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Genetic characterization of an unknown and endangered native population of the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae; Teleostei) in the Loboi Swamp (Kenya)

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ABSTRACT

Nuclear and mitochondrial DNA polymorphism were studied in a number of natural populations of the Nile tilapia *Oreochromis niloticus* (Cichlidae; Teleostei) from East Africa in order to determine the origin of a recently discovered population from a warm water spring, the Lake Bogoria Hotel Spring, an affluent of the Loboi Swamp. This population was initially considered to have been introduced from other sites within the region. Its significant and unique genetic variability (high microsatellite and mtDNA polymorphism, highly significant F_{st} values and the presence of private alleles) indicate however that it is an entirely new and formerly unknown natural population that had escaped earlier studies of this species. This natural population, that represents a significant genetic resource, is threatened by extensive human encroachment of the Loboi Swamp.

1. Introduction

The Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae; Teleostei), is a widespread species used in tropical aquaculture. Natural populations occur in Africa from Senegal to Egypt down to Tanzania. *Oreochromis niloticus* has been introduced to almost every tropical country in the world for aquaculture purposes. In this context, natural genetic resources are of great importance for improvement of aquaculture strains. The GIFT (Genetic Improvement of Farmed Tilapia) tilapia (Eknath et al., 1993; Eknath and Acosta, 1998) that originated from a mixture of natural populations (taken from Egypt, Ghana, Kenya – Lake Turkana – and Senegal) on which a strong selection program has been applied, is a good example of what can be obtained by exploiting natural genetic resources in breeding programs. Various studies have reported that the greatest proportion of genetic diversity in this species is concentrated in Rift Valley lakes and rivers of East Africa (Trewavas, 1983; Agnèse et al., 1997). Five out of the seven subspecies described by Trewavas are present in Uganda and Kenya: *O. n. niloticus* in the White Nile; *O. n. eduardianus* in Lakes Edward, Kivu, Albert and George; *O. n. vulcani* in Lake Turkana, *O. n. sugutae* in the River Suguta and *O. n. baringoensis* in Lake Baringo. East Africa is thus a potential source for development of highly diversified and regionally adapted aquaculture strains.

During a genetic survey of the various populations of *O. niloticus* present in Kenya, an unknown population was found in a warm water (36.2 °C) spring that discharges at the southern end of the Loboi Swamp system (Fig. 1). It is known as the Lake Bogoria Hotel Spring (21°N 372″, 03°E 052″ about 25 km south of Lake Baringo and 285 km north of Nairobi). The Loboi Swamp lies in the Loboi Plain within the Baringo–Bogoria half-graben (Ashley et al., 2004), a depressed block of land bordered by one major fault.

In the course of her exhaustive study of *O. niloticus* natural populations, Trewavas (1983) made no reference to the Loboi Swamp population in spite of the fact that it is relatively easy to locate. It occurs in a stream that crosses the tarmac road leading to Lake Bogoria Reserve and flows a few hundred meters into the Loboi Swamp through dense *Papyrus* and *Typha* vegetation. Initially, this population was considered to have been recently introduced from one of the already known Kenyan, Lakes Baringo or Turkana, River Suguta or even from Lake Victoria (the latter represents a non-native population introduced more than 50 years ago from Lakes Albert and Turkana) populations.

Occurrence of tilapia in hot water springs is not new. One subspecies of the Nile tilapia, *O. n. filoa* described from hot alkaline springs in the Awash system (Ethiopia) also inhabits hot pools (Trewavas, 1983). Elevated temperatures are well known to interfere with sex-ratios in fishes (Baroiller et al., 1995; Pavlidis et al., 2000;

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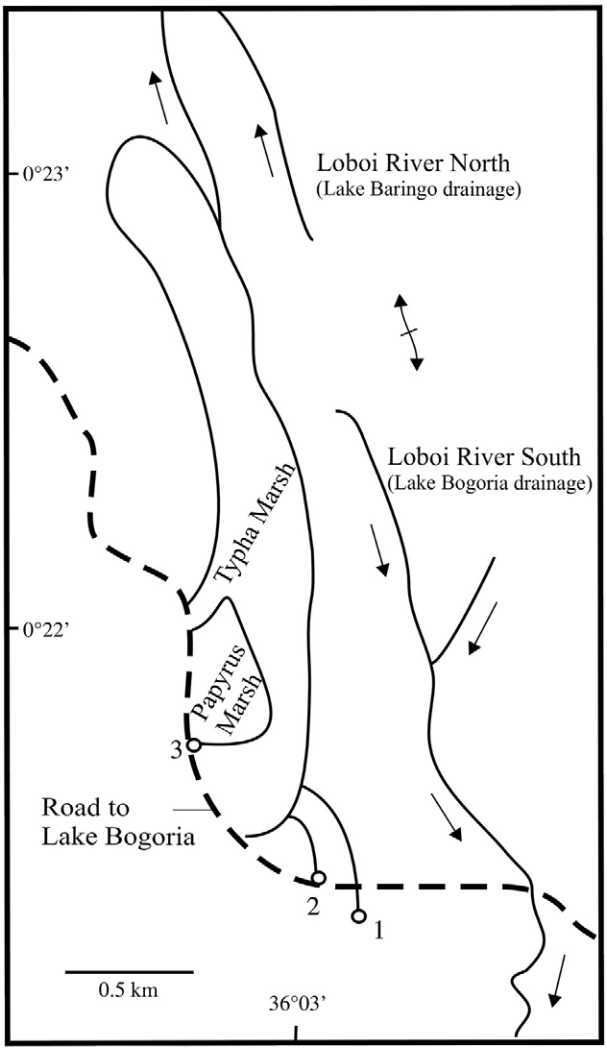

Fig. 1. Map of Loboi Swamp showing the *Papyrus* and *Typha* zones of the swamp, the Loboi River North (N) and South (S) and the springs feeding into the swamp: (1) Lake Bogoria Hotel Spring, (2) Chelaba Spring and (3) Turtle Spring.

Koumoundouros et al., 2002; Bezault et al., 2007), meristic characters (Lindsey, 1988) and shape plasticity (Georgakopoulou et al., 2007).

Effects of high temperatures on sex-ratios in tilapia have been well documented (Baroiller and D'Cotta, 2001). Temperature has been known to override the action of sex determining genes. Recently, Tessema et al. (2006) demonstrated that sensitivity of sex determination to temperature treatments is under genetic control. Sex determination in tilapia is an important research issue (Tuan et al., 1999; Mair et al., 1991) because development of monosex strains that yield higher productivity, without use of steroid hormones or hand sexing of fishes could be an important application for culture.

In this respect, the Loboi Swamp population represents an interesting natural model for the study of the effects of hot environments on sex-ratios and may be useful for understanding sex determination in the Nile tilapia. The first step is to determine the origin of this supposedly introduced population. To achieve this preliminary objective we carried out a study of genetic variation in mtDNA (sequence of partial D-loop region) and microsatellites to compare the Loboi Swamp population with the four other natural populations of *O. niloticus* in East Africa that could have been the source population.

2. Materials and methods

2.1. Sampling design

Fish specimens were captured using gill nets or scoop nets. Five populations (Table 1) were compared with the "Lake Bogoria Hotel Spring" sample from the Loboi Swamp. Four populations from Kenya and Uganda represented potential source populations that may have been introduced into the spring (Lakes Turkana, Baringo, Albert, and River Suguta). One additional sample of *O. niloticus* from River Senegal at Saint Louis (representing the subspecies *O. n. niloticus*) was used to evaluate overall levels of differentiation.

A fragment of muscle tissue was taken from each specimen and samples preserved in 95% ethanol for later DNA analyses. Where possible, voucher specimens were fixed in 4% formalin and later preserved in 70% ethanol and are presently stored at the National Museums of Kenya (NMK) in Nairobi or at the Musée Royal de l'Afrique Centrale (MRAC) in Tervuren, Belgium (Table 1).

2.2. DNA extraction, sequencing and microsatellite analysis

Total DNA was extracted using the GenElute Mammalian Genome DNA Miniprep Extraction Kit (reference G1N-350; Sigma) and samples conserved in Tris–EDTA buffer at −20 °C. A 450 bp fragment in the 5′ region of the D-loop was amplified in each sample using two primers: 5′-ACCCCTAGCTCCCAAAGCTA-3′ (forward) and 5′-CCTGAAGTAGGACCAGATG-3′ (reverse). Amplifications were performed in a final volume of 50 µl containing 0.25 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µM of each primer, 1× buffer and 10 units of *Taq* polymerase (Promega) and 5 µl of DNA in the elution buffer. Amplifications were realized as follows: pre-denaturation at 94 °C for 4 min followed by 35 cycles of denaturation–annealing–elongation (94 °C, 30 sec.; 52 °C, 1 min; 72 °C, 1 min) and a final elongation step of 72 °C for 5 min. Sequencing of the PCR products were carried out using an ABI3730 XL automatic DNA sequencer (Applied Biosystems) following the manufacturer recommendations.

The six nuclear microsatellite loci analyzed here had previously been employed to study a selective sweep event in *Sarotherodon melanotheron*, a brackish water tilapia (Agnèse et al., 2009. Four poly AC repeat loci namely *UNH860* (GenBank number G68195), *UNH887* (G68210), *UNH874* (G68202) and *UNH1003* (G68280) amplified according to the specifications of Carleton et al. (2002). The other two loci *Prl1AC* and *Prl1GT* are located in the promoter region of the Prolactin I gene (Swennen et al., 1992). Primers used for *PRL1AC* were 5′-CGTGTCTTGTGGGGAAA-3′ forward and 5′-CATTCCTGTTGCATCCATTCA-3′ reverse. Primers used for *PRL1GT* were 5′-GTTAGCCCCCTCCTCACTCC-3′ forward and 5′-CAGGTGT-GACGAGCAAGGT-3′ reverse. PCR amplifications were performed using identical protocols for all loci: a final volume of 10 µl consisting of 0.25 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µM of each primer, 1× buffer, 10 units of *Taq* polymerase and 1 µl DNA). PCR conditions were as follow: 3 min at 94 °C then 35 cycles of denaturing–annealing (91 °C, 30 s and 55 °C, 30 s) and a final elongation step at 72 °C for 5 min. Microsatellite polymorphisms were screened by migration through 6% polyacrylamide gels using an ALF sequencer (Pharmacia, LKB) and allele size determined by comparison with migration of two size markers, one larger and another smaller than the target microsatellites.

2.3. Data analysis

2.3.1. Sequence analyses

Sequences were aligned manually using BioEdit 5.09 (Hall, 1999). Genetic diversity estimates were computed using DnaSP version 4.00.5 (Rozas et al., 2003) and involved analysis of π, the average number of nucleotide differences per site between two sequences (Nei, 1987, equations 10.5 or 10.6), and k the average number of nucleotide difference between sequences (Tajima, 1983; equation

Table 1
Origin of samples analyzed (basin and population), subspecies (following Trewavas, 1983).

Basin	Population	Subspecies	GenBank no	Voucher specimens
L. Turkana	North Island	*O. n. vulcani*	EF016681 to EF016696	NMK-1639/ 1–11 NMK-1641/1–13
L. Albert	Butiaba Bay	*O. n. eduardianus*	FJ440577 to FJ440587 EF016671 to EF016679	NMK-1384/1–11
L. Baringo	Robertson Camp	*O. n. baringoensis*	FJ440604 to FJ440607 EF016697 to EF016708	95-027-P-0074-0084 (MRAC)
R. Suguta	Kapedo	*O. sugutae*	FJ440608 to FJ440610 EF016710 to EF016714	
Loboi Swamp	Lake Bogoria Hotel Spring		FJ440588 to FJ440603	NMK-1798/1–6
River Senegal	Saint Louis	*O. n. niloticus*	FJ440611 to FJ440619 EF016715 to EF016723	

Voucher specimens when available are currated at the National Museums of Kenya (NMK), or at the Musée Royal d'Afrique Centrale de Tervuren (MRAC).

A3). Aligned sequences were analyzed independently using maximum parsimony (MP), maximum likelihood (ML) and distance methods (DM). ML and DM tests were carried out in MEGA3 (Molecular Evolutionary Genetics Analysis) version 3.0 (Kumar et al., 2004), MP with PHYLIP 3.57 (Felsenstein, 1993). Bootstrap analysis (1000 replicates) was used (for ML, MP, and DM) to assess the relative robustness of branches (Felsenstein, 1985). Pair-wise sequence divergences between unique mtDNA haplotypes were calculated using the Kimura two-parameter model (Kimura, 1980). Consensus trees were obtained using PHYLIP 3.57 (Felsenstein, 1993).

A phylogenetic network was constructed by means of a median-joining network algorithm (Bandelt et al., 1999), as implemented in the Network program version 4.1.0 (available at http://www.fluxus-engineering.com/sharenet.htm).

2.3.2. Genotype analyses

Microsatellite data were checked for scoring errors due to stuttering or large allele dropout and presence of null alleles, using Micro-Checker software (Van Oosterhout et al., 2004). Intra-population genetic variability was measured by estimating observed heterozygosity (H_{obs}) and expected heterozygosity (H_{exp}) following Hardy–Weinberg proportions under the panmictic hypothesis. Both were carried out using GENETIX software version 4.02 (Belkhir et al., 2004) according to Nei's unbiased estimate (Nei, 1978). Linkage disequilibrium was tested using GENEPOP version 3.4 (Raymond and Rousset, 1995). Genetic differentiation among populations were estimated from Wright's F-statistics as proposed by Weir and Cockerham (1984), F_{st} and associated probabilities were estimated using a Markov chain method as provided in GENEPOP version 3.4 (Raymond and Rousset, 1995). The length of the Markov chain involved a burn in period of 1000 iterations and 100 batches of 1000 iterations thereafter. Bonferonni adjustments (Rice, 1989) were used to detect significant effects with an overall Type I error rate set at 0.05. A neighbour joining tree was constructed using un-weighted pair group method (Reynolds et al., 1983) using Populations 1.2.28 software available at the CNRS UPR9034 website www.cnrs-gif.fr/pge. One thousand replications were run to test the confidence of the topology of the resulting phylogenetic tree.

3. Results

3.1. MtDNA haplotypes

Twenty seven unique haplotypes were identified among the 79 partial D-Loop sequences assessed (GenBank accession numbers are given in Table 1). All sampled populations were polymorphic except for River Senegal where only a single haplotype was present in the 20 specimens examined there.

Sample size (n), number of observed haplotypes (Hob), number of polymorphic sites (p), average number of nucleotide differences (k) and nucleotide diversity (π) are presented in Table 2. Of the 27 haplotypes detected, 24 were unique to specific sample localities: 10 were only observed in Lake Turkana (total number of haplotypes sequenced $n=18$), two in Lake Albert ($n=20$), two in River Suguta ($n=10$), three in Lake Baringo ($n=16$), six in Lake Bogoria Hotel Spring ($n=16$) and only a single haplotype in River Senegal ($n=18$). The Lake Turkana population contained the highest number of haplotypes ($n=11$). High nucleotide diversity was observed for Lake Baringo ($p=37, k=16.527, \pi=0.049$) compared with the other samples (values never exceed $p=15$, $k=3.54$, $\pi=0.00595$). This result is congruent with Nyingi and Agnèse (2007). This study demonstrated that *O. niloticus* from Lake Baringo had been partially introgressed by mtDNA from *O. leucostictus* (Trewavas, 1983), although this introgression was apparently not accompanied by any nuclear gene transfer. Nyingi and Agnèse (2007) concluded that haplotypes of both species were now present in the *O. niloticus* population in Lake Baringo.

Phylogenetic relationships among all haplotypes observed based on MP, ML or MD methods were congruent. Fig. 2 presents a consensus NJ tree where bootstrap values obtained with MP and ML have been indicated. All haplotypes observed, except two, the haplotype from River Senegal and one haplotype from Lake Baringo, clustered in a single highly supported group (bootstrap value = 100). The single haplotype from River Senegal was highly differentiated from all others. This result is congruent with that of Rognon and Guyomard (2003). They reported that *O. niloticus* from West Africa (from R. Senegal to the Nile) has been introgressed with mtDNA from *O. aureus*. The haplotype observed in the River Senegal sample corresponds to this alien haplotype. The haplotypes from *O. niloticus* from Lake Baringo mentioned above, introgressed by *O. leucostictus* (Nyingi and Agnèse, 2007) also did not cluster with other Nile tilapia haplotypes.

Table 2
Genetic variability observed in 6 populations of *O. niloticus* based on the partial sequences of mtDNA control region: LBHS, Lake Bogoria Hotel Spring; n , sample size, Hob, number of different haplotypes observed — unique or private haplotypes are in brackets; p, number of polymorphic sites; k, average number of nucleotide differences and π, nucleotide diversity.

Population	n	Hob	p	k	π
1. Lake Tukana	18	11(10)	15	2.147	0.00595
2. Lake Albert	20	4(2)	3	0.300	0.00082
3. Lake Baringo	16	5(2)	37	16.527	0.04578
4. River Suguta	10	4(2)	6	2.000	0.00551
5. LBHS	16	7(6)	14	3.542	0.00976
6. River Senegal	18	1(1)	0	0.000	0.00000

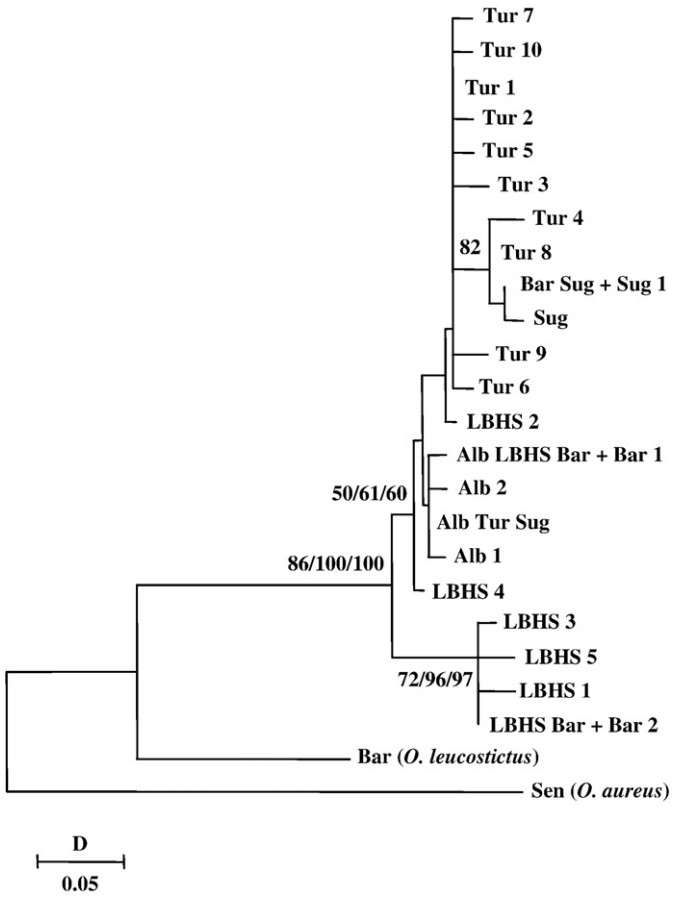

Fig. 2. NJ tree (based on a Kimura2 distance matrix) representing the phylogenetic relationships of the different D-Loop haplotypes of *O. niloticus* observed. Tur, Lake Turkana; Bar, Lake Baringo; Sug, River Suguta; LBHS, Lake Bogoria Hotel Spring; Alb, Lake Albert; Sen, River Senegal. Haplotypes Bar Sug (common to the Baring and Suguta samples) and Sug 1 only differed by a deletion and thus are considered as identical when computing the k2 distance. Haplotypes Alb LBHS Bar and Bar 1 on one hand and LBHS Bar and Bar 2 on the other hand were in the same situation. Numbers represent bootstrap values (1000 replicates) for ML/MP/DM respectively.

Of the seven haplotypes observed in the Lake Bogoria Hotel Spring population, one was shared with Lakes Baringo and Albert, but all others were unique.

Fig. 3 describes the relationships among all original East African haplotypes (mtDNA from Lake Baringo and River Senegal that originated from introgression were not represented) using a median-joining network (Bandelt et al., 1999). Haplotypes generally clustered according to their geographical origin. All 11 haplotypes

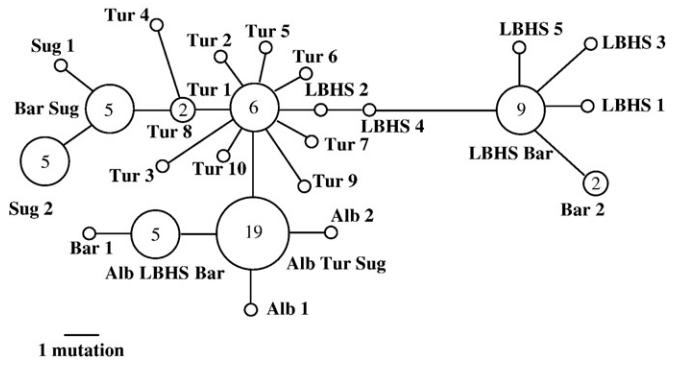

Fig. 3. Median-joining network among mtDNA haplotypes observed for non introgressed East African samples of *O. niloticus* only. The size of each circle is proportional to the haplotype frequency, abbreviations are the same as Fig. 2.

found in Lake Turkana grouped together. The same relationships were observed for Lake Albert and River Suguta haplotypes (only one haplotype clustered with Lake Albert and Turkana). Haplotypes from the Lake Bogoria Hotel Spring also clustered together except for a single haplotype that was shared with Lakes Baringo and Albert. Only haplotypes found at Lake Baringo clustered with multiple sites, with some clustering with the River Suguta haplotypes and others with Lake Albert or Lake Bogoria Hotel Spring.

For any given lake or river site, the most common haplotype present commonly occupied a central position in the network. This is seen commonly when there is a single ancestral haplotype and a few derived ones at a site. Reflecting this pattern, haplotypes Tur 6, AlbTurSug and Lob 6 are likely to be the ancestral haplotypes in Lake Turkana, Lake Albert and Lake Bogoria Hotel Spring populations, respectively.

3.2. Microsatellites analysis

156 specimens from six populations were screened for variation at six microsatellite loci. All loci analyzed were polymorphic in all populations with number of alleles ranging from 2 to 21. Locus *UNH887* was the least polymorphic with only 15 alleles, while locus *Prl1AC* was the most polymorphic with a total of 30 alleles. No scoring error was detected in the data due to either stuttering or large allele dropout using Micro-Checker. As expected, only PRL1GT and PRL1AC were found to be in linkage disequilibrium in an overall population comparison. The only highly significant result was for Lake Turkana. The most polymorphic population was Lake Turkana with 95 alleles while the least polymorphic was the Lake Baringo population with only 25 different alleles. Expected heterozygosities ranged from 0.198 (River Senegal, locus *UNH860*) to 0.924 (Lake Turkana, locus *UNH874*). The total range of F_{is} values showed many heterozygote deficiencies with 18 out of 36 (50%) being statistically significant ($P<0.05$) (Table 3) after Bonferonni correction. Using Micro-Checker, therefore, presence of null alleles could not be rejected. The relatively high number of private alleles observed is congruent with the highly statistically significant pair-wise F_{st} values recorded between population pairs that were all highly significant (Table 4).

A factorial correspondence analysis plot showed that individual genotypes were clustered into relatively distinct groups corresponding with the different populations (Fig. 4). The Senegal population was clearly differentiated from all East African populations. Among these, the Lake Bogoria Hotel Spring individuals were much more scattered, indicating higher nuclear differentiation among specimens. In contrast, the Lake Turkana genotypes were less dispersed even though this sample exhibited the highest gene diversity (95 alleles). This resulted from the fact that most alleles in this population occurred at low frequencies: (65.26%, *i.e.* 62/95 having frequencies <0.05) leading to lower individual differentiation in comparison with the Lake Bogoria Hotel Spring sample where only 5 from the 37 alleles observed (13.5%) were present at frequencies lower than 0.05.

4. Discussion

4.1. Genetic characterisation and origin of the Lake Bogoria Hotel Spring population

F_{is} values for many samples and loci showed heterozygote deficits (Table 3) that can best be attributed to either Wahlund's effect (Wahlund, 1928), presence of null alleles or high inbreeding rates.

The Wahlund's effect is a heterozygote deficit observed when two populations in Hardy–Weinberg (HW) equilibrium but with different allele frequencies are mixed. The new population resulting from this mixture in most cases no longer approaches HW equilibrium. This only concerns the first generation (at the moment the population

Table 3
Heterozygosity (observed and expected) and F_{is} values observed in the 6 samples of *O. niloticus*.

LOCUS		Lake Turkana	Lake Albert	Lake Baringo	Lake Bogoria Hotel Spring	River Suguta	River Senegal
	n	44	48	30	20	10	20
UNH860	H_{exp}	0.9056	0.3153	0.2594	0.3642	0.3642	0.1975
	H_{obs}	0.9070	0.2051	0.2333	0.2222	0.2222	0.0000
	F_{is}	0.010	**0.361**	0.117	**0.929**	0.439	**1.000**
	P	0.8308	0.0038	0.5045	0.0000	0.3412	0.0026
	A	21 (14)	5 (0)	2 (0)	7 (2)	3(0)	2(0)
UNH874	H_{exp}	0.9237	0.8861	0.8431	0.7245	0.7245	0.8421
	H_{obs}	0.8261	0.7143	0.3929	0.5714	0.5714	0.6842
	F_{is}	0.117	**0.208**	0.484	**0.456**	0.284	**0.213**
	P	0.1022	0.0047	0.547	0.0076	0.0778	0.0028
	A	20(3)	14(0)	6(0)	5(3)	5(1)	12(1)
UNH887	H_{exp}	0.7286	0.6911	0.6280	0.6633	0.6633	0.3750
	H_{obs}	0.7234	0.6889	0.1154	0.8571	0.8571	0.5000
	F_{is}	0.018	0.014	**0.660**	**0.128**	**−0.220**	−0.310
	P	0.2656	0.1558	0	0.0096	0.0103	0.2772
	A	8(0)	10(2)	3(0)	4(2)	4(0)	2(1)
UNH1003	H_{exp}	0.6998	0.6723	0.8472	0.7700	0.7700	0.3364
	H_{obs}	0.6190	0.5000	0.3600	0.4000	0.4000	0.1667
	F_{is}	0.127	**0.266**	**0.357**	0.240	**0.520**	**0.526**
	P	0.3497	0.0123	0	0.3537	0.0023	0.0428
	A	12(5)	8(2)	4(0)	2(0)	5(1)	4(4)
Prl1GT	H_{exp}	0.7200	0.5444	0.8691	0.6600	0.6600	0.5150
	H_{obs}	0.7021	0.5500	0.6538	0.5000	0.5000	0.1000
	F_{is}	0.036	0.002	0.163	**0.354**	0.291	**0.815**
	P	0.3397	0.622	0.266	0.0009	0.2043	0
	A	13(4)	13(4)	5(0)	7(2)	5(0)	3(1)
Prl1AC	H_{exp}	0.8990	0.8731	0.6816	0.8333	0.8333	0.8244
	H_{obs}	0.8000	0.8000	0.3200	0.3333	0.3333	0.8667
	F_{is}	**0.121**	0.096	0.625	**0.336**	**0.655**	−0.017
	P	0.0063	0.4442	0.3200	0.0015	0.0017	0.172
	A	21(6)	14(0)	5(0)	12(2)	7(0)	8(3)

n (number of specimens studied), H_{obs} (observed heterozygosity), H_{exp} (expected heterozygosity), F_{is} (coefficient of inbreeding), *P* (associate F_{is} probability), *A* (number of alleles observed in the sample with the number of private allele in brackets). Values in bold are statistically significant after Bonferonni correction (Rice 1989).

mixed). If the new (mixed) population survives and interbreeds at random, then, any subsequent populations will immediately return to HW equilibrium. If a Wahlund's effect is observed this can be either a transitory effect or may persist if individuals from the two original populations do not mate at random but practice assortative mating (this could be possible in case of different homing localities for example). On one hand it is difficult to consider that for most of the samples studied here that are not at HW equilibrium, we mainly observed transitory structures. On the other hand, in the present study, some localities sampled represent "small pools" in which it is difficult to imagine that more than one population (at least two differentiated populations) can co-exist. For example a Wahlund's effect is hardly possible in small basins such as Lake Baringo or River Suguta. The Lake Baringo is a shallow basin with a diameter of only 5 km, and the Suguta River itself is reduced in the dry season to a relatively short length (few kilometres even few hundred meters near the spring that gave rise to the river) and thus genetically different populations are unlikely to co-exist there.

Table 4
Matrix of pair-wise F_{st} measured between the six samples studied.

	Turkana	Albert	Baringo	LBHS	Suguta
Albert	0.139				
Baringo	0.202	0.235			
LBHS	0.204	0.290	0.246		
Suguta	0.127	0.223	0.246	0.243	
Senegal	0.277	0.328	0.375	0.351	0.365

LBHS, Lake Bogoria Hotel Spring. All the observed values were highly statistically significant ($P<0.001$).

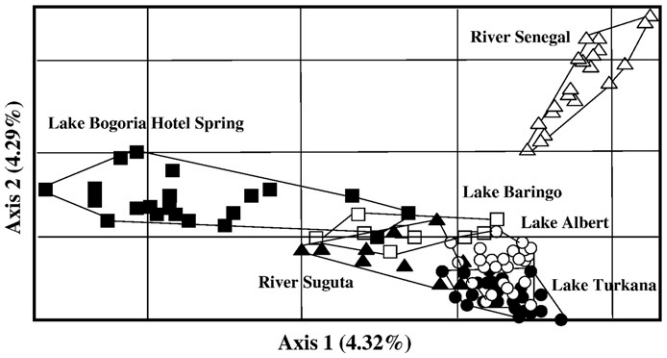

Fig. 4. Factorial correspondence analysis plot of microsatellite genotypes of *O. niloticus*.

Presence of null alleles can also explain the observed homozygotes excesses. Results obtained using Micro-Checker indicate that their occurrence is a possibility but, if null alleles were present they should have been evident at most loci among most populations. This would have been likely if for example, primers had been designed from one subspecies and failed to amplify some alleles (produce nulls) in others. This possibility is unlikely as the six microsatellite loci studied here have been employed successfully on *Sarotherodon melanotheron* (Agnèse et al., 2009) a related tilapia species.

In contrast, observed heterozygote deficits are more likely due to non-random sampling. Shoaling among tilapia species has been demonstrated in adult black-chinned tilapia (*Sarotherodon melanotheron* Rüppell 1852). Shoals observed within lagoons are usually composed of adult related individuals that chose to mate among themselves (Pouyaud et al., 1999). Similar deficits in heterozygotes have also been observed for *O. niloticus* by Bezault (2005), who demonstrated that they were due to presence of kin relationships among individuals in some fish shoals.

Measures of inter-population differentiation using F_{st} (Table 4) indicate that the Lake Bogoria Hotel Spring population was differentiated from all other populations of *O. niloticus* sampled in the region. This population was characterised by 10 private alleles and five haplotypes that were unique to this population. Both extent of mitochondrial and microsatellite differentiation were in the range of those observed among other naturally occurring discrete populations in the region (Lakes Baringo, Turkana and River Suguta). These observations did not correspond with what would have been expected if the Lake Bogoria Hotel Spring population had resulted from an "exotic" introduction. An introduced stock would likely show low polymorphism (resulting from a probable population bottleneck) and an absence (or very low number) of private alleles and unique haplotypes. Instead, nuclear and mitochondrial polymorphisms observed in the Lake Bogoria Hotel Spring population showed unique patterns of variation (compared with neighbouring populations). These observations suggest the high likelihood that the Lake Bogoria Hotel Spring population did not originate from an introduction from other neighbouring East African natural populations.

Another possibility is that the Lake Bogoria Hotel Spring population may have originated from a mixture of one regional *O. niloticus* population with another tilapiine species. This hypothesis results from the breadth of genotypes observed in the Lake Bogoria Hotel Springs (Fig. 4). This hypothesis is unlikely to be true however considering the number and nature of the different private haplotypes observed (Fig. 2). Under an "alien" origin hypothesis, private haplotypes would not be closely related to naturally occurring haplotypes in *O. niloticus* in the region. In the present case, the five private haplotypes found in the Lake Bogoria Hotel Spring population were closely related to other haplotypes observed in geographically closely related populations of *O. niloticus*. The most likely hypothesis therefore, is that the Lake Bogoria Hotel Spring population is native to the swamp.

It is curious, however, that Trewavas (1983) did not report this population considering its current relative accessibility. Possible reasons may be that at the time of her study (late 1970s), the road to the Lake Bogoria Reserve did not cross the spring at the same spot as it does today, at this time it was only a track (the Lake Bogoria reserve was only established in 1973 and the tarmac road even much later). In addition, it is possible that the spring may have been obscured from view at the time by extensive macrophytes. The area is a lot more open today since the burning and harvesting of *Papyrus* and *Typha* is common for roofing of houses and many local areas have been cleared for farming (Ashley et al., 2004).

4.2. How can we explain the occurrence of a second native and differentiated population in the Lake Baringo basin?

The fact that the Lake Bogoria Hotel Spring population is genetically distinct from the Lake Baringo population indicates that a barrier has prevented gene flow between the two populations at some stage. In order to explain the existence of two genetically differentiated *O. niloticus* populations in the Lake Baringo Basin, it is necessary to consider the history of the Loboi Swamp. Sediment studies by Ashley et al. (2004) indicated that the Loboi Swamp area was a poorly drained floodplain during the late Holocene. The origin of the swamp dates back to a period of higher rainfall in East Africa, the Little Ice Age (~700 BP) which led to higher lake levels in most East African lakes (Mohammed et al., 1995; Verschuren et al., 2000) and simultaneously to the establishment and expansion of the Loboi Swamp in the Loboi Plain (Ashley et al., 2004). The River Loboi (North) is the principal outlet from the swamp towards the Lake Baringo system while the River Loboi (South) divides off from the eastern side of the swamp from where it has been diverted for irrigation and flows now to the northern end of Lake Bogoria, (Fig. 1) an alkaline (pH>10) lake (Harper et al., 2003).

The swamp presents a physical barrier that may inhibit fish movement but in addition, it may constitute a chemical barrier. Ashley et al. (2004) noted that while the chemical composition of the Loboi River was similar to that of other major springs and groundwater discharges into the swamp, its composition at the outlet from the swamp was highly depleted of dissolved oxygen (~4% saturated D.O. in comparison to ~60% in the springs and groundwater discharges) due to high oxygen consumption as a result of aerobic decomposition of detritus from macrophytes in the swamp. It is, therefore, highly likely that the conditions within the swamp may not be suitable for tilapiine fish.

4.3. Period of isolation

With the observation that the Lake Bogoria Hotel Spring *O. niloticus* population has probably been isolated from the Lake Baringo *O. niloticus* population by the swamp for the last 700 years, it is appropriate to determine if the extent of genetic differentiation between the two populations reflects this time frame. If we consider the private haplotypes observed in the Bogoria Hotel Spring population, four of them are closely related (Fig. 3). Of which, it is highly likely that haplotype Lob 6 is ancestral to the others (Lob 1, Lob 3 and Lob 5). According to this hypothesis, this differentiation occurred after the two populations became isolated. Differences observed between these haplotypes varied from 0.27 % to 0.54% which following the evolutionary rate of mtDNA used by Rognon and Guyomard (2003) corresponds with a minimum isolation period of 96,000 to 150,000 years (using 5.6% or 3.6% of divergence per million of years). Whatever error range may be associated with these estimations, it is obvious that the extent of differentiation between haplotypes observed within the Lake Bogoria Hotel Spring is not compatible with a recent (700 years) origin for this population. If dating of the swamp formation is reasonably accurate, we may conclude that the existence of the spring preceded that of the swamp and that isolation of the Lake Bogoria Hotel Spring population was therefore much older than the origin of the swamp itself. According to this hypothesis, it is likely that the Lake Bogoria Hotel Spring population evolved before the swamp flowed into Lake Bogoria and not into Lake Baringo. The Loboi Swamp region has been shown to have been exposed to continuous perturbations that have caused changes in the drainage course of the Loboi River due to local tectonic movement (Owen et al., 2004).

4.4. The Lake Bogoria Hotel Spring population constitutes a new and endangered natural genetic O. niloticus resource

The utility of natural genetic resources of *O. niloticus* in aquaculture of this species has been demonstrated following the development of a synthetic strain based on a crossbreeding of different natural populations from Rivers Senegal, Volta, Nile and Lake Turkana (Eknath et al., 1993). This tilapia strain referred to as GIFT tilapia, has been the subject of a major family selection program and is now well known and established in Asia where it offers improved culture performance over other cultured *O. niloticus* stocks (Eknath and Acosta, 1998).

The Lake Bogoria Hotel Spring population therefore constitutes a new genetic resource for future breeding programs whose value has not been considered so far. This population offers new opportunities because it lives in relatively high temperatures (approx. 36 °C) and may have developed hypoxic resistance mechanisms as dissolved oxygen levels are generally low in hot waters. Special adaptation may also be present to regulate the sex-ratio since sex determination is known to be influenced by high temperatures (Baroiller and D'Cotta, 2001; Tessema et al., 2006) and hence potentially may offer a model for the study of sex determination in tilapiine fishes.

Our study involved sampling from only a single spring in the Loboi drainage, it did reveal a novel population. A further study should be carried out that expands this to other microhabitats in the Loboi system (springs, swamp and river) in order to determine the geographical extent of this population and perhaps identify others and then to characterise them both genetically and morphologically. At least two other springs should be investigated, the Chelaba Spring that is close to the Lake Bogoria Hotel Spring and the Turtle Spring that is located at the border of the papyrus marsh (Fig. 1). Such a study is crucial and urgent since these populations are threatened by both environmental and anthropogenic factors. The Loboi Swamp itself has receded in size by about 60% over a short period of 30 years due to expansion of irrigation via a ditch constructed in 1970 (Ashley et al., 2004; Owen et al., 2004). In addition, periodic avulsion events have caused changes to the courses of rivers in this region. The most recent was during the El-Nino induced heavy rains of 1997 that caused changes to the course of the Loboi River. The river that used to feed Lake Baringo partially changed its course and now also flows to Lake Bogoria. Changes in flow were also influenced by intensive encroachment by local farmers leading to weakening of stream banks (Harper et al., 2003, Owen et al., 2004).

An additional threat to local aquatic fauna is recent expansion of aquaculture. The Lake Bogoria human population (mainly composed of farmers) has started to diversify their livelihoods by construction of earthen ponds along the rivers and streams in the region. Fish farmers are now breeding different tilapia species (Nyingi and Agnèse, 2007) that could potentially hybridize naturally with or even replace the native population of *O. niloticus* like it has been reported in Lake Victoria where native species have disappeared.

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