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# Genetic differentiation of a primitive teleost, the African bonytongue *Heterotis niloticus*, among river basins and within a floodplain river system in Benin, West Africa

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Examination of eight microsatellite DNA loci revealed high levels of genetic differentiation among populations of the African bonytongue *Heterotis niloticus* from three river basins that constitute important fishing areas in Benin. Low levels of population genetic differentiation were detected within the Ouemé–Sô River floodplain system. These results have important implications for conservation and management of stocks supporting important inland fisheries in West Africa.



Key words: allelic diversity; Arapaimidae; conservation and management units; heterozygosity; Osteoglossiformes.

Inland fisheries provide a crucial source of protein for human diets in sub-Saharan Africa. In Benin, West Africa, inland fisheries account for *c*. 75% of the total fishery yield (FAO, 2003). Increased fishing pressure, use of unsustainable fishing methods, destruction of spawning habitats and reduction of floodplain habitat by dam construction and agriculture, however, are threatening inland fisheries in Benin (Hauber *et al.*, 2011*a*). Basic information for the conservation and management of inland fisheries in Benin is needed, including knowledge on the levels of genetic differentiation among stocks. Here, findings are reported for an analysis of population genetics of the African bonytongue *Heterotis niloticus* (Cuvier 1829), a fish that supports important commercial and subsistence fisheries in Benin and other African countries (Gbaguidi & Pfeiffer, 1996), and that has been introduced for aquaculture at several locations across Africa (Monentcham *et al.*, 2009). Despite its importance, *H. niloticus* has been little studied in Benin (Adite *et al.* 2005, 2006), and the present investigation is the first ever to examine population genetics of this fish.

Levels of genetic differentiation for *H. niloticus* were estimated among three river basins in Benin, where fishing is a traditional and important economic activity

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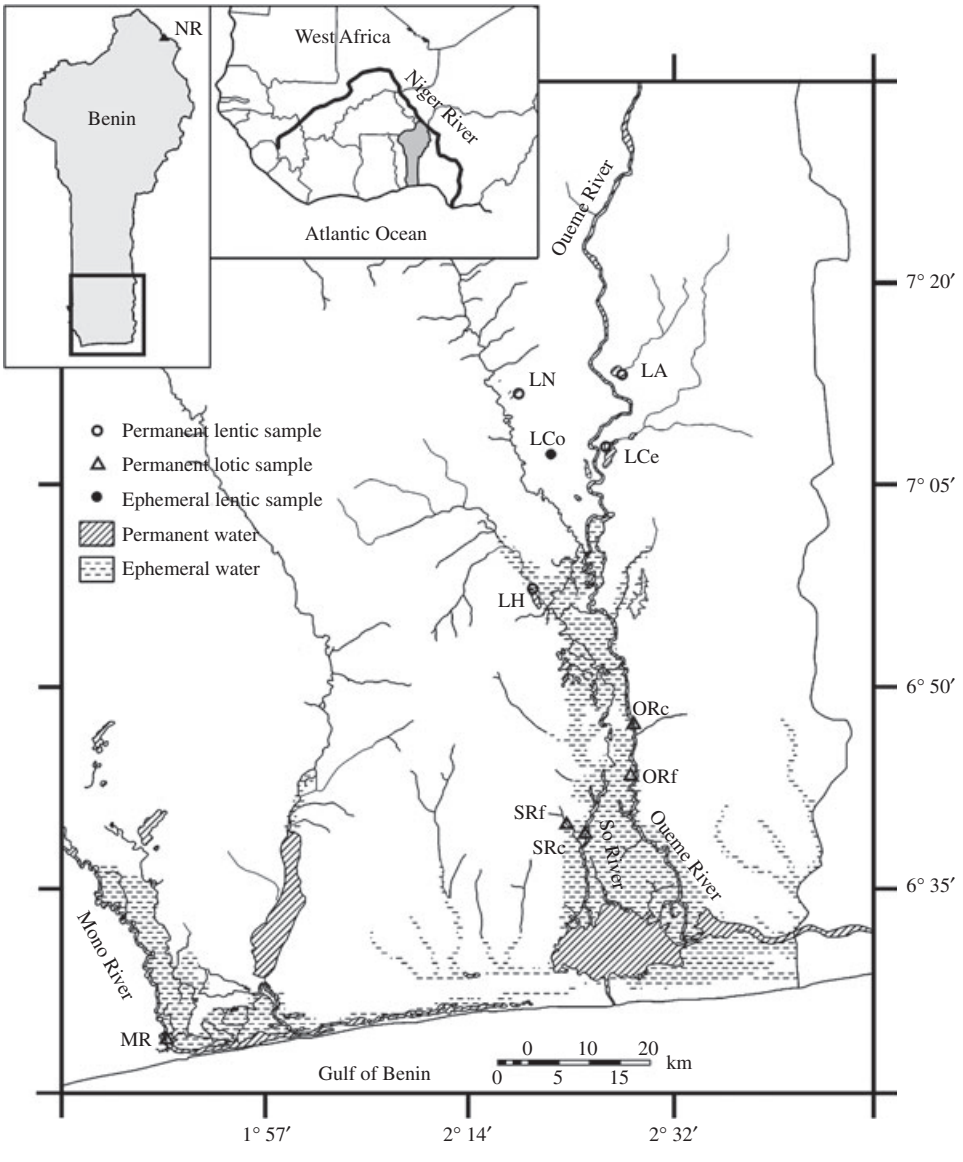


FIG. 1. Map showing the study region and localities sampled (NR, Malanville, Niger River; MR, Mono River; LN, Lake Nakava; LA, Lake Azilli; LCe, Lake Cele; LCo, Lake Codo; LH, Lake Hlan; ORc, Ouemé River on channel; ORf, Ouemé River near channel; SRc, Sô River on channel; SRf, Sô River near channel).

(Fig. 1): Malanville, a city on the Niger River in northern Benin (Hauber *et al.*, 2011*b*); the lower Mono River in south-west Benin (FAO, 2003); the Ouemé–Sô River floodplain system in south-central Benin (FAO, 2003). High levels of genetic differentiation are expected among these basins given the barriers to fish dispersal among them. In addition, levels of genetic differentiation among localities within the Ouemé–Sô River floodplain system were examined. The Ouemé and Sô Rivers flow

adjacent to each other through south-central Benin and share a common seasonal floodplain within the coastal region. Commercial fishing for *H. niloticus*, mostly using cast-nets and gillnets, occurs throughout the Ouemé–Sô River floodplain system, and exploited habitats include river channels, seasonally flooded habitats and lakes (FAO, 2003). Seasonal flooding facilitates dispersal of *H. niloticus* within the Ouemé–Sô River floodplain system (Adite *et al.*, 2006); thus, low levels of population genetic differentiation for this fish are expected within this system.

A total of 211 *H. niloticus* specimens were collected from nine localities in the Ouemé–Sô River floodplain system, one locality in the Mono River and one in the Niger River (Fig. 1 and Supporting Information Table S1). Within the Ouemé–Sô River floodplain system, samples were obtained from four permanent lakes (Hlan, Azilli, Nakava and Cele), an ephemeral lake that forms during flooding seasons (Codo), and one site in the main channel and one in the floodplain near the main channel of both the Ouemé and Sô Rivers.

Individuals were genotyped for six microsatellite loci as described by Carrera *et al.* (2011), and two new microsatellite loci: *Hni5* (5′-CAGGAAGATTTGCACCACCT-3′ and 5′-TGCCATTCTGGAAAAAGGAG-3′) and *Hni28* (5′-TCCCTGCAGTCTGA AACACA-3′ and 5′-AGACCCACAAGATCCAGGTG-3′). PCR products were run in an ABI 377 automated sequencer using GeneScan 400 HD ROX size standard (Applied Biosystems; www.appliedbiosystems.com) for sizing. Allele calling and sizing for each sample were performed with GeneScan 3·1.2 and Genotyper 2·5 (Applied Biosystems). To assess reproducibility, PCR and genotyping were repeated for 30% of the samples, randomly chosen.

Number of alleles, allelic richness, expected heterozygosity and $F_{IS}$ (measured as Weir and Cockerham's $f$) for each microsatellite per locality were calculated in FSTAT 2.9.3.2 (Goudet, 2002). Deviations of Hardy–Weinberg equilibrium (HWE) expectations were tested using the heterozygosity-based method implemented in GenoDive (Meirmans & Van Tienderen, 2004). POPGENE (Yeh *et al.*, 2000) was used to test non-random associations between pairs of microsatellite loci (Weir, 1979), and to conduct the Ewens–Watterson test for deviations from neutrality in individual markers (Manly, 1985). Presence of potential null alleles was tested with Micro-Checker (van Oosterhout *et al.*, 2004). Rarefaction analyses to assess whether estimates of allelic richness were affected by sample sizes were conducted in Allelic Diversity Analyzer (ADZE; Szpiech *et al.*, 2008).

Pair-wise $F_{ST}$ values with corresponding 95% C.I. were calculated with FreeNA (Chapuis & Estoup, 2007). This programme estimates $F_{ST}$ with and without correcting for the presence of potential null alleles. GenoDive was used to compute $P$-values of the $F_{ST}$ estimations without correcting for the presence of potential null alleles. POWSIM (Ryman & Palm, 2006) was used to assess the statistical power for detecting population differentiation within the Ouemé–Sô River system with the applied set of markers and sample sizes. Nine subpopulations were assumed using a combination of $N_e/t$ of 500/10 (leading to $F_{ST}$ *c.* 0·01), 750/10 (leading to $F_{ST}$ *c.* 0·007) and 1000/10 (leading to $F_{ST}$ *c.* 0·005). A total of 1000 replications were run for each level of differentiation. Isolation by distance for the samples from the Ouemé–Sô River system was tested using river distances between localities and the software programme Isolation By Distance. Web Service (IBDWS; Jensen *et al.*, 2005).

Structure 2·3.1 (Pritchard *et al.*, 2000) was used to examine the number of differentiated populations. Analyses were performed using three iterations for $K$ values

ranging from 2 to 10, with 500 000 steps and a burn-in of 125 000 steps, with all other settings set as default. Convergence on stable likelihoods was determined based on likelihood plots and comparison of results from multiple independent runs. Analyses were repeated for four possible model combinations: no admixture-correlated frequencies; admixture-correlated frequencies; no admixture-independent frequencies; admixture-independent frequencies. Three-dimensional factorial correspondence analyses (FCA) were conducted in Genetix (Belkhir *et al.*, 2000). *K*-means clustering analyses were conducted in GenoDive. This method clusters sampling localities into a number of groups ($K$) assigned *a priori* in a way that minimizes within-cluster diversity and maximizes among-cluster diversity. The pair-wise matrix of distances between individuals was based on Euclidean distances, using the hill-climbing method for $K$ values from 2 to 6. The programme reports results from three methods to determine the best $K$: (1) the Akaike information criterion (AIC), (2) the pseudo-$F$ statistic (pseudo-$F$) and (3) the Bayesian information criterion (BIC).

All genotypes were obtained for all individuals, with the exception of one locus for one individual. Reproducibility was 100% for samples that were repeated. Allelic diversity, heterozygosity and $F_{IS}$ results are shown in Supporting Information Tables S2 and S3. No linkage disequilibrium among any pairs of loci or deviations from neutrality at any locus was detected. Significant deviations from HWE expectations after Bonferroni correction ($P < 0{\cdot}001$) were observed in six of the 96 calculations: locus *Hni62* departed significantly from HWE in three localities, locus *Hni5* in two and locus *Hni47* in one. Micro-Checker results suggest the presence of potential null alleles for locus *Hni5* in six localities, locus *Hni62* in four localities, locus *Hni47* in two localities, and loci *Hni19*, *Hni52* and *Hni28* in one locality.

Average number of alleles was lowest (3·50) for the Niger River locality, intermediate (5·88) for the Mono River locality and highest (9·25) for the Ouemé–Sô River system (Supporting Information Table S3). Rarefaction analyses conducted in ADZE suggest sample sizes were sufficient to capture much of the allelic diversity present in each basin.

$F_{ST}$ estimates correcting for the presence of null alleles were very similar to estimates obtained without such correction (Table I), suggesting that potential null alleles, if present, would not substantially bias results. As expected from the initial predictions, $F_{ST}$ results indicated high genetic differentiation between populations from the three river basins: $F_{ST}$ values including the Niger River locality were the highest (average $F_{ST} = 0{\cdot}27$) with all comparisons significant, whereas $F_{ST}$ values between the Mono River and Ouemé–Sô River floodplain system were the second highest (average $F_{ST} = 0{\cdot}09$) with all comparisons significant. Also as expected from the initial predictions, low levels of genetic differentiation were observed between localities within the Ouemé–Sô River floodplain system (average $F_{ST} = 0{\cdot}03$). After Bonferroni correction ($P < 0{\cdot}001$), $F_{ST}$ pair-wise values were significant for only nine of 36 comparisons, all of which included locations that were ephemeral habitats (*i.e.* non-permanent populations): six included the Sô River floodplain (SRf), two Lake Codo and one the Ouemé River floodplain (ORf). Some of these significant $F_{ST}$, however, included zero or values close to zero in their 95% C.I. Nonetheless, significant $F_{ST}$ values may reflect stochasticity in the formation of temporary groups within ephemeral habitats. None of the comparisons between permanent lakes were significant suggesting that gene flow among them is sufficient to prevent marked local genetic differentiation. According to the POWSIM results, the probability of

TABLE I. Pair-wise $F_{ST}$ between the *Heterotis niloticus* localities (see Fig.1) examined with (above diagonal; with lower and upper 95% C.I. in parenthesis) and without (below diagonal) correction for null alleles*

| Locality | NR | MR | LN | LA | LCe | LCo | LH | ORc | ORf | SRc | SRf |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NR | | **0·307** (0·195–0·448) | **0·264** (0·112–0·412) | **0·220** (0·091–0·356) | **0·253** (0·106–0·403) | **0·293** (0·167–0·418) | **0·235** (0·137–0·320) | **0·257** (0·096–0·448) | **0·252** (0·146–0·380) | **0·319** (0·185–0·452) | **0·318** (0·175–0·473) |
| MR | **0·327** | | **0·072** (0·013–0·133) | **0·080** (0·023–0·064) | **0·081** (0·038–0·150) | **0·124** (0·071–0·174) | **0·090** (0·038–0·075) | **0·073** (0·026–0·125) | **0·063** (0·030–0·048) | **0·092** (0·033–0·148) | **0·122** (0·073–0·172) |
| LN | **0·260** | **0·072** | | 0·000 (0·000–0·006) | 0·014 (0·000–0·035) | 0·035 (0·005–0·065) | 0·006 (0·000–0·017) | 0·048 (0·017–0·086) | 0·010 (0·000–0·100) | 0·042 (0·000–0·112) | **0·060** (0·019–0·121) |
| LA | **0·218** | **0·087** | −0·006 | | 0·021 (0·008–0·034) | 0·025 (0·000–0·061) | 0·006 (0·001–0·012) | 0·030 (0·000–0·064) | 0·014 (0·000–0·031) | 0·045 (0·000–0·106) | **0·050** (0·010–0·108) |
| LCe | **0·257** | **0·095** | 0·009 | 0·016 | | 0·039 (0·004–0·085) | 0·020 0·000 0·055 | 0·042 (0·000–0·104) | 0·026 (0·006–0·052) | 0·023 (0·000–0·121) | **0·059** (0·008–0·144) |
| LCo | **0·297** | **0·131** | 0·029 | 0·017 | 0·039 | | 0·024 (0·000–0·053) | **0·085** (0·026–0·162) | **0·053** (0·008–0·100) | 0·006 (0·000–0·027) | 0·012 (0·000–0·034) |
| LH | **0·237** | **0·100** | 0·010 | 0·008 | 0·021 | 0·018 | | 0·039 (0·010–0·075) | 0·008 (0·000–0·017) | 0·031 (0·000–0·084) | **0·040** (0·004–0·091) |
| ORc | **0·259** | **0·081** | 0·045 | 0·024 | 0·046 | **0·090** | 0·040 | | 0·020 (0·000–0·048) | 0·076 (0·014–0·161) | **0·079** (0·031–0·168) |
| ORf | **0·256** | **0·070** | 0·011 | 0·014 | 0·028 | **0·049** | 0·008 | 0·018 | | **0·053** (0·000–0·125) | **0·049** (0·002–0·121) |
| SRc | **0·320** | **0·095** | 0·030 | 0·034 | 0·018 | −0·002 | 0·021 | 0·077 | **0·044** | | 0·020 (0·000–0·056) |
| SRf | **0·325** | **0·131** | **0·054** | **0·045** | **0·062** | 0·011 | **0·035** | **0·086** | **0·045** | 0·010 | |

*Bold numbers indicate pair-wise comparisons that were significant ($P < 0·001$) in the analyses without corrections for null alleles.

 

detecting a significant $F_{ST}$ of 0·01, 0·007 and 0·005 within the Ouemé–Sô River floodplain system was 99·8, 94·8 and 82·4%, respectively.

The other analyses conducted also indicated high genetic differentiation among the three river basins, and low genetic differentiation within the Ouemé–Sô River floodplain system. Structure analyses clearly showed the Niger River fish were genetically differentiated from those of all other localities regardless of the model and $K$ used. These analyses, however, did not show any clear differentiation when excluding specimens from the Niger River or from the Niger River + Mono River. Results from FCA that included all specimens clearly separated the three river basins, whereas analyses excluding Niger River specimens clearly distinguished the Mono River from the Ouemé–Sô River floodplain system localities (Fig. 2). When excluding Niger River and Mono River fish, FCA results did not reveal a clustering pattern within the Ouemé–Sô River floodplain system. $K$-means clustering analysis with $K = 3$ (best $K$ according to the AIC) separated the three basins. In analyses with $K = 4$ (best $K$ according to the pseudo-$F$) and $K = 5$ (best $K$ according to the BIC), the Niger River and Mono River still constituted separate clusters, and Ouemé–Sô River system localities were further divided. Finally, IBDWS analyses did not indicate a pattern of isolation by distance within the Ouemé–Sô system.

According to the results, barriers to dispersal appear to have effectively isolated populations of *H. niloticus* among the three basins examined. On the basis of the high levels of genetic differentiation observed among the three basins, *H. niloticus* from each basin should be considered a different management and conservation unit in Benin. The geographic scale at which significant genetic differentiation was detected, cautions against translocations of *H. niloticus* from different regions. This fish has been introduced for aquaculture at locations throughout Africa (Monentcham *et al.*, 2009). Translocations, however, could threaten the persistence of endemic genetically differentiated *H. niloticus* lineages through mechanisms such as competition, genomic introgression by hybridization and outbreeding depression, as has been observed in other freshwater fishes (Gilk *et al.*, 2004; Goldberg *et al.*, 2005; Littrell *et al.*, 2007; Muhlfeld *et al.*, 2009; Houde *et al.*, 2011).

Average allelic diversity and heterozygosity (3·5 and 0·34) for the *H. niloticus* Niger River sample in Malanville are extremely low compared to values reported for other freshwater fishes: 9·1 for average allelic diversity and 0·54 for heterozygosity (DeWoody & Avise, 2000). Accordingly, this population could be vulnerable to overexploitation, which may already be occurring in this region for some fish species (Hauber *et al.*, 2011*b*). Expanded genetic analyses in northern Benin, including a larger sample from Malanville and other regions of the Niger Basin, are needed to establish whether low genetic diversity is a characteristic of *H. niloticus* in this region. The Mono River locality also showed comparatively low average allelic diversity and heterozygosity (5·88 and 0·59), suggesting that this *H. niloticus* population may also be vulnerable to overexploitation. The sample from this locality, however, was also small, and larger samples should be analysed for a more robust interpretation.

Seasonal flooding appears to facilitate gene flow within the Ouemé–Sô River floodplain system, where, in general, low levels of genetic differentiation were detected among localities. Temporal stochasticity, however, may occur during the formation of groups within ephemeral habitats of the floodplain, as reported in another African fish, *Pseudocrenilabrus multicolor victoriae* (Schöller 1903), that

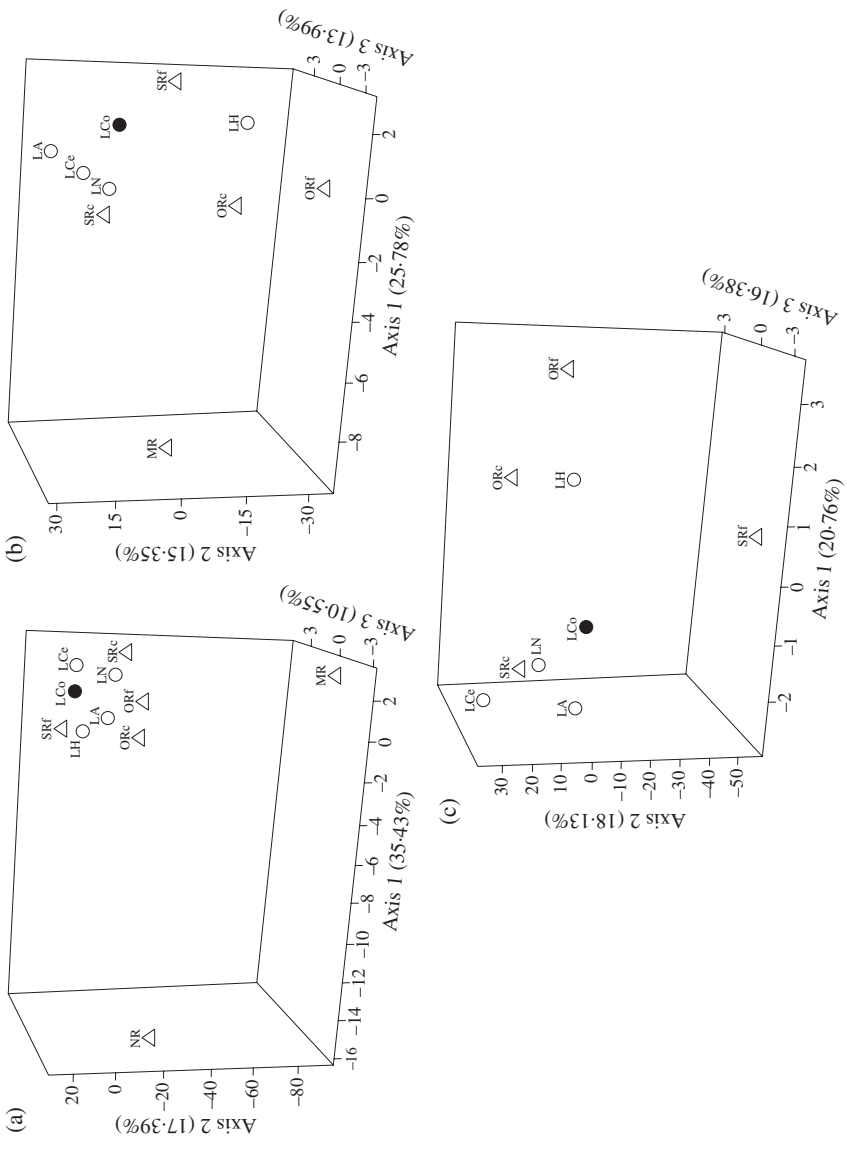


FIG. 2. Three-dimensional factorial correspondence analyses (3D-FCA) of *Heterotis niloticus* from (a) all localities, (b) excluding the Niger River locality and (c) excluding the Niger River and Mono River localities (see Fig. 1).

also occupies a river system with seasonal flooding (Crispo & Chapman, 2010). Nonetheless, according to the results, it seems appropriate to consider *H. niloticus* a single conservation and management unit within this ecosystem. Management of this stock should treat lakes, flowing channels and seasonal floodplain pools, as a set of interconnected essential habitats. Adite *et al.* (2006) propose that maintenance of sufficient numbers of spawning adults in the permanent lakes may be essential for sustainable fisheries in the Ouemé–Sô River floodplain. Genetic diversity of *H. niloticus* within the Ouemé–Sô River basin (average allelic diversity = 9·25; heterozygosity = 0·60) was greater than that observed in the other two basins, and comparable to the average reported for other freshwater fish species (DeWoody & Avise, 2000).

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## Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE S1. Localities sampled, their geographic coordinates, date of collection, and *Heterotis niloticus* sample sizes

TABLE S2. Summary information for the eight polymorphic microsatellite loci at each locality sampled

TABLE S3. Mean number of alleles, heterozygosity, and inbreeding coefficient for each locality

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