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Research Article

Earliest evidence of *Mus musculus* ssp. in Western Europe during the Late Neolithic (Tosina, Mantova, Northern Italy): new insights on the house mice migratory waves

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Abstract

New evidence arising from the study of the microtheriological materials from the Late Neolithic site of Tosina of Monzambano (Province of Mantova, northern Italy) shows that *Mus musculus* ssp. was present in Western Europe in the Late Neolithic — at least 1500 years earlier than previously clearly demonstrated. This discovery provides evidence for a first migratory wave, following human populations moving from east to west, of this highly synanthropic animal species. The data are consistent with the evidence of *Mus musculus musculus* — hypothesised to belong to a second wave of house mice reaching Europe — from the Romanian Copper Age site of Bucşani La Pod tell.

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Introduction

The Neolithic is undoubtedly the crucial moment for the realization of the socio-economic relationships that will characterize all cultural transitions of humanity.

The decision to settle permanently in selected areas and there to cultivate plants and breed animals, and at the same time to reduce the activities of hunting and gathering wild products, are the crucial steps of the Neolithic "revolution". This cultural change begins in the Near East, around 11–12000 years ago, when a group of human beings began cultivating cereals and legumes and took control of some wild ungulates (Vigne et al., 2011; Vigne, 2015). Only later, around 10500 years ago, did vegetables and animals begin to show traces of modifications resulting from human action. Agriculture/farming becomes the main source of sustenance only between 10000 and 9500 years ago (Cucchi et al., 2012).

The beginning of the Neolithic practices, above all the need to store plants and seeds in specific places, creates the conditions for commensalism that will lead different animals to adapt to the sharing of spaces with humans. Among these, one of the best known is the domestic mouse (*Mus m. domesticus* and *Mus m. musculus*). The domestic mouse is the subject of research as it is directly linked to the diffusion of Neolithic practices from their areas of origin in the Near East to the wider world (Cucchi et al., 2005, 2011, 2013, 2020).

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The domestic mouse migrates together with man, foodstuffs and domestic animals as a synanthropic animal favoured by adaptation to these conditions. It is therefore an indirect sign of the transmission of knowledge from the Near East to Western areas of Europe, areas that will be so profoundly changed by the process of Neolithization that we can truly speak of a Neolithic "revolution".

The spread of the domestic mouse to the West seems to have involved two distinct routes: 1- through the Mediterranean by means of the main islands. It seems to be linked to trade involving maritime transport. However, after an early arrival on Cyprus during the Neolithic period, the house mouse does not seem to reach the other main islands before the Bronze Age (Cucchi and Vigne, 2006). The old colonization, 5000 years BC, of western Europe from the north African cost propose by Castillo et al., 2001 have been rejected by the AMS dating that brings the samples forward to the seventh century BC/third century AD (Alcover et al., 2009). 2- Eastern Europe was the second route of "Neolithization" for Western Europe. Recent works have shown, without a shadow of a doubt, that the domestic mouse, probably already a second wave of the species, reaches Romania (La Pod tell site) during the Chalcolithic, 4500–3900 cal. BC (Cucchi et al., 2011, 2020). Cucchi et al., 2020 give a complete representation of the timing of the house mouse dispersal in Southwestern Asia and Eastern Europe starting from 40000 years ago to 3000 years ago. Beginning from the Upper Pleistocene feral communities in the central Asia the authors point out that the house mouse moves westward, at first slowly up to about 12000 years ago, then, with the increase of commercial exchanges linked with

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Table 1 – Tosina di Monzambano. List of ^{14}C dating of sector A (dating are made by two laboratories: LTL, CEDAD Università del Salento; DSH, CIRCE Università di Caserta) Calibrated using OxCal v3.10.

Sample	Laboratory	Radiocarbon age	Calibration 1σ	Calibration 2σ
US 144	LTL14339A	4974 ± 45	3800–3698 BC (68.2%)	3940–3860 BC (14.2%) 3820–3650 BC (81.2%)
US 127	DSH8237_C	5019 ± 45	3936–3872 BC (45%) 3810–3758 BC (38%) 3744–3714 BC (17%)	3947–3706 BC (100%)
US 110base	DSH8236_SE	5120 ± 47	3976–3931 BC (42%) 3876–3806 BC (58%)	4036–4022 BC (2%) 3994–3794 BC (98%)
US 139 c. 5	LTL14338A	5199 ± 45	4045–3965 BC (68.2%)	4230–4200 BC (3.4%) 4170–4090 BC (7.4%) 4080–3940 BC (84.6%)

the beginning of the neolithization process, it becomes more common in the sites of the Eastern Mediterranean invading Eastern Europe between 8500 and 6500 years ago.

In Western Europe, the definite presence of the house mouse has been established, with some doubts, from the Neolithic Cucchi et al., 2005, 2020. The data from Tosina (Lombardy, Northern Italy) highlight how migrations involving the transport of goods and foodstuffs from east to west involved the involuntary transport of small "guests", already perfectly adapted to life alongside human communities.

In any case, as pointed out by Cucchi et al., 2005, the probable reason for the lack of data showing the presence of house mice in Western Europe in contexts older than the Iron Age is that excavation methods did not involve fine sieving of excavated soils.

The site of Tosina

The Neolithic village of Tosina di Monzambano is located a few hundred metres west of the village of Monzambano in the province of Mantova (northern Italy) and a few kilometres south of the southeastern shore of Lake Garda at about 90 metres above sea level (Fig. 1). Tosina is an important late Neolithic site (Chassey-Lagozza culture) dated to about 6000 years ago (Poggiani Keller, 2014; Poggiani Keller et al., 2014; Bona, 2014; Lo Vetro, 2014; Castiglioni and Rottoli, 2014) (Tab. 1, Fig. 3), located in a very important geographical area on trade routes that linked Eastern Europe to Northeastern Italy and this latter area to Western Europe and central/southern Italy (Poggiani Keller, 2014; Lo Vetro, 2014).

The stratigraphic series of sector A-A1 consists of approximately one meter of sediments with archaeological importance. The late Neolithic levels are sealed between a 30 cm–40 cm layer of pluoghsoil (US 100) and a sterile layer of late glacial silts (US 106) (Fig. 2).

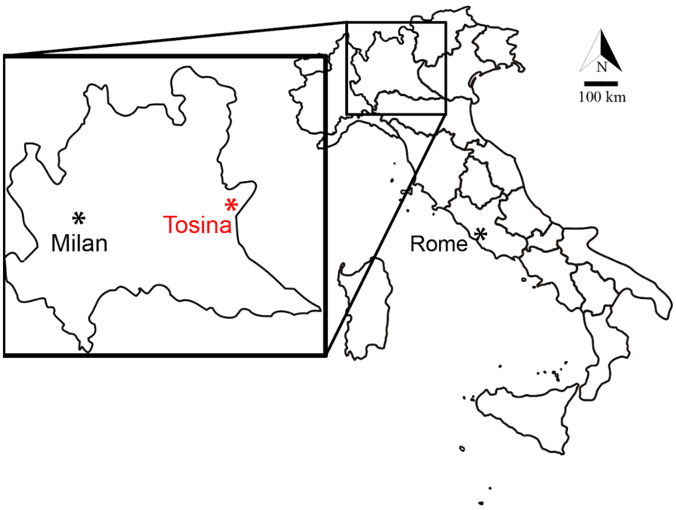

Figure 1 – Geographical location of the Late Neolithic site of Tosina di Monzambano.

Material and Methods

The material considered in this paper comes, as mentioned above, from the late Neolithic site (Chassey-Lagozza culture) of Tosina di Monzambano (province of Mantova, northern Italy) (Fig. 1) and, more specifically, from sectors A and A1 and from stratigraphic units attributed to the same chronological horizon (late Neolithic).

The study of the small mammals was carried out, where possible, to the species taxonomic level while the rest of the microvertebrates was mostly catalogued at the class or superclass level, although for some groups a lower level of determination was reached.

The small mammal samples have been identified following (Chaline et al., 1974) and Niethammer and Niethammer and Krapp, 1978, 1982, 1990.

The sediments had been sieved with a 1 mm mesh sieve and then they had been picked over to collect microvertebrate remains.

To establish the MNI (Minimal Number of Individuals) and the relative frequencies of the more-represented skeletal elements, only molar teeth were used for the rodents, whereas in the case of moles these counts considered also the siding of the same bones (for example: 3 left humeri and 2 right humeri mean the presence of at least 3 individuals).

The Tosina small faunal sample consists of 1474 remains of microvertebrates belonging to 4 classes (Amphibia, Mammalia, Aves and Reptilia) and to a superclass (Pisces) (Bona et al., 2018) (Tab. 2). One piece, a left hemimandible (catalogue number Tos-Micro 99), has been attributed to *Mus musculus* ssp. (Fig. 4).

Considering the type of archaeological deposit — open air — to avoid risks of contamination the following procedure was followed: 1- the ploughed soil and the top of the first stratigraphic unit in situ (US 105) were eliminated; 2- the sediment coming from rodent burrows, post holes and any other stratigraphic irregularities was not sieved. Furthermore, to confirm the uniformity of the context, a study of taphonomic aspects of bone accumulation was carried out on all the odontological and post-cranial remains of the micro-mammals along with a numerical study of the remains of all the microvertebrates, mainly following the work of Andrews, 1990; Fernàndez-Jalvo and Andrews, 1992; Fernàndez-Jalvo and Avery, 2015; Fernàndez-Jalvo et al., 2014, 2016.

The traces of predation and digestion on teeth, hemimandibles, maxillary and post-cranial bones were then recorded, focusing on their frequency with respect to the number of specimens recovered in order to compare the microfauna of the Tosina site with that of the sites considered in the above-cited works.

Taphonomic analysis

For this study, the taphonomic analysis turned out to be essential in demonstrating the quality of the work done during the excavation and sieving phases. It allowed us to show that the studied remains derive from ancient accumulations made mainly by predators and are not the result of accumulation of dead animals in recent den burrows.

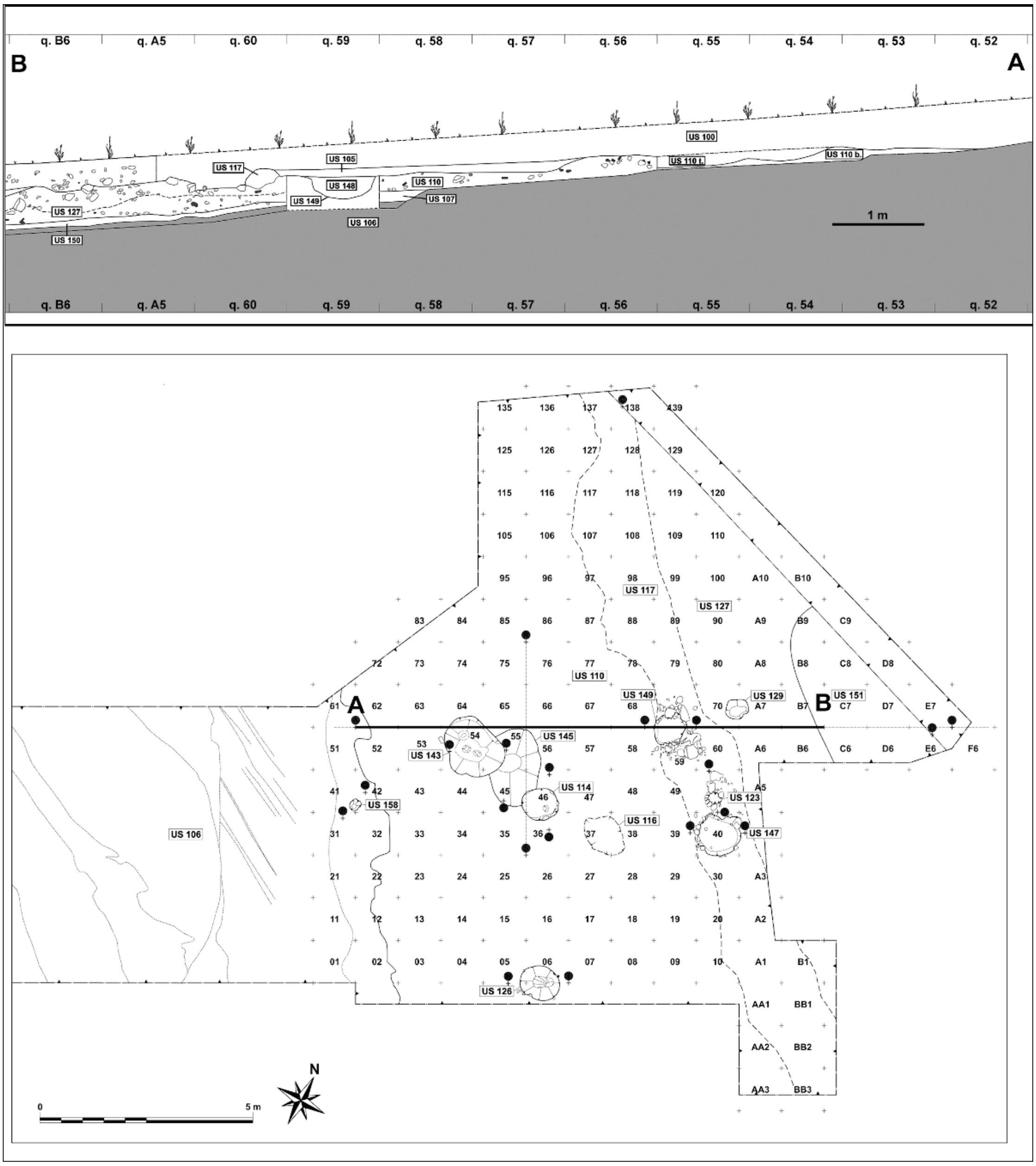

Figure 2 – Tosina di Monzambano. Section (A-B) and planimetry of the Sector A-A1. The stratigraphical section starts from the US100, the pluoghsoil, to the US 106, the sterile level.

Taphonomic analysis: results

The taphonomic analysis of the molars and incisors (following Andrews, 1990; Fernàndez-Jalvo et al., 2016) allowed us to show that traces of digestion are present on about 40% of the sample (Tab. 3). The majority of finds show a relatively low level of digestion. Some finds with a high degree of digestion are also present 3% of the sample (Tab. 3).

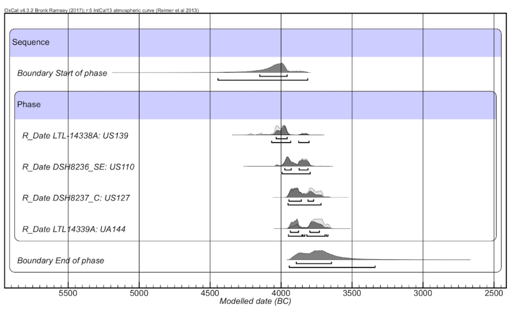

Figure 3 – Tosina di Monzambano. Four radiocarbon determinations (calibrated with OxCal 4.3 -Bronk Ramsey 2009-) from the area where the remains of *Mus musculus* ssp.were found.

The intraspecific analysis confirms the outcome, with the sole proviso that on average the larger animals (*Arvicola* — water vole — size) show a lower level of digestion than the smaller animals (*Microtus* size) (Tab. 4).

The degree of fragmentation of the hemimandibles is quite high and only 5 of 22 specimens still retain teeth (Tab. 5).

Study of the post-cranial bones of Rodentia and Eulipotyphla shows that only one humerus out of 106 long bones is preserved intact, most of the other long bones being represented by fragments of diaphyses or unfused epiphyses. Post-cranial bones show, overall, some degree of digestion in almost 50% of the sample.

Taphonomic analysis: discussion

The degree of digestion of the teeth and the extent of fracturing of the hemimandibles and post-cranial bones seem to indicate the action of a medium- to small-size predator that often shatters the hemimandibles of its prey (net of any other taphonomic processes such as trampling, weathering, etc.). Specifically, the degree of digestion shown by the incisors and molars — low to moderate — would indicate predators of

Table 2 – Tosina di Monzambano. List of number of small vertebrate remains by stratigtaphic units).

	US 107 t	US 107	US 110	US 127	US 150	US 152	US 153	US 168	Total	%	% of taxa
Aves	2	5	11	2	3	6			29	1.97	1.97
Pisces	42	21	111	49		91		3	317	21.51	24.08
Ciprinidae				7					7	0.47	
Tinca tinca	1		4	4		4			13	0.88	
Esox lucius	1		3	2					6	0.41	
Squalius cephalus			10			1			11	0.75	
Barbus plebejus			1						1	0.07	
Reptilia	30	39	250	98	3	104		4	528	35.82	36.84
Emys orbicularis	1	5	5	1		3			15	1.02	
Amphibia	20	17	72	46	1	77		2	235	15.94	16.28
Bufo bufo			2						2	0.14	
Pelophylax sp.			3						3	0.20	
Mammalia											20.83
Cricetidae											
Arvicola amphibius	2		31	29	3	14	2		81	5.50	
Myodes glareolus			4	3		5			12	0.81	
Microtus (Terricola) savii			2	1					3	0.20	
Microtus sp.			9	1		7			17	1.15	
Muridae											
Apodemus gr. *sylvaticus/flavicollis*		1	8	3		1			13	0.88	
Mus domesticus				1					1	0.07	
Gliridae									0		
Glis glis			1	3		1			5	0.34	
Muscardinus avellanarius				1					1	0.07	
Soricidae											
Crocidura suaveolens		1		1					2	0.14	
Neomys fodiens			1						1	0.07	
Sorex araneus				1					1	0.07	
Erinaceidae											
Erinaceus europaeus	1		1	1		1			4	0.27	
Talpidae											
Talpa sp.	1		1	1		2			5	0.34	
Talpa caeca			1			2			3	0.20	
Talpa europaea		1	4	2		4			11	0.75	
Chiroptera			1						1	0.07	
Rodentia post cranial	16	15	24	51		37			143	9.70	
Soricomorpha post cranial	1					2			3	0.20	
Total	118	105	560	308	10	362	2	9	1474	100	100

category 3 such as tawny owl (*Strix aluco*) or eagle owl (*Bubo bubo*) (Fernàndez-Jalvo and Andrews, 1992). According to the data collected, the eagle owl can be excluded and so the tawny owl seems to be the most probable predator.

Other important traces have also been detected on the post-cranial finds: traces of bio-erosion due to vegetal action represented by small meandering furrows along the cortical bone (also observed on some incisors); traces of fracturing due to mechanical action of a predator; traces of combustion, with three elements showing clear traces of burning that are difficult to interpret (Tab. 6).

The set of data acquired confirms that the microfauna collected from the Late Neolithic levels of the Tosina di Monzambano site is the result of an ancient accumulation due to the action of one or more predators, probably nocturnal category 3 raptors (Fernàndez-Jalvo and Andrews, 1992).

Environmental Analysis

The faunal assemblage from the Tosina site (Tab. 2) allows us to make suggestions about the environmental conditions of the surrounding area during the Late Neolithic.

Table 3 – Tosina di Monzambano. Small mammal molars and incisors degree of digestion according to the categories described by Fernàndez-Jalvo and Andrews (1992). Stratigraphic units (US) reported are all late Neolithic in age. (n=number of specimens; %= percentage of the number of specimens).

Molars		US 107 + 107 t	US 110 + 110 t	US 113	US 127	US 150 + 152 + 153	Total
non-digested	**n**		23		29	11	63
	%		53.5		74.4	78.6	63.6
light	**n**	1	13	1	7	2	24
	%	50	30.2	100	17.9	14.3	24.2
moderate	**n**	1	5		3		9
	%	50	11.6		7.7		9.1
heavy	**n**		2			1	3
	%		4.6			7.1	3
extreme	**n**						
	%						
Incisors							
non-digested	**n**	4	18		18	8	48
	%	66.7	58.1		58.1	50	57.1
light	**n**	2	9		7	6	24
	%	33.3	29		22.6	37.5	28.6
moderate	**n**		2		6	2	10
	%		6.4		19.3	12.5	11.9
heavy	**n**		2				2
	%		6.4				2.4
extreme	**n**						
	%						

The rodents in the sample are mostly from species that favour closed to covered environments with limited open areas. The *Arvicola amphibius* (water vole) along with various species of soricomorpha, amphibians and the marsh turtle (*Emys orbicularis*) attests to the presence of important wetlands or marshes.

The presence of two species of moles (*Talpa europea* and *Talpa caeca*) allows us to understand how both well-developed soils and poorly-developed soils were present.

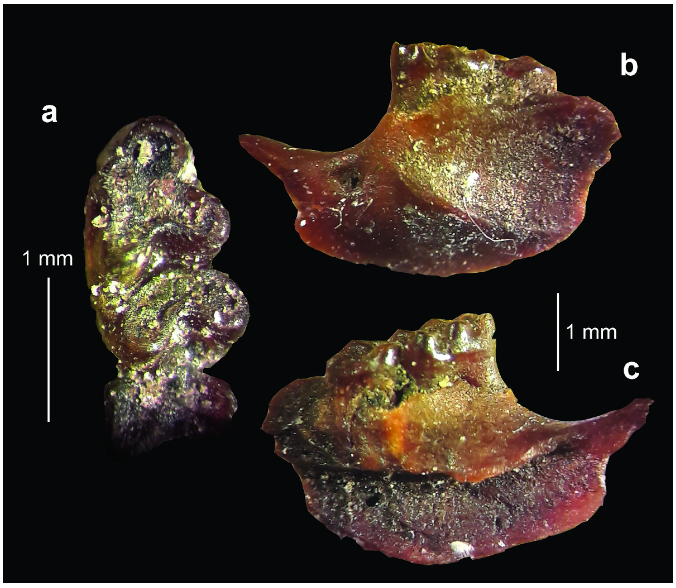

Figure 4 – Tosina di Monzambano. *Mus musculus* ssp. left hemimandible (Tos-Micro 99). a) m/1-2 particular in occlusal view; b) hemimandible, buccal side; c) hemimandible, lingual side. Scale bars: 1 mm.

Table 4 – Tosina di Monzambano. Small mammal teeth digestion rate (Fernàndez-Jalvo and Andrews, 1992) (n=number of specimens; %= percentage of the number of specimens).

		Digested		Not digested	
		n	%	n	%
Arvicola amphibius	US 107 t	2	100		
	US 110	13	41.9	18	58.1
	US 127	7	22.6	24	77.4
	US 139			1	100
	US 150	1	25	3	75
	US 152			14	100
	US 153	1	50	1	50
	Total	24	28.2	61	71.8
Apodemus gr. *sylvaticus-flavicollis*	US 107			1	12.5
	US 110	4	50	4	50
	US 127	1	33.3	2	66.7
	US 152			1	100
	Total	5	38.5	8	61.5
Myodes glareolus	US 110	1	25	3	75
	US 127	1	33.3	2	66.7
	US 152		0	5	100
	Total	2	16.7	10	83.3
Microtus (Terricola) savii	US 110	2	100		
	US 127	1	100		
	Total	3	100		
Glis glis	US 110	1	100		
	US 127			3	100
	US 113	1	100		
	US 152	1	100		
	Total	3	50	3	50

The fish include both more lacustrine species such as the tench (*Tinca tinca*) along with species more typical of riverine environments such as the pike (*Esox lucius*). Perhaps the latter were caught in the nearby Mincio river.

The presence of this faunal accumulation due to the combined action of men and birds of prey makes it possible to hypothesize that sector A and A1 represented a marginal area of the village, probably marshy with riparian vegetation, where the inhabitants discarded some of their waste.

Palynological (Zanon, 2014) and carpological (Castiglioni and Rottoli, 2014) analyses confirm the presence of an extensive wood, with the most represented specie are *Corylus* and *Quercus* ssp., with cultivated areas adjacent to the site. The level of the water of the lacustrine basin showed seasonal variations testified by both from the presence of pollen from aquatic plants and algae and from the presence of carpological remains which highlight the action of mineralization processes favoured by the occasional presence of water.

At the current state of research it is not possible to propose a wider view of the environmental conditions of northern Italy during the late Neolithic.

Mus musculus ssp.

The most significant specimen among the identified fauna is a house mouse (*Mus musculus* ssp.) left hemimandible (Tos-Micro 99).

The Tos-Micro 99 sample consists of an incomplete left hemimandible (state of fragmentation D), with slight traces of digestion, still bearing the first and second molars (Fig. 4).

The first molar is 1.519 mm long and 0.927 mm wide.

The tubercle of the mesial lobe of the first molar is tetralobic but with a more trilobic shape typical of the *M. m. domesticus* subspecies (Orsini et al., 1983) (Fig. 4). The absence in Western Europe of reports

Table 5 – Tosina di Monzambano. Small mammal hemimandible breakage degree according to the categories described by Andrews (1990) (number of specimens).

		A	B	C	D	E	E+
Arvicola amphibius	US 110						1
	US 127					3	
	US 153				1		
Apodemus gr. *sylvaticus-flavicollis*	US 107				1		
	US 110				2	4	
	US 127				1		
	US 152					1	
Myodes glareolus	US 110					1	
Mus musculus ssp.	US 127				1		
Rodentia	US 107					1	
Crocidura suaveolens	US 107		1				
	US 127					1	
Neomys fodiens	US 110		1				
Sorex araneus	US 127		1				
Chiroptera	US 110			1			

of wild mice of the genus *Mus* prior to the arrival of the first specimens in association with *Homo sapiens* allows us to attribute the find to *Mus musculus* ssp. According to morphological features proposed by Orsini et al., 1983 and to the considerations about the geographical dispersal of *Mus m. musculus* proposed by Cucchi et al., 2011, 2020 it is possible tentatively to attribute Tos-Micro 99 to *Mus m. domesticus*, an Eastern species.

The archaeological and zoological implications of this find are very important. For the first time in a well excavated and sieved sediments Neolithic site in Western Europe, the presence of this human-commensal species has been demonstrated.

As many works point out (e.g.: Cucchi et al., 2005, 2013, 2020), the house mouse is very soon closely associated with Neolithic man and all his activities.

Conclusion

The diligence of the excavation and the taphonomic study of the microvertebrates recovered certify the provenance of the *Mus musculus* ssp. specimen from the late Neolithic context of Tosina di Monzambano (MN).

This discovery, although represented by a single find, allows us to move back by about at least 1500 years the first clear evidence of the domestic mice that followed men and their foodstuffs into Western Europe (Cucchi et al., 2005).

It is interesting to note that the greater attention being paid by scholars to the study of small mammal fauna is giving very important results in the field of the interpretation of the spread of domestic mice in Western Europe. The synanthropic habit of this species is important for the

Table 6 – Tosina di Monzambano. Small mammal post-cranial modifications (n= number of specimens; %= percentage of the number of specimens).

	US 107 + 107 t		US 110 + 110 t		US 127		US 152		Total	
	n	%	n	%	n	%	n	%	n	%
Digested	6	54.5	8	27.6	7	70	13	76.5	34	50.8
Not digested	5	45.5	21	72.4	3	30	4	23.5	33	49.2
Traces of vegetal erosion	1				2				3	
Burned	1		2						3	
Predatory mechanical action	1		2		1		2		6	

understanding of commercial routes and the westward spread of new ideas.

The Tosina di Monzambano find allows us to make a logical link to the important *Mus m. musculus* remains found in a burned house of the Romanian Chalcolithic (Bucşani La Pod tell) on the boundary between Eastern and Western Europe. This represented, according to (Cucchi et al., 2011), the second invasion of house mice of the genus *Mus* that displaced a previous wave of the subspecies *Mus m. domesticus* (the find of Tosina probably represents one of these first colonizers) and pushed it westwards.

The trade exchanges mediated through sites like Tosina favoured the penetration of domestic mice into Western Europe as early as the late Neolithic.

The present work shows that more attention towards the excavation and recovery of microfauna from difficult excavation contexts such as prehistoric open air sites will allow us to increase our knowledge and more fully understand the phenomenon of the spread of the house mouse to the west — and that of the associated phenomenon of Neolithization.

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