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RESEARCH ARTICLE

Genetic structure and gene flow in an endangered native tilapia fish (*Oreochromis esculentus*) compared to invasive Nile tilapia (*Oreochromis niloticus*) in Yala swamp, East Africa

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Abstract The introduction of invasive Nile tilapia (*Oreochromis niloticus*), and the rapacious predator Nile perch (*Lates niloticus*), into Lake Victoria resulted in a decline in population sizes, genetic diversity and even extirpation of native species which were previously the mainstay of local fisheries. However, remnant populations of native fish species, including tilapia, still persist in satellite lakes around Lake Victoria where they may coexist with *O. niloticus*. In this study we assessed population genetic structure, diversity, and integrity of the native critically endangered Singidia tilapia (*O. esculentus*) in its refugial populations in the Yala swamp, Kenya, and contrasted this diversity with populations of the invasive tilapia *O. niloticus* in satellite lakes (Kanyaboli, Namboyo and Sare) and Lake Victoria. Based on mtDNA control region sequences and eight nuclear microsatellite loci, we did not detect any mtDNA introgression between the native and the invasive species in Lakes Kanyaboli and Namboyo, but did find low levels of nuclear admixture, primarily from *O. niloticus* to *O. esculentus*. Some genetic signal of *O. esculentus* in *O. niloticus* was found in Lake Sare, where *O. esculentus* is not found, suggesting it has recently been extirpated by the *O. niloticus* invasion. In both species, populations in the satellite lakes are significantly genetically isolated from each other, with private mtDNA haplotypes and microsatellite alleles. For *O. niloticus*, genetic diversity in satellite lakes was similar to that found in Lake Victoria. Our data imply a low frequency of immigration exchange between the two populations of *O. esculentus* and we suggest that the populations of this endangered species and important fisheries resource should be conserved separately in Lakes Kanyaboli and Namboyo and with high priority.

Keywords Admixture · Lake Victoria basin · Nile tilapia · Population genetic structure · Singidia tilapia · Yala swamp

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Introduction

The collapse of native fisheries and population declines of endemic species caused by purposeful fish introductions to Africa's largest lake, Lake Victoria, is one of the most famous biodiversity disasters (Kaufman 1992; Pringle 2005). First introduced sometime between the 1920s and 1960s, by the 1980s two invasive species dominated the Lake Victoria fishery: the Nile perch *Lates niloticus* (L.) and the Nile tilapia *Oreochromis niloticus* (L.) (Balirwa et al. 2003; Pringle 2005). These exotic species have dramatically altered the original ecosystem and consequently led to the extinction of hundreds of indigenous Lake Victoria fish species, including native tilapia species (Ogutu-Ohwayo 1990; Witte et al. 1992a, 1992b; Lowe-McConnell 2000; Goudswaard et al. 2002; Balirwa et al. 2003). For example, prior to the invasion of *Nile perch* and *O. niloticus*, the Singidia tilapia *O. esculentus* Graham was

the most important fisheries species in Lake Victoria (Balirwa et al. 2003). Today, *O. esculentus* has disappeared from Lake Victoria (Twongo 1995; Aloo 2003), dramatically declined throughout the region, and is now classified as a "critically endangered" species on the IUCN Red List (Twongo et al. 2006).

Remnant populations of the native tilapia species *O. esculentus* still persist in satellite water bodies in the Lake Victoria catchment (Loiselle 1996; Aloo 2003). Such peripheral lakes are thought to play an important role in conservation and speciation of fishes in the Africa Rift Valley (e.g. Mwanja et al. 2001; Abila et al. 2004; Genner et al. 2007). Formed through cycles of drying and refilling characteristic of Lake Victoria's geology history (Bishop and Trendall 1966), satellite lakes function as contemporary refugia and therefore have special significance for conservation of Lake Victoria's fauna (Ogutu-Ohwayo 1990; Kaufman and Ochumba 1993; Maithya 1998; Mwanja et al. 2001). For example, some recent studies of fishes in satellite Lake Kanyaboli indicated that it may conserve cichlid species richness and genetic diversity that is threatened or extirpated in Lake Victoria proper (Kaufman and Ochumba 1993; Aloo 2003; Abila et al. 2008).

Kenya's largest freshwater wetland, the Yala swamp, covers about 175 km^2 and houses three satellite lakes: Kanyaboli, Namboyo and Sare (Fig. 1). The Yala swamp formed in the Pleistocene, when water level changes and river flow reversals characteristic of that era (Johnson et al. 1996; Elmer et al. 2009) infilled former lakes with silt, slowed water flows, and allowed the establishment of rooted plants and reeds and eventually a swamp ecosystem (Aloo 2003). The swamp hosts numerous fish species, though generally at low abundance because dissolved oxygen is limiting (Aloo 2003; Abila et al. 2004).

The invasive *O. niloticus* has managed to establish itself in all the satellite lakes in the Yala swamp (Aloo 2003; Jembe et al. 2006). Ecological, genetic and physiological characteristics make *O. niloticus* well-suited to commercial fisheries and also a successful invader of ecosystems throughout the tropical and subtropical world (Canonico et al. 2005). They are omnivorous, have a high fecundity and a long life span, and grow large and quickly, and therefore can out compete native fish from feeding and breeding grounds (Ogutu-Ohwayo 1990; Lowe-McConnell 2000; Goudswaard et al. 2002). Further, *O. niloticus* is a more flexible and efficient algae feeder than *O. esculentus*, which likely contributes to its competitive success (Batjakas et al. 1997). Invasive *O. niloticus* also often hybridize with the native tilapia species in the natural environment, further displacing endemic species (Agnèse et al. 1998; Canonico et al. 2005; D'Amato et al. 2007).

Using random amplified polymorphic DNA (RAPD) as a molecular marker, Mwanja and Kaufman (1995) detected hybrids of *O. niloticus* and *O. esculentus* from satellite lakes near Lake Victoria. The authors suggested that no pure stocks of *O. esculentus* remain in the satellite lakes (Mwanja and Kaufman 1995). However, based on variation in three microsatellite and 24 allozyme loci, Agnèse et al.

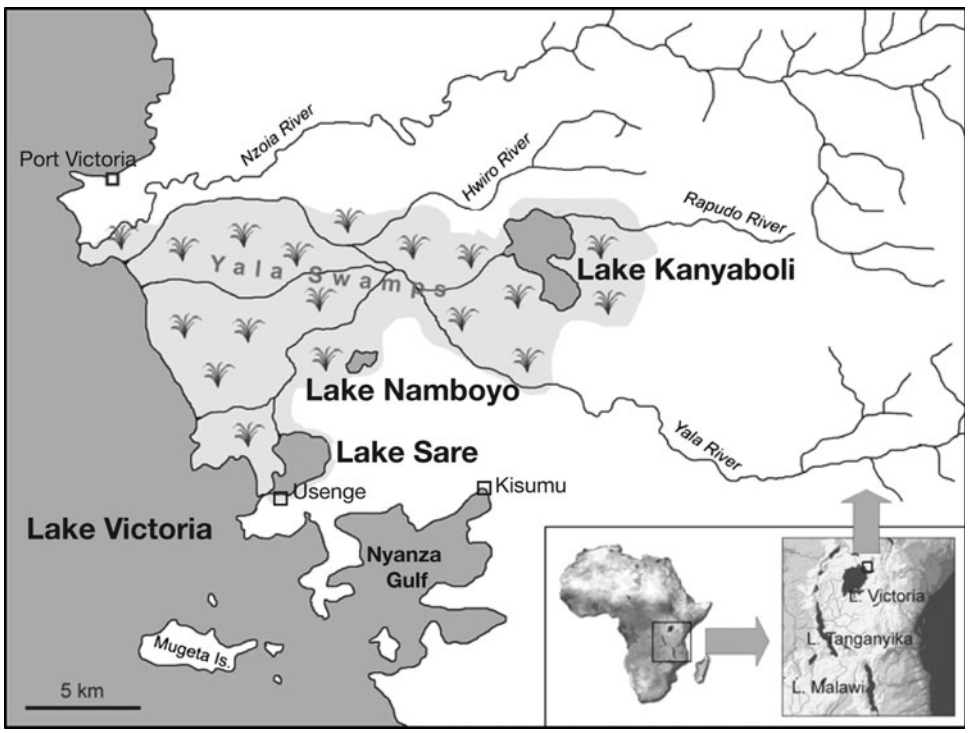

Fig. 1 A map of Yala swamp showing the locations of Lake Victoria including the Nyanza Gulf (0°30′S, 35°15′E), and the three satellite lakes, Lakes Kanyaboli (00°04′30″N, 34°09′36″E), Namboyo (00°00′23″N, 34°05′09″E) and Sare (00°02′36″S, 34°03′32″E) (from Abila et al. 2004)

(1999) suggested "purity" of *O. esculentus* refugial populations in Lake Kanyaboli. Knowledge of the genetic make up of *O. esculentus* and the population genetic structure of *O. niloticus* will be crucial for conservation and management of the endangered *O. esculentus* (O' Connell and Wright 1997; Okumus and Çiftci 2003; Mwanja 2004). Further, the identification of genetically diverse populations may provide knowledge on extant variability and characteristics associated with differences in life-history traits of the native tilapia species, which may be important for fisheries sustainability (Canonico et al. 2005).

The objective of the present study was to quantify levels of extant genetic diversity and connectivity among refugial populations of the native, endangered *O. esculentus* and to contrast this with the invasive species, *O. niloticus*. Additionally, we aimed to identify populations of *O. esculentus* that have not received genetic introgression from *O. niloticus* and could be the focus of future conservation and management efforts of this critically endangered African fish species.

Materials and methods

The study area

The study was carried out in the Nyanza Gulf, the eastern-most portion of Lake Victoria, as well as in three satellite lakes near Lake Victoria in Kenya (Fig. 1). Nyanza Gulf has an area of approximately 1,920 km^2 in a total north-south length of approximately 60 km, and is shallow, with a mean depth of only 6 m. Lake Victoria samples of *O. niloticus* were obtained within the Nyanza Gulf (0°30′S, 35°15′E), but *O. esculentus* could not be collected there (see below). The Yala swamp (0°00′S–0°30′S, 34°30′E–35°15′E, 1,134 m above sea level) lies immediately to the east of the Gulf and is bordered to the north by the Nzoia River and to the south by the Yala River (Fig. 1). Three main satellite lakes are found in the Yala swamp: Kanyaboli, Namboyo, and Sare.

Lake Kanyaboli (00°04′30″N, 34°09′36″E; 10.5 km^2; average depth: 2.5 m; maximum depth: 4.5 m) is the largest of the satellite lakes and the most remote from Lake Victoria (Crafter et al. 1992; Fig. 1). It is separated from Lake Victoria by extensive papyrus swamps that seem to inhibit faunal exchanges between the two lakes. It has average dissolved oxygen level of 7.3 mg O_2 l^{-1} (Aloo 2003). Native *O. esculentus* coexists with introduced *O. niloticus* in this lake and population size of *O. esculentus* was found to be high (an average catch per canoe of 26 kg; Opiyo 1991). No records of Nile perch have been documented in Lake Kanyaboli corroborating that it has been isolated from Lake Victoria at least since the 1950s.

Lake Sare (00°02′36″S, 34°03′32″E) is continuous with, and discharges its water directly into, Lake Victoria (Fig. 1). Lake Sare is about 5 km^2 in area, 5 m deep at its centre, and has average dissolved oxygen level of 8.1 mg O_2 l^{-1} (Aloo 2003). This lake no longer houses *O. esculentus*, probably due to predation pressure by Nile perch, which is established there (Aloo 2003).

Lake Namboyo (00°00′23″N, 34°05′09″E) is located between lakes Kanyaboli and Sare (Fig. 1). It is a small lake of about 0.01 km^2 with a depth of about 17 m, and has an average dissolved oxygen level of 4.8 mg O_2 l^{-1}. *Oreochromis esculentus* coexists with *O. niloticus* in the lake and Nile perch is not present (Aloo 2003).

Sample collection

We collected adult fish samples (minimum body length of both species: 15 cm) of *O. niloticus* and *O. esculentus* in February 2009 by gill netting. *Oreochromis niloticus* were collected from Lake Victoria and the three satellite lakes (Fig. 1). *Oreochromis esculentus* samples were only collected from two satellite lakes, Kanyaboli and Namboyo (Fig. 1), because the species is extirpated from Lake Victoria and Lake Sare (Aloo 2003; P.O. Angienda, personal observation). Thirty individuals of each species were collected from each lake.

The collected specimens comprised of approximately equal ratio of male and female for both species at each sampling site (*O. niloticus*: Lake Victoria—male:female = 13:17, Lake Kanyaboli—16:14, Lake Namboyo—15:15, Lake Sare—12:18; *O. esculentus*: Lake Kanyaboli—15:15, Lake Namboyo—14:16). Sexing was conducted by visual inspection of the genital papillae area of every sampled fish in the field. We confirmed that all the fishes were fully developed enough to sex them precisely (P.O. Angienda, personal observation). The two species were readily identified in the field based on morphology: *O. esculentus* are distinguished from *O. niloticus* by being smaller in body size with small heads and whitish coloured ventrally and reddish coloured dorsally (Fig. 2). It is almost impossible to identify hybrids between the species based on morphology (Lowe-McConnell 2000).

Fin clips were obtained for genetic analysis and immediately preserved in 95% ethanol. Genomic DNA was extracted by sodium chloride and ethanol precipitation method following proteinase *K* (10 mg/ml) digestion (Bruford et al. 1998).

Mitochondrial DNA sequencing

Approximately 850 bp of mitochondrial DNA control region was amplified using primers L-Pro-F (Meyer et al. 1994) and 12S5R (5′ GGC GGA TAC TTG CAT GT 3′).

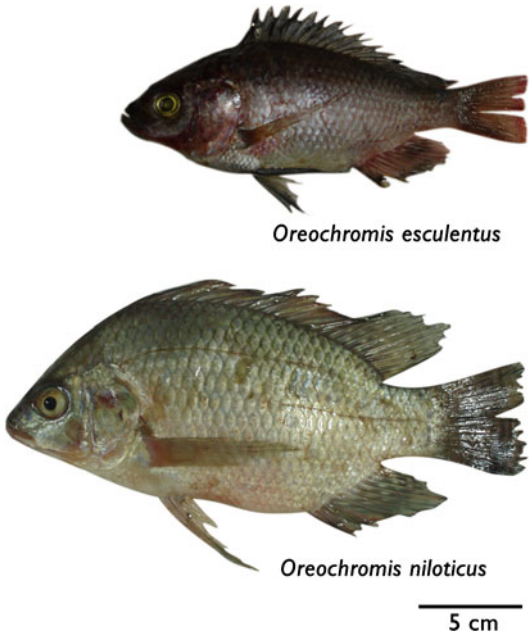

Fig. 2 Micrograph of *O. niloticus* and *O. esculentus* shows the differences in shape, colour and size of the two study species

We chose the control region because it has high mutation accumulation that is useful for resolution of the population structure of a species. PCR amplification was performed in a reaction volume of 20 µl, which comprised 1X PCR buffer, 25 µM of each dNTP, 0.5 µM of each of the forward and reverse primers, 0.1 U *Taq* polymerase (Genaxxon) and 100–200 ng of DNA template. The following thermal conditions were used: an initial denaturation phase at 94°C for 5 min followed by 35 cycles with a denaturation phase at 94°C for 30 s, an annealing phase at 49–58°C for 30 s, an extension phase at 72°C for 90 s, followed by a final extension phase at 72°C for 10 min in Perkin Elmer GeneAmp PCR 9700 (Norwalk, CT). After size confirmation by gel electrophoresis, amplified PCR products were purified enzymatically with Exonuclease I (Fermentas) and FastAP (Shrimp Alkaline Phosphatase, Fermentas) following manufacturer's directions. The purified mtDNA fragments were subject to direct sequencing in the forward and reverse directions using the same forward and reverse primers as in the PCR and the BigDye Terminator 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All reactions for DNA sequencing were run on an ABI 3130*xl* automated DNA sequencer (Applied Biosystems). Forward and reverse sequences were assembled in SEQUENCHER version 4.2.2., edited using CHROMAS version 2.0 computer software and aligned in CLUSTAL W version 1.83 (Thompson et al. 1994). Sequences are publicly available under GenBank accession numbers HQ152986-HQ153030.

Microsatellite genotyping

We used primers that were originally developed for constructing a genetic linkage map for the African cichlid fish species, *Astatotilapia burtoni* (Sanetra et al. 2009). Eight polymorphic nuclear microsatellite loci were chosen from eight different linkage groups from Sanetra et al. (2009): Abur30, Abur51, Abur110, Abur28, Abur4, Abur18, Abur25 and Abur41. Forward primers were labeled with a fluorescent dye (6-FAM, HEX or NED). Reaction were carried out in 20 µl volumes which comprises 1X PCR buffer, 25 µM of each dNTP, 0.5 µM of each of the forward and reverse primers, 0.1 U *Taq* polymerase (Genaxxon) and 100–200 ng of DNA using the same PCR conditions as for mtDNA amplification (annealing temperature of 55°C). PCR products were diluted in formamide HiDi and electrophoresed in an ABI 3130xl automated sequencer. Fragment sizes were compared to ROX 500 bp size standard (ABI) as determined using GENOTYPER software (Applied Biosystems).

Statistical analyses

Mitochondrial DNA control region

To determine levels of extant genetic diversity in *O. esculentus* and *O. niloticus*, the number of polymorphic sites, number of mtDNA haplotypes, haplotype diversity (h) and nucleotide diversity (π) were calculated for each population as well as for the entire pooled population of each species using ARLEQUIN version 3.01 (Excoffier et al. 2005). TCS version 1.21 (Clement et al. 2000), which utilizes the statistical parsimony method described in Templeton et al. (1992), was used to construct the haplotype network to investigate the phylogenetic relationships among the mtDNA haplotypes of each species. Deletion mutations were treated as a fifth state. Ambiguous connections in the haplotype network generated by TCS were resolved following the criteria outlined in Crandall and Templeton (1993).

To examine intra- and inter-specific genetic differentiation between populations, exact tests for population differentiation (Raymond and Rousset 1995) as well as calculation of pair-wise estimates of F_{ST} (Weir and Cockerham 1984) were carried out using ARLEQUIN. The 95% significance levels for pair-wise intra- and inter-specific population comparisons were adjusted using a Bonferroni correction.

Microsatellites

Analysis with MICRO-CHECKER (van Oosterhout et al. 2004) was performed to check against errors due to null

alleles, drop out and stutter using the Brookfield's (1996) Eq. (1) at 95% confidence level. To assess the microsatellite diversity in both species, the number of alleles per locus (N_a), observed (H_O) and expected heterozygosity (H_E) were calculated using GENEPOP version 4.0 (Rousset 2008). Multilocus tests for Hardy–Weinberg Equilibrium (HWE), F_{IS} estimates, and intra- and inter-specific population genetic differentiation were also performed in GENEPOP. Genetic variation was analyzed in two dimensional individual based factorial correspondence analyses (FCA) in GENETIX version 4.03 (Belkhir et al. 1996).

Genetic admixture between *O. niloticus* and *O. esculentus* at microsatellite loci was assessed using an individual-based Bayesian cluster approach as implemented in STRUCTURE version 2.3.1 under a model of admixed ancestry among populations and correlated allele frequencies (Pritchard et al. 2000; Falush et al. 2003) assuming two populations ($K = 2$). The MCMC was run for 500,000 generations to estimate Q after 200,000 generations were discarded as burn-in. A 90% probability interval around Q was used for individual admixture values. We ran the analysis three independent times to check for convergence on similar values. The three runs arrived at identical values of ln ($P|D$) ($\pm \leq 0.1$).

Results

Genetic diversity: mtDNA

Sixty individuals of *O. esculentus* sampled from two satellite lakes contained eight different haplotypes (Fig. 3 *lower*). Four haplotypes were unique to Lake Namboyo and three haplotypes were unique to Lake Kanyaboli. Only one haplotype was shared between localities. Lake Namboyo had higher haplotype (0.816) and nucleotide (0.004) diversity than Lake Kanyaboli (Table 1).

One hundred twenty *O. niloticus* sampled from three satellite lakes and Lake Victoria contained 37 different mtDNA haplotypes (Fig. 3 *upper*). Haplotype diversity for *O. niloticus* was highest in Lake Victoria (0.829) and slightly

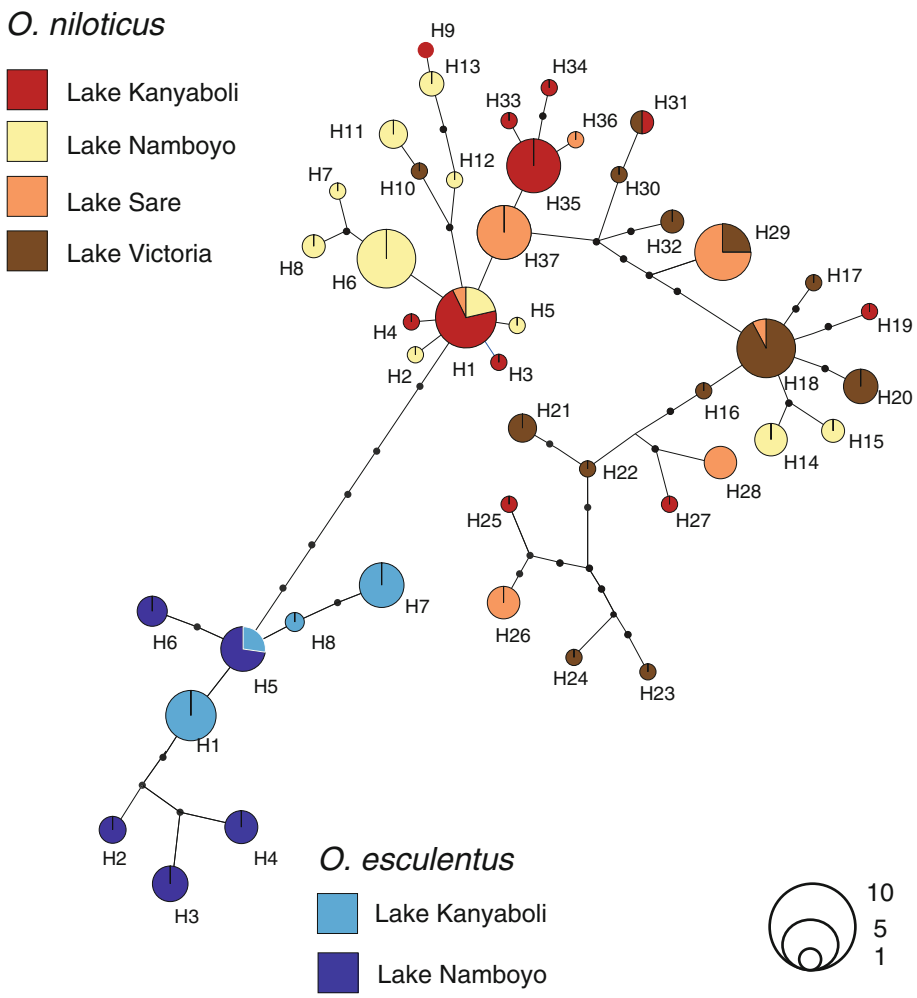

Fig. 3 Haplotype networks of *O. niloticus* and *O. esculentus* based on the mtDNA control region (850 bp). Each node in the network represents a single mutational step between haplotypes. Small black circles indicate intermediate haplotypes that are not present in our samples, but are inferred mutations in these networks

lower in each of the three satellite lakes (0.77–0.8; Table 1). Nucleotide diversity for *O. niloticus* was 0.008 for the Lake Victoria population and ranged from 0.005 to 0.012 in satellite lake populations.

The haplotype networks of *O. niloticus* and *O. esculentus* could be connected by six mutational steps (*O. niloticus*-H1 to *O. esculentus*-H5) and no haplotypes were shared between the two species (Fig. 3).

Genetic diversity: microsatellites

All populations of both species were in Hardy–Weinberg equilibrium for the loci sampled, except for two populations of *O. niloticus* (Table 1), which may be non-panmictic. *Oreochromis niloticus* showed low presence of null alleles in Abur18 (8%) in Lake Victoria and Abur25 (11%) in Lake Kanyaboli. *Oreochromis esculentus* also showed low level of null alleles in Abur18 in Lake Namboyo (9%). All other populations and loci showed no evidence of null alleles. The low presence of null alleles and lack of consistency across populations and loci means all loci are suitable for subsequent analyses (Dakin and Avise 2004).

Levels of allelic richness and heterozygosity were similar across populations and species. The number of alleles per locus ranged from 6 to 13 in populations of *O. esculentus* and 4–15 in populations of *O. niloticus*. For *O. esculentus*, the population in Lake Kanyaboli had higher H_O (0.81 ± 0.209) than in Lake Namboyo (0.795 ± 0.11) while *O. niloticus* had the highest mean observed heterozygosity (H_O) in Lake Namboyo (0.783 ± 0.146; Table 1). Contrary to expectation, heterozygosity and allelic richness were not much higher for the *O. niloticus* population in Lake Victoria than in the satellite lakes where population sizes should be smaller.

Similar to the results of the mtDNA control region, all populations of both species had private alleles (i.e. alleles that were found in only one population; Allendorf and Luikart 2007). The highest number of private alleles was observed in Lake Namboyo for *O. niloticus* (12) while for *O. esculentus* Lake Kanyaboli had a higher number of private alleles (5) than Lake Namboyo (2) (Fig. 4).

Population differentiation

For mtDNA and nuclear markers, population differentiation was highly significant between all pairs of populations within and between species (Table 2). F_{ST} values for intraspecific comparisons of mtDNA among the four *O. niloticus* populations ranged from 0.164 to 0.221 and 0.244 for between the two *O. esculentus* populations. F_{ST} values for mtDNA among populations between species ranged from 0.177 to 0.287 (Table 2).

Although F_{ST} values for microsatellites showed highly significant population differentiation in both species, they were always lower than mtDNA and ranged from 0.02 to 0.069 among the four *O. niloticus* populations and was 0.057 between the two *O. esculentus* populations (Table 2).

Admixture between invasive and native species

Factorial correspondence analysis (FCA) of eight microsatellite genotypes revealed two distinct genetic groups

Table 1 Summary of the level of genetic diversity in four *O. niloticus* and two *O. esculentus* populations at both mtDNA control region and eight microsatellite loci

	Species	*O. niloticus*				*O. esculentus*	
	Lake	L. Victoria	L. Namboyo	L. Kanyaboli	L. Sare	L. Namboyo	L. Kanyaboli
mtDNA	*N*	30	30	30	30	30	30
	Number of haplotypes	13	11	11	7	5	4
	No. of polymorphic sites	27	24	30	35	7	4
	Haplotype diversity (*h*)	0.829	0.8	0.77	0.77	0.816	0.655
	Nucleotide diversity (π)	0.008	0.005	0.006	0.012	0.004	0.002
Microsatellites	*N*	24	24	24	24	24	24
	N_a	7.625 (±2.134)	7 (±3.505)	8.375 (±2.875)	7.25 (±1.982)	7.125 (±3.137)	8.125 (±4.155)
	H-W tests (*P*)	0.386 (±0.022)	0.368 (±0.024)	**0.000 (±0.000)**	**0.000 (±0.000)**	0.305 (±0.020)	0.476 (±0.029)
	H_E	0.733 (±0.116)	0.713 (±0.133)	0.788 (±0.077)	0.734 (±0.135)	0.745 (±0.104)	0.771 (±0.129)
	H_O	0.768 (±0.138)	0.783 (±0.146)	0.726 (±0.145)	0.697 (±0.114)	0.795 (±0.110)	0.810 (±0.209)
	F_{IS}	−0.0504	−0.1012	0.0810	0.0497	−0.0720	−0.0514

N sample sizes, N_a observed mean number of alleles across eight loci, *H-W tests* (*P*) *P* values for multilocus tests for Hardy–Weinberg Equilibrium (HWE), H_E mean expected heterozygosity, H_O mean observed heterozygosity, F_{IS} inbreeding coefficient. Bold denotes significant departures from HWE

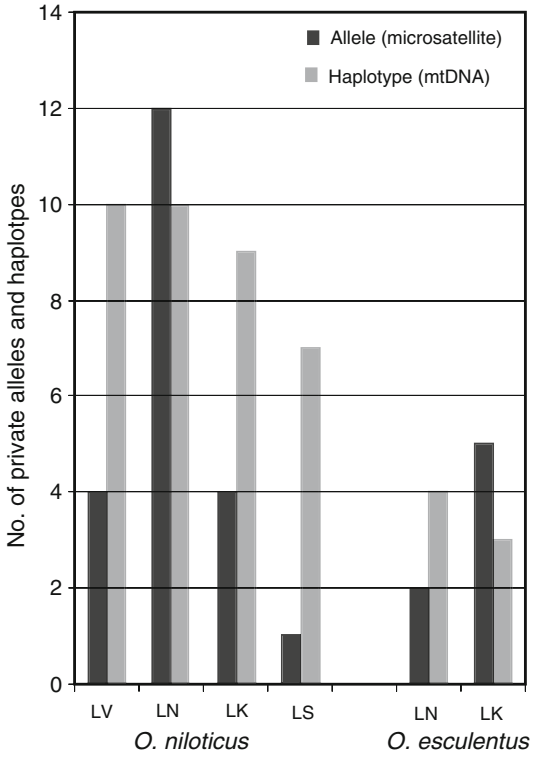

Fig. 4 A number of private microsatellite alleles and private mtDNA haplotypes present in four lakes and two lakes of *O. niloticus* and *O. esculentus*, respectively. *LV* Lake Victoria, *LN* Lake Namboyo, *LK* Lake Kanyaboli, *LS* Lake Sare

representing the two species and separated along the *x*-axis (6.83%). The *y*-axis (3.51%) separated individual microsatellite genotypes within populations (Fig. 5).

Structure analyses that were used to detect the proportion of nuclear genetic admixture between *O. niloticus* and *O. esculentus* showed no hybrid genotypes (i.e. no individuals for which Q values were $\sim$0.5; Fig. 6). Instead, most individuals were 'purely' representative of one or the other species (i.e. Q close to 1 for *O. niloticus* and Q close to 0 for *O. esculentus*). Six individuals of *O. esculentus* had 90% probability intervals that extend more than 30% out of a pure species Q value, which suggests a degree of genetic

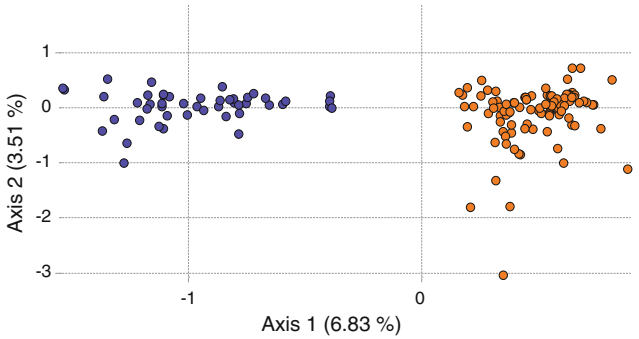

Fig. 5 Factorial correspondence analysis of microsatellite allele variation in *O. niloticus* (*black*) and *O. esculentus* (*grey*) shows that the two species are distinct and hold similar amounts of genetic variation

introgression from *O. niloticus* into those individuals (Q ranges from 0.09 to 0.34 $\pm$ probability interval). These six individuals of *O. esculentus* had three different *O. esculentus* mtDNA haplotypes (H1, H5 and H7). Two individuals of *O. niloticus* showed some admixture ($Q = 0.78$ and 0.95 $\pm$ probability interval) and had two different *O. niloticus* mtDNA haplotypes (H17 and H36). Population level admixture was very low and indicated genetic isolation of the two species. Both populations of *O. esculentus* were >97% membership to the *O. esculentus* genetic cluster for microsatellite loci and had no *O. niloticus* mtDNA haplotypes. All four populations of *O. niloticus* were >98% characteristic of the *O. niloticus* genetic grouping and had no *O. esculentus* mtDNA haplotypes.

Discussion

Satellite lakes as refugia for endemic fishes

The cichlid fish species flock in Lake Victoria experienced one of the worst mass extinctions of the 20th century (Barel et al. 1985). Lake Victoria alone originally contained over 500 indigenous species of cichlids. Mainly due to anthropogenic influences, particularly the introduction of exotic

Table 2 Population differentiation (i.e. F_{ST} values) between four populations of *O. niloticus* (*O. n;* in *normal font*), two populations of *O. esculentus* (*O. e*; in *bold*), and six populations of both species (in *italics*) from mtDNA control region sequences (below diagonal) and microsatellite loci genotypes (above diagonal)

	Victoria (*O. n*)	Namboyo (*O. n*)	Kanyaboli (*O. n*)	Sare (*O. n*)	Kanyaboli (*O. e*)	L. Namboyo (*O. e*)
Victoria (*O. n*)	–	0.051	0.069	0.05	*0.147*	*0.174*
Namboyo (*O. n*)	0.185	–	0.02	0.03	*0.137*	*0.163*
Kanyaboli (*O. n*)	0.199	0.188	–	0.034	*0.122*	*0.142*
Sare (*O. n*)	0.164	0.212	0.221	–	*0.151*	*0.165*
Kanyaboli (*O. e*)	*0.257*	*0.272*	*0.287*	*0.287*	–	**0.057**
Namboyo (*O. e*)	*0.177*	*0.192*	*0.207*	*0.207*	**0.244**	–

All pairwise comparisons from mtDNA and microsatellites were significantly differentiated ($P < 0.001$)

species such as Nile perch and *O. niloticus*, hundreds of endemic species, including the native tilapia *O. esculentus*, went extinct (Twongo 1995; Balirwa et al. 2003). Recent studies on satellite lakes in the Lake Victoria region have lead to the discovery of fish species richness and genetic diversity previously not sampled from Lake Victoria (Chapman et al. 2002; Mwanja 2004; Abila et al. 2004, 2008). This demonstrates that satellite lakes and other small water reservoirs surrounding Lake Victoria are playing a critical role in the evolution and conservation of the region's ichthyofauna in this era of anthropogenically induced extinction. Primarily this occurs by isolated habitats with ecological conditions different from Lake Victoria (Chapman et al. 2002; Aloo 2003) and that are not yet invaded by aggressive introduced species. Thus, these surrounding small waterbodies act as refugia during the contemporary biodiversity crisis in a manner analogous to their historical role as refugia during the late Pleistocene desiccation of Lake Victoria (Abila et al. 2008; Elmer et al. 2009; present study).

Our study assessed the genetic integrity and population structure of the endangered *O. esculentus* in its known refugium, the Yala swamp. We identified some low-level nuclear genetic admixture from *O. niloticus* to *O. esculentus*. Nonetheless, *O. esculentus* retains a strong spatially isolated population structure between satellite lakes and we found no introgression of non-native mtDNA. Therefore we argue that these refugial habitats should be given high priority for conservation of this native tilapia species. We discuss this in detail below.

Genetic structure and diversity of endangered tilapia *O. esculentus*

For the native critically endangered species *O. esculentus*, our genetic analyses revealed two discrete populations based on microsatellite and mtDNA data. Nuclear genetic differentiation was low (~6%) but statistically significant between lakes. MtDNA differentiation was higher and also statistically significant between populations, and only one haplotype was shared between populations of the satellite lakes. This is indicative of low gene flow between the two satellite lakes Kanyaboli and Namboyo. These are the only two lakes in the Yala swamp which still contain native tilapia *O. esculentus*, which have been extirpated from Lake Victoria proper.

This lack of gene flow is most likely due to physical isolation between the two populations of *O. esculentus*, which are separated by approximately 10 km of wetland and papyrus swamps. In addition, the eco-physiological properties of these two lakes are different, which could also limit gene flow between the two populations by local adaptation or physiological avoidance (Chapman et al. 2002; Crispo and Chapman 2008). Lake Namboyo has more dilute waters with lower dissolved oxygen content relative to Lake Kanyaboli because there is no mixing between Lake Namboyo and the main swamp (Aloo 2003). Lake Namboyo is also much deeper than Lake Kanyaboli and thus contains a different proportion of littoral habitat. Tellingly, the fish species composition of these two lakes is quite different, which also indicates habitat isolation: Lake Kanyaboli is home to more than ten species, including three native tilapia species and invasive *O. niloticus*; Lake Namboyo houses only four species, including two native tilapias (Aloo 2003).

Each population of *O. esculentus* had private mtDNA haplotypes and microsatellite alleles. Despite the fact that Lake Namboyo is much smaller than Kanyaboli and native tilapias are in low abundance in Lake Namboyo relative to Kanyaboli (Aloo 2003), *O. esculentus* from Lake Namboyo showed higher haplotype diversity than the population from Lake Kanyaboli. Fishing pressure has been shown to reduce genetic diversity in other fish species (Hauser et al. 2002) and heavy fishing in Lake Kanyaboli (Aloo 2003) may be reducing its genetic diversity. However, microsatellite allelic richness in Lake Kanyaboli was higher than in Lake Namboyo. Overall microsatellite polymorphism in both satellite lakes is rather high (Table 1). Unfortunately comparisons of genetic diversity in satellite lakes versus Lake Victoria proper was not possible because

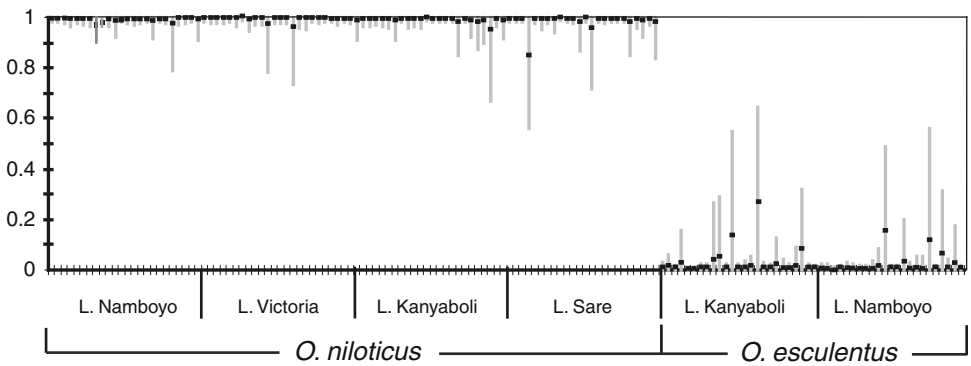

Fig. 6 The distribution of individual membership coefficients ($Q \pm 90\%$ probability intervals) of *O. niloticus* and *O. esculentus* genotypes in the lakes identified through microsatellite analyses. Each sample along the *x* axis represents an individual. There is a slightly greater level of admixture in *O. esculentus*

O. esculentus has been extirpated from that habitat (Twongo 1995; Aloo 2003). Nevertheless, remnant populations of *O. esculentus* still exist in sympatry with the introduced *O. niloticus* in the satellite lakes, perhaps because of the absence of Nile perch there (Aloo 2003).

Population genetics of invasive tilapia *O. niloticus*

The invasive tilapia *O. niloticus* was also highly genetically differentiated among all the populations at mtDNA and microsatellite loci. Our study identified four genetically distinct populations in the three satellite lakes and Lake Victoria. Both mitochondrial DNA and microsatellite markers revealed private haplotypes and alleles that were restricted to each of the four lakes. As with *O. esculentus*, the genetic structuring of the introduced *O. niloticus* is most likely caused by the fact that the four lakes are geographically isolated from one another, thus preventing movement of individuals between populations and thereby diminishing gene flow. Introduction of *O. niloticus* into the Lake Victoria basin began as early as 1924 (Trewavas 1983). However, widespread introduction of this species has been predominant during the last half century (Beauchamp 1958; EAFFRO 1964). Thus, our data suggest that the relatively high mtDNA diversity in *O. niloticus* (Table 1) could reflect multiple and widespread introductions of this species into the lakes. This pattern of high genetic diversity driven by multiple and successive introductions has also been found in the recently introduced populations of cichlid fish species, *Cynotilapia afra* in the Lake Malawi National Park (Zidana et al. 2009). Our data also suggest that duration of $\sim$60 or 90 years could be sufficiently long enough for the introduced populations to have genetically diverged from one another via genetic drift. The strong founder effects during colonization into new habitats may also contribute to the substantial genetic differentiation among the populations. This population differentiation caused by strong genetic drift has been shown in recently established European populations of the invasive Chinese mitten crab (*Eriocheir sinensis*) in only a few generations (Herborg et al. 2007).

Satellite lakes tended to have slightly lower genetic diversity and allelic richness at microsatellite markers than Lake Victoria, probably due to population bottlenecks, and/or smaller effective population sizes (Nei et al. 1975). Nonetheless, the difference in genetic diversity between Lake Victoria and the satellite lakes was not substantial. Further, mtDNA nucleotide diversity was not generally higher in Lake Victoria than in the satellite lakes though haplotype diversity was found to be (Table 1). This concurs with previous similar studies on haplochromine cichlids (Abila et al. 2004, 2008) that found similar levels of genetic diversity in satellite lakes as in Lake Victoria, although supposedly larger population sizes for Lake Victoria would have suggested that it should have a higher genetic diversity (Ewens 1972). Mwanja et al. (2001) also concluded that measures of diversity varied as to whether they were higher or lower in satellite lakes relative to Lake Victoria, depending on what types of molecular markers were being analyzed.

Admixture between invasive *O. niloticus* and endangered native *O. esculentus*

Mitochondrial and nuclear markers indicated that the two tilapia species remain highly, though not completely, genetically distinct. However, there was indication of low-level asymmetric nuclear introgression from the invasive species to the endemic. There was no evidence for introgression of mtDNA across the species boundary, as is expected to occur via occasional hybridization and backcrossing (Barton and Hewitt 1985; Redenbach and Taylor 2003). In fact, even low levels of historical or contemporary hybridization of freshwater fishes that are barely or indiscernible in nuclear DNA profiles can still be evidenced by mtDNA introgression (e.g. secondary contact of subspecies, Elmer et al. 2008), which was not present in our data. However, we did identify very low levels of genetic admixture at microsatellite loci, particularly from *O. niloticus* into *O. esculentus* (Fig. 6). This suggests that even in the refugial lakes *O. esculentus* is subject to low level hybridization with invasive *O. niloticus* and its genetic integrity may be in peril. We cannot currently discern whether or not this reduces the fitness of critically endangered *O. esculentus*.

The invasive species *O. niloticus* showed some extremely low introgression of nuclear alleles from *O. esculentus*. Though we were not concerned with the genetic integrity of this successful invasive species, genetic analyses allow for more direct testing of population history than does census. Interestingly, the population of *O. niloticus* with the highest proportion of introgression from *O. esculentus* (though still low, at $Q = 0.98$), was in satellite Lake Sare where *O. esculentus* is absent (Aloo 2003). Lake Sare used to be connected to Lake Victoria and generally shares its species composition. However, the presence of invasive species Nile perch and *O. niloticus* has resulted in local extirpations and all native tilapia species are now absent in Lake Sare. Our data suggest that *O. esculentus* was in fact historically present in Lake Sare but is now eliminated, possibly due to the arrival of invasive species. Alternatively, *O. niloticus* introgressed with *O. esculentus* prior to arriving in Lake Sare, though the contemporary population genetic structure and low gene flow among satellite lakes suggests this is unlikely.

The successful establishment of invasive species into new habitats could be facilitated by introgressive hybridization with native species (Hänfling 2007). The adaptive evolution of an invasive species may be promoted via genetic exchanges with native species or by competitive replacement of native species by ecologically or physiologically superior hybrids (i.e. hybrid vigour; Hänfling 2007). However, the very low degree of asymmetric nuclear introgression from the native species *O. esculentus* to the invasive species *O. niloticus* suggests that hybrid vigour is not facilitating the biological invasion. Further, *O. niloticus* and other aquaculture tilapia have proven almost worldwide to be very successful invaders because of ecological competitiveness and physiological tolerance (Canonico et al. 2005). Nonetheless, genome-wide approaches to assessing a possible role of introgressed adaptive alleles from native to invasive tilapia may provide important information about how and when *O. niloticus* flourishes in newly colonized habitats.

Previous attempts to study hybridization in Lake Victoria tilapia species led to conflicting results. Using RAPD markers, Mwanja and Kaufman (1995) found genetic signals of asymmetric introgression in the Lake Victoria region, with more genetic contribution from *O. niloticus* into *O. esculentus* (6.72% of bands) than the reverse (0.91%). This finding generally concurs with our own. However, based on variation in three microsatellite and 24 allozyme loci, Agnèse et al. (1999) suggested that *O. esculentus* from Lake Kanyaboli are genetically 'pure' in relation to *O. niloticus*. Since previous studies were based on relatively small sample sizes and genetic markers not ideally suited to identifying introgression, a re-evaluation of the question of hybridization between invasive and native tilapia species in the Lake Victoria basin, using suitable and sensitive molecular markers for detecting hybridization (Frankham et al. 2004), is timely. Our data contribute further evidence to the hypothesis that there is a low level of introgression from *O. niloticus* into *O. esculentus*, which likely threatens the conservation of this endangered species.

Since *O. esculentus* and *O. niloticus* are phylogenetically closely related (although they are not sister taxa; Klett and Meyer 2002), both species might share certain alleles and/or allele size classes at microsatellite loci because of shared evolutionary history. While some allele size classes are shared between species, our multilocus data indicated only low levels of mixture and discrete genetic grouping of both species at all loci examined. No mtDNA haplotypes were found to be shared by these two species (see Fig. 3) and the sequence divergence in the mtDNA control region between the species was considerable (uncorrected *p*-distance 13.9 %). Multilocus microsatellite data completely distinguish both species at the population and individual level (see Fig. 6). When there is admixture, as inferred from individual level analyses, it is asymmetrical in the anticipated direction from invasive *O. niloticus* into declining *O. esculentus* (Fig. 6).

A better understanding of historical and contemporary levels of hybridization between *O. esculentus* and *O. niloticus* would be aided by additional temporal and/or geographical samples, particularly for *O. esculentus*. Unfortunately the low abundance and restricted contemporary distribution of the endangered species *O. esculentus* (Jembe et al. 2006) makes this a difficult task. Comparing population genetic structure of native *O. esculentus* before and after an introduction of *O. niloticus* will provide insights into a direct connection of admixture between these two species. In addition, contrasting population structure of *O. esculentus* in Lake Victoria region, where there is potential for admixture with *O. niloticus*, to that from a native and genetically "pure" source population lacking contact with *O. niloticus* will gain insights into how much the genome of *O. esculentus* has been "polluted" by *O. niloticus* in Lake Victoria region. However, it is also possible that "pure" *O. esculentus* no longer exists because its geographical distribution is always overlapped with *O. niloticus* (Trewavas 1983).

Conservation genetics and its implications

Multiple lines of evidence—the presence of private alleles in the populations, restricted gene flow among the populations, and low levels of genetic exchange between the two *Oreochromis* species—strongly suggest that both species are represented by a network of relatively discrete genetic units that are geographically restricted. Therefore the refugial populations of *O. esculentus* in satellite lakes Kanyaboli and Namboyo should be managed as distinct and significant conservation units (Ryder 1986), since the species is already on the Red List of critically endangered species and only survives in few refugial habitats (Twongo et al. 2006).

The low level of introgression between *O. esculentus* and *O. niloticus* within the sampled lakes suggests that the satellite lakes harbor relatively "pure" *O. esculentus*. Thus the Yala swamp is an important refugium for this critically endangered species. The satellite lakes, particularly Lake Kanyaboli, are unique and important biological resources because they are comprised of fish species that populated Lake Victoria before the introduction of Nile perch. The indigenous fish species *O. esculentus* and *O. variabilis* which formed the mainstay of the tilapia fishery in Lake Victoria in the 1950s and 1960s but are now extirpated, are found in abundance in Lake Kanyaboli (Mavuti 1989). Our findings suggest there is an urgent need for the genetic assessment of other species of native tilapia,

O. leucostictus and *O. variabilis,* which still thrive in satellite lakes in the Yala swamp.

Our findings are moderately good news for conservation of this critically endangered species, as they indicate that there is only minor "genetic pollution" from the invasive tilapia species. The two populations of *O. esculentus* should therefore be managed as distinct conservation units, given the species' extremely limited contemporary geographic distribution (Jembe et al. 2006). Any anthropogenic impacts that may aid gene flow between satellite lakes and Lake Victoria should be minimized (Ryder 1986). To achieve this, conservation and management of the Yala swamp ecosystem should be accorded top priority to safe guard this critical genetic resource. Habitat distribution of *O. esculentus* (i.e. shallow water; Kudhongania and Cordone 1974) is not usually overlapped with Nile perch (i.e. deep water). Therefore, the primary cause of the disappearance of this native fish has been suggested to be competitive dominance by *O. niloticus* (Goudswaard et al. 2002) and major limnological changes to Lake Victoria (e.g. increased cyanobacteria prevalence, which is unpalatable to *O. esculentus*, Batjakas et al. 1997). Since Nile perch has not invaded Lakes Kanyaboli and Namboyo, efforts should be made to safeguard the lakes from invasion by this voracious predator. Human activities in the Yala swamp that pose a great threat to the future ecological integrity of this wetland system should be discouraged, including wetland 'reclamation' for agriculture, dam building and cage farming of non-native species. Unfortunately, physical alteration of tropical habitats and the invasion of non-native species have led to the extirpation or extinction of many species before they are even understood.

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