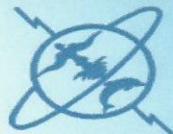


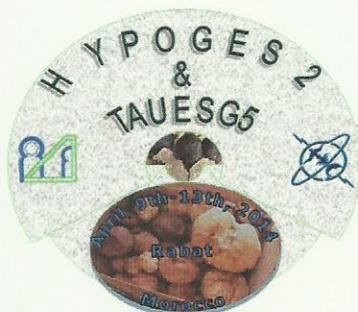


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FIRST EVIDENCE OF GENETIC DIFFERENTIATION OF *TUBER AESTIVUM/UNCINATUM* POPULATIONS FROM TURKEY

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Key words: Internal transcribed spacer, phylogeny, genetic variability, molecular biology

Truffles belonging to the *T. aestivum/uncinatum* species complex (hereinafter *T. aestivum*) are collected all over Europe (Paolocci et al. 2004), and also found in North-Africa (Jeandroz et al. 2008) and Asia (Song et al. 2005). Differently, the two most appreciated truffle species, *T. magnatum* and *T. melanosporum*, are patchily distributed in limited areas in southern Europe. During the last ice age both these species likely experienced a population bottleneck and it has been hypothesized that their refugia were located in their southernmost geographic ranges (Rubini et al. 2005; Riccioni et al. 2008, Murat et al. 2004). Conversely, whether *T. aestivum* also suffered from a similar bottleneck remains to be elucidated. Studies are also needed to test whether and to what extent *T. aestivum* specimens of different provenance are genetically different. Being one of the southernmost areas of its distributional range, Turkey may represent a natural reservoir a strain biodiversity for this species.

With the aims of assaying the possibility of typing populations according to their geographic origin and searching for possible phylogeographic signals, here we investigated the extent and distribution of genetic variability of *T. aestivum* ascocarps from Turkey and compared their genetic profiles with those from specimens recovered all over Europe.

To this end, a total of 44 *T. aestivum* truffles from five natural sites located in the Anatolian peninsula (Turkey), was genotyped by sequencing the ITS region of the rDNA. Additionally, 124 ITS sequences from *T. aestivum* samples collected all over Europe were retrieved from GenBank and considered in the phylogenetic analyses.

Sequence alignment revealed a high level of intraspecific polymorphism of the ITS region compared to other truffle species such as *T. melanosporum*, *T. magnatum* and *T. borchii* (Murat et al. 2004, Mello et al. 2005, Bonuso et al. 2010). Considering all the 168 *T. aestivum* samples, a total of 52 haplotypes were identified. In Turkish populations 29 haplotypes were identified and, interestingly, only 2 of them, present in these populations at very low frequency, were shared with other European populations. Moreover, most of the exclusive Turkish haplotypes (25/27) were population-specific. A preliminary phylogenetic analysis showed that most of the Turkish haplotypes form a well supported cluster that includes only few haplotypes from other countries. This suggests a possible phylogeographic differentiation of *T. aestivum* populations from the Anatolia region of Turkey.

Overall, these results let us argue that the Anatolian peninsula may represent a hotspot of strain biodiversity for *T. aestivum* and that Turkish populations could be typed according to their geographic origin. SSR (simple sequence repeat) analyses are in progress to gain more insight into the extent and distributional patterns of *T. aestivum* genetic diversity over its geographical range.

Acknowledgement:

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INTRODUCTION

Truffles belonging to the *T. aestivum/uncinatum* species complex (hereinafter *T. aestivum*) are collected all over Europe (1), and also found in North-Africa (2) and Asia (3). Differently, the two most appreciated truffle species, *T. magnatum* and *T. melanosporum*, are patchily distributed in limited areas in southern Europe. During the last ice age both these species likely experienced a population bottleneck and it has been hypothesized that their refugia were located in their southernmost geographic ranges (4, 5, 6). Conversely, whether *T. aestivum* also suffered from a similar bottleneck remains to be elucidated. Studies are also needed to test whether and to what extent *T. aestivum* specimens of different provenance are genetically different. Being one of the southernmost areas of its distributional range, Turkey may represent a natural reservoir a strain biodiversity for this species.

With the aims of assaying the possibility of typing populations according to their geographic origin and searching for possible phylogeographic signals, here we investigated the extent and distribution of genetic variability of *T. aestivum* ascocarps from Turkey and compared their genetic profiles with those from specimens recovered all over Europe.

MATERIALS AND METHODS

A total of 44 *T. aestivum* truffles from five natural sites located in the Aegean region of Turkey (Fig. 1) was considered. After a morphological control according to Montecchi and Sarasini (7), truffles were genotyped by sequencing the ITS region of the rDNA (1). ITS sequence visualization, editing and assembly were performed using FinchTV 1.3.1 (<http://www.geospiza.com>) and Geneious 4.8.5 (<http://www.geneious.com>). Additionally, 124 ITS sequences from *T. aestivum* samples collected all over Europe were retrieved from GenBank and considered in the phylogenetic analyses. For these analyses the software MEGA 6 (8) and the neighbor-joining method were adopted. Bootstrap test was performed using 1000 replicates. Haplotypes and their relative frequencies were calculated by the software Arlequin 3.11 (<http://cmpg.unibe.ch/software/arlequin3>). Some *de novo* analyzed *T. aestivum* samples from Turkish populations exhibited a G:C rich region in their ITS2 sequence, reported to interfere with sequencing reactions (1). Therefore this region was excluded from the sequence alignment and the downstream phylogenetic analyses.

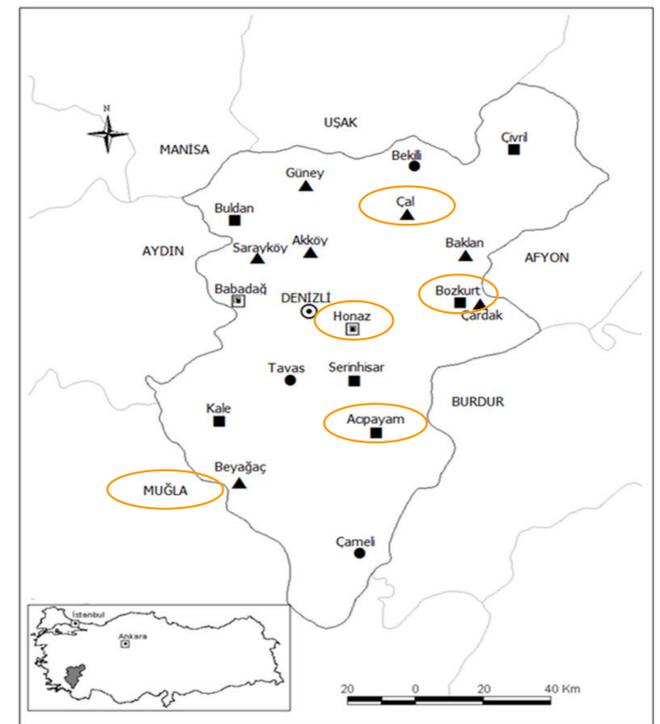


Figure 1 - *T. aestivum* collection sites in the Aegean region

Table 1 - Relative frequencies and specificity of *T. aestivum* ITS haplotypes in Turkish population

Haplotype no.	Relative frequencies in Turkish populations					Records in other countries
	Bozkurt	Acipayam	Honaz	Cal	Muğla	
1	0.0833	0	0	0	0	no
2	0.167	0	0	0	0	no
3	0.167	0	0	0	0	no
4	0.0833	0	0	0	0	no
5	0.0833	0	0	0	0	no
6	0.0833	0	0	0	0	no
7	0.0833	0	0	0	0	yes
8	0.0833	0	0	0	0	no
9	0.0833	0	0	0	0	no
10	0.0833	0	0.0909	0	0	no
11	0	0.75	0	0	0	no
12	0	0.25	0	0	0	no
13	0	0	0.0909	0	0	no
14	0	0	0.0909	0	0	no
15	0	0	0.0909	0	0	no
16	0	0	0.0909	0	0	no
17	0	0	0.0909	0	0	yes
18	0	0	0.0909	0	0	no
19	0	0	0.0909	0	0	no
20	0	0	0.0909	0	0	no
21	0	0	0.0909	0	0	no
22	0	0	0.0909	0.7	0	no
23	0	0	0	0.1	0	no
24	0	0	0	0.1	0	no
25	0	0	0	0.1	0	no
26	0	0	0	0	0.286	no
27	0	0	0	0	0.143	no
28	0	0	0	0	0.143	no
29	0	0	0	0	0.429	no

RESULTS

Considering all the 168 *T. aestivum* samples, a total of 52 ITS haplotypes were identified. In Turkish populations 29 haplotypes were identified (Tab. 1) and, interestingly, only 2 of them (n. 7 and 17) were shared with other European populations. Moreover, most of the exclusive Turkish haplotypes (25/27) were specific to single populations (Tab. 1). A preliminary phylogenetic analysis based on haplotypes alignment (Fig. 2) showed that most of the Turkish haplotypes form a well supported cluster (blue-shaded) that includes only few haplotypes from other countries. This suggests a possible phylogeographic differentiation of *T. aestivum* populations from the Anatolia region of Turkey.

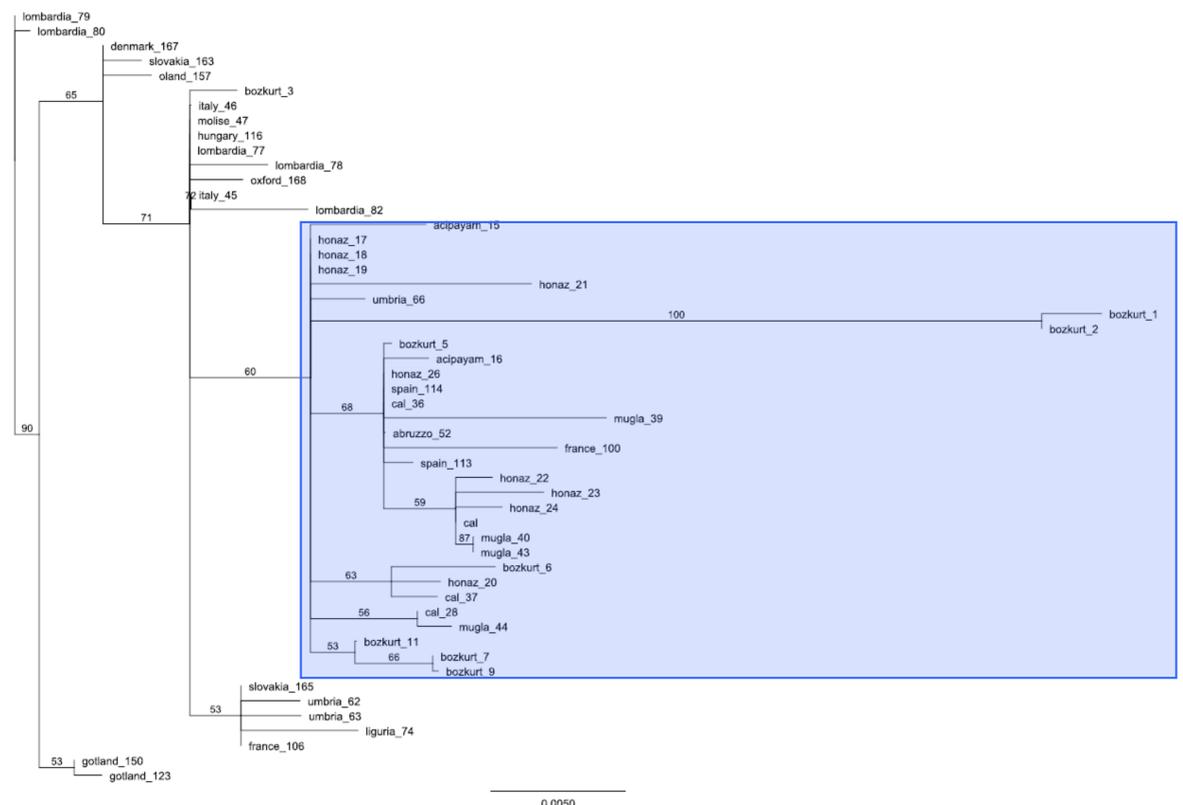


Figure 2 – Neighbor-joining tree of the 52 ITS haplotypes. Haplotypes are indicated with the main geographic area and an id number. The blue-shaded area represents the cluster which most of the Turkish haplotypes belong to.

CONCLUSIONS

Overall, these results let us argue that the Aegean region may represent a hotspot of strain biodiversity for *T. aestivum* and that Turkish populations could be typed according to their geographic origin. SSR (simple sequence repeat) analyses are in progress to gain more insight into the extent and distributional patterns of *T. aestivum* genetic diversity over its geographical range.

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