

## 2. The Utilization of Allozymes to Study Population of Tilapias

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### Abstract

We summarize here the results of four studies at the Genetics Laboratory of the Centre de Recherches Océanologiques (CRO), Côte d'Ivoire, using allozymes to study tilapia populations. The first study showed that the genus *Tilapia* (*sensu stricto*) has the largest number of ancestral characters and confirmed the hypothesis that the mouthbrooders (*Sarotherodon* and *Oreochromis*) arose from the substrate spawners (*Tilapia*). In addition, the results confirmed the hypothesis that all mouthbrooders arose from a single speciation event and that species of the genus *Sarotherodon* appear to have been the first to split from the substrate spawner group because, in the resulting phylogenetic tree, they are situated between the species of the genera *Tilapia* and *Oreochromis*.

The second study dealt with the genetic characterization of *O. niloticus* populations. These were clustered into three major groups: first, composed of the Nile drainage (the Nile and Lake Edward) and the Kenyan Rift Valley populations (the Suguta river and Lakes Baringo and Turkana); second, the Ethiopian Rift Valley populations (the Sodore hot springs and Lakes Awasa, Koka and Ziway); and third, West African populations, Lake Chad and the Chari, Niger, Volta and Sénégal rivers. We suggest that *O. niloticus* originated from the Nile, from where individuals have been able to colonize independently East and West Africa.

The third study revealed that the non-native Lake Victoria population of *O. niloticus* possess alleles common to both Nile and Kenyan populations and probably resulted from a mixing of these two. From this study, it seems highly improbable that there has been hybridization between *O. niloticus* and *O. esculentus*.

The fourth study revealed the presence of natural hybrids of *T. guineensis* and *T. zillii* in Lake Ayamé, Côte d'Ivoire: probably-caused by their confinement and close association in this artificial lake.



## Studies

### 1. Phylogenetic relationships among three tilapiine genera<sup>a</sup>.

The classification of Tilapias is controversial. Thys van den Audenaerde (1970, 1971, 1978, 1980) grouped all Tilapias under the genus *Tilapia*, within which he created a number of subgenera. Trewavas (1966, 1973, 1980, 1981, 1982a,b) divided the genus *Tilapia (sensu lato)* into three genera, *Tilapia*, *Sarotherodon* and *Oreochromis*, based on their meristic, morphometric and ethological characteristics and especially on their reproductive behavior: genus *Tilapia* are substrate spawners; genus *Sarotherodon*, paternal or biparental mouthbrooders; and genus *Oreochromis*, maternal mouth-brooders. Trewavas (1983) produced a detailed monograph on the mouthbrooding genera.

The evolution of the reproductive behavior of tilapias is also open to debate. Trewavas (1982a) suggested that a mouthbrooding ancestor diverged from the ancestral group of substrate spawners to give rise to the biparental mouthbrooders (*Sarotherodon*), and then the maternal mouthbrooders (*Oreochromis*) (Fig. 1). An alternative hypothesis, proposed by Peters and Berns (1978, 1982), is that the present mouthbrooders have different ancestors and were the result of multiple evolutionary events from substrate spawners. According to their hypothesis, maternal mouthbrooders were the first to split from the substrate spawners.

To investigate speciation in *Tilapia (sensu lato)*, 24 enzyme loci were studied in five species of the genus *Oreochromis*, four species of the genus *Sarotherodon*,

11 species of the genus *Tilapia (sensu stricto)* and four other African cichlid species genera. Results showed a close correspondence between the clustering of species and the genus to which they belong. Species of the genus *Tilapia (sensu stricto)* are found at one end of the tree. Three *Sarotherodon* species are found at the middle along with a clustering of species of the genus *Oreochromis*. *S. melanotheron* is found at the other end of the tree (Fig. 2a). The addition of other cichlid species (*Chromidotilapia guntheri*, *Chylochromis duponti*, *Hemichromis fasciatus*, *Pelmatochromis buettikoferi* and *Tylochromis jentinki*) did not change the previous phylogenetic structure (Fig. 2b).

This phylogenetic tree shows that the species of the genus *Tilapia (sensu stricto)* could be considered to have the largest number of ancestral characters of all the species of *Tilapia (sensu lato)* proposed by Thys van den Audenaerde. Indeed, the five species added to the tree are all grouped within *Tilapia (sensu stricto)*. The fact that *Tilapia (sensu stricto)* can be considered to have the largest number of ancestral characters confirms the hypothesis that mouthbrooders (*Sarotherodon* and *Oreochromis*) arose from substrate spawners. In addition, these results confirm the hypothesis that mouthbrooders have a *common* ancestor. The phylogenetic trees obtained clearly showed the clustering of species by genus. If multiple evolutionary events had given rise to the mouthbrooders, this clustering of species by genus would not be observed. The observation is consistent with the hypothesis that a single speciation event gave rise to all mouthbrooders. Moreover, as suggested by Trewavas

<sup>a</sup>Phylogenetic relationship among 21 species of three tilapline genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. L. Pouyaud and J.-F. Agnèse. 1995. J. Fish Biol. 47: 26-38.

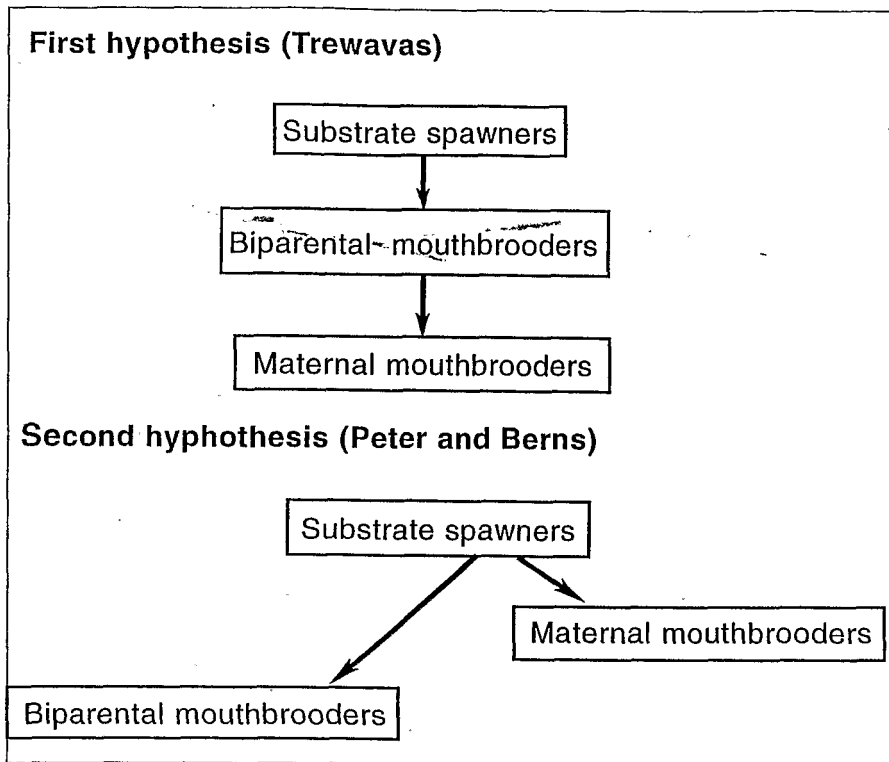


Fig. 1. Alternative hypotheses for the reproductive behavior of tilapias. Arrows indicate the origin of groups.

(1980), species of the genus *Sarotherodon* appear to have been the first to split from the substrate spawners group, since they are situated in between the species of the genus *Tilapia* and *Oreochromis* of the phylogenetic tree.

Although these results clarify the phylogenetic relationships among these species of the three genera *Tilapia* (*sensu stricto*), *Sarotherodon* and *Oreochromis*, questions remain concerning the relationships between *S. melanotheron* and all other species. This species is genetically closer to species of the genus *Oreochromis* than those of the genus *Sarotherodon*. Further studies on a larger

number of species in *Tilapia* (*sensu lato*) will be needed to elucidate the evolutionary event(s) that gave rise to this species.

## 2. Genetic differentiation among natural populations of *Oreochromis niloticus*<sup>b</sup>.

Among all tilapia species, the Nile tilapia (*Oreochromis niloticus*) is the most commercially important species. The natural range of *O. niloticus* includes the Awash, Benue, Chari, Gambia, Niger, Nile, Sénégal and Volta rivers and many lakes like those of the Rift Valley: Albert, Baringo,

<sup>b</sup>J.-F. Agnèse, B. Adépo-Gourène, E.K. Abban and Y. Fermon. Heredity: genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). (In press).

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Edward, George, Kivu, Tanganyika and Turkana. Trewavas (1983), using morphometrical analysis described seven subspecies: *O. niloticus niloticus* from West Africa and Nile; *O. n. baringoensis* from Lake Baringo; *O. n. cancellatus* from the Awash river system in Ethiopia; *O. n. eduardianus* from Lakes George, Edward and Tanganyika; *O. n. filoa* from the hot springs of the Awash river, *O. n. sugutae* from Suguta river in Kenya and *O. n. vulcani* from Lake Turkana. Seyoum and Kornfield (1992a) described a new subspecies, *O. n. tana* from Lake Tana in Ethiopia, based upon its genetical characteristics.

In Africa, *O. niloticus* populations from several sources have been introduced into rivers: e.g., in Côte d'Ivoire where a farmed strain (resulting from the interbreeding of individuals from the Nile and Volta basins) is now present in all major rivers. Cameroon, Guinea and Benin also have some introduced populations which, as in Côte d'Ivoire, resulted from the escape of captive broodstock. In many of these situations, it seems that this species did not cause the disappearance of any other species, particularly any related tilapia species. However, this was not the case in Lake Victoria where *Oreochromis niloticus* was introduced in the late 1950s (Kaufman 1992). This species and introduced Nile perch (*Lates niloticus*) have been implicated in the disappearance of a closely related species, *O. esculentus*, which originally supported the lake's most important fishery (Ogutu-Ohwayo 1990).

Although *O. niloticus* has a wide distribution and is of great economic importance, the genetic characterization of natural populations has not been thoroughly accomplished. This could be of great importance for research on strains for aquaculture, for the protection of endangered populations (for example, those in Lake Baringo or rivers like the

Suguta river), and for making biogeographical inferences. There has been some genetic characterization of strains used in aquaculture (McAndrew and Majumdar 1983; Basiao and Taniguchi 1983; Maracanas et al. 1995). Recently, some studies have been made on natural populations: Seyoum and Kornfield (1992a, 1992b) made a study on East African populations and Rognon (1993) and Rognon et al. (in press) studied West African populations.

Our study was the first in which the natural populations of *O. niloticus* representing all the subspecies described in the major basins (the Awash, Niger, Nile, Sénégal, Suguta and Volta rivers and Lakes Baringo, Chad, Edward, Tana and Turkana) were investigated. This was to facilitate subspecies or population characterization and biogeographic inferences. We analyzed the genetic differentiation among seventeen natural populations using allozymes. Sixteen of the 25 loci studied were polymorphic. Dendrograms showed that the populations are clustered into three major groups: the first composed of the Nile drainage (the Nile and Lake Edward) and the Kenyan Rift Valley populations (the Suguta river and Lakes Baringo and Turkana); the second composed of the Ethiopian Rift Valley populations (the Sodre hot springs and Lakes Awasa, Koka and Ziway); and the third, West African populations (Lake Chad and the Chari, Niger, Sénégal and Volta rivers) (Fig. 3).

The main difference between our results and Trewavas's (1983) nomenclature is the genetic differentiation observed in *O. n. niloticus*. All the West African populations (Chad, Niger, Sénégal and Volta basins) appear closely related, whereas populations from the Nile are closely related to the East African populations (the Suguta river and Lakes Baringo, Edward and Turkana). Her

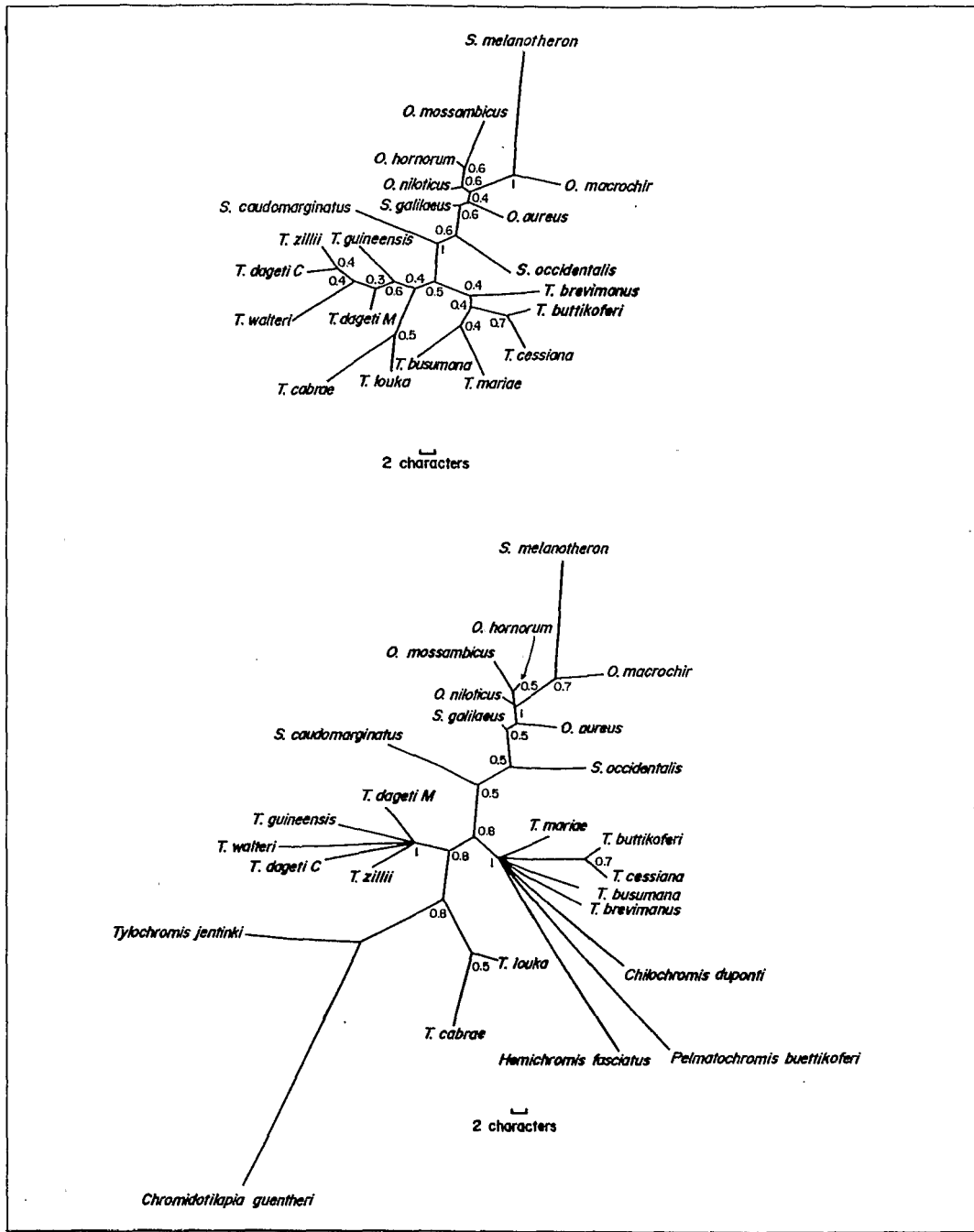


Fig. 2. Genetic relationships (adapted from Pouyaud and Agnèsè 1995): (a) among 20 tilapia species and (b) between these species and five species of other genera. Lengths of the different branches of the networks have been calculated by a parsimony algorithm (MIX) and are based on the number of allelic differences (characters). *T. guineensis* C and *T. guineensis* M represent two different populations of this species from Côte d'Ivoire and Mali, respectively.

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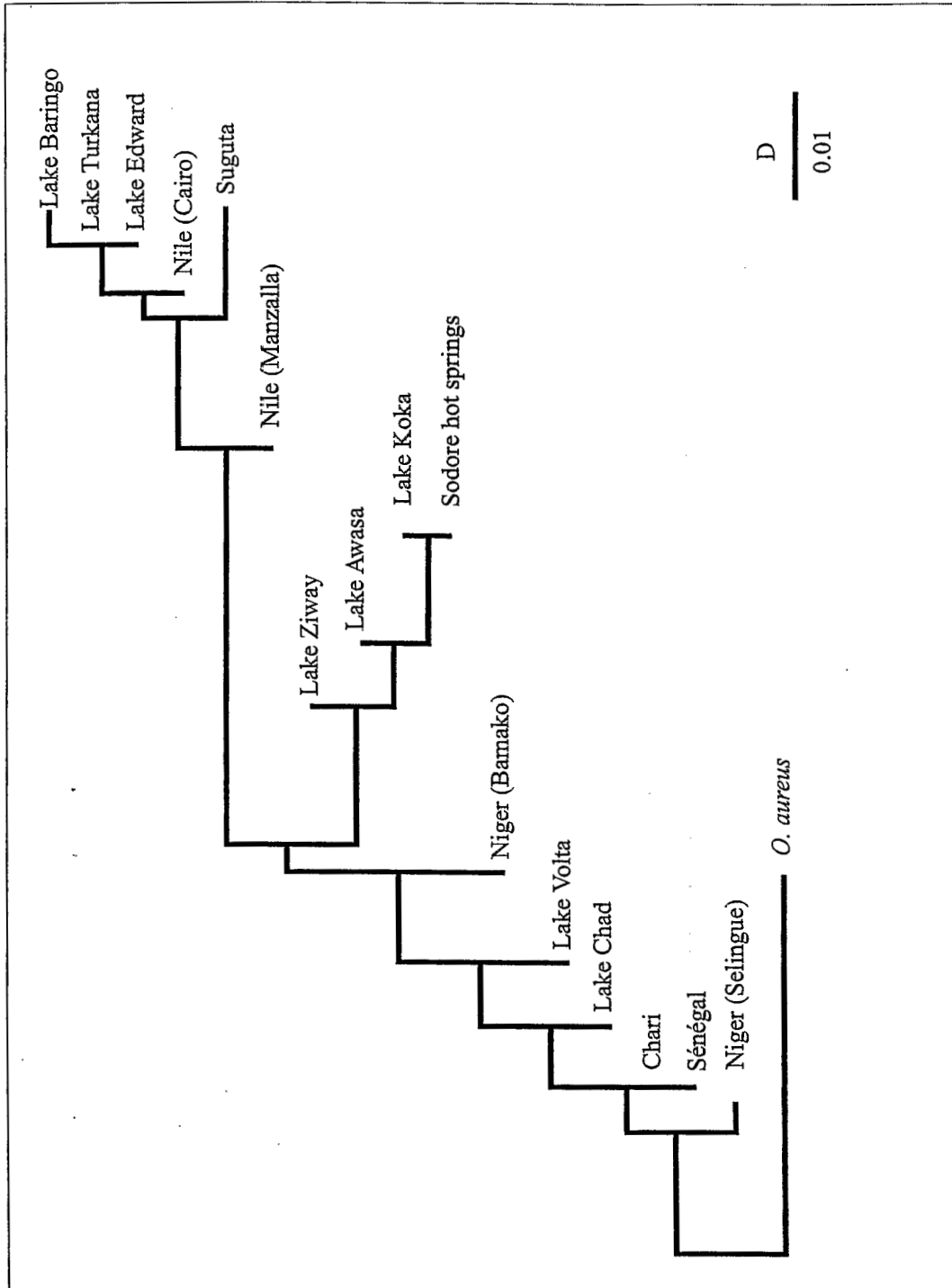


Fig. 3: Genetic relationships among different populations of *Oreochromis niloticus* with *O. aureus* for comparison) (adapted from Agnès et al., in press) using the neighbor-joining method on a Nei (1978) genetic distances matrix.

subspecific nomenclature, based on morphological differences, is not consistent with the data from our genetic analyses.

The distribution of tilapia species and populations in Africa depends upon historical events (climatic change, geological change such as volcanic events, etc.). With our results, it became possible to reconstruct some of the past events that led to the current distribution of *O. niloticus* populations. Colonization is often accompanied by bottleneck effects, due to the generally small size of populations that gain access to one basin from another. These bottlenecks intensify genetic drift and lead to the loss of genetic diversity. In these conditions, populations located at, or close to, the origin of the species are those which have undergone the fewest bottlenecks. If we consider polymorphism (P), the populations that we studied can be divided in two groups: the Nile and West African populations (subspecies *O. n. niloticus*) with P values never less than 0.04 and the East African populations (all other subspecies) with P values never greater than 0.04 (Fig. 4). Further results, from a mitochondrial DNA study, showed that the Nile population can be considered as the more polymorphic one compared to the West African populations. These observations led us to hypothesize that the area of origin of *O. niloticus* could be the Nile. From this area, individuals have been able to colonize, independently, East and West Africa (Fig. 5). To elaborate further on this colonization model, more data on the genetic differentiation of populations are needed.

### 3. Genetic characterization and possible origin of the *Oreochromis niloticus* population of Lake Victoria<sup>c</sup>.

*Oreochromis niloticus* was introduced into Lake Victoria during the late 1950s (Kaufman 1992). This species and introduced Nile perch, *Lates niloticus*, are implicated in the disappearance of *O. esculentus*, which originally supported the lake's most important tilapia fishery (Ogutu-Ohwayo 1990).

Genetic characterization of *O. niloticus* from Lake Victoria was performed to look for evidence of its hybridization with *O. esculentus* and to explore the origins of the Lake Victoria *O. niloticus*. *O. niloticus* specimens were collected from Usenge and Kusa, in the Kenyan part of Lake Victoria, from Lake Baringo, and from the Nile at Manzalla. *O. esculentus* specimens were collected from Lake Kanyaboli, a small lake very near Lake Victoria. Nineteen loci were analyzed and a phylogenetic tree was produced.

The two populations of Lake Victoria appeared phylogenetically close to the Nile population (Lake Manzalla) (Fig. 6). Only two polymorphic loci were observed from the Lake Victoria populations. The second allele of Aat-3 was present in the Nile population but absent in the Kenyan (Lake Baringo) population. The second allele of Pgi-1 was absent in the Nile population but present in the Kenyan (Lake Baringo) population. The Lake Victoria population possess alleles common to both Nile and Lake Baringo populations and probably results from a mixing of these two populations. No specific allele of *O. esculentus* was found in *O. niloticus* from Lake Victoria. Hence,

<sup>c</sup> Paper in preparation by J.-F. Agnès, B. Adépo-Gourène, J. Owino and R. Amán.

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there has probably been no hybridization between these two species.

#### 4. Interspecific hybridization of autochthonous tilapia in Côte d'Ivoire<sup>d</sup>

Recently, there was a case of hybridization between sympatric autochthonous species of *Tilapia*, *T. guineensis* and *T. zillii* in an artificial lake, Lake Ayamé in Côte d'Ivoire (Pouyaud 1994). It is easy to distinguish these species on their pharyngeal teeth (Teugels and Thys van den Audenaerde 1992) or on the coloration of the caudal fin (Pouyaud 1994). Specimens collected in Lake Ayamé displayed typical species-specific and intermediate color patterns.

Twenty-five loci were analyzed in more than 100 specimens from the lake. Seven were polymorphic and two were discriminating between these two species. See Fig. 7 for results of a factorial analysis of the different genotypes observed in Lake Ayamé. G stands for specimens of *T. guineensis*, Z for *T. zillii* and H for undetermined individuals. The results show that the H individuals were genotypic intermediates of *T. guineensis* and *T. zillii* species. Most of them were heterozygotes at the discriminating loci. This hybridization was probably a result of their confinement and close association in the artificial lake. Of five artificial lakes in Côte d'Ivoire where both species are present, we found such in four.

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<sup>d</sup> Paper submitted to *Environ. Biol. Fish.* by L. Pouyaud, P. Berrebi and J.-F. Agnès.



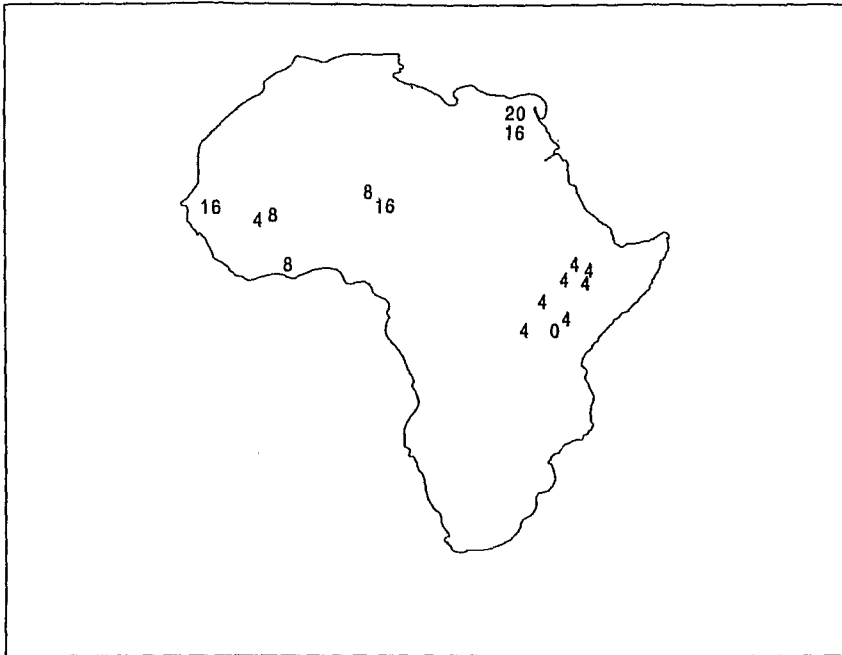


Fig. 4. Values of P (number of polymorphic loci observed divided by the number of all loci studied) in percent, observed in all the populations investigated.

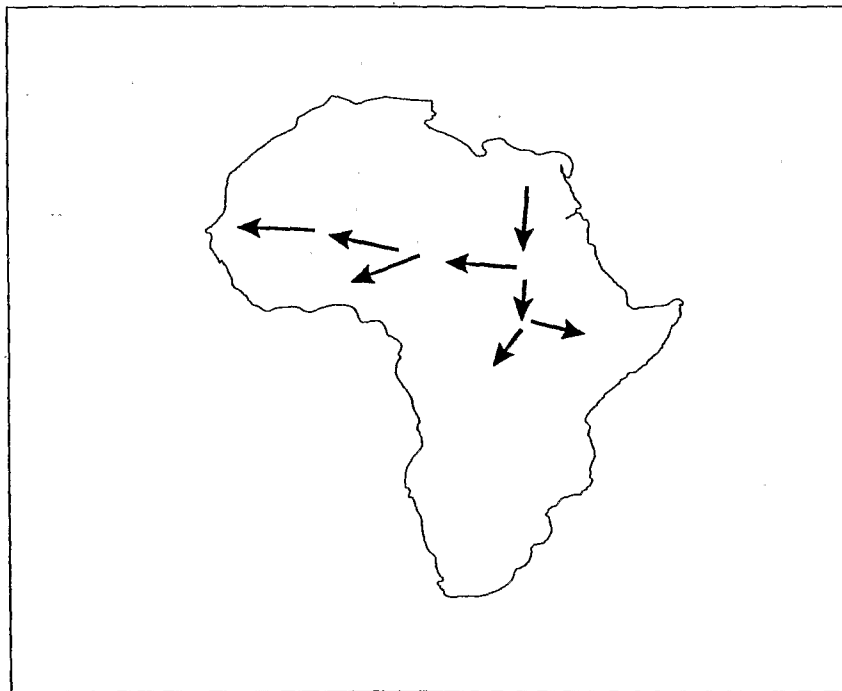


Fig. 5. Possible colonization events that led to the current distribution of *Oreochromis niloticus*. From the Nile, individuals have independently colonized East and West Africa.

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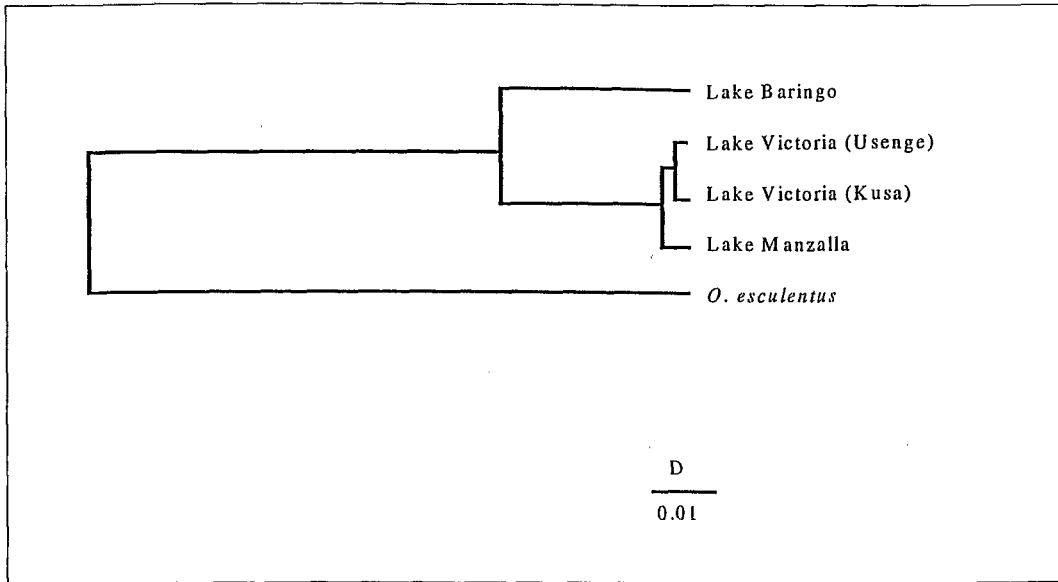


Fig. 6. Dendrogram (Nei 1972) genetic distances and UPGMA, showing genetic relationships among Lake Victoria, Kenyan and Nile populations of *Oreochromis niloticus*, and between this group of populations and *O. esculentus* from Lake Kanyaboli.

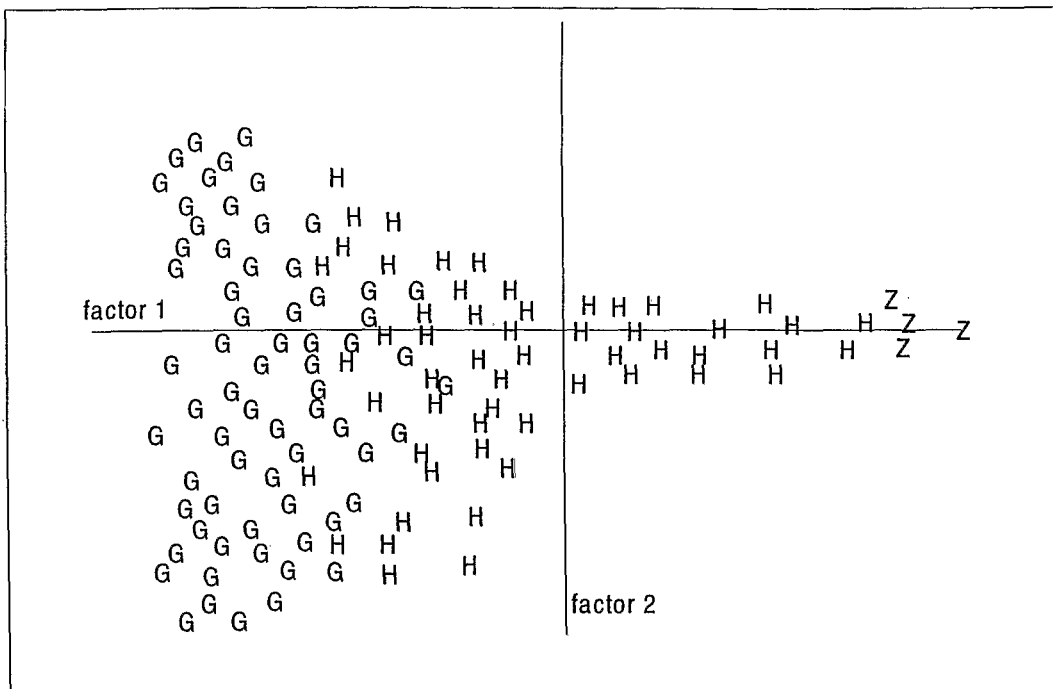


Fig. 7. Factorial analysis of *Tilapia guineensis* (G), *T. zillii* (Z) and undetermined specimens (H). Allozymes data were submitted to a correspondance analysis to assess overall relationships between samples. Multi-locus data input consisted of an unweighed sample x allele matrix, with each sample defined by its allelic counts at the loci.

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