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Rapid Species Identification of Morphologically Similar Cetacean Species *Kogia sima* and *K. breviceps* by High-resolution Melt Analysis

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Abstract.- Due to their morphological similarities, the differentiation of pygmy (*Kogia breviceps*) and dwarf (*K. sima*) sperm whales is problematic. Here we report a quick and efficient method for distinguishing the two species based on differences in high-resolution melt (HRM) curves of amplicons from the mitochondrial DNA control region. *K. sima* and *K. breviceps* generated distinct melt curve profiles as molecular fingerprints for species typing. The mitochondrial identities of the 38 samples were all correctly typed using HRM analysis. It is estimated that a 30-sample identification work of *Kogia* spp. can be accomplished in 2-3 h using the HRM PCR assay without regular full sequencing process. The assay substantially simplifies the required work of definitive species assignment before stranding data are used in distributional and ecological studies.

Keywords: *Kogia*, HRM, mitochondrial DNA control region, cetacean, species identification.

INTRODUCTION

One of the important challenges facing modern biology is to develop accurate and reliable methods for the rapid identification of species in numerous fields of study, such as taxonomy, epidemiology, forensics, archeology, and ecology. The differentiation of pygmy (*Kogia breviceps*) and dwarf (*K. sima*) sperm whales (nomenclature after Rice, 1998) has been difficult due to the morphological similarities between the two species (Caldwell and Caldwell, 1989; Jefferson *et al.*, 1993; Wursig *et al.*, 2000). *Kogia* spp. are porpoise-like and robust with a distinctive underslung lower jaw. *K. breviceps* lacks teeth in the upper jaw, while *K. sima* may have up to three pairs of vestigial teeth in this position, and *K. breviceps* reach a maximum size of about 3.8 m and a weight of 450 kg, while *K. sima* are smaller at 2.7 m and 272 kg (Jefferson *et al.*, 1993). The height and position of the dorsal fin of the two species are distinguishable (Fig. 1). Although the characters mentioned above could be used to differentiate these two species, they are probably not separable under certain circumstances. Considering the numbers of *Kogia* strandings for which accurate species identification has not been possible because of damage to the carcass, age- and size- related confusion, or inexperienced stranding network personnel, it was recommended that definitive species assignment of *Kogia* whales should be made before stranding data are used in distributional and ecological studies (Chivers *et al.*, 2005).

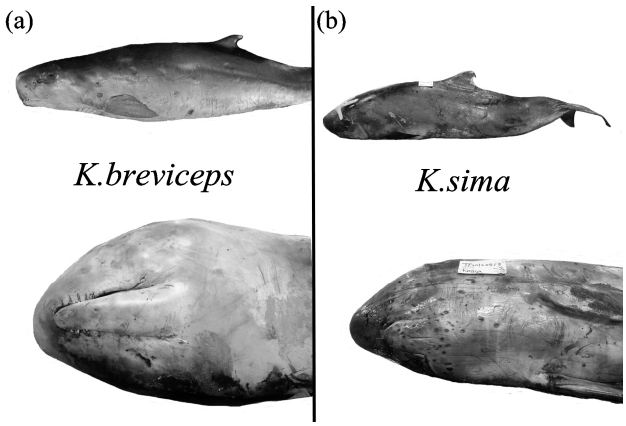

Fig. 1. Lateral view of the full body and ventral view of the head in *Kogia* spp.: (a) pygmy sperm whale (*K. breviceps)*, (b) dwarf sperm whale (*K. sima).*

The molecular methods that have already been used in identification of *Kogia* spp. include electrospray ionization mass spectrometry (ESI-MS) (Duffield *et al.*, 2003) and DNA sequencing (Ross

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et al., 2003; Chivers *et al.,* 2005). ESI-MS is a simple and time-efficient technique and it has been successfully applied to distinguish between *K. breviceps* and *K. sima* by the different molecular weights in myoglobin and hemoglobin alpha-chain. However, the equipment for ESI-MS is not widely available in every institute. Another limitation is that the data of ESI-MS cannot provide further information (*e.g*. DNA sequence) for biological and other research. DNA sequencing is currently the most frequently used approach used in molecular identification of *Kogia* at the species level. Sequencing DNA requires experienced personnel and is relatively costly and time consuming, which can be prohibitive for large numbers of samples. High-resolution melt (HRM), developed by Wittwer *et al*. (2003), has advantages over other genotyping technologies. It has already been used for the identification of PCR product (from 50 bp to 1 kb) of different microorganisms and humans (reviewed in Reed *et al.,* 2007) and arthropods (Malewski *et al.,* 2010). HRM is more cost-effective than sequencing or TaqMan-probe-based real-time PCR, and is a fast, accurate, and simple method used for identifying genetic variation in nucleic acid sequence. HRM analysis starts with PCR product in the presence of a saturating dsDNA binding dye (*e.g.* LCGreen^{R} Plus). The dye has high fluorescence when it binds to dsDNA, and has low fluorescence when it is unbound. When the dsDNA dissociates into single strands with increasing temperature, the dye is released, causing a change in fluorescence. The HRM instrument can collect the fluorescence data and make melting curves. Different DNA sequences will generate distinct melting curve according to their sequence, length or GC content. So the melting curves are used as molecular fingerprints for species typing. The saturation dyes provide consistent saturation of dsDNA to offer reliable and sensitive data during melting curve acquisition (Reed *et al.,* 2007).

Here we report an efficient and reliable method for distinguishing the two species based on differences in the HRM curve of PCR products of mitochondrial DNA (mtDNA) control region. This approach can be used to verify field identification of stranded *Kogia* spp. and it also makes it possible to retroactively identify species assignment for previous strandings where photographs and measurements were not available or assignment was questionable but voucher specimens (skin, muscle or blood) have been collected and archived.

Materials and methods

Sample collection, DNA extraction, and PCR procedure

We examined 38 muscle or skin samples from *Kogia* spp. found stranded along the coasts of Taiwan. They included 9 *K. breviceps* and 21 *K. sima* identified by morphology, and 8 unidentified *Kogia* sp. Genomic DNA was extracted from 20 mg of tissue using QuickExtract™ DNA Extraction solution 1.0 (Epicentre Biotechnologies) following the manufacturer's instructions. Each sample was placed in a tube containing 0.5 mL of QuickExtract™ Solution and heated at 65°C for 15 min to make sure that the solution functioned completely. Then the tube was transferred to 98°C and incubated for 2 min to obtain PCR-ready DNA. We designed a reverse primer (DLP4H-fast2: 5'-AGCGGGWTRYTGRTTTCACGCGGCATG-3') with high Tm that is suitable for the fast (two-step) PCR protocol. And a forward primer (DLP1.5: 5'-TGTAAAACGGCCAGTTCACCCAAAGCTGRA RTTCTA-3') designed in a previous study (Dalebout *et al.,* 1998) was used in conjunction with it (Fig. 2). The primers target sequence within the highly variable mtDNA control region of cetaceans. PCR was performed in a reaction containing 1 µL both forward and reverse primers (10 µM), 25 µL TAQXpedite Universal 2X MasterMix (Epicentre Biotechnologies), 10-100 ng of DNA template, and 18-22 µL sterile water to make a total volume of 50 µL. The PCR was carried out as follows: an initial denaturation step at 98 °C for 50 s followed by 25 cycles of a denaturation step at 95 °C for 10 s and an annealing/extension step at 65°C for 20 s, and a final extension step at 68°C for 3 min. PCR was completed within 30 min resulting in a 420 bp fragment. Species identification of these specimens was confirmed by DNA sequencing. A pair of primers (DLP1.5 sequencing: ACGACGGCC AGTTCACCCAAAGCTG; DLP4H-fast sequencing: AGCGGGTTGCTGGTTTCACGCGGCATG) for sequencing was designed. The sequence analysis

was conducted with the BLAST Search option in GenBank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and DNA surveillance (http://www.dna-surveillance.auckland.ac.nz).

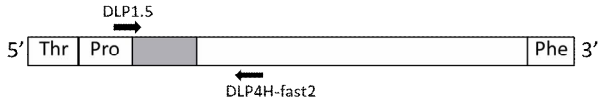

Fig. 2. A schematic map of the mtDNA control region and the primer binding sites. The shaded region is the hypervariable region in cetaceans.

HRM analysis

Immediately after PCR amplification, 1 µL of LCGreen^R plus dye (Idaho Technology) was added into 9 µL of each PCR product to make a final volume of 10 µL. Each sample was heated in a glass capillary on LightScanner^R 32 (Idaho Technology) at 95°C for 1 min and then cooled to 40°C for 2 min. The HRM data were generated by increasing the temperature from 70°C to 95°C at 0.3°C/s and recording fluorescence. The fluorescence data were converted into melt profile charts and analyzed as fluorescence versus temperature graphs using LightScanner^R 32 software provided by the manufacturer. In normalization, temperature ranges on each side of the melting transition were chosen and the data points from a sample were scaled between 0 and 100% fluorescence. The normalized data were then calculated into a difference plot by selecting one *K. sima* sample as the reference curve and subtracting the fluorescence of each sample relative to this reference curve. This step clustered samples automatically into groups that have similar melting curves. HRM analysis for each sample required ~2 min.

Results

The designed primer pairs amplified a clear single band with expected size in electrophoresis. The species of every specimen was confirmed by sequencing and following sequence analysis using BLAST and DNA surveillance. The 9 *K. breviceps* and 21 *K. sima* identified by morphology were all confirmed, and all of the 8 unidentified *Kogia* sp. were determined as *K. sima*. Therefore there were 9 *K. breviceps* and 29 *K. sima* in these 38 tested samples. The raw data collected during the HRM had a range of initial fluorescence readings and it was difficult to properly analyze results (Fig. 3a). The selection of pre- (79.2 °C) and post- (82.8 °C) melt regions was used to align and normalize data correctly to build normalized data. The melting curves of these two species were distinct in normalized data (Fig. 3b). For advanced calculation of the difference plot that is a form of HRM analysis facilitating normalized data visualization, one *K. sima* was selected as a baseline and the position of each sample relative to the baseline was plotted against the temperature (Fig. 3c). The profiles of the two species were clearly distinguishable. The mitochondrial identities of the 38 samples were all correctly typed using HRM analysis.

Discussion

In this study, a HRM PCR diagnostic assay was developed to distinguish between *K. breviceps* and *K. sima* based on nucleotide differences in the mtDNA control region. The HRM PCR assay was 100% accurate, and it is less expensive and more rapid than full sequencing. The new designed primer pair and PCR protocol significantly reduced the total PCR cycling time from approximately 1.5 h to less than 30 min. It is estimated that a 30-sample identification work of *Kogia* spp. can be accomplished in 2-3 h using the HRM PCR assay without gel electrophoresis, PCR clean-up, sequencing reactions, chromatogram editing, sequence alignment, and analyses. Additional advantages of this assay are that very small quantities of sample are needed and degraded material could be used for this analysis. Samples for this assay are easily obtained in the field or from archived tissues. Given the problems encountered with field identification of *Kogia,* species confirmation from strandings and from stored tissue collections can be achieved using this simple and time-efficient technique, as HRM gives unambiguous assignment for these two species. The assay would be a useful diagnostic tool for preliminary screening if large numbers of samples need to be identified. The post-HRM PCR product could be used for subsequent sequencing if necessary. The 420 bp amplicon of highly variable

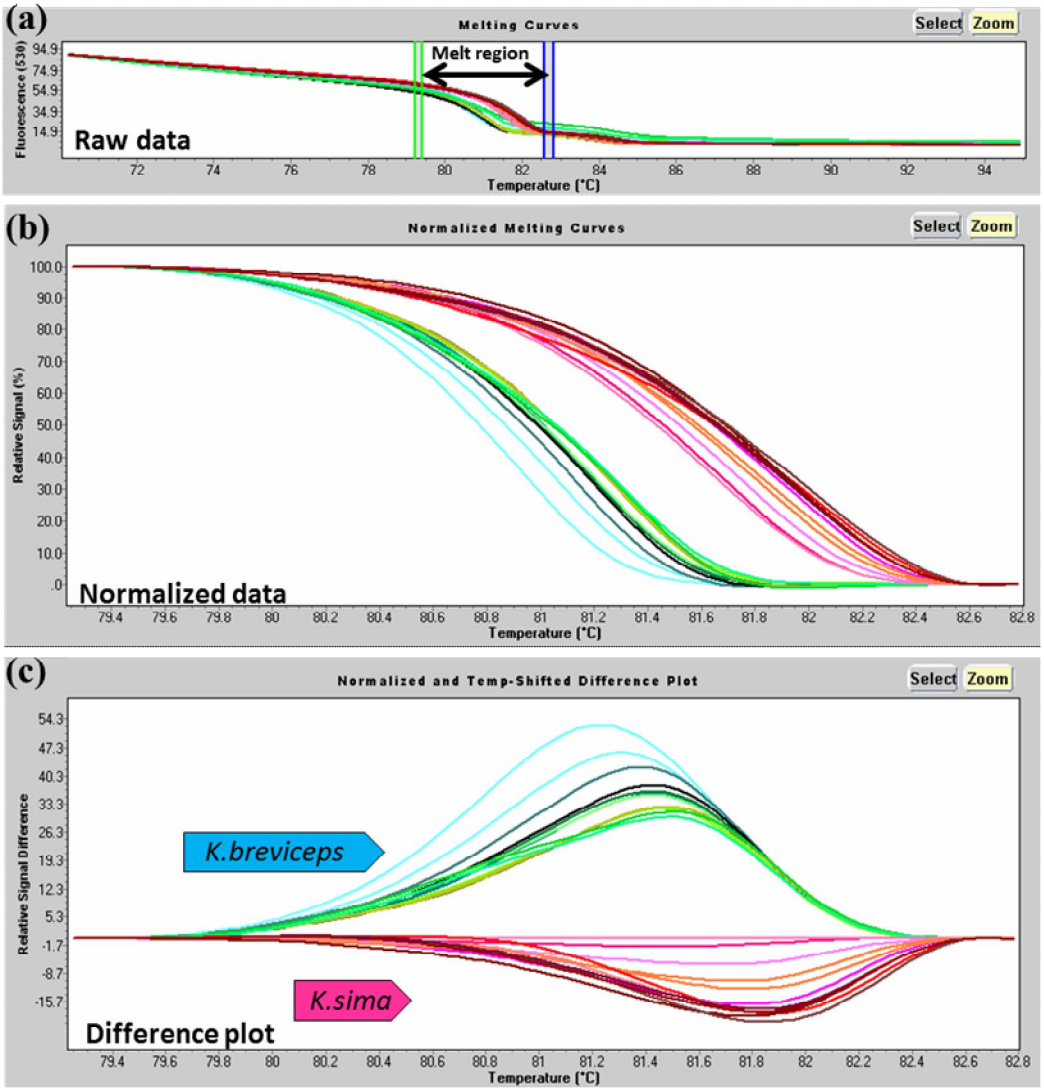

Fig. 3. Analysis of HRM data from the mtDNA control region of *Kogia* spp. Different species were highlighted in different colors. *K. breviceps* = red to orange, *K. sima* = blue to green. (a) Raw data showing the pre-melt (vertical green line), melt and post-melt (vertical blue line) regions. (b) Normalized data derived from the raw data plots showing more detailed view of the selected region. (c) Difference plot derived from the normalized data showing two distinct melting profiles.

mtDNA control region of cetaceans can provide genetic variation information for not only species identification but also population differentiation.

Chivers *et al.* (2005) reported that *K. sima* could be divided into two clades, one in the Atlantic Ocean and one in the Indo-Pacific Ocean, while all *K. breviceps* haplotypes from different ocean basins were in one clade when mtDNA cytochrome *b* gene and control region were used for analysis. Besides, the pairwise genetic distances between *K. breviceps* and the two *K. sima* clades were similar. Even though the *Kogia* specimens used in this study were collected from strandings only along the coasts of Taiwan, we suppose the assay can be successfully used for differentiation of the *Kogia* specimens from the Atlantic Ocean although more research is needed. Maldini *et al.* (2005) suggested that the species composition in the stranding database

reflects the composition of species found in live animal surveys and advocates for the usefulness of stranding data as a source of information when other data are not available. When systematic survey is difficult due to factors such as size, surfacing behavior, or pelagic life history, the stranding data may better represent the occurrence frequency of some species. The distribution of *Kogia* spp. is known primarily from stranding records of animals due to the shyness and difficulty in species identification from a far distance at sea. Before stranding data are used in distributional and ecological studies, the accurate species assignment of *Kogia* was recommended, and the presence of voucher specimens from most strandings and the efficiency of HRM PCR assay make species assignment of these stranding events now feasible. For future study, the assay has potential for the differentiation of other species with similar appearance, such as *Tursiops truncatus* and *T. aduncus*, *Feresa attenuata* and *Peponocephala electra*, and *Delphinus capensis* and *D. delphis.*

Acknowledgements

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References

Caldwell, D.K. and Caldwell, M.C., 1989. In: *Handbook of marine mammals* (eds. S.H. Ridgway and R. Harrison), Academic Press, San Diego, California, pp. 235-260.

Chivers, S.J., Leduc, R.G., Robertson, K.M., Barros, N.B. and Dizon, A.E., 2005. *Mar. Mam. Sci.*, **21:** 619-634.

Dalebout, M.L., Van Helden, A., Van Waerebeek, K. and Baker, C.S., 1998. *Mol. Ecol.*, **7:** 687-694.

Duffield, D.A., Barros, N.B., Espinoza, E.O., Ploen, S., Gulland, F.M.D. and Heyning, J.E., 2003. *Mar. Mam. Sci.*, **19:** 395-399.

Jefferson, T.A., Leatherwood, S. and Webber, M.A., 1993. *FAO species identification guide: Marine mammals of the world*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Maldini, D., Mazzuca, L. and Atkinson, S., 2005. *Pac. Sci.*, **59:** 55-67.

Malewski, T., Draber-Monko, A., Pomorski, J., Los, M. and Bogdanowiez, W., 2010. *Int. J. Legal Med.*, **124:** 277–285.

Reed, G.H., Kent, J.O. and Wittwer, C.T., 2007. *Pharmacogenomics*, **8:** 597-608.

Rice, D.W., 1998. *Marine mammals of the world: systematics and distribution.* Society of Marine Mammalogy, Lawrence, Kansas.

Ross, H.A., Lento, G.M., Dalebout, M.L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A.G., Lavery, S. and Baker, C.S., 2003. *J. Hered.*, **94:** 111-114.

Wittwer, C.T., Reed, G.H., Gundry, C.N., Vandersteen, J.G. and Pryor, R.J., 2003. *Clin. Chem.*, **49:** 853–860.

Wursig, B., Jefferson, T.A. and Schmidly, D.J., 2000. *The marine mammals of the Gulf of Mexico*. Texas A&M University Press, College Station, Texas.

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Adult Density, Abdominal Status and Resting Preference of *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract.- *Culex quinquefasciatus* is responsible for the spread of many dreadful diseases in many parts of the World. In the present study, adult density, abdominal status and resting habit of *C. quinquefasciatus* were monitored from February through June, 2012. For the data collection, living rooms and offices in Sargodha city were selected. Out of 6007 specimens, 2748 (45.74%) were males and 3259 (54.25%) were females. The highest density was observed in the month of April. During the whole study period, the density of females was higher than males. There was no significant difference between the number of male and female captured at each trapping dates. However, densities of adult mosquitoes differ significantly across trapping dates. There was a weak positive correlation between the temperature and density of mosquitoes. However, the density of adult mosquitoes was negatively affected by humidity. The most preferable site for the females in the living rooms and offices was walls. It is concluded

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that as the adult density of *C. quinquefasciatus* was higher during the month of April, so it is suggested that proper steps should be taken before April to minimize the spread of *C. quinquefasciatus* in the study area.

Keywords: *Culex quinquefasciatus*, resting patterns, adult density.

Culex quinquefasciatus Say, 1823 is one of the most commonly occurring and widely dispersed species throughout the World (Weinstein *et al.*, 1997; Hill and Connelly, 2012). This species shows highly endophilic and anthropophilic behaviour (Barbosa and Regis, 2011). It remains in close proximity to breeding sites or to their hosts (Weinstein *et al.*, 1997). It is an important vector of many pathogens including various types of human diseases such as vectors of West Nile virus (WNV), Saint Louis encephalitis in Southern United States (Foster and Walker, 2002; Gosh *et al.*, 2012), Western Equine encephalitis and Japanese encephalitis (Goddard *et al.*, 2002). It is able to transmit filarial nematode *Wuchereria bancrafti* in tropical Africa and South East Asia and Rift Valley fever (RVF) in Africa (Foster and Walker, 2002). In tropical areas, very high population densities of *C. quinquefasciatus* have been established because of the environmental factors that hold up a large number of breeding sites and rapid biological growth. Factors such as climate, seasonality, presence of micro-habitats for breeding and factors related to anthropogenic behaviour are known to be responsible for causing changes in the abundance, behavior and population dynamics of *C. quinquefasciatus* (Blank, 1992; Igbinosa, 1989; Impoinvil *et al.*, 2008; Kim *et al.*, 2010; Muturi *et al.*, 2008; Stoops *et al.*, 2007). Temperature, relative humidity, seasonality and water quality are considered as predicators of mosquito species distribution (Impoinvil *et al.*, 2008; Pemola and Jouhari, 2005).

Present study was aimed at recording the temporal variation in the density, the feeding and resting preferences of adult *C. quinquefasciatus*. This study will be helpful in efficient control of this potentially dangerous vector in the study area.

Materials and methods

The data was recorded from the living rooms and offices of Sargodha city from February through June, 2012. A total of 20 sampling sites (10 living rooms and 10 offices) were selected for the sampling. Mosquitoes were collected weekly using aspirator tube. Thirty minutes were spent at each catching site to collect the mosquitoes resting on different surfaces (*i.e.*, walls, objects and doors). Collected mosquitoes were brought to the laboratory in plastic bottles (3.5 cm high and 1.5 cm wide) and identified up to species level using standard identification keys (Barraud, 1934; Christopher, 1933). The density of adult males and females, number of fed, unfed, gravid and semi gravid females during each trapping date was recorded. The resting preferences of both sexes were also observed. Parametric tests were used on normally distributed data. The number of males and females collected during different trapping dates were compared using t-test. However, Kruskal-Walis test was used to compare the number of male/female mosquitoes within trapping dates. Analysis of variance (ANOVA) was used to compare the number of fed, unfed, gravid and semi-gravid mosquitoes at different trapping dates. ANOVA was also used to compare the number of male/female mosquitoes captured from walls, doors or objects. All statistical analysis was performed using SPSS Version, 16.

Results and discussion

In total, 6007 specimens of *C. quinquefasciatus* were collected. Of the total catch, 2748 (45.74%) were males and 3259 (54.25%) were females. The highest adult density of both sexes was recorded during the month of April (Table I). Mosquitoes were found to be very sensitive to temperature changes. It has been reported that adult female mosquitoes digest blood faster and feed more frequently in warmer climates (Gillies, 1953). However, the temperature above 34°C generally has a negative impact on the survival to vector and parasites (Rueda *et al.*, 1990). In April the average temperature was 33°C so the number of mosquitoes was highest. Furthermore, in April there was little rain fall in Sargodha (13 mm). During the dry

season little rainfall may create new habitats or breeding sites for the mosquitoes and can play a role to enhance densities of mosquitoes. However, heavy rainfall may eliminates the breeding places of mosquitoes, through flooding, thus this may decrease the densities of mosquitoes (Kelly-Hope *et al.*, 2004). The lower densities of mosquitoes in May and June (Table I) may be due to high temperature during these months in the study area. The density of females was higher than males throughout the study period. It was collected from the offices and living rooms where activity of human was high. Females suck blood of human to fulfill their dietary requirement. The results of the study coincide with the findings of Kaliwal *et al.* (2010), who also reported higher density of females as compared to the male mosquitoes.

Table I.- Density of adult male and female *C. quinquefasciatus*.

Month	Week	Males	Females
February	1st	12	12
	2nd	19	19
	3rd	35	27
	4th	57	34
March	1st	59	39
	2nd	69	58
	3rd	79	112
	4th	104	198
April	1st	519	602
	2nd	424	491
	3rd	350	412
	4th	409	460
May	1st	212	235
	2nd	145	213
	3rd	96	158
	4th	62	70
June	1st	41	56
	2nd	29	32
	3rd	14	19
	4th	13	12
Total		**2748**	**3259**

When the density of males and females (captured at each trapping date) were compared, non-significant difference was observed (T-value = -0.47; P = 0.643). However, there was significant difference in the density of males/females collected at different trapping dates (Kruskal-Wallis test = 16.70; df = 4; P = 0.002 for males and Kruskal-Wallis test = 16.47; df = 4; P = 0.002 for females).

Out of the total females, the number of fed, unfed, semi-gravid and gravid was 1333 (40.90%), 1215 (37.28%), 536 (16.44%) and 193(5.92%) respectively. Results of ANOVA showed that there was no difference in the number of fed, unfed, gravid and semi-gravid within trapping dates (df = 3, 12; F = 0.21; P = 0.0886). However, significant difference was found in the number of fed, unfed, gravid and semi-gravid across the trapping dates (df = 4, 12; F = 18.80; P = 0.001).

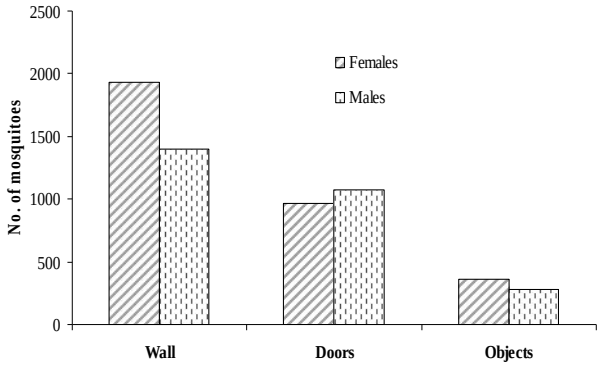

Fig. 1. Resting preferences of male and female *C. quinquefasciatus.*

The most preferable resting site for both sexes was walls followed door and objects (Fig. 1). This result is contrary to the findings of Kaliwal *et al.* (2010) who reported in their study that *C. quinquefasciatus*, especially females, prefer hanging objects. There was a weak positive correlation between temperature and the density of mosquitoes (Pearson correlation = 0.196; P = 0.752). However, negative correlation was observed between humidity and abundance of mosquitoes (Pearson correlation = -0.556; P = 0.33). Humidity affect the mating behaviour, oviposition and seeking of host (Wu *et al.*, 2007). According to Chaves and Kitron (2010) moisture play an important role in oviposition and mosquitoes may move and find oviposition sites easily. But the fact become negatively correlated due to the other environmental factors such as

excessive rain fall as it may washed out the mosquitoes's eggs and oviposition sites.

References

Barbosa, R.M.R. and Regis, L.N., 2011. *Mem. Inst. Oswaldo Cruz, Rio de Janerio*, **106**: 451-455.

Barraud, P.J., 1934. *Fauna British India. Diptera, vol. V. Culicidae. Tribes Megarhinini and Culicini.* Taylor and Francis, London, pp. 463.

Blank, J.L., 1992. In: *Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction* (eds. T.E. Tomasi and T. Horton). Comstock Publishing Associates, Ithaca, New York, pp. 186-212.

Chaves, L.F. and Kitron, U.D., 2010. *Bull. entomol., Res.*,**10**:1-9

Christopher, S.R., 1933. *The fauna of British India including Ceylon and Burma (Diptera: Culicidae).* Taylor and Francis, London, pp. 564.

Foster, W.A. and Walker, E.D., 2002. In: *Medical and veterinary entomology* (eds. G. Mullen and L. Durden). Academic Press. New York, NK, pp. 245-249.

Gillies, M.T., 1953. *East Afr. med. J.*, **30**:129-135.

Goddard, L.B., Roth, A.E., Reisen, W.K. and Scott, T. W., 2002. *Emerg. Infect. Dis.*, **8**: 1385-1391.

Ghosh, A., Chowdhury, N. and Chandra, G., 2012. *Indian J. med. Res.*, **135**: 581-598

Hill, S. and Connelly, C.R., 2012. *Southern house mosquito* Culex quinquefasciatus *Say*. University of Florida Press. pp. 1-5. www.edis.ifas.ufl.edu/pdf

Igbinosa, I.B., 1989. *J. appl. Ent.*, **107**: 325 -330.

Impoinvil, D. E., Keating, J., Mbogo, C.M., Potts, M. D., Chowdhury, R.R. and Beier, J.C., 2008. *J. Vector Ecol.*, **33**: 107-116.

Kaliwal, B.M., Kumar, A., Shanbhag, A.B., Dash, A.P. and Javali, S.B., 2010. *Indian J. med. Res.*, **131**: 711-719.

Kelly-Hope, L.A., Purdie, D.M. and Kay, B.H., 2004. *J. med. Ent.*, **41**: 133-150.

Kim, H.C., Chong, S.T., Nunn, P.V. and Klein, T.A., 2010. *Entomol. Res.*, **40**: 136-144.

Muturi, E.J., Mwangangi, J., Shililu, J., Jacob, B. G., Charles, M., Githure. J. and Novak, R.J., 2008. *J. Vector Ecol.*, **33**: 56-63.

Pemola, D.N. and Jauhari, R.K., 2005. *J. appl. Biosci.*, **31**: 105-113.

Rueda, L.M., Patel, K.J., Axtell, R.C. and Stinner, R.E., 1990. *J. med. Ent.*, **27**: 892-898.

Stoops, C.A., Shinta, Y.R.G., Sismadi, P., Elyazar, I. R.F., Bangs, M.J. and Sukowati, S., 2007. *J. med. Ent.*, **44**: 543-553.

Weinstein, P., Laird, M. and Brown, G., 1997. *Exotic and endemic mosquitoes in New Zealand as potential arbovirus vectors.* Occas. paper, Ministry Health, Wellington, New Zealand, pp. 16.

Wu, Y.Y., Sun, C. P., Gao, J.G., Zhang, J., Huang, D. F. and Song, F.P., 2007. *J. agric. Sci. Technol.*, **9**: 98-103.

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Biochemical Divergence of Glucose, Urea, Uric Acid, Lactate Dehydrogenase, Total Proteins and Alpha Amylase in Six Varieties of Local Silkworm

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Abstract.- Main objective of this study was to estimate some biochemical parameters (glucose, urea, uric acid, lactate dehydrogenase, total protein and alpha amylase) of indigenous silkworm groups. Local varieties were reared under standard protocols. From among local varieties, the highest level of urea was found in White Haratee (6.52 mg/dl), whereas Lemon Khorasan variety showed low level (4.01 mg/dl) ($P<0.05$). The highest level of lactate dehydrogenase (LDH) was recorded in Lemon Haratee and White Haratee (19.56 IU/L), and Lemon Khorasan showed low level (0.00 IU/L) compared with other varieties ($P<0.05$). On the basis of obtained dendrograms, the silk worm varieties were divided into three distinct groups.

Keywords: Silkworm, biochemical parameter, cluster analysis, Iranian Silkworm.

Nowadays, there are few indigenous silkworm groups in some countries that are basis for various breeding programs. These varieties, adapted to local conditions, must meet criteria for commercial production.

Accurate identification and characterization of indigenous groups is very important for sericulture improvement. Measurement and comparison of biochemical parameters of silkworm's haemolymph in local groups led to identification of these groups. If these local silkworm groups perform better than the current lines, then these can be distributed to farmers (Seidavi, 2010).

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Previous studies have shown that the biochemical parameters of silkworm heamolymph have an important role in its performance (ESCAP, 1993). Main objectives of this study were to estimate some biochemical parameters (glucose, urea, uric acid, lactate dehydrogenase (LDH), total protein and α-amylase) of indigenous silkworm groups, and compare these with indigenous Iranian silkworms based on biochemical characteristics.

Materials and methods

This study was conducted on six local silkworm varieties (1) Lemon Khorasan, (2) Lemon Haratee, (3) White Haratee, (4) Yellow Haratee, (5) Pink Khorasan and (6) Baghdadi in Iran Silkworm Research Center. This region has a mean temperature of 17ºC, annual rainfall of 1450 mm, latitude and longitude 49º 36´ and 34º 16´ and -6.9 altitudes from sea level.

The most favorite conditions found for moth emergence was 25ºC and 75% relative humidity in hatching room for 12 days. Disease-free eggs of the local silkworm varieties were reared according to standard protocols. Silkworm eggs were exposed to 18 h light- 6 h darkness for 6 days. After change of egg color, the eggs were placed on complete darkness for three days. In the morning of 4th day, light was supplied, and the eggs hatched. Standard procedure of ESCAP (1993) was followed for rearing of silkworm. Young silkworm were fed on chopped leaves and paraffin-covered paper and the adults were fed on leaves and branches.

The haemolymph was collected from the 5th instar by cutting abdominal proleg in 1.5 ml tube containing a few granules of phenylthiourea to prevent melanization. After 10 min centrifugation at 10000 rpm, the supernatant was preserved at -20ºC until analysed, whereas the pellets were discarded. The supernatant was used for estimation of the various biochemical components *viz.*, glucose, urea, uric acid, LDH, total protein and α-amylase using commercial kits (Thomas, 1998; Seidavi, 2011).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) with Tukey's studentized range (HSD) test in a complete randomized design at α=0.05 to determine if the differences found between the treatments were significant.

Results and discussion

Obtained results are summarized in Table I.

Glucose

The amount of glucose in six local varieties varied between 2.00 and 8.52 mg/dl. The highest level of glucose was in White Haratee and Yellow Haratee (8.52 mg/dl), and the lowest was in Lemon Haratee variety (2.00 mg/dl) The statistical differences between varieties for this trait were significant ($P<0.05$).

Urea

The amount of urea in six local varieties ranged between 4.01-6.52 mg/dl. The highest level of urea was White Haratee (6.52 mg/dl), and Lemon Khorasan variety (4.01 mg/dl) remained at lower level than other varieties. The statistical differences between varieties for this trait were significant ($P<0.05$).

Uric acid

From obtained results, it is showed that amount of uric acid in six studied local varieties included between 0.45-2.35 mg/dl. Among studied local varieties, the highest level of uric acid belonged to Yellow Haratee (2.35 mg/dl), and Lemon Khorasan and Baghdadi variety (0.45 mg/dl) remained at lower level than other varieties. Other varieties were between these two groups. Meanwhile statistical differences between studied varieties for this trait were significant ($P<0.05$).

LDH

The LDH concentration in six local varieties ranged between 0.00-19.56 IU/L. The highest level was record in Lemon Haratee and White Haratee (19.56 IU/L). The statistical differences between studied varieties for this trait were significant ($P<0.05$).

Total protein

The total protein in six local varieties ranged between 4.76-23.17 g/dl. The highest level of total protein was in Lemon Khorasan (23.17 g/dl), and the lowest in White Haratee variety (4.76 g/dl) The statistical differences between studied varieties for this trait were significant ($P<0.05$).

Table I.- Biochemical components (Mean±SEM) in heamolymph of six studied local silkworm varieties*.

Parameter	Unit	Variety					
		Lemon Khorasan	Lemon Haratee	White Haratee	Yellow Haratee	Pink Khorasan	Baghdadi
Glucose	[mg/dl]	8.02±0.02^{a}	2.00±0.00^{c}	8.52±0.29^{a}	8.52±0.29^{a}	5.51±0.28^{b}	2.50±0.28^{c}
Urea	[mg/dl]	4.01±0.01^{c}	3.01±0.01^{d}	6.02±0.57^{a}	5.01±0.01^{b}	6.52±0.28^{a}	4.51±0.28^{bc}
Uric acid	[mg/dl]	0.45±0.02^{c}	0.50±0.00^{c}	1.45±0.54^{b}	2.35±0.02^{a}	0.55±0.02^{c}	0.45±0.02^{c}
LDH	[IU/L]	0.00±0.00^{c}	19.56±0.29^{a}	19.56±6.06^{a}	10.53±0.29^{b}	2.00±0.00^{c}	19.06±0.58^{a}
Total protein	[g/dl]	23.17±10.91^{a}	6.07±0.03^{b}	4.76±0.03^{b}	5.81±0.01^{b}	5.86±0.03^{b}	5.76±0.03^{b}
α-amylase	[IU/L]	23.07±0.58^{b}	30.60±3.17^{a}	21.07±3.46^{b}	5.51±0.28^{c}	9.03±0.57^{c}	10.03±0.03^{c}

*Means in each row followed by the same letters are not significantly different at α=0.05.

α- amylase

α-amylase in six local varieties ranged between 5.51-30.60 IU/L. The highest level of Alpha α-amylase was a Lemon Haratee (30.60 IU/L), while lowest in Yellow Haratee variety (5.51 IU/L) The statistical differences between studied varieties for this trait were significant (P<0.05).

As Mohammadis and Prasanna (2003) stated cluster analysis refers to "a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster". The results presented here establish its usefulness in realizing a better projection of the genetical differences between silkworm strains with different yield potentials. Researchers emphasized that the high genetic variation might not give always a high genetic diversity in the inbreeding population of same species.

Figure 1 shows classification of six varieties of silkworm based on glucose, urea, uric acid, LDH, total protein, and α-amylase parameters into three distinct groups. At cross 4.24, two clusters were formed which were divided into subgroups at cross of 3.20. Frequent divisions were also observed in major groups. First group included Lemon Khorasan variety. Second group included other varieties.

There are many reports about silkworm genetics, nutrition, and biochemistry (Seidavi, 2011), however there is not efficient reports about silkworm biochemistry characteristics in local varieties at Iran till now.

As indicated earlier, our final analysis and conclusion has been based on the average linkage between groups or UPGMA. Chatterjee and Data (1992) have shown that UPGMA yields more accurate results for classification purposes than other hierarchical methods. Thus, the present paper also presents the result of other clustering approaches (Salehi-Nezhad *et al.*, 2010).

These biochemical components are important in identification and performance of silkworm, since these components have positive correlations with silkworm performance.

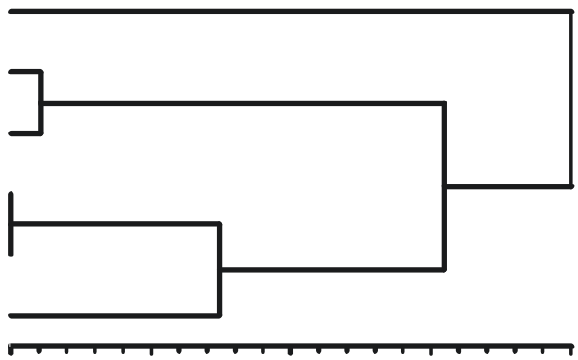

Fig. 1. Cluster analysis of 6 local studied silkworm varieties based on all studied heamolymph biochemical parameters. 1, Khorasan Lemon; 2, Lemon Haratee; 3, White Haratee; 4, Yellow Haratee; 5, Pink Khorasan; 6, Baghdadi

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References

Chatterjee, S.N. and Datta, R.K., 1992. *Genetics laboratory*, Central Sericultural Research and Training Institute, Mysore-570008, Karnataka, India.

Cruz, C.D. and Regazzi, A.J., 2001. Modelos biométricos aplicados ao melhoramento genético. 2nd ed, Imprensa Universitária, 390p.

ESCAP, 1993. *Principle and techniques of silkworm breeding*. United Nations, New York.

Mohammadis, A. and Prasanna, B.M., 2003. *Crop Sci*., **43**, 1235-1248.

Rohlf, F.J., 1998. *NTSYSpc: numerical taxonomy and multivariate system*, version 2.02e. Exeter Software, New York.

Salehi-Nezhad, M., Mirhosseini, S.Z., Gharahveysi, S., Mavvajpour, M. and Seidavi, A.R., 2010. *Biotechnology*. **9**: 149-156.

Seidavi, A.R., 2011. *Afr J. Pharm. Pharmacol*., **5**: 2435-2443.

Sneath Phasokal, R.R., 1973. *Numerical taxonomy*. Freeman, San Francisco, CA.

Thomas, L., 1998. *Clinical laboratory diagnostics*. 1st ed. Frankfurt TH-Books verlagsgesellschaft.

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Fish Biodiversity of River Swat

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Abstract.- The fish fauna of river Swat has been briefly discussed. Fifty (50) species of freshwater fishes have been recorded from the river Swat from its origin in Kalam up to the confluence of river Kabul and Swat at Charsadda. All the species are high Asian and South Asian but no West Asian species has been recorded. Present study adds six (6) new records from river Swat namely *Barilius modestus*, *Barilius vagra*, *Gagata pakistanica, Clupisoma garua, Eutropiichthys vacha*, and *Xenentodon cancilla.* This paper includes names of all the fishes recorded from river Swat and its tributaries up to May, 2011.

Key Words: Fish Biodiversity, River Swat, Bajur, new records.

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The river Swat commences at Kalam with the confluence of Ushu and Utror Rivers and flow for about 160 km across the valley up to Chakdara. The total length of the river is 250 km from Kalam to its confluence with Kabul River near Charsadda. Many large and small tributaries join the river along its course as mentioned in the project report of Environmental Protection Society (EPS) Swat.

The fishes were collected from the river Swat at different localities and from the feeding streams of river swat present in Bajur Agency namely Salarzai stream, Nawagai stream and Mamund stream. Study is conducted from 2004 to 2010 with intervals. Although collections from these areas were done every year but special efforts were made in 2004, 2009 and 2010.

The fish fauna of this river has been described by Hussain and Shah (1960), Ahmad and Mirza (1963a,b), Ahmad (1965), Mirza (1973a), Rafique and Javed (2002), and Mirza (2007). The present paper is mainly based on authors' collection and papers published by Rafique and Javed (2002) and Mirza (2007).

Methods and materials

The fishes were collected from the river Swat at different localities and from the feeding streams of river swat present in Bajur Agency namely Salarzai stream, Nawagai stream and Mamund stream from 2004 to 2010. They were fixed in 10% formalin and then shifted into 70% alcohol. Fishes were identified by using standard taxonomic keys viz. Fishes of the Punjab, Pakistan (Mirza and Sandhu, 2007), Fishes of the world (Nelson, 2006), The freshwater fishes of the Indian region (Jayaram, 1999), Inland Fishes of India and Adjacent Countries (Talwar and Jhingran, 1991) and Pakistan ki Taza Pani ke Machliyan (Mirza, 1990).

SYSTEMATIC ACCOUNT

Order Salmoniformes
Family Salmonidae

1. *Onchorhynchus mykiss* (Walbaum)
This species is restricted to the upper part of river Swat above Madayan. It is represented by two specimens caught from Bahrain.

2. *Salmo trutta fario* Linnaeus
This species has been reported by Mirza (2007). It is also confined to upper part of the river.

Order Cypriniformes
Famil Cyprinidae

Subfamily Cultrinae

3. *Chela cachius* (Hamilton)
This species is not present in the present collection although reported by Mirza (2007).

4. *Salmophasia bacaila*(Hamilton)
Two specimens of this fish were collected in 2008 and one specimen in 2010 from lower swat.

5. *Salmophasia punjabensis (Day)*
This species was also reported by Mirza (2007) from lower part of river Swat**.** It has also been collected by us in large numbers from streams of Bajur Agency in 2004. Previously this fish was identified as *Salmostoma punjabensis.*

Subfamily Aspidoparinae

6. *Aspidoparia morar* (Hamilton)
This specie is not present in our collection. It was reported by Mirza (2007) from lower part of river Swat.

Subfamily Rasborinae

7. *Amblypharyngodon mola* (Hamilton)
This specie is also reported by Mirza (2007) from lower part of river Swat.

8. *Barilius pakistanicus* Mirza and Sadiq
This species is widely distributed in the upper and lower part of the river including Bajur agency. In our collection it is represented by large number of specimens.

9. *Barilius modestus* (Day)
This fish has been reported for the first time from river Swat. Many specimens of this fish were collected in 2010 from streams of Bajur Agency which means it is an inhabitant of upper swat.

10. *Barilius vagra* (Hamilton)
The species was collected from the streams of Bajur Agency in 2004. In present survey we could not find any specimen neither from Swat nor Bajur Agency. We are reporting it for the first time from upper part of river Swat.

11. *Devario devario* (Hamilton)
This specimen is not present in our collection and has been reported by Mirza (2007) from lower part of Swat River.

12. *Rasbora daniconius* (Hamilton)
This species was also reported by Mirza (2007) from lower part of the river.

Subfamily Barbinae

13. *Labeo diplostomus* (Heckel)
This species is recorded by us from the lower part of the river that is from the area below Mingora. It was also collected in large number from a stream in Sherabad Ugdo, near Makki Masjid, New Tableeghee Markaz, Swat in the year 2004. This fish has also been misidentified and reported as *Labeo dero* by Rafique and Javed (2002).

14. *Puntius chola* (Hamilton)
This species is also restricted to the lower part of the river Swat and has been collected by us from village Kota and Thana.

15. *Puntius conchonius* (Hamilton)
This species is represented by many specimens collected from seasonal tributaries of the river Swat at village Kota, Thana, Chakdara, Barikot etc. it is frequently available in streams of Bajur agency as well.

16. *Puntius sophore* (Hamilton)
This species was first recorded in 2004 from

swat city and chakdara by us. It is also represented by many specimens collected from various areas of lower part of the river in 2009-10.

17. *Puntius ticto* (Hamilton)
This species was collected from the streams of Bajur agency where it is abundantly present. Mirza (2007) has reported it from lower part of river Swat.

Subfamily Torinae

18. *Naziritor zhobensis* (Mirza)
This species is not present in our collection. This was reported by Mirza (2007) from lower part of the river.

19. *Tor macrolepis* (Heckel)
In the present collection this species is represented by three specimens collected from Chakdara. In 2004 it was captured from sheradad village near tableeghi markaz. Rafique and Javed (2002) has reported this species as *Tor putitora*.

Subfamily Garrinae

20. *Crossocheilus diplocheilus* (Heckel)
This species is represented by many specimens in the present collection. The species is widely distributed in the lower part of the Swat and all around the valley. It is also well established in streams of Bajur agency. This fish has previously been reported as *Crossocheilus latius and Crossochilus latius diplocheilus* as well but according to the new understanding they are all the same species and is now identified as *Crossochilus diplocheilus.*

21. *Garra gotyla* (Gray)
This species is also represented by many specimens in our collection. The fishes of this species are very abundant during summer season. It is also widely distributed in lower swat and adjoining area. We were not able to collect it from upper swat and Bajur agency which means it is found only in high water temperature areas.

Subfamily Schizothoracinae

22. *Racoma labiata* McClelland and Griffith
This species is represented by several specimens in the present survey and is widely distributed to the lower part of the river Swat. It is also found in the upper part of the river that is above Mingora.

23. *Schizopyge esocinus* (Heckel)
This species is now very rare in River Swat. Although in the present collection no specimen of this species was captured, but two specimen of this species were previously collected from river Swat in 2004 from Barikot and are present in the Fisheries Lab. Department of Zoology, University of Peshawar. Mirza (2007) has reported this species from the lower part of Swat river whereas Rafique and Javed (2002) had collected a single specimen from Mingora bridge. This fish was previously named as *Schizothorax esocinus.*

24. *Schizothorax plagiostomus* (Heckel)
This is the most abundant species of the river. In the present collection several specimen of this species are colleted by authors from lower and upper part of the river including Bajur agency. It is widely distributed in the valley and adjoining area.

Subfamily Cyprininae

25. *Carassius auratus* (Linnaeus)
This is an exotic species which is now very well established in the area. Both small and large water bodies contain fishes of this species. This species is also restricted to the lower part of the river. In 2004 we were not able to collect this species from Bajur agency but during the last few years it has established itself very well there. Last year we were able to capture dozens of specimens from all the three streams of Bajur agency.

26. *Cyprinus carpio* Linnaeus
This also is an exotic species and is well established in the area. This species is also restricted to the lower part of the river where the water temperature do not falls below 5°C.

Family Nemachielidae

27. *Acanthocobitis botia* (Hamilton)
This species has been collected many times from river swat and its tributaries in past few years. About 14 specimens are present in our lab. It was also reported by Mirza (2007) from lower part of the river.

28. *Schistura alepidota* (Mirza and Banarescu)
Several specimens of this species are present in the present collection. We have collected all the specimens from lower part of the river that is below Mingora as well as from the streams of Bajur agency. Mirza (2007) has reported this species from upper Swat. Rafique and Javed (2002) has reported it from river Swat and adjoining areas.

29. *Schistura naseeri* (Ahmad and Mirza)
This species was reported by Mirza (2007) from upper Swat and Rafique and Javed(2002) from river Swat, Hazara and Kashmir.

30. *Schistura prashari* (Hora)
This species is present in our collection from lower Swat. Mirza (2007) reported it from upper part of the river.

31. *Triplophysa naziri* (Ahmad & Mirza)
The present collection contains several specimens of this species. These specimens are captured by the authors from both upper and lower part of the river. Mirza has also reported it from the same area. Rafique and Javed (2002) has most probably described this species as *Schistura naziri*

32. *Triplophysa choprai* (Hora)
This species has been reported by Mirza (2007) from upper part of the river although we were not able to collect this species in 2009 and 2010. Many specimens were collected in 2004 from Barikot and Chakdara and are present in Fisheries Lab. Dept. of Zoology, University of Peshawar.

Order Siluriformes
Family Bagridae

33. *Mystus bleekeri* (Day)
The species has been reported by Mirza (2007) from lower part of the river Swat. It has been collected many times from the lower most part of river swat near Charsadda.

Family Sisoridae

34. *Gagata cenia* (Hamilton)
The species has also been reported by Mirza (2007) from lower part of the river Swat.

35. *Gagata pakistanica* (Mirza)
The species has recently been collected from River Khyali near Charsadda in May 2011. Earlier Mirza (1999) has reported it from downstream of Terbela Dam, River Chenab and Qadirabad. A total of eight specimens were collected with the maximum length of 11 cm. it is also a new record from river swat.

36. *Glyptosternum reticulatum* McClelland & Griffith
It is also present in our collection. Only a single specimen of this species was collected from a perennial tributary at Matta in 2010. However previously it was captured from Madyan. The fish is restricted to upper part of the river that is above Mingora.

37. *Glyptothorax cavia* (Hamilton)
This species has been reported by Mirza (2007) from lower part of the river. In our collection of 2009-10 no specimen of this species is present. However few specimens of this species were colleted from Chakdara (lower part of river Swat) in 2004 which are present in Fisheries Lab. Dept. of Zoology, University of Peshawar.

38. *Glyptothorax naziri* Mirza & Naik
This species has been reported by Mirza (2007) from lower part of the river.

39. *Glyptothorax punjabensis* Mirza & Kashmiri
A single specimen of this species is collected from the lower part of the Swat River near Thana bypass. But about 40 specimens were collected from three streams of bajur agency which shows its abundance in the upper areas.

40. *Glyptothorax stocki* Mirza & Nijssen
The present collection contains one specimen of this species collected from river Swat near Landakay that is from lower part of the river.

41. *Glyptothorax sufii* Bashir & Mirza
This species is not present in our collection and has been reported by Mirza (2007) from lower Swat.

Family Schilbidae

42. *Clupisoma naziri* Mirza & Awan
This species has been reported by Mirza (2007) from river Swat near Charsadda. The present collection contain no specimen of this fish but many specimen collected by students in 2006 from river Swat near Charsadda are present in Fisheries Lab. Dept. of Zoology, University of Peshawar.

43. *Clupisoma garua* (Hamilton)
This species is not reported by Mirza (2007). The specimens of this species collected by our students in 2006 from river Swat near Charsadda are present in Fisheries Lab. Dept. of Zoology, University of Peshawar.

44. *Eutropiichthys vacha* (Hamilton)
This species is also not reported by Mirza (2007) and has been collected by students of Fisheries, Dept. of Zoology, University of Peshawar in 2006. At present three specimens of this species are present in Fisheries Lab. Dept. of Zoology, University of Peshawar.

Order Channiformes
Family Channidae

45. *Channa gachua* (Hamilton)
The specimens were collected in large number from the streams of Bajur agency but only two specimen of this species were collected from a perennial tributary at village Kota that is from lower part of the river. This species is also reported by Mirza (2007) from lower Swat and Rafique and Javed(2002) from Barikot area of Lower Swat. Population of this species is constantly at a decline as now it is rarely found in lower part of river swat.

46. *Channa punctata* (Bloch)
This species is represented by many specimens collected by the authors from various spot from lower part of the river as well as from Bajur agency. This species is also reported by Mirza (2007).

Oreder Beloniformes
Family Belonidae

47. *Xenentodon cancila* (Hamilton)
Three specimens of this fish were collected by us for the first time in 2004 from chakdara although in the present collection no specimen was captured. Mirza (2007) had also reported this fish from lower part of the river on the basis of our collection in 2004. Specimens of this species are present in Fisheries Lab. Dept. of Zoology, University of Peshawar. This fish is now very rare in the river because of poisoning and electrofishing practices in the upper part of the river.

Order Mastacembeliformes
Family Mastacembelidae

48. *Mastacembelus armatus* (Lacepede)
There are several specimen of this fish collected in the present survey as well as in many previous surveys. This species is widely distributed in the perennial tributaries of lower part of the river Swat. These

tributaries possess a little warmer water as compared to the main river. A type of side canal of the main river which is called Iraab is the suitable habitat for this fish. Iraab actually receive warm water of irrigation from rice field. The species has also been collected from all the streams of Bajur agency in large number.

Order Perciformes
Family Belontidae

49. *Colisa fasciata* (Bloch & Schneider)
This species is not present in our collection and has been reported by Mirza (2007) from region below Mingora that is from lower part of the river.

50. *Colisa lalia* (Hamilton)
This species is also not present in our collection and has been reported by Mirza (2007) from region below Mingora that is from lower part of the river.

Discussion

The fishes recorded from the river Swat comprise both small sized and large sized fishes. Large sized fishes like *Racoma labiata, Schizothorax plagiostomus, Mastacembelus armatus, Tor macrolepis, Cyprinus carpio* and trout species are consumed as food hence considered more important economically. Although small sized fishes have little fisheries importance as described by Talwar and Jhingran (1991) and Mirza and Sandhu (2007) but according to modern understanding they are equally important being an integral part of the food chains and food webs of the riverine ecosystem. They are part of the biodiversity, hence should be given due importance. Some of the small sized fishes like *Puntius, Barilius, Schistura* and *Colisa* etc. are beautiful ornamental fishes, kept alive in aquaria and bear great economic value. If proper marketing is done, these fishes may reduce the national expenditure on import of ornamental fishes.

According to our own observations and discussion with local people of swat, *Tor macrolepis* is becoming endangered in the river Swat due to the use of inhuman fishing technique like electrocuting, dynamiting etc. Although a hatchery has been constructed in Chakdara for Mahasher (*Tor macrolepis)* but being an important game and food fish it is a strong candidate for being cultured in lower Swat area. Fisheries department and NGOs operating in Swat shall pay immediate attention to this important fish.

As discussed by Rafique and Javed (2002), *Schizothorax plagiostomus*, being a frequently occurring fish in river Swat can be commercialized by establishing its rearing ponds in which it could be reared under semi controlled conditions. We add to this list the name of *Clupisoma naziri* and *Clupisoma garua* which can also be commercialized as quality of its flesh is far better than other species and it gets very good price in the market. In the culture ponds/ areas of *Schizothorax plagiostomus* other fishes like *schizopyge esocinus* and *Racoma labiata* which have become rare in the river can be re-established, if not for the commercial use, at least for the conservation of the biodiversity of the area.

Table I.- Maximum length and weight of some important food fishes from river swat in present collection.

Name of fish	Condition	Maxi. weight (gm)	Maxi. length (cm)
Carassius auratus	Preserved	170	19
Channa gachua	Preserved	10	9.5
Channa punctatus	Preserved	170	22.5
Crossocheilus diplocheilus	Preserved	50	14.3
Clupisoma garua	Preserved	95	23.5
Clupisoma naziri	Preserved	70	23.5
Cyprinus carpio	Preserved	95	18.5
Eutropiichthys vacha	Fresh specimen	190	28
Labeo diplostomus	Preserved	135	22
Mastacembelus armatus	Preserved	120	35
Mystus bleekeri	Preserved	30	14.5
Oncorhynchus mykiss	Preserved	116	21.5
Racoma labiata	Fresh specimen	345	30
Salmo trutta fario	Preserved	26	13.5
Schizothorax plagiostomus	Fresh specimen	400	32
Tor macrolepis	Preserved	70	18.5

The most important aspect of the present study is that it includes six (6) new records of fish species which were not recorded previously from river swat. These are *Barilius vagra, Barilius modestus, Gagata pakistanica, Clupisoma garua, Eutropiichthys vacha* and *Xenentodon cancilla*. Thus the fish fauna of river Swat is now updated

and is represented by fifty (50) species, thirty four (34) genera, ten (10) families and seven (7) orders of Teleostean fishes in this paper.

References

Ahmad, N, D., and Mirza, M. R., 1963a. *Pak. J. Sci*., **15:** 75-81**.**

Ahmad, N.D. and Mirza, M.R., 1963b. *Scientist (Karachi),* **6:** 153-161**.**

Ahmad, N., 1965. *Trout in Swat Valley*. Govt. Printing Press, West Pakistan, Lahore

Hussain, A. and Shah, S.Z.A., 1960. *Agric. Pakistan,* **11**: 301-310.

Jayaram, K.C., 1999. *The freshwater fishes of the Indian region*. Narendra Publishing house: New Delhi.

Mirza, M.R., 2007. *Biologia-Pakistan,* **53:** 109-112.

Mirza, M.R., Parveen, A. and Javed, M.N., 1999. *Punjab Univ. J. Zool*., **14:** 01-04.

Mirza, M.R. 1990. *Pakistan ki taaza pani ki machlian* (Urdu). Urdu Science board, Lahore

Mirza, M.R., 1973. *Biologia (Pakistan),* **19:** 119-144.

Mirza, M. R. and Sandhu, A.A., 2007. *Fishes of the Punjab Pakistan*. Polymer publications: Lahore.

Nelson, J.S., 2006. *Fishes of the world.* (4th edition). John Wiley & Sons, N.Y.

Rafique, M., and Javed, H.I., 2002. *Rec. Zool. Surv. Pakistan*, **14:** 43-48.

Talwar, P.K. and Jhingran, A.G., 1991. *Inland fishes of India and adjacent countries*, Vol. 1 and 2. Oxford & IBH publishing Co. Pvt. Ltd: New Delhi.

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