



## THE INCIDENCE OF NATURAL HYBRIDIZATION OF TWO STRAINS OF CLIMBING PEARCH *ANABAS TESTUDINEUS* (BLOCH, 1792)

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### ABSTRACT

An investigation was conducted to find out the possibilities of escaping of introduced strain of *Anabas testudineus* (commonly known as Thai Koi) from cultured ponds to nearby open waters, intra-species breeding between escaped Thai Koi and local Bangladeshi Koi, and the production of a putative hybrid from their cross breeding in nature. Among the farm owners, 92.31% admitted the incidence of escaping of cultured Thai Koi to the nearby water. Thai Koi farms were located in vulnerable places of which 53.85% were near beels and 38.46% near rivers. Farms with no fence barrier were 46.15% and 53.85% were very poorly fenced. Up to  $25.38 \pm 7.99\%$  of the total daily catch of the local anglers consisted of exotic Koi (with black spots on body). From the daily catch analysis, 17.40% of the sample specimens had exotic varieties. Morphological analysis of the spotted Koi resulted in 66.70% of fish characterizing for neither local, nor exotic, and therefore, 11.61% of the total catch was adjudged to be intra-specific putative hybrid of local and Thai Koi. In addition, several morphometric, meristic and nonparametric characteristics of local, Thai and putative hybrid Koi were found to be significantly different ( $p < 0.05$ ).

**Key words :** *Anabas testudineus*, intraspecific natural hybridization, exotic Koi, Bangladesh.

### INTRODUCTION

Interbreeding of mutually exclusive natural populations of a species is termed as intraspecific hybridization. Such populations attain considerable genetic differentiations and breeding between them often found to create heterotic effects in the offspring. The climbing perch fish, *Anabas testudineus* (Bloch, 1792), locally known as Koi in Bangladesh, is a prominent fresh water indigenous species. This commercially and nutritionally valuable species is found as different strains based on geographical localities in Bangladesh, India, Thailand, Myanmar, Sri Lanka, Nepal and other Asian countries (Talwar and Jhingran, 1991). Because of similar environmental conditions, trans-boundary movement of fishes has become a common practice in the Asian countries for aquaculture purposes (Rahman, 2005). Thai Koi was first introduced in Bangladesh in 2002 (BFRI, 2006) which is recognized as a strain of *A. testudineus*. Bleeker (1855) described this species as *A. oligolepis* based on a larger body size and absence of spots at the base of the caudal fin (Axelrod, 1974). The name *A. oligolepis* is no longer valid and *A. testudineus* is widely used for both the indigenous and Thai strains.

Strains being genetically slightly differentiated, they readily interbreed if they are brought into the same habitat conditions. Being conspecific, their mating with the local population in the nature may not be unexpected, if the exotic ones escape to the natural water bodies of the area. Thai strain can mate with

indigenous strains and can produce the intraspecific hybrids in the natural waters of Bangladesh. Biswas (2009) reported the mating and successful offspring production scenario in hatchery tank after hormonal induction. There are other records of the incidence of interspecific and intraspecific natural hybridization of various fishes (Leary *et al.*, 1983; Strauss, 1986; Hauser *et al.*, 2006). The local strain is likely to be endangered by introgression of Thai Koi gene pool if Thai strain escapes from the culture systems and reproduces in natural waters.

There has not been any attempt known yet on the study of the risk of escape of Thai Koi into natural waters and the consequent natural hybridization especially to the concern of conservation of indigenous Koi in Bangladesh. It is time now to find out the scenario of the particular species composition in the natural waters whether the local strain is being affected by the exotic strain. In the present investigation, the objectives were to find out the incidence of mixing of Thai and local Koi and the consequent intraspecific hybridization in the particular natural waters in Khulna district of Bangladesh.

### MATERIALS AND METHODS

The possibility of natural hybridization between local and Thai Koi was undertaken in the beels (natural depression) of Pipremari and Putimari in Khulna district of Bangladesh (Fig. 1). Prior to this study, the

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taxonomic characteristics of local and Thai Koi were determined from the collected specimens as proposed by (Biswas and Shah, 2009). In the present investigation two beels were selected which are very close to the Thai Koi farms.

Firstly, visit and surveys were made on 13 Koi farms in the selected regions of Khulna followed by an interview of the farmers for information of the incidence of escape of Thai Koi from the ponds into beels or rivers. Secondly, interviews and discussions were also conducted with eight local anglers/fishermen who catch Koi from those beels, especially by hook and line, net and bamboo traps, etc. Finally, the beel catches were analyzed to assess the variety of catch composition. In total 70 catches were analyzed over four months (September-December, 2010). Subsequently, analysis and identification of the 'putative hybrid' were done by comparing the taxonomic characteristics (morphometric, meristic and qualitative) of local and Thai Koi. The crossed product termed as 'putative hybrid' was determined by a limited number of parameters. To compare the morphological characters among the local, Thai and putative hybrids, one-way ANOVA (parametric test) and Kruskal-Wallis test (nonparametric) were done using SPSS 12.0 for Windows.

## RESULTS AND DISCUSSION

**Risk of escape of Thai Koi :** Most of the culture ponds of Thai koi were located in vulnerable places prone to escape to the natural waters where 53.85% were near the beel, 38.46% near the river and only 7.69% were closed protected ponds. Among the surveyed ponds 46.15% was found having no fence barrier and 53.85% having poorly netted (Fig. 2). Among the respondents Koi farmers, 92.31% informed about incidence of escape of fish up to some extent from their ponds whereas the rest of the farmers (7.69%) did not provide any evidence of escape (Fig. 3).

Preventing escape of Thai strains from culture ponds is difficult as they can easily crawl over land using their spiny opercula and tail during rainy season (Liem, 1987; Davenport and Matin, 1990; Sakurai *et al.* 1993). Rahman (2005) has also reported cases of introduction of non-indigenous fish in Bangladesh over last decades through purposeful floodplain stocking or inadvertent escape from aquaculture systems.

**Beel catch composition :** Local anglers mentioned that they assumed 25.38±7.99% of their daily total catch from open water to have consisted of black spotted Koi which is similar to Thai Koi. The local Koi is clearly distinguished from Thai ones by the absence of such black body spots (Hoq, 2006; Biswas and Shah, 2009). An intraspecific hybrid may or may not have black spots on their body surface depending on penetrance and expressivity of the gene like that in the single strain

of Thai Koi. Therefore, in substantiating hybrids from the natural waters it was crucial to distinguish between local, Thai and putative hybrids from the beel catches. Taxonomic studies, especially meristic and qualitative traits were considered for distinguishing them. During the primary screening, 17.40±19.20% of black spotted Koi (either Thai or putative hybrids) was found in beel catch (Fig. 4). As we took every daily catch, which was real amount harvested by regular anglers, into count, the unequal catch quantity and the fluctuated catch composition resulted in higher and unusual standard deviation of the number of spotted Koi, which might come when, for instance, one day we found ten catch and the other day we observed only one catch.

**Morphometric and meristics analysis :** Before morphological analysis of only spotted Koi, the local and Thai Koi were taxonomically compared and the local Koi was found without body spots whereas Koi having body spots all over the surface could be sharply categorized into ones having profuse black spots (92.3%) and having limited spots (7.7%) (Biswas and Shah, 2009). The color was yellowish at the pelvic fin region (63.3%) or whitish to yellowish (36.7%) in local Koi, but in case of Thai Koi it was only whitish (46.2%) or whitish to yellowish (53.8%). Another significant distinguishing feature observed was the number of anal spine which was 10 (9.97±0.32) in local Koi but 9 (9.08±0.39) in Thai Koi. We found only one local specimen that had anal spines <10 among 42 samples (2.38%) and on the other hand >9 anal spines were found in only two Thai specimens among 38 samples (5.26%).

From the taxonomic differences between local and Thai Koi, it is assumed that the specimens with body spots and more than nine anal spines (>9) could be primarily typed as neither Thai nor local Koi which can be termed as putative hybrid. Though with only one cross character unequivocal separation of natural hybrid cannot be strong, it could be considered as having an important basis in a cross as both the characters are not morphometric (one is qualitative and the other is meristic). Such qualitative-meristic character combination, which is heritable, is likely to distinguish hybrids more precisely. Based on this key identification, comparisons were made between local and Thai Koi with respect to other traits. Unlike Thai ones, no dense spot was found on the body surface of the putative hybrid specimens, but limited body spots were observed in 90.0% of the total sample and there were several black lines (10%) over the body surface. Color at pelvic fin base region was also mixed, such as yellowish (25%), whitish to yellowish (55%) and whitish (20%) in the putative hybrids (Table 1). The Thai Koi did not show yellowish color at pelvic fin region even in mature stage in the study period (Biswas and Shah, 2009). Therefore, the yellowish color was reckoned to may have also been inherited from local Koi in the putative

hybrids. Among these nonparametric characteristics, body spots and color at pelvic fin region were found significantly different ( $p < 0.05$ ) in the local, Thai and putative hybrid. The proportion of some morphometric characters showed significant differences among local, Thai and putative hybrids ( $p < 0.05$ ) (Table 2). Significant differences were also found among some meristic characters ( $p < 0.05$ ) (Table 3).

In the present study, through analysis of the morphological characteristics, 66.7% of the collected spotted koi from the beel catches were adjudged to be neither Thai nor local Koi. Therefore, 11.61% of the total sample (converted from spotted koi to the total catches) was calculated to be plausible natural intra-specific hybrids collected from the beels.

Though protein or DNA electrophoresis and DNA barcoding are considered to be the modern and unequivocal methods of differentiating strains or hybrids and species identification respectively (Ramaseshaiah and Dutt, 1984; Hassan *et al.*, 2005; Hauser *et al.*, 2006; Strauss (1986); Welsh and Cincotta (2004), Hebert, *et al.* (2003), and Arami, *et al.* (2011), morphological features are also used for discriminating the natural hybrids and identification of species. Thai and local Koi can naturally breed in open water, as they are the two conspecific strains occurring both in fresh and brackish water over the range of their distribution

(Davenport and Matin, 1990; Riehl and Baensch, 1991). The hybridization in traditional waters can bring introgression of genes in the endemic strains. Therefore, the risk of destruction of the gene pool of the native Koi through introgression is rather increasing in the waters of Bangladesh. Hauser *et al.* (2006) reported similar incidence in two strains of *Salmo trutta* in natural waters after getting the evidences of natural hybridization. Negative perception can be arisen in the market demand if endemic fish losses their identity. Such attitude is often seen among the local consumers in the market. Hauser *et al.* (2006) and Boyd (2008) reported such negative impact on *Salmo trutta* in U.K., and on Rainbow Trout in Australia respectively. For controlling these types of consequences strict adherence to the rules and regulations in line with the Fisheries Policy of the country is highly necessary. The present survey revealed a neglecting and violating attitudes by the Koi farmers. If this trend continues, the endemic Koi may be totally disappear in near future. The findings of this investigation may provide a significant message to the concern of endemic *A. testudineus* of Bangladesh. Though the incidence of natural infraspecific hybridization of local and Thai Koi was studied in specific areas, it could be an alarm for other parts of the country. Therefore, suggestion is made for carrying out a research applying molecular genetic technology in other natural waters over the country.

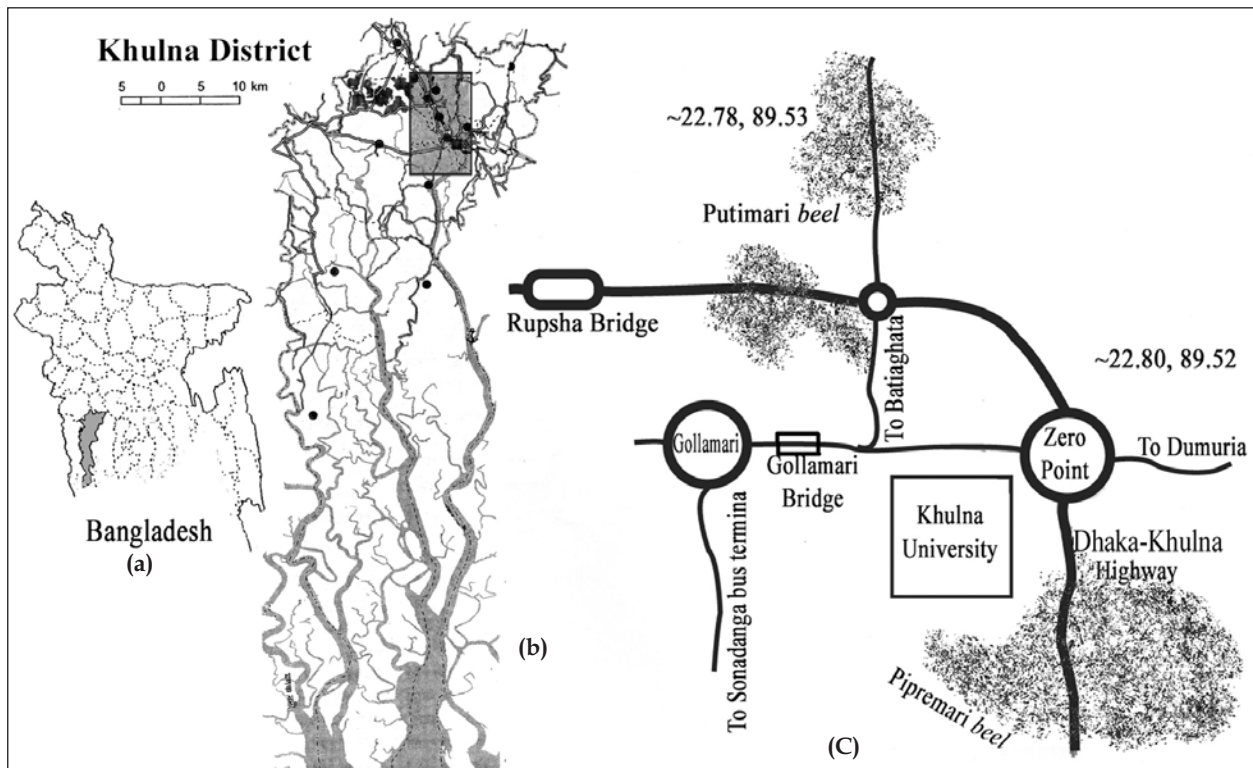


Fig. 1 : The locations of two beels surveyed for natural hybridization Thai and local koi in Khulna, Bangladesh (a = Bangladesh, b = Khulna, c = beel in cluster dots).

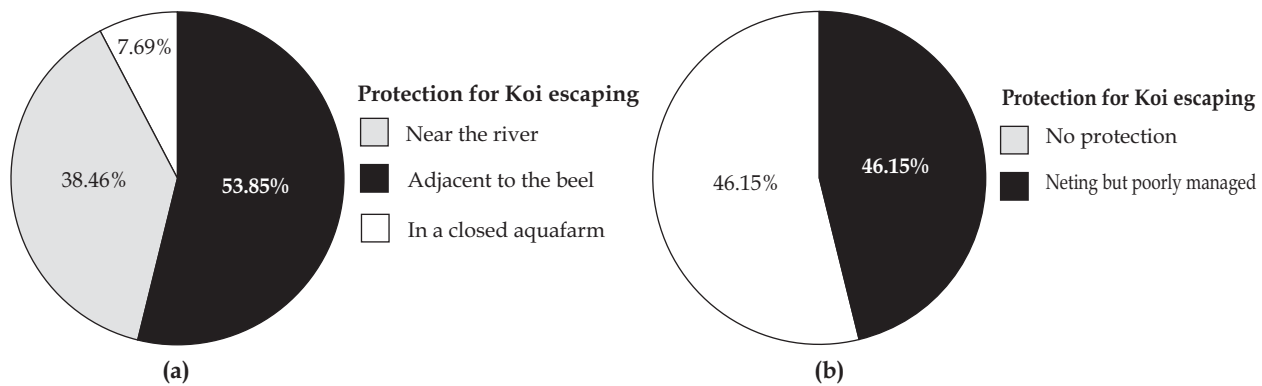


Fig. 2 : The location of Thai Koi culture pond (a) and the status of protection from escaping (b) in the local areas of Khulna.

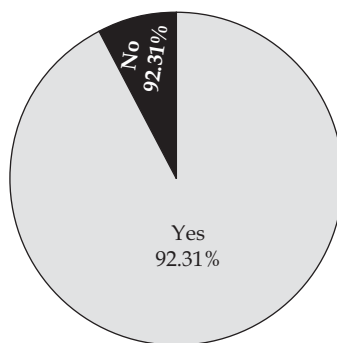


Fig. 3 : The incidence of Thai Koi escaping from the farmer's observation.

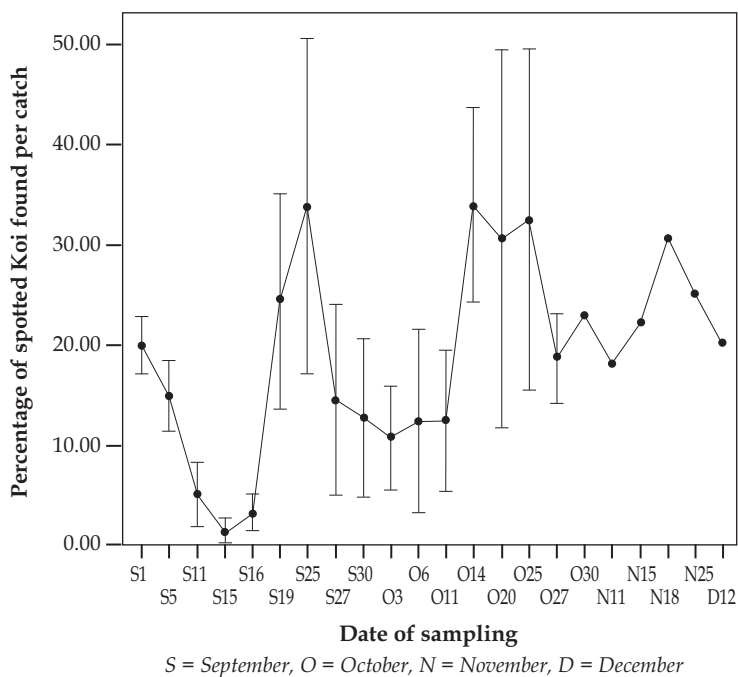


Fig. 4 : The percentage of catch of body spotted Koi.



Table 1: Comparison of nonparametric traits among the local, Thai and putative hybrid Koi.

Strains	Body spot (%)				Spot on caudal fin base (%)				Colour at pelvic fin (%)		
	Absent	Less	More	B. lines	Absent	B1	B2	A	W	W-Y	Y
Local	100	0.0	0.0	0.0	16.7	16.7	63.3	3.3	0.0	36.7	63.3
Thai	0.0	7.7	92.3	0.0	19.2	11.5	46.2	23.1	46.2	53.8	0.0
Hybrids	0.0	90.0	0.0	10.0	10.0	10.0	70.0	10.0	20.0	55.0	25.0

(B: Black, B1: Black spot at one side, B2: Black spot on both side, A: Albino, W: Whitish, Y: Yellowish)

Table 2: Comparison of morphometric proportion among the local, Thai and putative hybrid Koi.

Strains	TL:SL *	SL:HL	SL:BD *	SL:SDB *	SL:FDL *	HL:ED *	HL:SnL *
Local	1.26 ± 0.03 <sup>b</sup>	3.03 ± 0.16 <sup>ab</sup>	2.84 ± 0.24 <sup>a</sup>	2.07 ± 0.09 <sup>a</sup>	2.94 ± 0.14 <sup>a</sup>	4.19 ± 0.40 <sup>a</sup>	4.26 ± 0.35 <sup>a</sup>
Thai	1.23 ± 0.03 <sup>a</sup>	3.12 ± 0.19 <sup>b</sup>	3.27 ± 0.32 <sup>b</sup>	2.24 ± 0.21 <sup>b</sup>	3.16 ± 0.18 <sup>b</sup>	4.83 ± 0.68 <sup>b</sup>	4.94 ± 0.68 <sup>b</sup>
Hybrid	1.24 ± 0.08 <sup>a</sup>	2.99 ± 0.25 <sup>a</sup>	3.05 ± 0.40 <sup>c</sup>	2.05 ± 0.14 <sup>a</sup>	3.00 ± 0.27 <sup>a</sup>	4.43 ± 0.23 <sup>a</sup>	4.59 ± 0.44

(TL: Total length, SL: Standard length, HL: Head length, BD: Body depth, FDB: 1st dorsal fin base length, ED: Eye diameter, LnL: Snout length; \*Significantly different at 5% level; Differences in superscript letter denote significant variation ( $p < 0.05$ ) in means)

Table 3: Comparison of meristic characters among the local, Thai and putative hybrid Koi.

Strains	AS *	DS	DR *	PcR	CaR	SUL *
Local	9.97 ± 0.32 <sup>b</sup>	16.90 ± 0.61 <sup>a</sup>	8.77 ± 0.43 <sup>a</sup>	15.50 ± 0.57 <sup>a</sup>	16.40 ± 0.56 <sup>a</sup>	17.20 ± 1.06 <sup>b</sup>
Thai	9.08 ± 0.39 <sup>a</sup>	17.08 ± 0.48 <sup>a</sup>	9.69 ± 0.55 <sup>b</sup>	15.65 ± 0.89 <sup>a</sup>	16.54 ± 0.71 <sup>a</sup>	16.08 ± 0.98 <sup>a</sup>
Hybrid	10.10 ± 0.31 <sup>b</sup>	17.17 ± 0.58 <sup>a</sup>	9.50 ± 0.51 <sup>b</sup>	15.42 ± 1.24 <sup>a</sup>	16.33 ± 0.65 <sup>a</sup>	16.75 ± 1.06 <sup>b</sup>
	SLL *	SAL	SBL *			
Local	10.60 ± 0.93 <sup>a</sup>	4.00 ± 0.26 <sup>b</sup>	10.80 ± 0.76 <sup>b</sup>			
Thai	11.15 ± 1.35 <sup>ab</sup>	3.88 ± 0.33 <sup>ab</sup>	9.73 ± 0.67 <sup>a</sup>			
Hybrid	11.67 ± 0.65 <sup>b</sup>	3.75 ± 0.45 <sup>a</sup>	10.58 ± 1.83 <sup>b</sup>			

(AS: Anal spine, DS: Dorsal Spine, Dr: Dorsal fin ray, PcR: Pectoral fin ray, CaR: Caudal fin ray, SUL: Scale on upper lateral line, SLL: Scale on lower lateral line, SAL: Scale above lateral line, SBL: Scale below lateral line. \*Significantly different at 5% level; Differences in superscript letter denote significant variation ( $p < 0.05$ ) in mean)

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