31 ± 03^{b}	1.17 ± 02^{a}
G1	1.40 ± 03^{c}	1.27 ± 03^{b}	1.07 ± 03^{a}
H1	1.36 ± 03^{c}	1.23 ± 04^{b}	1.10 ± 02^{a}
I2	1.27 ± 04^{c}	1.15 ± 03^{b}	1.09 ± 03^{a}
J2	1.43 ± 02^{c}	1.32 ± 02^{b}	1.18 ± 02^{a}
K2	1.30 ± 03^{c}	1.20 ± 03^{b}	1.11 ± 02^{a}
L2	1.28 ± 02^{c}	1.16 ± 03^{b}	1.02 ± 02^{a}
M2	1.53 ± 02^{c}	1.42 ± 02^{b}	1.24 ± 01^{a}
N2	1.34 ± 02^{c}	1.23 ± 03^{b}	1.04 ± 03^{a}

The values in the same row followed by different superscripts are significantly different ($P < 0.05$).

Table 3. The observed pooled within group correlation matrix among 14 characters of Truss Network data

	A1	B1	C1	D1	E1	F1	G1	H1	I2	J2	K2	L2	M2	N2
A1	1.00													
B1	-0.32	1.00												
C1	0.03	-0.15	1.00											
D1	0.06	0.13	-0.02	1.00										
E1	-0.06	-0.05	0.12	-0.14	1.00									
F1	-0.14	0.18	-0.05	0.02	-0.19	1.00								
G1	0.22	-0.08	0.10	-0.01	0.01	-0.10	1.00							
H1	0.42	-0.05	0.04	0.04	-0.20	-0.27	0.09	1.00						
I2	0.04	-0.01	0.12	0.24	-0.01	0.10	0.05	0.25	1.00					
J2	-0.13	0.15	-0.01	0.18	-0.09	0.25	0.03	0.02	0.22	1.00				
K2	-0.14	0.11	0.05	0.20	0.12	0.33	0.00	-0.17	0.54	0.16	1.00			
L2	0.14	-0.22	0.24	0.17	-0.28	0.20	0.00	0.17	0.28	0.43	0.08	1.00		
M2	0.10	-0.25	0.42	0.02	0.35	-0.02	0.08	0.07	0.17	0.20	0.14	0.38	1.00	
N2	0.08	-0.18	0.36	-0.00	0.39	-0.21	-0.01	0.17	0.17	-0.13	0.10	-0.03	0.36	1.00

Table 4. Eigenvalues, percentage of variance and DFA loading of Truss Network characters. High loading characters indicated in bold types

Function	1	2
Eigenvalues	283.81	1.14
% of variance	99.60	0.40
Canonical correlation	0.99	0.73
A1	0.658*	0.125
M2	0.436*	-0.052
C1	0.344*	0.021
J2	0.343*	0.319
L2[(a)]	0.300*	0.296
G1[(a)]	0.208*	0.058
D1[(a)]	0.181*	0.166
N2[(a)]	0.116*	0.114
F1[(a)]	0.113*	0.036
I2	0.122	0.741*
H1	0.181	0.532*
K2	0.171	0.490*
B1	0.162	-0.262*
E1[(a)]	0.082	-0.196*

*Largest absolute correlation between each variable and any discriminate function; [(a)] This variable not used in the analysis.

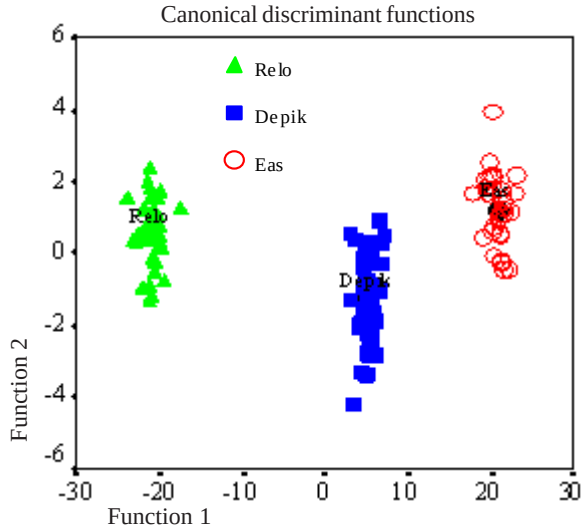

Figure 2. Scatterplot of Function 1 against Function 2 of truss network characters for three presumed *Rasbora* taxa.

Table 5. The pairwise group comparison for 14 characters of Truss Network data based on Mahalanobis F-statistics

Synonym species	Eas	Depik
Depik	2215.5 (0.00)	-
Relo	14503.7 (0.00)	6880.2 (0.00)

The values in parentheses are significant level.

species comparison were significantly different (Table 5). As could be extrapolated from the previous analysis, Eas and Depik were more closely related to each other than they were with Relo.

DISCUSSION

In general, two quantitative morphological analysis able to differentiate the three presumed species of *Rasbora* into separate groups, however, the degree of differentiation varied. Based on univariate analysis, all truss characters were significantly different among Rasbora group, indicating that these characters were completely distinguished the samples into three separate groups where the Relo characters were lower than the other groups. It mean that the mean values between Depik and Eas was closer compared to Relo. In addition, based on the multivariate analysis (DFA), the Depik and Eas showed considerable low degree of overlap, while Relo was more distant. The truss network data was succesful in distinguishing the three groups; this method completely discriminated the three groups, however, the distance between Depik and Eas was closer compared to their individual distances with Relo as observed also in univariate analysis.

According to Dwivedi and Dubey (2012) the truss network is more useful and an effective strategies for the descriptions of shape, have a better data collection and a diversified analytical tools in comparison to that of traditional morphometric method,

in order to discriminate phenotypic stock because the configuration of the constructed landmarks covers the entire fish body with no loss of information and therefore it is more sensitive to change (Lim 2008). This method has been also successfully utilized to differentiate and identify stock of the horse mackerel *Trachurus trachurus* (Murta *et al*. 2008) and the Japanese threadfin bream *Nemipterus japanicus* (Lim 2008).

Within the same species, size can vary with the time of the year and can be greatly affected by environmental factors, i.e. nutritional status and temporal variation of water condition. However, the effect of size could mask delicate and more fascinating biological patterns of variation among suites of variables (Rohlf & Bookstein 1987). Thus, difference in shape is more important factor to distinguish homogenous groups of fishes. Generally, the morphometric approaches revealed that the head and caudal region were the major characters for distinguishing the groups. Probably this is related to differences in food preferences and swimming activities.

Morphometric Versus Genetic Approach in Rasbora. According to Pamilo and Nei (1988), the genetic tree constructed from DNA sequence does not necessarily agree with the species tree constructed from morphological data, and one of the essential factors that cause this difference is genetic polymorphism in the ancestral species. For example, the phenotypic variation between *Carcinus maenas* and *Pachygrapsus marmoratus* was not concordant with mtDNA genetic data (Silva & Paula 2008). A similar observation was also found in many crab species (Reuschel & Schubart 2006; Brian *et al*. 2006).

Based on previous report by Muchlisin *et al*. (2012), Eas and Depik have a very close relation genetically. A similar result was demonstrated from morphometric data in the present study, indicating that the morphometric data is in agreement with genetic data from previous study. A similar observation has been documented by Trabelsi *et al*. (2004) who found high correlation between the phylogenetic tree and biometric data within lagoon sand smelt (*Atherina lagunae*) species.

Evolutionary changes in genetic and morphometric characters have been known to be differentially affected by many factors including environmental conditions. Lim (2008) reported that the morphological characters were changing more rapidly than genetic characters when the author investigated the genetic structure of the Japanese threadfin bream (*Nemipterus japonicus*) along the Peninsular Malaysia coast.

The traditional morphometric method is still considered as a useful tool for fish identification. The method has achieved remarkable success in identification of evolutionary related species and provides a strong foundation and starting point for all current work on fish taxonomy (Stiasny *et al.* 1996). However, morphological appearances are often affected by environmental factors and developmental stages, and thus could lead to misidentification (Teletchea 2009). Therefore, the genetic method is useful to overcome this problem. According to Dawnay *et al.* (2007), when morphology is unsatisfactory in species identification, the genetic data attempts to provide additional important information which cannot be detected by traditional method. Therefore, a holistic approach by a combination of genetic and morphometric methods should be applied for a better understanding of the taxonomic status of any newly investigated taxon.

It was confirmed that Eas and Depik belong to the same species, *R. tawarensis*, and Relo was a distinct species, by a lower extent morphometric data from present study which was supported by the genetic data from previous report of Muchlisin *et al*. (2012).

Rasbora tawarensis is an endemic and threatened fish species in Lake Laut Tawar, Aceh Province. In addition, Lumbantobing (2010) has proposed three others species of Rasbora endemic to Aceh waters, i.e. *R. nodulosa in* Tripa area (Nagan Raya and Aceh Barat Daya Districts), *R. kluetensis* in Kluet area (Aceh Selatan District) and *R. truncata* in Alas area (Aceh Tenggara and Aceh Singkil Districts). However, no information in regard to bio-ecology of others endemic Rasbora in Aceh waters, hence further comprehensive studies are crucially needed to evaluate and record detail information on endemic fishes in Indonesia, particularly in Aceh.

REFERENCES

Barriga-Sosa IDLA, Jimenez-Badillo MDL, Ibanez AL, Arredondo-Figuuero JL. 2004. Variability of tilapias (*Oreochromis* spp.) introduced in Mexico. Morphometric, meristic and genetic characters. *J Appl Ichthyol* 20:7-14. http://dx.doi.org/10.1111/j.1439-0426.2004.00445.x

Baur H, Leuenberger C. 2011. Analysis of ratios in multivariate morphometriy. *Syst Biol* 60:813-825. http://dx.doi.org/10.1093/sysbio/syr061

Brian JV, Fernandes T, Ladle RJ, Todd PA. 2006. Patterns of morphological and genetic variability in UK populations of the shore crab, Carcinus maenas Linnaeus, 1758 (Crustacea: Decapoda: Brachyura). *J Exp Mar Biol Ecol* 329:47-54. http://dx.doi.org/10.1016/j.jembe.2005.08.002

Dawnay N, Ogden R, McEwing R, Carvalho GR, Thorpe RS. 2007. Validation of the carcoding gene COI for use in forensic genetic species identification. *Forensic Sci Int* 173:1-6. http://dx.doi.org/10.1016/j.forsciint.2006.09.013

Dwivedi AK, Dubey VK. 2013. Advancements in morphometric differentiation: a review on stock identification among fish populations. *Rev Fish Bio Fisher* 23:23-39. http://dx.doi.org/10.1007/s11160-012-9279-1

Erguden D, Turan C. 2005. Examiniation of genetic and morphologic structure of sea bass (*Dicentrarchus labrax* L., 1758) population in Turkish coastal waters. *Turk J Vet Anim Sci* 29:727-733.

Espinosa-Lemus V, Arredondo-Figueroa JL, Barriga-Sosa IDLA. 2009. Morphometric and genetic characterization of tilapia (Cichlidae: Tilapiini) stocks for effective fisheries management in two mexican reservoirs. *Hidrobiologica* 19:95-107.

Hermida M, Fernandeza JC, Amaroa R, Miguel ES. 2005 Morphometric and meristic variation in Galician threespine stickleback populations, northwest Spain. *Exp Bio Fish* 73:189-200. http://dx.doi.org/10.1007/s10641-005-2262-0

Jayasankar P, Thomas PC, Paulton MP, Mathew J. 2004. Morphometric and genetic analysis of Indian Mackerel (*Rastrelliger kanagurta*) from Peninsular Indian. *Asian Fish Sci* 17: 201-215.

Kaupinis A, Bukelskis E. 2010. Morphological and genetic variation in vandace (*Coregonus albula*) in the Lake of Lithuania. *Acta Zool Lituan* 20:51-60. http://dx.doi.org/10.2478/v10043-010-0008-5

Kocovsky P, Adams JV, Bronte CR. 2009. The effect of sample size on the stability of principal component analysis of truss-based fish morphometric. *T Am Fish Soc* 138:487-496. http://dx.doi.org/10.1577/T08-091.1

Lim TY. 2008. Biology of the *Betta pucnax* group from Johore. [Thesis]. Universiti Sains Malaysia, Penang.

Lumbantobing DN. 2010. Four new species of the *Rasbora trifasciata*-Group (Teleostei: Cyprinidae) from Northwestern Sumatra, Indonesia. *Copeia* 2010:644-670. http://dx.doi.org/10.1643/CI-09-155

Muchlisin ZA, Fadli N, Siti-Azizah MN. 2012. Genetic variation and taxonomy of Rasbora group (Cyprinidae) from Lake Laut Tawar, Indonesia. *J Ichthyol* 52:284-290. http://dx.doi.org/10.1134/S0032945212030034

Muchlisin ZA, Musman M, Siti Azizah MN. 2010. Length-weight relationships and condition factors of two threatened fishes, *Rasbora tawarensis* and *Poropuntius tawarensis*, endemic to Lake Laut Tawar, AcehProvince, Indonesia. *J Appl Ichthyol* 26:949-953. http://dx.doi.org/10.1111/j.1439-0426.2010.01524.x

Muchlisin ZA, Siti-Azizah MN. 2009. Diversity and istribution of freshwater fishes in Aceh water, Northern-Sumatra, Indonesia. *Int J Zool Res* 5:62-79. http://dx.doi.org/10.3923/ijzr.2009.62.79

Murta AG, Pinto AL, Abaunza P. 2008. Stock identification of horse mackerel (*Trachurus trachurus*) through the analysis of body shape. *Fish Res* 89:152-158. http://dx.doi.org/10.1016/j.fishres.2007.09.026

Palma J, Andrade JP. 2002. Morphological study of *Diplodus sargus*, *Diplodus puntazzo* and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. *Fish Res* 57:1-8. http://dx.doi.org/10.1016/S0165-7836(01)00335-6

Pamilo P, Nei M. 1988. Relationships between gene trees and species trees. *Mol Biol Evol* 5:568-583.

Pinheiro A, Teixeira CM, Rego AL, Marques JF, Cabral HN. 2005. Genetic and morphological variation of Solea lascaris (Risso 1810) along the Portuguese coast. *Fish Res* 73:67-78. http://dx.doi.org/10.1016/j.fishres.2005.01.004

Poulet N, Berrebi P, Crivelli AJ, Lek S, Argillier C. 2004. Genetic and morphometric variations in the pikeperch (Sander lucioperca0 of a fragmented delta. *Arch Hydrobiol* 159:531-554. http://dx.doi.org/10.1127/0003-9136/2004/0159-0531

Reuschel S, Schubart CD. 2006. Phylogeny and geographic differentiation of Atlanto-Mediterranean species of the genus Xantho (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses. *Mar Biol* 148:853-866. http://dx.doi.org/10.1007/s00227-005-0095-1

Reyes-Valdez CA, Ruiz-Campos G, Camarena-Rosales F, Castro-Aguirre JL, Bernardi G. 2011. Population morphometric variation of the endemic freshwater killifish, Fundulus lima (Teleostei: Fundulidae), and its coastal relative F. parvipinnis from the Baja California Peninsula, Mexico. *Rev Fish Biol Fisher* 21:543-558. http://dx.doi.org/10.1007/s11160-010-9175-5

Rohlf JF, Bookstein FL. 1987. A comment of on shearing as a method for "size correction". *Syst Zool* 36:356-367. http://dx.doi.org/10.2307/2413400

Setijaningsih L, Arifin OZ, Gustiano R. 2007. Karakterisasi tiga strain ikan gurame (*Osphronemus gouramy*) berdasarkan metode truss morfometriks. *J Iktiol Indo* 7:23-30.

Silva IC, Paula J. 2008. Is there a better chela to use for geometric morphometric differentiation in brachyuran crabs? A case study using *Pachygrapsus marmoratus* and *Carcinus maenas*. *J Mar Biol Assoc UK* 88:841-853. http://dx.doi.org/10.1017/S0025315408001483

Stiasny MLJ, Parenti LR, Johnson GD. 1996. *Interelationship of fishes*. San Diego: Academic Press.

Teletchea F. 2009. Molecular identification methods of fish species: reassessment and possible applications. *Rev Fish Biol Fisher* 19:265-293. http://dx.doi.org/10.1007/s11160-009-9107-4

Trabelsi M, Maamouri F, Quignard JP, Boussayd M, Faure E. 2004. Morphometric or morpho-anatomal and genetic investigations highlight allopatric speciation in Western Mediterranean lagoons within the *Atherina lagunae* species (Teleostei, Atherinidae). *Est Coast Shelf Sci* 61:713-723. http://dx.doi.org/10.1016/j.ecss.2004.07.011