The Journal of Animal & Plant Sciences, 25(3): 2015, Page: 825-835
ISSN: 1018-7081

MICROSATELLITE-BASED SIBSHIP RECONSTRUCTION AND ESTIMATION OF GENETIC RELATEDNESS IN THE ENDANGERED *LABEO CALBASU* (HAMILTON 1822) (CYPRINIDAE: CYPRINIFORMES)

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ABSTRACT

The microsatellite genotyping technique was used to reconstruct sibship and estimate pairwise relatedness among individuals of *Labeo calbasu* collected from four natural and one hatchery stocks**.** The mean values for the number of alleles, observed and expected heterozygosity and polymorphic information content of the five microsatellite markers used for genotyping 165 individuals were 6.00, 0.622, 0.743 and 0.699 respectively. Deviation from Hardy-Weinberg expectation was detected at four of the five loci. The number of reconstructed half-sib family was lowest in the Hatchery sample. The number of full-sib family was lowest in the Jamuna river sample and highest in the Haor sample. The numbers of reconstructed families were fewer in the real sample compared to the simulated sample of the same number of unrelated individuals. The mean relatedness coefficients r_{xyW} was found to be highest in the Jamuna river sample and lowest in the Hatchery sample, though no difference was observed in mean r_{xyLR} values of the five samples. The misclassification rates estimated based on the relatedness coefficients were found to be quite high, ranging from 11.17 (Unrelated-Fullsib) to 32.02% (Halfsib-Fullsib) and more loci need to be analyzed for accurate separation of related individuals from unrelated ones.

Key words: Polymorphism, Microsatellite, Relatedness, Family Reconstruction, *Labeo calbasu*

INTRODUCTION

Information on family relationships and genetic relatedness among individuals of a population has practical implications in behavioral ecology as well as population and conservation genetics (Blouin, 2003); and is particularly important for breeding programs planned for stock enhancement (Hansen and Jensen, 2005). The rate of inbreeding in a captive stock can be controlled by avoiding mating between related individuals. Due to external fertilization and a high fecundity, in most fishes, it is difficult to trace back the parents as well as the sibs and identify individuals based on genetic relationships. This is believed to be one of the major causes of inbreeding problem in a fish hatchery. Molecular marker based reconstruction of sibship and estimation of genetic relatedness are considered potential alternatives to inferring the degree of genetic relationships among individuals identified as full-sibs, half-sibs and unrelated pairs (Kanno et al., 2011) that can be used to choose mating individuals to minimize inbreeding (Lynch and Ritland, 1999). These marker based methods allows estimation of sibship groups within the sampled offspring, reconstruction of the genotypes of the inferred parents, making inferences about the mating system, dispersal behavior in offspring and estimation of effective population size where sampling of spawning parents is difficult or impossible but sampling of a sufficient number of their offspring is feasible (Wang, 2002; Wang, 2004; Herbinger et al., 2006; Wang and Santure, 2009; Hudy et al., 2010).

Labeo calbasu, has been categorized as an endangered species in Bangladesh (IUCN, 2000) due to reduction in availability in the nature. Moreover, unlike the other three major carp species such as rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*), *L. calbasu* has not been used as a regular species in the carp polyculture system. Therefore, its abundance could not be compensated through commercial aquaculture and restocking programs, though it has had a high potential. Sahu et al. (2007) for example, reported better performance by *L. calbasu* in growth and production parameters compared to catla and mrigal in a carp polyculture system. Of late, however, this species has been seen to be bred to a limited scale in hatcheries owing to consumer preference and species

diversification. As a threatened species, the population structure and other ecological parameters are vulnerable to change that might be reflected in the estimates of genetic variability and genetic relatedness among the individuals. Therefore, these parameters need to be estimated and incorporated into the stock management and stock improvement plans.

Several reports are available on the analysis of population genetic structure of *L. calbasu* using different methods (Mostafa et al., 2009; Hossain et al., 2010; Saha et al., 2010; Hasan et al., 2013). Truss network, analysis exhibited high isolation in morphometrics (Hossain et al., 2010) and microsatellite analysis exhibited a high level of genetic differentiation (Hasan et al., 2013) among the *L. calbasu* populations in Bangladesh. Saha et al. (2010) reported bottlenecks in the Halda and Jamuna rivers, and one hatchery populations of *L. calbasu*. All these reports however lack information about family structure and genetic relatedness among the individuals. In this study, we assessed kinship or relatedness among the individuals of four natural and one hatchery stocks of the endangered carp *L. calbasu* based on offspring genotype data by two approaches: (1) evaluating the ability to resolve three levels of kinship- full-sib, half-sib and unrelated in the samples using three estimates of relatedness coefficients r_{xyW} (Wang 2002) and r_{xyLR}(Lynch and Ritland, 1999) and r_{xyQG} (Queller and Goodnight, 1989); (2) reconstructing sibship among the same individuals based on microsatellite genotyping. We found fewer half-sib families and lower relatedness values in the hatchery stock compared to the wild stocks.

MATERIALS AND METHODS

Collection of samples and isolation of genomic DNA: Fin clips were collected from a total of 165 mature *L. calbasu* samples, 33 each from five sources: the Jamuna river, the Padma river, the Halda river, Tola *Haor* and a hatchery, and preserved in 95% ethanol in separate microfuge tubes and stored at -20ºC. The sampling locations are shown in a map of Bangladesh in Figure 1. Genomic DNA was isolated from each sample following standard protocol of proteinase-K digestion, phenol:chloroform:isoamyl alcohol extraction and ethanol precipitation (Hasan et al., 2013).

Microsatellite genotyping: We used five heterologous microsatellite markers: *Lr10, Lr21, Lr24, Lr26* developed from *Labeo rohita* (Das et al., 2005), and *CcatG1* developed from *Catla catla* (Naish and Skibinski, 1998) (Table 1). The methods of microsatellite genotyping have been detailed in Hasan et al. (2013).

Statistical analysis of microsatellite data: For quantifying genetic variability we calculated the number of alleles, observed heterozygosity (*Ho*), expected heterozygosity (*He*) and the polymorphic information content (PIC) for each locus in the complete set of samples using CERVUS version 3.0.3 (Kalinowski et al., 2007). The same software was used for exact test for deviation from Hardy-Weinberg expectation at the five loci based on genotype frequencies of 165 individuals.

For estimating the degree of genetic relationship among all pairs of individuals we calculated three relatedness coefficients: r_{xyW} (Wang, 2002), r_{xyLR} (Lynch and Ritland 1999) and r_{xyQG} (Queller and Goodnight, 1989) using the software COANCESTRY version 1.0.1.2 (Wang, 2011). For evaluating the bias of $r_{xyW,}$ r_{xyLR} and r_{xyQG} values for unrelated, half-sibs and full-sibs categories, the observed allele frequency estimated from the genotype data of all the 165 individuals were used to generate the relatedness coefficient of 5000 pairs of individual under each of the three relationship categories: Full-sibs (FS), Half-sibs (HS) and unrelated (UR). The mean values for the relatedness coefficients obtained from the simulated genotype data and those expected under three different relationship categories- 0.5 (Full-sib), 0.25 (Half-sib) and 0.0 (unrelated) were subjected to two-tailed t-tests to examine if there was any significant difference. The rates of misclassification for assigning individuals to different relationship categories were estimated following Blouin et al. (1996) based on a cut-off point set at the average of the simulated $r_{xyW,}$ r_{xyLR} and r_{xyQG} values for the two levels of relationship categories.

As, the relatedness estimates of two samples showing different levels of heterozygosity cannot be directly compared, we made comparisons between the distribution of observed pairwise relatedness (r_{xy}) values among pairs of individuals within each sample with that expected in a sample of simulated unrelated individuals from a population with the same allele frequencies following Hansen and Jensen (2005). For that purpose, we generated the genotypes of 250 unrelated individuals for each population by using the genotype data of the corresponding sample both for population 1 and population 2 in the HYBRIDLAB v1.0 program (Nielsen et al., 2006). We then calculated the relatedness among all the pairs of individuals totaling 31125 [n(n-1)/2] pairs per population. Comparisons between the real and simulated pairwise relatedness coefficients were undertaken using a Mann-Whitney *U*-test.

We reconstructed half- and full-sib families without information on parental genotypes as per maximum likelihood method implemented in the software COLONY 2 (Jones and Wang, 2010). We assumed a tentative 2.5% error rate for all loci, both for allelic dropouts and erroneous sizing of alleles. We compared the family configuration of each sample with that of a sample of the same number (n=33) of unrelated individuals simulated from the empirical allele frequency data by using the HYBRIDLAB v 1.0 program (Nielsen et al., 2006). We followed Hansen and Jensen (2005) inference for significance in family construction: the

sibship reconstruction of a sample was considered 'significant' if the number of half- and full-sib families was lower in the real samples compared to the samples of simulated unrelated individuals and if there were more individuals in one or more of the full-sib families generated from real individuals than were observed in any of the full-sib 'families' generated from the simulated unrelated individuals.

RESULTS AND DISCUSSION

Variability in the microsatellite loci: We have characterized five heterologous microsatellite DNA markers, four developed from *Labeo rohita* and one developed from *Catla catla* in *L. calbasu*. The variability measures estimated across the five microsatellite loci are shown in Table 1. A total of 30 alleles were detected in 165 fish originating from four natural and one hatchery populations. The number of alleles ranged from 4 to10 with a mean (±SE) of 6.00 ±1.048. The locus-specific *Ho* ranged from 0.558 (*Lr24*) to 0.673 (*Lr10*) with a mean (±SE) of 0.622±0.023 and H_E ranged from 0.580 (*Lr24*) to 0.877 (*CcatG1*) with a mean (±SE) of 0.743±0.049. Significant deviations from Hardy-Weinberg expectation were detected at four loci. The strength of a molecular marker for analyzing genetic diversity is evaluated on the basis of its polymorphic information content (PIC). The PIC of a marker is estimated from the number and frequency of alleles which provides an indicator of genetic diversity. The five markers were found to be highly informative as the PICs were equal or >0.500 (Botstein et al., 1980) supporting the suitability for analysis of genetic diversity in *L. calbasu*.

Genetic relatedness: To evaluate the bias for relatedness towards the expected value of 0.5 for full-sib, 0.25 for half-sib and 0 for unrelated, we used three estimates of relatedness coefficients: r_{xyW}, (Wang, 2002) r_{xyLR}(Lynch and Ritland, 1999) and r_{xyQG} (Queller and Goodnight, 1989). The mean values for the three estimators obtained from 5000 pairs of individuals under each of the three relationship categories are presented in Table 2. The mean values for r_{xyW} and r_{xyLR} were in agreement with the theoretical distributions of 0.5, 0.25 and 0 for full-sib, half-sib and unrelated categories respectively (two tailed probability: ≥0.3482 for r_{xyW} and ≥0.1804 for r_{xyLR}). However, the two-tailed t tests revealed a highly significant difference between the mean relatedness r_{xyQG} obtained from the simulated genotype and that expected under the full-sib category (theoretical r_{xy}=0.5) (P=0.0001). Further, we used these three estimators for estimation of misclassification rates in grouping the individuals based on the r_{xy} values. The mid-point of the means of the three categories under comparison (UR-FS, UR-HS and FS-HS) were used as cut-off point as per Blouin et al. (1996) to classify the individuals and to estimate the Type I (unrelated misclassified as full-sibs and half-sibs, half-sibs into full-sibs) and Type II (full-sibs and half-sibs misclassified as unrelated and full-sibs as half-sibs) error rates in classifying the individuals.

The misclassification rates were higher between HS-FS and HS-UR category pairs for all estimators (Table 3). The misclassification rates for $r_{xyW,}$ r_{xyLR} and r_{xyQG} between HS-FS pairs were 33.34, 31.50 and 31.02 and between HS-UR were 28.56, 32.08 and 30.35 respectively. Lowest misclassification rate was found between FS-HS for r_{xyW} (12.84%), between UR-HS for r_{xyLR} (12.24%) and between FS-HS for r_{xyQG} (13.30%) (Table 3). Varied levels of Type I and Type II errors have been reported in classifying fishes into the first order relationship categories based on relatedness coefficients derived from microsatellite genotyping (Fontane and Dodson, 1999; Bentzen et al., 2001; Porta et al., 2006; Kozfkay et al., 2008). The rates of misclassification between different kinship levels decrease with increased number of loci from 10 to 30 (Bentzen et al., 2001) and increased levels of average heterozygosity (H_E) at the loci (Blouin et al., 1996). Similarly, Robinson et al. (2013) had shown that bottleneck and reduction in genetic variation could decrease the accuracy of estimating relatedness from genetic data. A relatively high level of misclassification that we have obtained in our study might be attributed to the less number of microsatellite loci used for the analysis and low level of average heterozygosity (mean H_E=0.743) as well as bottlenecks in the population. Tapio et al. (2010) identified three factors such as the number markers, genetic variability of the markers and the population structure that affect the efficacy of the methods used in the calculation of relatedness between individuals.

Among the five populations, the mean r_{xyW} value of the Jamuna was found to be the highest and that for the Hatchery was found to be the lowest while the mean r_{xyLR} and r_{xyQG} values for all the populations were more or less similar (Table 4). As the means cannot be directly compared due to differences in heterozygosities of the populations, we compared the distributions of r_{xyW} for full-sib, half-sib and unrelated individuals determined as per Blouin et al. (1996). We selected r_{xyW} because this estimator gave a relatively more varied means for the populations compared to the other two estimators. The distribution of relatedness coefficient r_{xyW} values under the three relationship categories in five different populations of *L. calbasu* obtained from real data are plotted along with relatedness of 250 simulated unrelated individuals in Figure 2. Plots of distribution of observed pairwise relatedness values for all the samples were not exactly similar to the distribution of simulated unrelated individuals. There appeared to be tendencies towards an excess of both low and high relatedness values when compared with the distribution of simulated relatedness

values. The low relatedness values were more prominent than those of the high relatedness values in the Hatchery population. However, the Mann-Whitney *U* test could not detect any significant difference (probabilities ≥0.192) between the observed and simulated distributions in all the samples.

Microsatellite DNA markers, due to the high mutation rate, wide distribution in the genome and simple Mendelian inheritance, have the potential to construct a pedigree on the basis of genotype data. For example, Toro et al. (2002) observed a strong correlation between the genealogical co-ancestry and the relatedness (r_{xyLR}) estimated from the simulated genotypic data based on as low as 10 microsatellite loci. Using the heterologous microsatellite markers developed from *Labeo rohita* and *Catla catla* we have estimated relatedness of various degrees among the individuals of five different populations of *L. calbasu*. Our study revealed the existence of excess low relatedness values (r_{xy}) among real individuals compared to the values for simulated unrelated genotypes. The scenario was most prominent in the hatchery sample that had also been reflected in mean relatedness estimates (Table 4). Low values of relationships are expected when parents are not related and higher values are expected in inbreeding situations. The lower relatedness values observed in the hatchery samples can be explained by recent introduction of unrelated individuals in the system.

Family reconstruction: We reconstructed full-sib and half-sib families using Wang's (2004) method which showed that the number of half-sib families ranged from five (Hatchery) to eight (Haor) and the number of full-sib families ranged from nine (Jamuna) to 13 (Haor) (Figure 3a, c, e, g, and i). We also reconstructed full- and half-sib families from the same number (#33) of unrelated individuals simulated from the gene frequency of the original samples to test the significance of family reconstruction according to Hansen and Jensen (2005). The number of half-sib families in the simulated and real samples were same in the Padma and the Haor populations (Figures 3 d, g) whereas, the number of half-sib families were higher than those of their respective real samples of the Jamuna, Halda and the Hatchery populations (Figures 3b, f ,j). The number of full-sib families reconstructed within the simulated samples were higher than those of their respective real samples in all the populations. In addition to these, several of the putative full-sib families of the real samples included far more individuals than were observed in the simulated data in all the populations. For example, four of the nine full-sib families under the Jamuna sample contained higher number of individuals (4-9) than the highest value (3) obtained for the simulated data (Figures 3a, b).

According to Hansen and Jensen (2005), the family reconstruction is considered to be significant if the number of families reconstructed from the simulated genotypes were higher than those constructed from original genotype data. Reconstruction of families involving simulated unrelated individuals, based on the allele frequencies of the respective samples, confirmed that much higher numbers of families that include fewer individuals would be expected as compared to the real samples.

For instance, the highest number of individuals within a full-sib family in the simulated hatchery (HT) sample was two (Figure 3i), whereas four full-sib families in the real hatchery sample consisted of four to six individuals (Figure 3j). Among the natural populations, the Jamuna river, for example, a maximum of nine individuals were found in a full-sib family, whereas the maximum number of individuals in the full-sib families reconstructed from the simulated genotype was three. The low numbers of families found in the real hatchery samples were not due to random grouping of individuals, but should be considered significant (Hansen and Jensen, 2005). Hansen et al. (2006) reported fewer numbers of half- and full-sib families in hatchery samples compared to the river counterparts in *Catla catla*.

Table 1. Genetic variability at five microsatellite loci in 165 individuals of *L. calbasu* collected from four natural and one hatchery stocks in Bangladesh. *Ho*: heterozygosity observed, H_E: heterozygosity expected, PIC: polymorphic information content, F_{IS}: inbreeding coefficient, HWEP: exact probability for deviation in Hardy-Weinberg expectation.

Locus	No. Alleles	*Ho*	H_E	PIC	F_{IS}	HWEP
Lr10	4	0.673	0.723	0.672	0.012	0.00660*
Lr21	6	0.636	0.808	0.776	0.134	0.00006*
Lr24	5	0.558	0.580	0.499	-0.078	0.00580*
Lr26	5	0.667	0.727	0.689	-0.055	0.13000
CcatG1	10	0.576	0.877	0.862	0.330	0.00009*
Mean±SE	6±1.04	0.622±0.023	0.743±0.049	0.699±0.061	0.068±0.075	

* Indicates significant deviation from Hardy-Weinberg expectation after sequential Bonferroni corrections (initial k=5)

In summary, we have characterized genetic variability at five loci in the endangered *L. calbasu*. Four of the five loci showed nonconformity to Hardy-Weinberg equilibrium that is a concern for the population. The marker based estimators of genetic relatedness did not show any sign of skewed distribution rather mostly followed the trend of unrelated individuals. Some variations were observed in family sizes of the real sample ranging from one to nine though the highest family size was four for the simulated samples. The power of the three estimators used in the present study was very similar in classifying individuals in to full-sib, half-sib and unrelated categories. We recommend developing homologous primers for this endangered species and using a larger number of loci to estimate relatedness and reconstruct sibship among individuals of different source populations.

Table 2. Mean (±SE) r_{xyW}, r_{xyLR} and r_{xyQG} values estimated from simulated genotypes of 5000 pairs of individuals under each relationship category [a,b]

	FS	HS	UR
r_{xyW}	0.4968±0.010	0.2509±0.010	0.0000±0.011
	(p=0.3482)	(p=0.776)	(p=0.9976)
r_{xyLR}	0.4952±0.011	0.2509±0.011	0.0013±0.009
	(p=0.1804)	(p=0.7835)	(p=0.6392)
r_{xyQG}	0.4870±0.010	0.2452±0.010	0.0021±0.010
	(p=0.0001*)	(p=0.1566)	(p=0.5474)

[a] Simulation of genotype under each relationship category was performed based on allele frequency distribution of 165 individuals of *L. calbasu* (FS=Full-sib; HS=Half-sib; UR=Unrelated)
[b]The nominal p value (α=0.05) was subjected to sequential Bonferroni corrections for multiple comparisons (initial k=3). * Significant

Table 3. Estimated misclassification (error) (%) among full-sibs (FS), half-sibs (HS), and unrelated (UR) of *L. calbasu* on the basis of r_{xyW} (Wang, 2002), r_{xyLR} (Lynch and Ritland, 1999) and r_{xyQG} (Queller and Goodnight, 1989) using the midpoint of the means of the two distributions as the assigned threshold

Type I error	**UR as FS (%)**	**UR as HS (%)**	**HS as FS (%)**
r_{xyW}	16.26	15.18	33.34
r_{xyLR}	12.76	12.24	31.50
r_{xyQG}	17.15	13.67	31.02
Type II error	FS as HS (%)	FS as UR (%)	HS as UR (%)
r_{xyW}	12.84	15.68	28.56
r_{xyLR}	13.06	18.20	32.08
r_{xyQG}	13.30	16.00	30.35

* The cut-off values were set at the mean of the average simulated r_{xyW}, r_{xyLR} and r_{xyQG} values under each category as per Blouin et al. (1996).
Midpoints are as follows:
r_{xyW}: FS as HS: 0.374; FS as UR: 0.248; HS as UR: 0.125;
r_{xyLR}: FS as HS: 0.373; FS as UR: 0.248; HS as UR: 0.126
r_{xyQG}: FS as HS: 0.3646; FS as UR: 0.2432; HS as UR: 0.1233

Table 4. Mean r_{xyW} (Wang, 2002), r_{xyLR} (Lynch and Ritland, 1999) and r_{xyQG} (Queller and Goodnight, 1989) values of five different populations of *L. calbasu* estimated by COANCESTRY (Wang, 2011).

Population	**r_{xyW} (Mean±SE)**	**r_{xyLR} (Mean±SE)**	**r_{xyQG} (Mean±SE**
Jamuna	0.0394±0.016[a]	-0.0312±0.0135[a]	-0.005±0.013
Padma	-0.0316±0.014[b]	-0.0317±0.0112[a]	-0.027±0.012
Halda	-0.0375±0.016[b]	-0.0312±0.0128[a]	-0.022±0.014
Haor	-0.0375±0.014[b]	-0.0312±0.0122[a]	-0.026±0.013
Hatchery	-0.1817±0.019[c]	-0.0312±0.0151[a]	-0.023±0.019

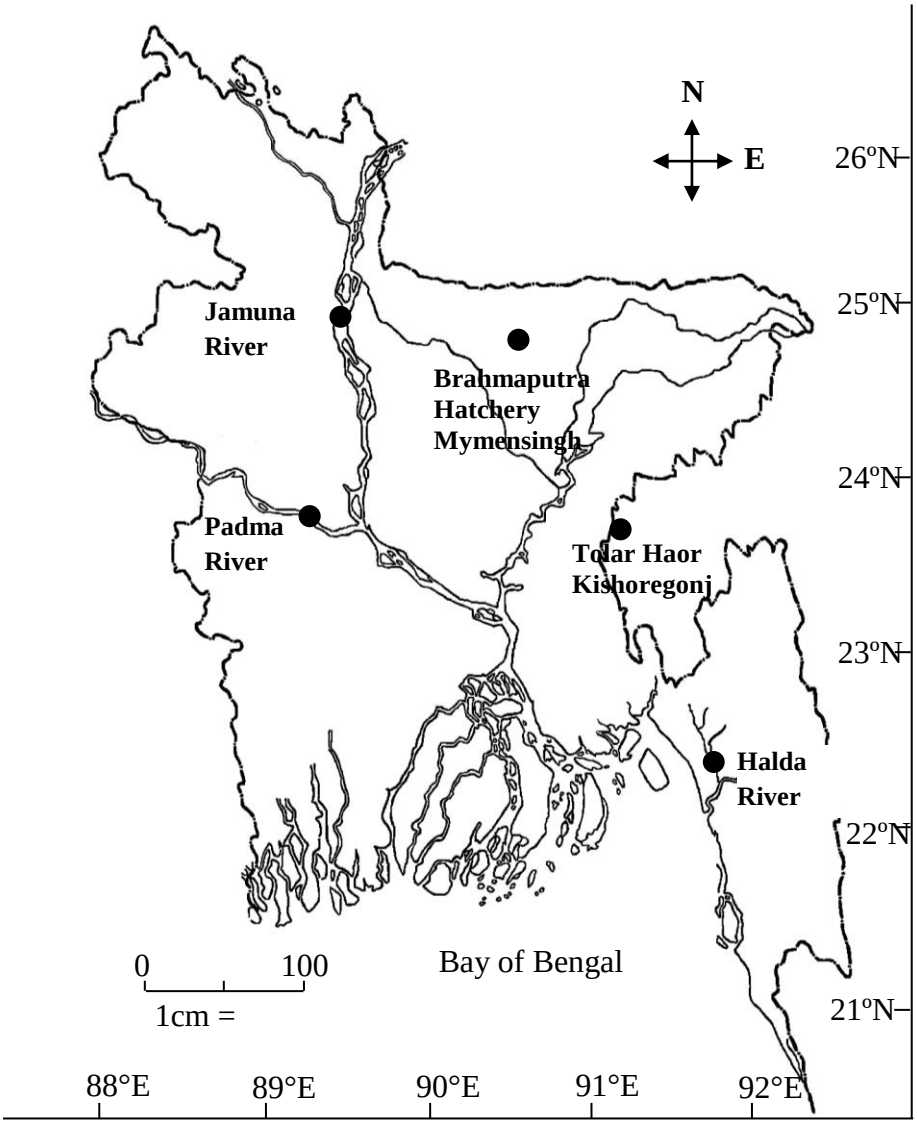


Fig. 1. Map of Bangladesh showing five different sampling sites.

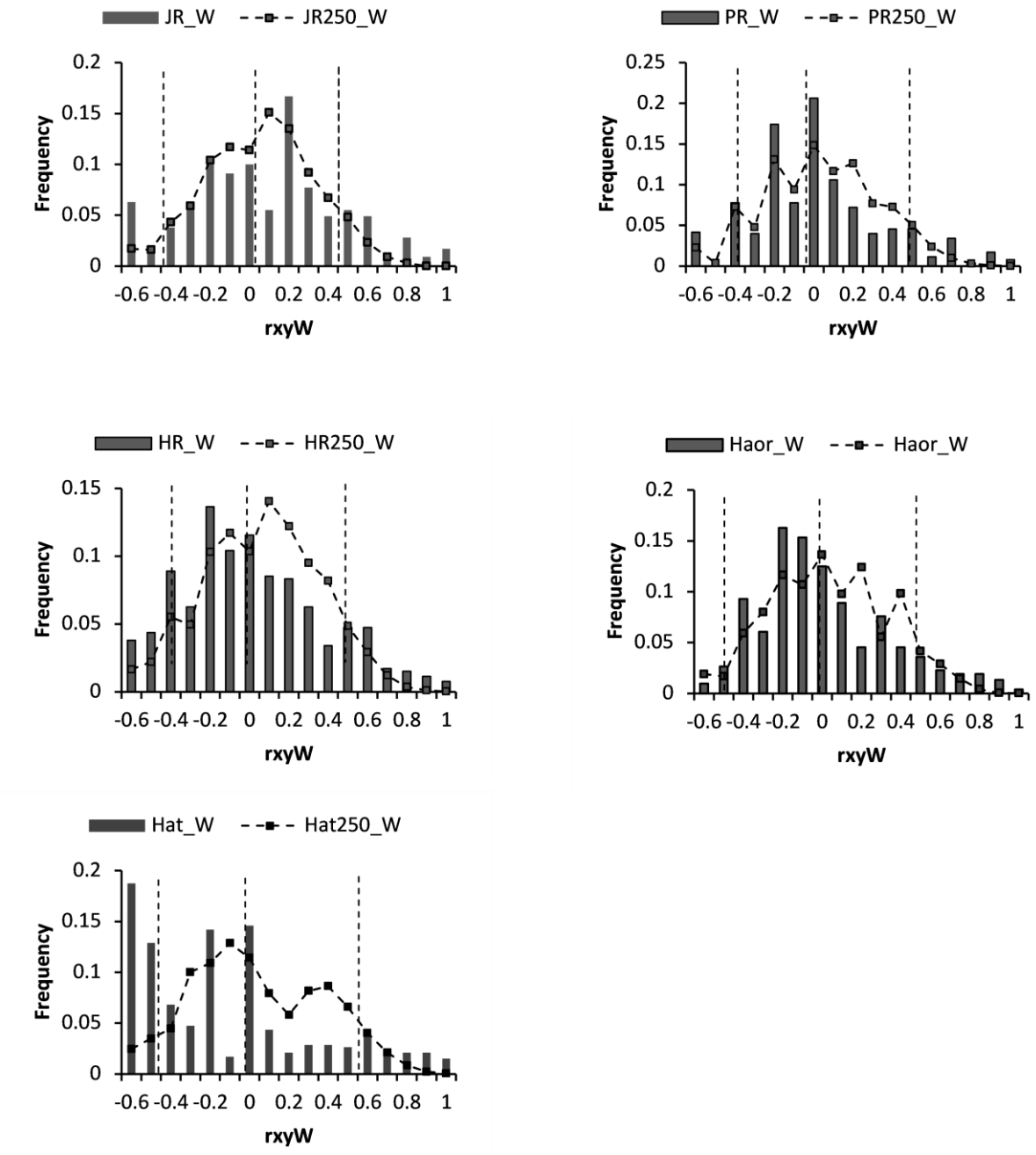
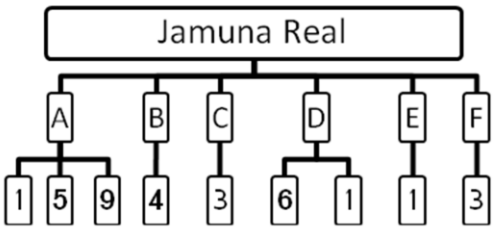
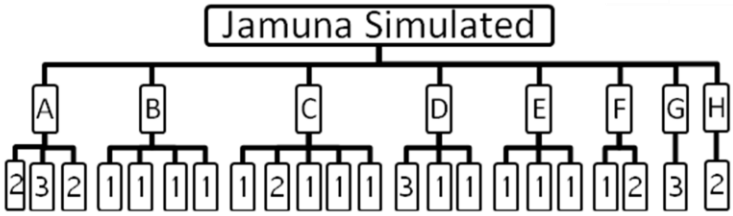


Fig. 2. Frequency distribution of pairwise relatedness r_{xyW} (Wang, 2002) values between individuals of five *L. calbasu* populations. The curves show the frequency distribution of pairwise relatedness values between 250 simulated unrelated individuals, whereas the bars show the frequencies of observed relatedness values. The broken vertical lines denote the 5, 50 and 95% quantiles of the simulated distributions of relatedness values.

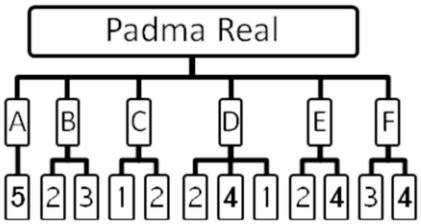
(a)


Jamuna Real
A B C D E F
1 5 9 4 3 6 1 1 3

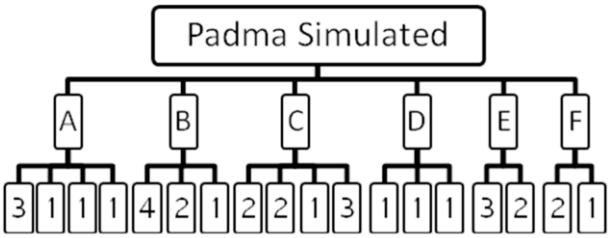
(b)


Jamuna Simulated
A B C D E F G H
2 3 2 1 1 1 1 1 2 1 1 1 3 1 1 1 1 1 1 2 3 2

(c)


Padma Real
A B C D E F
5 2 3 1 2 2 4 1 2 4 3 4

(d)


Padma Simulated
A B C D E F
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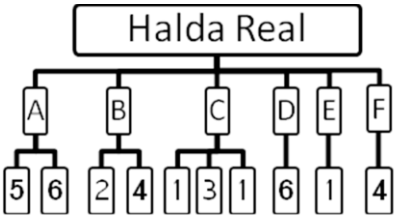
(e)


Halda Real
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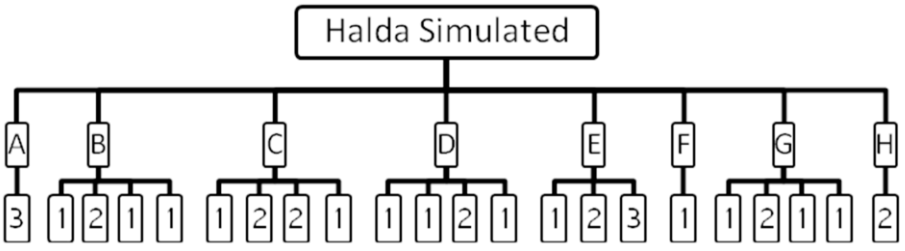
(f)


Halda Simulated
A B C D E F G H
3 1 2 1 1 1 2 2 1 1 1 2 1 1 2 3 1 1 2 1 1 2

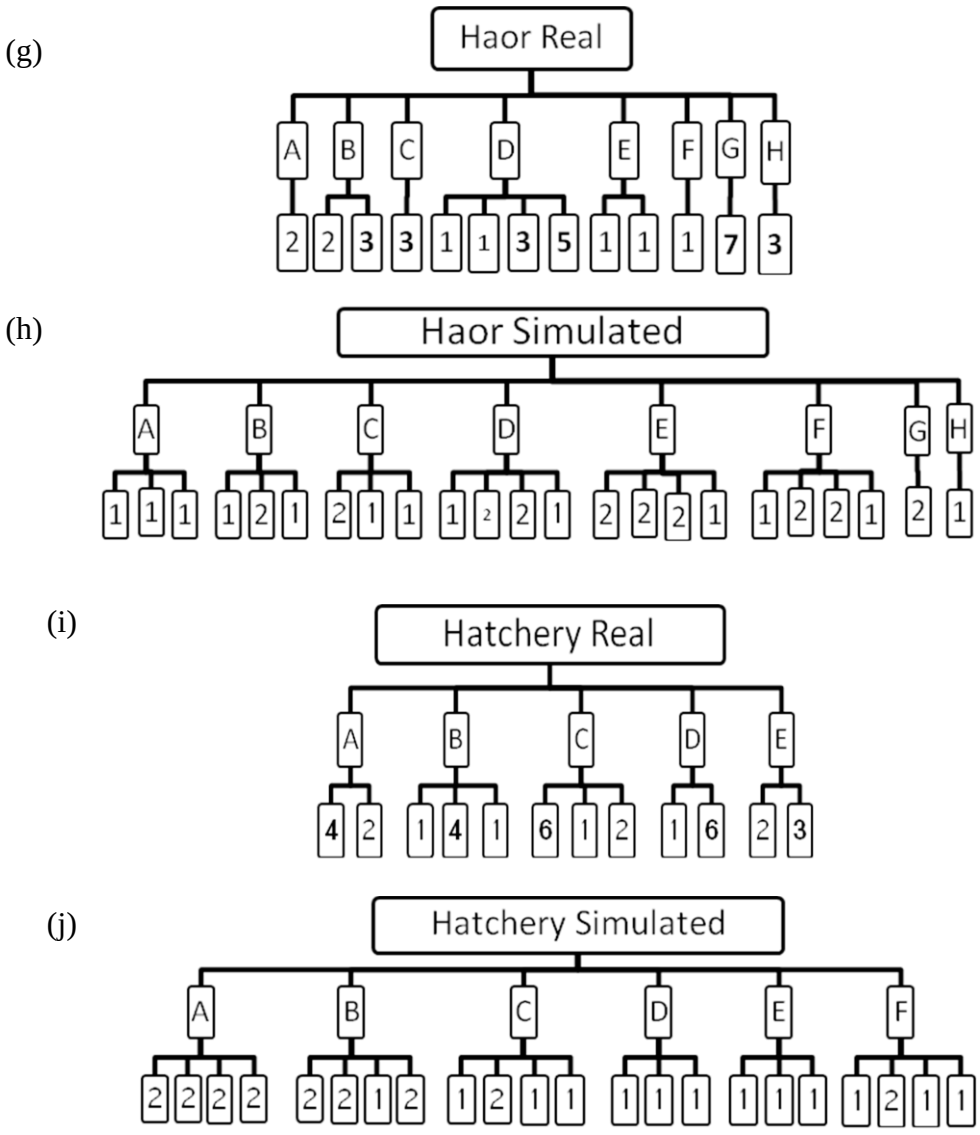


Fig.**3**. **Results of sibship reconstruction grouping the individuals into full-sib families under the corresponding half-sib families (cluster). The upper hierarchy of the figures denotes the source of the sample, the second hierarchy denotes the half-sib families (cluster) and the third hierarchy denotes individual full-sib families nested within half-sib families. The number of individuals included to each full-sib family is mentioned. The bold numbers under the full-sib category of the real sample indicate full-sib family sizes that are higher than those observed in the sibship reconstruction based on simulated unrelated individuals. (a) Sibship reconstruction based on real** Jamuna river (JR) **individuals (b) simulated unrelated JR individuals, (c) real Padma river (PR) individuals (d) simulated unrelated PR individuals (e) real Halda river (HR) individuals (f) simulated unrelated H**R **individuals, (g) real Haor individuals (h) simulated unrelated Haor individuals, (i) real Hatchery individuals (j) simulated unrelated Hatchery individuals.**

Acknowledgements: This research was supported by United States Department of Agriculture (USDA) through "*Ex situ* conservation of some indigenous fishes of Bangladesh by selecting the best stock through DNA markers" project (BGARS-120).

REFERENCES

Bentzen, P., J. B. Olsen, J. E. McLean, T. R. Seamons and T. P. Quinn (2001). Kinship analysis of pacific salmon: insights into mating, homing,

and timing of reproduction. J. Hered. 92:127-136.

Blouin, M.S. (2003). DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol. Evol., 18:503–511.

Blouin, M.S., M. Parsons, V. Lacaille and S. Lotz (1996). Use of microsatellite loci to classify individuals by relatedness. Molec. Ecol. 5: 393-401.

Botstein, D., R. L. White, M. Skolnick and R. David (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Das, P., A. Barat, P. K. Meher, P.P. Ray and D. Majumdar (2005). Isolation and characterization of polymorphic microsatellites in *Labeo rohita* and their cross species amplification in related species. Molec. Ecol. Notes 5: 231-233.

Fontane, P.M. and J. J. Dodson (1999). An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites. Molec. Ecol. 8: 189-198.

Hansen, M. M. and L. F. Jensen (2005). Sibship within samples of brown trout (*Salmo trutta*) and implications for supportive breeding. Conserv. Genet. 6: 297-305.

Hansen, M.M.**,** V. Simonsen, K.L.D. Mensberg, M.R.I. Sarder and M.S. Alam (2006). Loss of genetic variation in hatchery-reared Indian major carp, *Catla catla*. J. Fish Biol.69: 229-241.

Hasan, M. R., M. Nahiduzzaman, M. A. R. Hossain and M. S. Alam (2013). Population genetic structure of an endangered carp, *Labeo calbasu* (Hamilton, 1822) revealed by heterologous microsatellite DNA markers. Croat. J. Fish. 71: 65-73.

Herbinger, C.M., P.T. O'reilly and E. Verspoor (2006). Unravelling first generation pedigrees in wild endangered salmon populations using molecular genetic markers. Molec. Ecol. 15:2261–2275.

Hossain, M.A.R., M. Nahiduzzaman, D. Saha, M. U. H. Khanam and M. S. Alam (2010). Landmark based morphometrics of an endangered carp kalibaus, *Labeo calbasu* stocks. Zool. Studies 49: 556-563.

Hudy, M., J. A. Coombs, K. H. Nislow and B. H. Letcher (2010). Dispersal and within-stream spatial population structure of brook trout revealed by pedigree reconstruction analysis. Trans. Am. Fish. Soc.139:1276–1287.

IUCN (2000). Red Book of Threatened Fishes of Bangladesh. Islam M. A., M. Ameen, A. Nishat, (Eds.) The World Conservation Union, Dhaka, Bangladesh. 116 pp.

Jones, O. R. and J. Wang (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data**.** Molec. Ecol. Resour. 10: 551-555.

Kalinowski, S.T., M. L. Taper and T.C.Marshall (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molec. Ecol. 16(5): 1099–1106.

Kanno, Y., J. C. Vokoun and B. H. Letcher (2011). Sibship reconstruction for inferring mating systems, dispersal and effective population size in headwater brook trout (*Salvelinus fontinalis*) populations. Conserv. Genet. 12:619–628.

Kozfkay, C.C., M.R. Campbell, J.A. Heindel, D. J. Baker, P. Kline, M.S. Powell and T. Flagg (2008). A genetic evaluation of relatedness for broodstock management of captive, endangered Snake river sockeye salmon *Oncorhynchus nerka*. Conserv. Genet. 9(6):1421-1430.

Lynch, M. and K. Ritland (1999). Estimation of pairwise relatedness with molecular markers. Genetics, 152:1753-1766.

Mostafa, M.G., A.S. I. Ahmed, M.G. Mustafa, M.G. Rabbane, M.N. Islam and S.M. Rafiquzzaman (2009). Genetic diversity of wild and farmed Kalibaus (*Labeo calbasu*, Hamilton, 1822) by RAPD analysis of the genomic DNA. Ribarstvo. 67(2): 41-52.

Naish, K.A. and D.O.F. Skibinski (1998). Tetranucleotide microsatellite loci for Indian major carp. J. Fish Biol. 53: 886-889.

Nielsen, E., L. Arvebach and P. Kotlicki (2006). Hybridlab (version 1.0): a program for generating simulated hybrids from population samples. Molec. Ecol. Notes 6: 971–973.

Porta, J., J. M. Porta, G. Martinez-Rodriguez and M.C. Alvarez (2006). Genetic structure and genetic relatedness of a hatchery stock of Senegal sole (*Solea senegalensis*) inferred by microsatellites. Aquaculture, 251: 46-55.

Queller, D.C. and K. F. Goodnight (1989). Estimating relatedness using molecular markers. Evolution 43:258-275.

Robinson, S. P., L.W. Simmons, and W. J. Kennington. (2013). Estimating relatedness and inbreeding using molecular markers and pedigrees: the effect of demographic history. Molec. Ecol. 22: 5779-5792.

Saha, D., M. Nahiduzzaman, , S. Akter, M.N., Islam, M.A.R. Hossain and M.S. Alam (2010). Bottleneck in the endangered kalibaus, *Labeo calbasu* (Cyprinidae: Cypriniformes) populations in Bangladesh revealed by microsatellite DNA marker analysis. Genes Genom. 32:47-53.

Sahu, P.K., J. K Jena, . P.C. Das, S. Mondal and R. Das (2007). Production performance of *Labeo*

calbasu (Hamilton) in polyculture with three Indian major carps *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) with provision of fertilizers, feed and periphytic substrate as varied inputs. Aquaculture, 262 (2-4): 333–339.

Tapio, I., M. Tapio, , M-H.,Li, R. Popov, Z. Ivanova and J. Kantanen (2010). Estimation of relatedness among non-pedigreed Yakutian cryo-bank bulls using molecular data: implication for conservation and breed management. Genet. Sel. Evol. 42:28

Toro, M., C. Barragan, C. Ovilo, J. Rodriganez, C. Rodriguez and L. Silio (2002). Estimation of coancestry in Iberian pigs using molecular markers. Conserv. Genet. 3:309-320.

Wang, J. (2002). An estimator of pairwise relatedness using molecular markers. Genetics 160: 1203-1215.

Wang, J. (2004). Sibship reconstruction from genetic data with typing errors. Genetics 166: 1963-1979.

Wang, J. (2011). COANCESTRY: A program for simulating, estimating and analyzing relatedness and inbreeding coefficients. Molec. Ecol. Resour. 11: 141–145.

Wang, J. and A.W. Santure (2009). Parentage and sibship inference from multi-locus genotype data under polygamy. Genetics 181: 1579-1594.