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Roman trade relationships at Sagalassos (Turkey) elucidated by ancient DNA of fish remains

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Abstract

The excavations of Roman and Early Byzantine contexts at the town of Sagalassos (Turkey) yielded fish remains belonging to species that do not occur near the site. The modern geographical distribution of the identified fish indicates trade with various regions of Anatolia, the Mediterranean coast, Egypt and/or the Levant. Trade with Levant and Egypt is evident throughout the period by the presence of *Clarias*, a catfish living amongst others in the Nile and Levant. Mitochondrial DNA analysis was successfully carried out on modern populations of this species from Turkey, Syria, Israel and Egypt. Several variable regions were discovered on the mitochondrial control region containing polymorphisms that distinguish the haplotypes. Primer sets were designed to amplify small fragments of ancient DNA containing these informative regions. Ancient fish DNA could be successfully extracted, amplified and sequenced. The analyses indicate that the catfish bones belong to *Clarias gariepinus* and that they originated from the lower Nile. In addition, this study sheds light on the understanding of the modern distribution of *C. gariepinus* in Anatolia.

Keywords: Ancient DNA; Archaeozoology; *Clarias gariepinus*; Mitochondrial DNA; Turkey; Trade

1. Archaeological relevance of the analyses

Analyses of fossil mitochondrial DNA (mtDNA) from archaeological sites have focused on human remains (e.g. Refs. [22,48]) while studies on other vertebrates, plants [46] and prokaryotes [64] remain rare. Ancient DNA from animal bones has been used to understand the domestication origin of populations, their diversity and evolution (e.g. Ref. [52] for cattle; Ref. [21] for rabbits). Several studies concentrate on the molecular identification of species that are closely related and difficult to distinguish using osteomorphological and osteometrical characters. Examples are the distinction between sheep and goat [29], and between six wild goose species and domestic goose [4]. Similarly, extractions of ancient DNA from 9000 to 20 years old salmon bone were undertaken by Butler and Bowers [7] in an attempt to refine identifications of archaeozoological salmonid remains from the Pacific Northwest of North America. In one case—a subrecent sample that was estimated to be 12–20 years old—amplification of a 119 base pair (bp) long region showed that the DNA sample was from *Oncorhynchus*, probably sockey salmon *Oncorhynchus nerka*. Burbidge [6] failed to extract mtDNA from prehistoric dentaries and maxillae of bluefin tuna (*Thunnus thynnus*), possibly because of the bone's texture or because of soil contaminants leaching into the bone [9]. More recently, mtDNA analyses have been carried out to distinguish species of the Serranidae family excavated at the Cooke Islands [32].

The aim of the DNA-analysis presented in this paper is to establish the geographic origin of a catfish species

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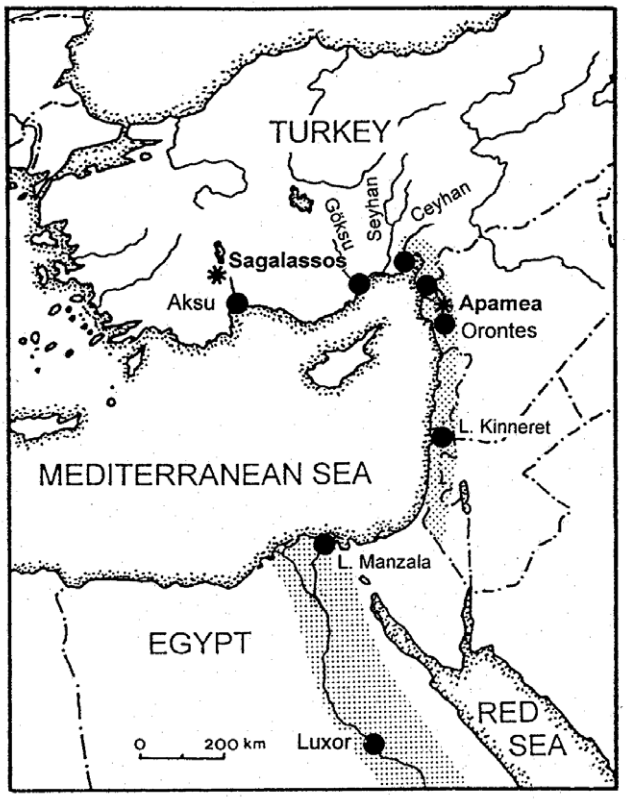

Fig. 1. Current original distribution of *C. gariepinus* in the Eastern Mediterranean area, location of the Sagalassos (Turkey) and Apamea (Syria) archaeological sites, and sampling localities of modern tissue for DNA analysis, except Mali and Senegal (see Table 1).

found throughout Roman and Early Byzantine levels of the town of Sagalassos (Turkey). This classical town is situated in western Anatolia at a distance of approximately 110 km north of the coastal town of Antalya (Fig. 1). Sagalassos boastfully announced its status of first town of the region of Pisidia on its inscriptions. More than a decade of interdisciplinary archaeological research has indeed illustrated how the town successfully exploited its various agricultural and mineral resources, exemplified by its important pottery production activity between late Hellenistic times and the 7th century AD. As a result, the town functioned as the pivot of a regional and supra-regional economic exchange pattern [34,62]. Faunal remains have been systematically studied since the beginning of the excavations in 1991 (e.g. Refs. [10,11,55]). The fish remains identified thus far indicate that none of the species are of a local origin. The majority of the remains belong to Anatolian freshwater fish, followed by Mediterranean species and fish designated as 'exotic freshwater species' [57,60]. A survey of the present-day ichthyofauna of the region has been carried out to better document the modern distribution of fishes. Such information is needed to establish the origin of the species identified at the site [59]. Presently, the Ağlasun River near the site lacks fish, except for some rare rainbow trout that escaped from fish farms. All the Anatolian freshwater species found at the site were probably derived from regions to the north, west or east of Sagalassos, with the possible exception of *Vimba vimba*, a cyprinid that is also found in the Aksu River (ancient Kestros). This river formed the eastern boundary of Sagalassos' territory in antiquity [63] and is running south towards the Mediterranean coast. Seven marine fish taxa were identified thus far, three of which occur exclusively in the Mediterranean Sea. The four other species are found in both the Black Sea and the Mediterranean Sea, but it is assumed that all the marine fish came from the latter area. Good roads were available in the direction of the Mediterranean Sea [31] and commercial contacts existed primarily with major contemporary cities of Pamphylia according to the study of coins [43] and ceramics [34]. A third category of fishes are the so-called 'exotic freshwater species'. They comprise the Nile perch *Lates niloticus* and a catfish of the genus *Bagrus* [60], which indicate that trade connections existed with Egypt. Two additional taxa in this category are tilapia (tribe Tilapiini) and a catfish of the genus *Clarias*. The distribution of these fish extends beyond Africa and includes parts of Asia Minor. Four tilapia species occur in the Levant, of which *Tilapia zillii* has the northernmost distribution (the Litani basin in Lebanon, coastal rivers of Palestine and the Jordan basin) [26]. The catfish genus *Clarias* is represented in the Near East by *Clarias gariepinus*, whose natural distribution is traditionally described as extending as far as the Orontes and Ceyhan basin in Turkey [25,45]. The wide modern distribution of *Clarias* and the tilapia species hampers the establishment of the former trade connections; archaeological and epigraphic data indicate that contacts with both regions existed. Connections with northeast Africa are indicated by coins from Alexandria found at Sagalassos and by finds of Sagalassian red slip ware in Egypt and Sudan. Tableware and oinophoroi from Sagalassos were found at Alexandria, Memphis, the Fayoum, Kellia and Hermopolis Magna in Egypt and as far as Pharas in Sudan [34 p. 288, 35]). Contacts with the Syro-Palestinian area are illustrated by imperial mints from Antiocheia found at Sagalassos, and by Sagalassian tableware and oinophoroi found at Antiocheia, Hama, Tel Anafa and Kapharnaon in Galilaea [34 p. 288, 41,42]. The epigraphic data also indicate contacts with both regions; the aristocracy of Sagalassos served almost exclusively the Roman army and the provincial administration of Syria, Palestina and Egypt during the Imperial period [13,14].

At the start of this mtDNA study it was accepted that the natural distribution of *C. gariepinus* stopped at the Ceyhan. During freshwater fish surveys, it appeared, however, that the species is present much further to the west [59]. It was attested in the Seyhan River, the Tarsus

Table 1
Location by country and river basin of the contemporary sampling sites of *C. gariepinus* and *C. anguillaris*

Location	Code	Species	Coordinates	*n*	Haplotypes
Turkey					
Aksu River	Ta	*C. gariepinus*	N 36° 50′, E 30° 55′	5	Ta (5)
Göksu River	Tg	*C. gariepinus*	N 36° 20′, E 33° 55′	5	Togc (5)
Ceyhan River	Tc	*C. gariepinus*	N 36° 40′, E 35° 40′	5	Togc (5)
Orontes River	To	*C. gariepinus*	N 36° 05′, E 36° 00′	5	Togc (5)
Syria					
Orontes	Sy	*C. gariepinus*	N 33° 40′, E 36° 25′	10	Sy1 (3), Sy2 (4), Sy3 (1), Sy4 (2)
Israel					
Lake Kinneret	Is	*C. gariepinus*	N 32° 45′, E 35° 30′	7	Is1 (1), Is2 (3), Is3 (1), Is4 (1), Is5 (1)
Egypt					
Lake Manzala	Em	*C. gariepinus*	N 31° 12′, E 31° 54′	7	E1 (1), E2 (1), E4 (2), E6 (1), E7 (1), E8 (1)
Nile River (Luxor)	El	*C. gariepinus*	N 25° 40′, E 32° 40′	4	E3 (2), E4 (1), E5 (1)
Senegal					
Senegal River	Se	*C. gariepinus*	N 16° 30′, W 15° 30′	1	Sel (1)
	CaS	*C. anguillaris*		1	CaS
Mali					
Niger River	Ma	*C. gariepinus*	N 12° 45′, W 07° 30′	1	Ma1 (1)
	CaM	*C. anguillaris*		1	CaM

Abbreviation: *n*, number of samples collected.

River and near Akgöl, which is part of the Göksu basin and further west in the Acısu and Aksu River (Fig. 1). Initially it was unclear if this wider distribution was the result of a natural colonization of coastal basins or if recent human introduction was involved. Poor sampling and the lack of a commercial fishery in freshwater may explain why the presence of *C. gariepinus* remained unreported in several Mediterranean basins of Anatolia. If the species occurred here naturally in ancient times, then the source from the *C. gariepinus* in Sagalassos might have been relatively close to the site (lower reaches of the Aksu basin). In the meantime, archaeozoological evidence has become available indicating that clariids were absent in the past from basins west of the Ceyhan (see discussion below). There is no phylogeographical evidence available on *C. gariepinus* in the region as previous genetic studies had only dealt with the populations of the African continent [1,2].

2. Material and methods

Modern material of *C. gariepinus* (Clariidae, Siluriformes, Teleostei) was collected at representative sites including the Aksu, Göksu, Ceyhan, and Orontes rivers in Turkey, the Orontes River in Syria, Lake Kinneret belonging to the Jordan basin in Israel, Lake Manzala and the Nile River at Luxor in Egypt, the Senegal River in Senegal, and the Niger River in Mali (Table 1). The closely related species *C. anguillaris* from the latter two basins were also incorporated into the study [1]. Mitochondrial DNA (mtDNA) was extracted from fin tissue samples of these modern populations and the control region (780 bp) was amplified using specific primers by the polymerase chain reaction (PCR) and sequenced as previously published [2]. Aligned sequences were used to identify short, highly variable regions and to design primers for amplification of fragments from the ancient material (see further discussion) (Fig. 2). A nested clade analysis (NCA) [49] of a truncated fragment of 435 bp was performed on an unrooted minimum spanning network (MSP) drawn by hand and using the MSP output of ARLEQUIN v. 2.0 [44]. The null hypothesis that there was no association between the haplotypes and geographical locations at different genealogical levels was tested. The nesting design was constructed following the rules described by Templeton et al. [50]. The program GEODIS v. 2.0 [37] was used for implementing the calculations of the Euclidian distance measures and their statistical significance. Interpretation of the contingency tests followed an updated version of the inference key of Templeton [49] (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm). A median network [3], which includes mutational information, was also prepared. In addition to the fin tissue samples, two modern bone samples were provided and processed as a blind test.

The Clariidae remains found at Sagalassos (1490–1600 m a.s.l., 37°40′N; 30°31′E) were collected with special care to prevent contamination. They comprise various skeletal parts, but pectoral spines are relatively abundant. These bones were preferred for analysis since their osteomorphology is diagnostic at the genus level [15,19]. All the clariid pectoral spines found at Sagalassos belong to the genus *Clarias* and not to

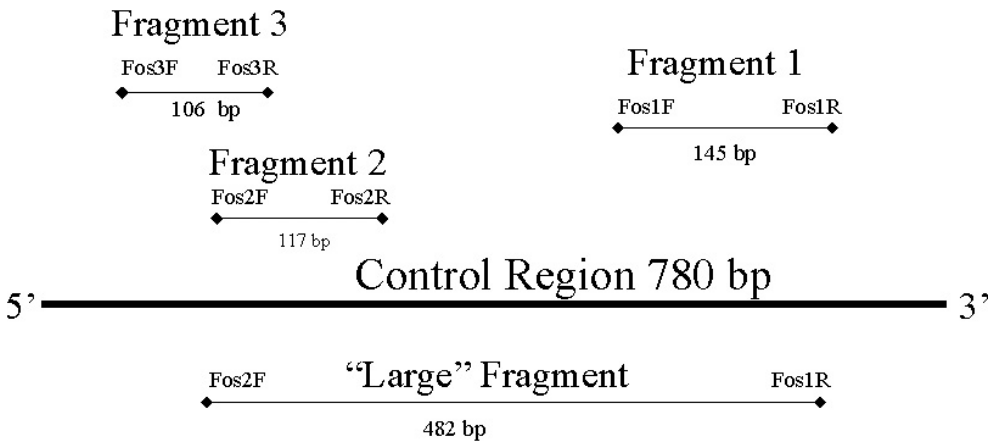

Fig. 2. Amplification strategy and primer designations for the four control region fragments, including the full length 780 bp sequence, of *C. gariepinus*.

Table 2
Subfossil skeletal elements analyzed, including their osteomorphological identification, the nature of the PCR amplification of the three short mtDNA fragments (+: successful; −: unsuccessful) and the genetic identification

Provenance and sample no.	Skeletal element	Bone id.	Mitochondrial control region amplification			Genetic id.
			Fragment 1	Fragment 2	Fragment 3	
Sagalassos						
1	Pectoral spine	*Clarias* sp.	−	−	−	–
2	Pectoral spine	*Clarias* sp.	−	+	−	*C. gariepinus*
3	Pectoral spine	*Clarias* sp.	−	−	−	–
4	Pectoral spine	*Clarias* sp.	−	−	−	–
5	Articular	Clariidae	−	−	−	–
6	Pectoral spine	*Clarias* sp.	−	−	−	–
7	Pectoral spine	*Clarias* sp.	+	+	−	*C. gariepinus*
8	Pectoral spine	*Clarias* sp.	+	+	−	*C. gariepinus*
9	Pectoral spine	*Clarias* sp.	−	−	−	–
10	Parasphenoid	Clariidae	−	−	−	–
11	Articular	Clariidae	−	+	−	*C. gariepinus*
12	Pectoral spine	*Clarias* sp.	−	−	−	–
13	Pectoral spine	*Clarias* sp.	−	+	−	*C. gariepinus*
14	Pectoral spine	*Clarias* sp.	−	−	−	–
15	Pectoral spine	*Clarias* sp.	−	−	−	–
16	Pectoral spine	*Clarias* sp.	−	+	−	*C. gariepinus*
Apamea						
–	Eight pectoral spines	*Clarias* sp.	−	+	−	*C. gariepinus*

Heterobranchus, another genus that is represented in the Nile. In the Levant only *C. gariepinus* occurs whereas in the Nile both this species and *C. anguillaris* are found [51]. Identification of bone finds at species level is possible when the vomer toothplate is preserved [18] but no such elements were found at Sagalassos. It appears that the Clariidae found at sites along the Egyptian Nile comprise *Heterobranchus* only very exceptionally and that the vomer toothplates found all belonged to *C. gariepinus* [16]. The other species, *C. anguillaris*, is also relatively rare in Egypt currently (W. Van Neer, 1983–1987, personal observation). For the reasons mentioned above, it is very likely that the clariid remains discovered at Sagalassos are from *C. gariepinus*. The analyzed samples from Sagalassos are indicated in Table 2. In addition, eight pectoral spines were investigated from Apamea (35°30′N; 36°25′E), a Byzantino-Islamic site in Syria. These remains date to the 6th–7th centuries AD and belong to *C. gariepinus* that was exploited extensively during and after the seasonal floods of the Orontes [54].

The second phase of the DNA study was conducted in a separate pressurized laboratory in order to avoid contamination of the subfossil material from Sagalassos and Apamea. Pipettors, labcoats, equipment, and plastics were purchased new for the purpose of this

project. Extraction and amplification procedures were conducted using filter-tip pipette tips and were carried out in separate rooms with a one-way flow of material to ensure no modern sample or amplified product ever entered the extraction area. Blank extractions were always included to control for the presence of contamination. All equipment was thoroughly cleaned, rinsed with sodium hypochlorite, and exposed to UV light prior to use. Subfossil material from the Apamea site is considerably more abundant than that found so far at Sagalassos. For this reason, Apamean material was used in the initial extractions in order to refine methods. Standard protease phenol/chloroform methods as well as silica extraction were attempted in parallel on the same material. The novel extraction procedure used was a slightly modified version of that used successfully for the amplification of Pleistocene equid bones [23]. Bone was ground to a fine powder with a clean mortar and pestle. Subsequently, 0.5 g of powder was incubated overnight at 55 °C in 2 ml of extraction buffer (5 M guanidinium thiocyanate, 0.1 M Tris–HCl, pH 6.4, 0.02 M EDTA, and 1.3% Triton X-100). The bone powder was pelleted by centrifugation, and the supernatant recovered. A volume of 35 µl of a SiO_2 suspension was added and incubated for 10 min at room temperature. Following centrifugation, the SiO_2 pellet was rinsed twice with buffer (5 M guanidinium thiocyanate, 0.1 M Tris–HCl, pH 6.4, 0.02 M EDTA), twice with 70% ethanol and left to air dry. DNA was eluted from the silica in 50 µl of TE. PCR amplifications were carried out in a volume of 40 µl, using 10 µl of the eluate. Standard extraction methods did not yield successful amplification reactions, whereas successful amplifications were achieved with two out of eight extractions of bones from Apamea using the silica method above. Amplification reactions were transported, unopened, to a separate laboratory for subsequent analysis and sequencing. Aliquots were subjected to electrophoresis through 2% agarose gels to assess success and quality of the amplification. Products of the successful amplification reactions were purified using Jetsorb (Genomed) and sequenced directly, as previously described [2], in order to avoid complications due to cloning and or amplification artefacts. Subsequent attempts were made to extract and amplify DNA from 16 subfossil bones from Sagalassos, using the silica method exclusively. After all historic samples had been processed, two modern bones (Mb1 and Mb2) were extracted in the same manner, in order to confirm the ability to extract and amplify DNA from fish bone and to compare amplification results from ancient versus modern material. Initial amplification of the control region from modern *Clarias* specimens produced a fragment of 780 nucleotides [2]. Based on this sequence, we identified three short, yet highly variable regions within the control region that were bounded by more conserved areas suitable for PCR primer design. The primer pair Fos1F (TAGAATCACTTTCACTTGGC) and Fos1R (AAAGGGTATGCACTTGATAGAG) would produce an amplified product of 145 base pairs (bp) (fragment 1). Similarly, Fos2F (CTTTTAAGACGAAGAAATTGAAGCC) and Fos2R (CAAGGTTGGTGGTCTCTTAC) would yield a 117 bp fragment (fragment 2), and Fos3F (AACATTACATTCAATTGTACCCG) with Fos3R (AAGGAAATATTTGTGTGTGCAG) a 106 bp fragment (fragment 3) (Fig. 2). A large fragment of 482 bp (435 bp without the primers) would be produced with the primers Fos2F and Fos1R. All sequences have been deposited in GenBank under accession numbers AF390154–AF390173.

3. Results

The complete sequence of the control region (780 bp) was obtained from modern specimens collected across the region (Fig. 1). Nucleotide diversity estimates were 8.32 ± 4.35 for the Nile River sample, 3.19 ± 1.78 in Lake Kinneret and 2.62 ± 1.39 for the Syrian Orontes sample. Only a single haplotype was observed from each of the four sampling sites in Turkey, which were all nearly identical to the most common haplotype from Syria. Specimens from river systems or geographic regions were generally found clustered together, although the Nile River specimens were the exception. The two west African *C. anguillaris* specimens clustered together along a single branch that is distinct from *C. gariepinus* found in the same region (data not shown). Despite this distinction within one region, North African specimens of these two species have mtDNA more similar to each other than to *C. gariepinus* from eastern or southern Africa (see Refs. [2,39]), making the genus paraphyletic. NCA, which attempts to differentiate between historic and more recent events by incorporating distance, frequency and mutational information, indicated that two clades had significant outcomes (Fig. 3). First, there was restricted dispersal with isolation by distance in clade 1-9, which includes Syria and the four sites in Turkey. Second, clade 4-1 (Egypt, Israel and Mali) exhibited evidence of allopatric fragmentation.

In the case of the subfossil material from Apamea, Syria, we successfully amplified fragment 2 from two out of eight specimens. Sequence analysis indicated that these specimens were identical to the most common contemporary Syrian haplotype. No successful amplification was obtained using the primer sets for fragments 1 or 3.

Of the 16 subfossil specimens from Sagalassos, we were able to amplify at least one of the fragments from six of these. Fragment 2 was the most successfully amplified, followed by fragment 1, while no successful amplifications were obtained from subfossil material for fragment 3 (Table 2). The combination of the reverse

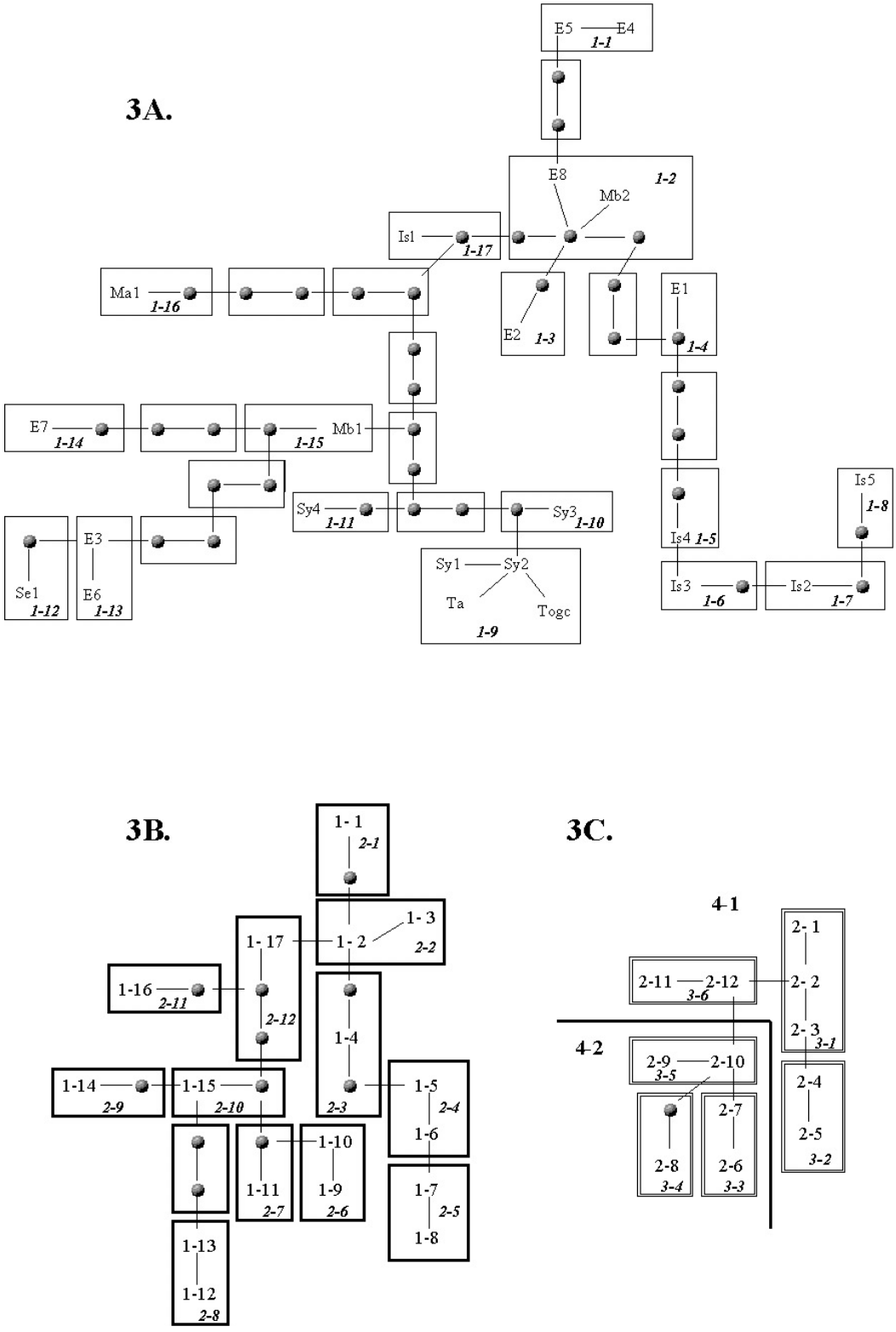

Fig. 3. Nested cladogram among mtDNA haplotypes of the contemporary populations of African catfish, *C. gariepinus,* in the eastern Mediterranean area. This figure was based on a truncated dataset of 435 bp, corresponding to amplification using the Fos2F–Fos1R primer set in order to include the two modern bone amplifications. Genotype labels are abbreviated as follows: E, indicates Nile River at Luxor and Lake Manzala (Egypt); Sy, Syria; Is, Israel; Se, Senegal; Ma, Mali; Mb1 and Mb2, two blind modern bone specimens (see also Table 1). (A) MSP and 1-level clades; (B) 2-level clades; (C) 3- and 4-level clades. Note that Asia Minor and North African specimens are genetically related and differ only slightly from North African *C. anguillaris* (Ca-M). Note also the similarity between Turkish and Syrian specimens, reflecting their geographic position at the edge of the species distribution.

primer for fragment 1, with the forward primer for fragment 2, would be expected to produce a 482 bp fragment ('large' fragment) (Fig. 2). Significantly, none of the amplifications using subfossil material with this primer combination yielded successful results while those using the final modern bone extractions did indeed produce this expected product. Sequences obtained from the subfossil amplifications were aligned with the truncated modern sequences (175 bp) in order to construct a median network (Fig. 4). Three *Clarias* haplotypes were identified from the subfossil material at Sagalassos. The most common of these three (found in four out of six specimens), was identical to a haplotype of *C. gariepinus* found only in the Nile River. The remaining two haplotypes were unique and differed from this common Nilotic form by mutations at single unique positions. The modern bone fragments also yielded unique sequences not previously identified. Based on the position of these haplotypes in the interior of the network, they can be assigned to the genus *Clarias*. In fact, the identity of these specimens were purposely kept unknown until after the sequence analysis was completed, at which time Mb1 was revealed to be *C. anguillaris*, from Qena, Egypt (26°10′N; 32°43′E), and Mb2 as *C. gariepinus* from the High Dam Lake, Egypt.

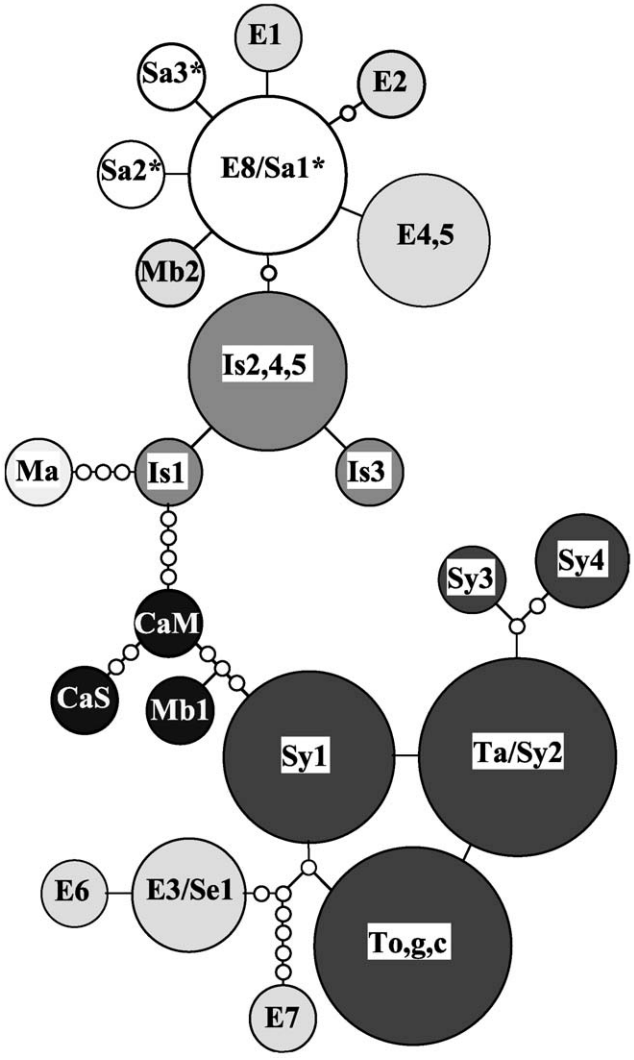

Fig. 4. Reduced median network based on the mtDNA control region sequence (175 bp common to both subfossil and modern amplifications) of *C. gariepinus* and *C. anguillaris* specimens. The circles represent genetic haplotypes, their size being proportional to their frequency. The lines connecting the circles indicate single mutational steps. Haplotypes E4 and E5 from Fig. 3 collapse to E4; similarly, haplotypes Is2, Is4, and Is5 collapse to Is2. Labels refer to sampling locations: E, Egypt; Is, Israel; Ma, Mali; Se, Senegal; Sa*, Sagalassos, Turkey; Sy, Syria; To, Orontes River, Turkey; Tg, Göksu River, Turkey; Tc, Ceyhan River, Turkey; CaS and CaM refer to specimens of the closely related species *C. anguillaris*, from Senegal and Mali, respectively. Two modern catfish bones, Mb1 and Mb2 from Egypt were included as a blind test, yielding unique sequences later confirmed morphologically as *C. anguillaris* and *C. gariepinus*, respectively. Haplotypes are also shaded to indicate common geographic origin of specimens; the black circles with white lettering represent the three *C. anguillaris* specimens. The subfossil Sagalassos specimens cluster together with modern Egyptian populations; three haplotypes were identified among the six subfossil fragments successfully sequenced. The most common of these was identical to modern specimens found in Lake Manzala, Egypt; the remaining two differ by single unique point mutations. Subfossil material from the archaeological site at Apamea, Syria, was identical to a modern specimen from Syria.

4. Discussion

The genetic structuring of modern African catfish (*C. gariepinus*) generally shows a correlation with geography, differentiating by river system in the eastern Mediterranean region. The exception to this is the diverse nature of the Egyptian population with haplotypes spread across the median network (Fig. 3). NCA indicating fragmentation of populations in northern Africa is consistent with the wet/dry cycling in the Sahara during the Pleistocene [30]. The fact that the Syrian specimens cluster with African rather than with Israeli specimens argues against a simple, singular northward migration pattern. Rather, our results may either indicate separate temporal or geographic origins of the Israeli and Syrian populations from two different Egyptian clades. Simulations based on coalescent theory [8] have shown that older haplotypes tend to lie towards the interior of networks. As range expansion occurred, it is possible that ancestral forms may have become widespread. Therefore, it seems likely that the lower Nile system represents an older population, from which small numbers colonized surrounding areas, giving rise to populations of lower diversity through founder effects. The Mediterranean Sea was at least 100 m lower than present on several occasions during the Pleistocene [5]. These conditions may have allowed Nilotic specimens to expand northward through freshwater connections now submerged or on massive freshwater runoff from the Nile during wet palaeoclimatic periods. Alternatively, the slip tectonic fault between the Gulf of Aqaba, via the Jordan, Litani, and Orontes valleys might have provided a stepping stone vehicle for northward migration.

Several typical freshwater African elements are known from the Levant up to the Orontes (e.g. *Hippopotamus*, *Crocodylus* and *Trionyx*) [36]. Further sampling is required to improve the power of resolution in this system.

The clustering of modern Turkish and Syrian specimens is of considerable interest. At present, we cannot determine if all the current Turkish populations are the result of a recent range expansion (consistent with reduced diversity and similarity to Syria) or an anthropogenic artefact. If human introduction did indeed occur, the stock source appears to have been from the region situated at the mouth of the Orontes and Ceyhan River in Southeast-Turkey and Northwest-Syria [36]. There is a clear divide in fish fauna between the more western Seyhan and the Ceyhan/Orontes system, which has been modified by modern channels between the Ceyhan and Seyhan rivers (Krupp, personal communication). In future it may be possible to address this question with more sensitive (hypervariable) genetic markers. On a more local scale, the present DNA-analysis has not been able to address the question whether the *C. gariepinus* presently found south of Sagalassos in the Aksu River are ancient populations or if they represent rather recent human introductions. However, archaeozoological evidence from the site of Kilise Tepe indicates that the modern populations found west of the Ceyhan River probably are all recent introductions. The site of Kilise Tepe (36°29′N; 33°35′E), situated along the Göksu basin, has yielded fish bones dated to the Bronze Age, the Iron Age and the Byzantine period. From the earliest period onwards, marine fish have been exported to the site from the Mediterranean coast, which is at approximately 40 km south. In that coastal area, *C. gariepinus* is found currently in the Akgöl [20]. The catfish can be easily captured, even by hand during the spawning season, and it is therefore strange that it is not found in the oldest occupation levels of Kilise Tepe when marine fish was already brought in. The first finds of *Clarias* at the site (in a Late Iron Age context) coincide with the presence of the Nile perch *L. niloticus*, which is a typical Nilotic species [56].

In total, six out of 16 subfossil bones from the Sagalassos site provided DNA that could be successfully amplified, predominantly with the primer set 2. Although this does not correspond precisely with fragment size, the results are consistent with the strength of amplifications using modern material. The limited amplification success of these small fragments from the ancient material is also consistent with its authenticity. Due to the extensive damage it incurs, ancient DNA is highly fragmented into pieces of extremely limited length [33]. Successful amplification of fragments greater than 200 bp in length from authentic ancient material is therefore highly unlikely. Significantly, only the extraction from the modern bones yielded the 482 bp product using the Fos1R and Fos2F primer combination. Contamination of the ancient material from modern sources would be expected to result in successful amplification of this 482 bp fragment. Additional support for the authenticity of our results stems from the fact that three haplotypes were identified from subfossil material, two of which were unique. The identification of the modern bone sample Mb1 as *C. anguillaris* also has important ramifications. Although the sequence from this specimen does not form a monophyletic group with the *C. anguillaris* from West Africa, it is very similar and is found in a nearby region of the network (Fig. 4). The inclusion of Mb1 in our analysis strongly suggests that the subfossil material represents *C. gariepinus*, rather than *C. anguillaris*, since all contemporary *C. anguillaris* are found in areas of the network quite removed from subfossil haplotypes. The identification of modern bone sample Mb2 as closely related to the Egyptian *C. gariepinus* haplotype E8, is logical (Figs. 3 and 4).

Further evidence exists that the conditions at the high altitude location of Sagalassos were favourable to the preservation of DNA. Successful amplification of DNA from human remains has also been achieved from material at the site [24] and such success has amongst others been linked to temperature [47]. In the case of the catfish bones, rapid burial of refuse may have helped avoid moist, aerobic conditions that would have lead to a more complete degradation of DNA.

5. Conclusions

The identity of the Apamea specimens with a contemporary Syrian haplotype indicates that the material was of local origin, as might be expected from the archaeological and historic context. Material from the Apamea site exists in sufficient quantity that it is possible to examine haplotype frequency changes over the last 1500 years, providing an extremely valuable opportunity to compare simulation studies to natural populations. The identity, or near identity, of Sagalassos sequences to those from the Nile River provides strong support that the material was in fact imported from the Nile region. Our results thus support the extensive archaeological evidence suggesting trade relationships between the occupants of Sagalassos and the Nile valley during Roman and Early Byzantine times.

It appears that *Clarias* catfish was the major species imported at Sagalassos from Egypt, whereas *Bagrus* and *L. niloticus* arrived only sporadically at the site. Given the large distance, it is obvious that these Nilotic fishes must have been transported in preserved form, probably as sun or smoke-dried specimens. These two curing methods were most commonly used in Egypt since prehistoric times. Early archaeological evidence for fish smoking is available from the 12,000 years BP site of

Makhadma, Middle Egypt [58,61]) and numerous iconographic data for smoking and sun-drying are known from the pharaonic period [17]. Curing allowed storage of fish products for several months, and made them suitable for long-distance transport as well. It is worth noting that the practice of sun-drying may in fact have limited the degree of DNA damage that occurred in our samples since this is largely a hydrolytic process. Due to the possible presence of PCR inhibitors in smoke, sun-dried specimens may be much more amenable to successful DNA amplification compared to smoke-dried ones. Trade of Nilotic fish in the Eastern Mediterranean region is documented since the Chalcolithic for the southern Levant, but the evidence is more abundant from the Bronze Age onward [28]. During that period fish from the Nile were transported as far as Lebanon, Cyprus [40] and Jordan [53]. In Roman times, fish from the Nile were transported as far as Italy and also a large part of the Egyptian grain was transported to the Imperial capital of Rome as tax or surplus production, forming part of the annona redistribution system [38]. Nile perch was found in a 2nd century AD context at Ostia, the port of Rome (Van Neer, unpublished), and it is likely that the spine of *Clarias* sp. found at Vallerano, 10 km south of Rome, also has a Nilotic origin [12].

On many sites in the Near East where *Clarias* bones have been found in association with Nilotic fish (*L. niloticus*, *Synodontis* and *Bagrus*), it has been assumed that the *Clarias* were of local origin since they occur naturally in the region (e.g. Ref. [27]). It remains to be verified whether these catfish were indeed local or if a fraction has been imported from the Nile. A strategy for the amplification of fossil mtDNA of *Clarias* is now available which may, together with hypervariable microsatellite loci, help to elucidate those issues.

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