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Palaeoecological and palaeoenvironmental reconstruction of the Mediterranean Europe during the Early and Middle Pleistocene based on fossil ungulates dietary adaptations

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Flavia Strani
1241886

Supervisor: Co-advisors:
Raffaele Sardella   Daniel DeMiguel
                                                   Luca Bellucci

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Eucladoceros dicranios. Artwork by Flavia Strani

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Riassunto

Il Pleistocene Inferiore e il Pleistocene Medio sono stati caratterizzati da due grandi eventi climatici: l'inizio dei cicli glaciali del Quaternario che hanno instaurato un graduale trend verso climi più freddi e aridi, e la transizione del Pleistocene Medio (*Early-Middle Pleistocene Transition*) con il passaggio da cicli glaciali regolati da una periodicità di 41 kyr a cicli con periodicità di 100 kyr. Entrambi gli eventi hanno avuto grandi conseguenze sugli ecosistemi terrestri dell'Emisfero boreale, portando in Europa meridionale alla progressiva scomparsa degli ambienti tipicamente subtropicali del Neogene superiore, sostituiti da habitat sempre più aridi e aperti. La risposta degli organismi terrestri a questi cambiamenti climatici è importante per comprendere come variazioni del clima presenti e future, possono influenzare gli habitat della regione del Mediterraneo.

Dall'inizio del cenozoico, i mammiferi si sono evoluti e diversificati per occupare una grande varietà di nicchie ecologiche in ambienti che vanno dalla tundra ai deserti, dalle steppe alle foreste tropicali. Tra i grandi mammiferi moderni, gli ungulati erbivori sono particolarmente suscettibili ai cambi di vegetazione in quanto la loro dieta è intrinsecamente legata alla disponibilità di risorse vegetali. Le abitudini alimentari degli ungulati fossili rappresentano quindi un'importante fonte di informazioni riguardo la struttura degli habitat del passato e la loro evoluzione a seguito di variazioni climatiche.

In questa tesi, le condizioni paleoambientali di quattro siti del Pleistocene Inferiore e Medio della penisola italiana e della penisola iberica, vengono analizzate e ricostruite mediante l'altezza della corona dei molari (ipsodontia) e i pattern di usura dentaria di artiodattili e perissodattili fossili. Dall'esame delle diete a adattamenti alimentari di questi gruppi di ungulati, è stato possibile acquisire anche nuove conoscenze sulla ripartizione delle nicchie ecologiche e sui meccanismi di sfruttamento delle risorse adottati da questi mammiferi durante il Pleistocene. È stato inoltre

sviluppato e testato un nuovo strumento per lo studio delle tracce di microusura presenti sullo smalto dei denti.

Dopo il passaggio dal Pliocene al Pleistocene, la regione centrale della penisola italiana è stata caratterizzata da ambienti molto eterogenei con la presenza sia da zone umide/paludose, foreste e praterie aperte. In questo contesto le numerose specie di ungulati simpatrici adottavano un ampio range di abitudini alimentari come riscontrato nelle faune fossili del sito di Coste San Giacomo (2.1 Ma; Anagni, penisola italiana centrale). In questa località taxa di taglia più piccola mostrano morfologie e pattern di usura dentaria tipica di brucatori con una dieta più selettiva, mentre animali di dimensioni maggiori risultano avere uno spettro di adattamenti più ampio con taxa brucatori, pascolatori o a dieta mista. Con il progressivo sviluppo del trend verso climi più freddi e più aridi, si osserva una propagazione degli ambienti aperti nella penisola. A seguito della transizione tra Gelasiano e Calabriano gli ungulati fossili mostrano un range più ristretto di abitudini alimentari come attestato nella località del Pleistocene Inferiore (~ 1.8 Ma) di Olivola (Aulla, penisola italiana centrale). In questo sito la maggior parte dei taxa mostra gradi di usura dentaria riconducibili a una dieta mista con l'assenza di ungulati strettamente brucatori. I cervidi in particolare che durante il Gelasiano consumavano prevalentemente risorse vegetali tenere e poco abrasive in ambienti boschivi, durante il Calabriano modificano le loro abitudini alimentari includendo il consumo di piante più abrasive per trarre vantaggio dalla diffusione di ambienti più aperti.

Le praterie aperte erano ancora l'elemento dominante degli ambienti dell'Europa meridionale durante il tardo Pleistocene Inferiore. A seguito del MIS 22, e del così detto "*0.9 Ma event*", si registra un incremento della stagionalità nella regione del Mediterraneo come attestato dall'alto numero di ungulati a dieta mista stagionale presenti nel livello EVT7 della sezione di Vallparadís (bacino di Vallès-Penedès, penisola iberica NE). Questa intensificazione della stagionalità potrebbe aver portato a considerevoli cambiamenti periodici della qualità di risorse vegetali disponibili. In

questo scenario è possibile che i mammiferi erbivori tendessero ad ampliare il proprio *dietary breadth* includendo più tipologie di piante (più e meno abrasive) nella propria dieta. Una marcata stagionalità ha rappresentato un elemento chiave anche per gli ecosistemi della penisola italiana dopo la fase terminale della transizione del Pleistocene Medio. Questi ambienti che in Italia Centrale (Fontana Ranuccio, bacino di Anagni) hanno agito come habitat-rifugio per popolazioni di ominidi fossili, erano caratterizzati da ambienti eterogenei che spaziavano da foreste a spazi più aperti come comprovato dalla relativa abbondanza di cervidi brucatori e dalla presenza di taxa con una dieta molto o fortemente ricca di piante erbacee. La discrepanza osservata tra i risultati ottenuti dei pattern di usura dentari a lungo e breve termine nelle popolazioni di uro fossile (*Bos primigenius*) suggeriscono che una forte stagionalità con rigidi periodi avversi potrebbe aver spinto questi grandi animali a nutrirsi periodicamente anche di risorse vegetali sub-ottimali rispetto alla loro dieta abituale.

Utilizzando alcuni dei dati raccolti durante il progetto di ricerca, è stato inoltre sviluppato un programma open access (*MicroWeaR*) per migliorare la metodologia corrente di analisi dei pattern di microusura dentaria. Il software, scritto in linguaggio di programmazione R, è un tool liberamente scaricabile che permette di quantificare e classificare in modo semi-automatico le tracce microscopiche di usura. Il programma è stato testato su foto di superfici dentarie appartenenti a due mammiferi erbivori fossili (un cervide e un primate) ottenute con uno stereomicroscopio e un microscopio elettronico a scansione. I risultati ottenuti dall'analisi effettuata con *MicroWeaR* sono consistenti con quelli acquisiti tramite utilizzo di altri software di elaborazione di immagini. *MicroWeaR* rappresenta quindi un valido strumento per uno studio rapido e preciso delle abitudini alimentari di taxa fossili.

Abstract

The Early and Middle Pleistocene were characterised by two major climatic events: the onset of the Quaternary glacial cycles which led to a gradual trend towards cooler and more arid conditions and the Early-Middle Pleistocene Transition (EMPT) with cycles changing from a 41 kyr to 100 kyr periodicity. Both events had great consequences on Northern Hemisphere terrestrial ecosystems, and in Southern Europe, led to the progressive disappearance of the late Neogene subtropical environments and to the spread of more arid open habitats. The response of past biota to these climatic changes is important to understand how present and future climate shifts can affect the structure of Mediterranean habitats.

Since the beginning of the Cenozoic era, mammals evolved and diversified to occupy a variety of ecological niches in different environments spanning from the cold tundra to arid deserts, from steppes to rainforests. In modern terrestrial large mammals, herbivorous ungulates are particularly susceptible to vegetation change as their feeding behaviours are intrinsically linked to plant resource availability. Dietary adaptations of fossil ungulates thus represent an important source of information about past habitats and their evolution following climatic variations.

In this dissertation, palaeoenvironmental conditions of four Early and Middle Pleistocene sites of the Italian and Iberian Peninsula are reconstructed by means of molar crown height (hypsodonty) and dental wear patterns of fossil artiodactyls and perissodactyls. By examining ungulate dietary proclivities, new insights on the niche partitioning and resource exploitation mechanisms of this group during the Pleistocene are also provided. Moreover, a new tool to investigate dental microwear patterns in fossil taxa is here tested and described.

After the Plio/Pleistocene transition, heterogeneous environments characterised the Central Italian Peninsula, with the occurrence of wetlands, closed canopies and open grasslands. In this context, numerous ungulates could live in sympatric conditions by adopting a wide range of feeding

behaviours as reported from the Early Pleistocene fossil assemblage of Coste San Giacomo (2.1 Ma; Anagni, Central Italian Peninsula). Smaller taxa were generally selective feeders while larger animals had more diverse dietary adaptations spanning from browsers to mixed feeders to grazers. As the trend towards cooler and more arid climates continued, open landscapes spread further in the peninsula. Following the Gelasian/Calabrian transition, fossil ungulates display a narrower range of feeding behaviours as attested in the Early Pleistocene (~ 1.8 Ma) locality of Olivola (Aulla, Central Italian Peninsula). In this site, most taxa display a mixed diet with no occurrence of strict browsing signals. Cervids in particular during the Gelasian consumed predominantly soft plant resources in wooded environments, but during the Calabrian shifted their diet to include more abrasive items taking advantage of the diffusion of more open habitats.

Open grasslands were still a dominant element of the Southern European environments during the late Early Pleistocene. After MIS 22, the so-called "0.9 Ma event", an increase in seasonality is recorded in Mediterranean Europe as attested by the high number of seasonal mixed feeders in layer EVT7 from the Vallparadís Section (Vallès-Penedès Basin, NE Iberian Peninsula). This increment of seasonality may have led to considerable periodic changes in the quality of food items available to herbivores which in adverse seasons widened their dietary breadth feeding on both soft and more abrasive plant resources. Seasonality also played a key role in shaping the ecosystems of the Italian Peninsula after the end of the EMPT, which acted as refugium habitats for archaic human populations. In the Middle Pleistocene site of Fontana Ranuccio (Anagni basin, Central Italian Peninsula), the relative abundance of browsing cervids and the presence of taxa with a grass-rich mixed or grazing diet point to heterogeneous environments spanning from forests to more open landscapes. The pronounced discrepancy between long-term and short-term dental wear patterns of the large aurochs *Bos primigenius* suggests the occurrence of marked seasonality with harsh adverse periods in which this species was forced to feed even on sub-optimal food items.

Using the collected dental microwear data, an open access program to improve current 2D microwear analysis has been designed. The software written in R environment is a free tool, *MicroWeaR*, which allows for a semi-automatic quantification and classification of microwear features. The program has been tested on pictures of two fossil herbivorous mammals (a cervid and a primate) taken with a stereomicroscope and a scanning electron microscope. Results are consistent with those obtained with other image processing software, thus *MicroWeaR* represents a robust program for precise and cheaper dental microwear analysis, and a valid tool to investigate dietary behaviours of fossil taxa.

Chapter 1

Introduction

The fossil record represents a vital source of information about past environments, animals and their evolution. Interactions between fossil organisms and their habitats as well as between the organisms themselves, provide valuable data about palaeoenvironmental conditions and the response of the Earth biota to past climatic changes. Given the present impact of human activities on ecosystems, the study of how plants and animals were affected by habitat alterations in ancient times is pivotal to understand how to better manage the impact of current and future environmental changes, especially in light of the current global warming (Pereira et al., 2010; Urban, 2015).

The Pleistocene represents an epoch of considerable climatic turmoil, which has greatly affected the habitats of the Northern Hemisphere in general and of the European continent in particular. The Mediterranean Europe acted as both a crossroad and an environmental refugium for many vertebrate taxa (including also relatives of modern *Homo*; Di Vincenzo et al., 2017) during the glacial phases of the interglacial/glacial cycles that marked this epoch (Schmitt, 2007). Understanding how the habitats of this region were shaped by Pleistocene climatic changes has always been a topic of great interest to palaeoecologists (e.g. Kahlke et al., 2011; Manzi et al., 2011).

More specifically, in the last 5 Ma, the Earth's climate system has testified two major events: the beginning of the Quaternary glacial cycles that marked the Plio-Pleistocene transition (2.6 Ma), and the Middle Pleistocene Revolution (MPR) or Early-Middle Pleistocene Transition (EMPT) (ca. 1.4-0.4 Ma) (Lisiecki and Raymo, 2005, 2007). The first signs of the onset of these cycles can be traced back to the late Pliocene when, around 3.1 Ma, continental ice sheets began to increase and shrink in size in the Northern Hemisphere (Raymo et al., 1989 and references therein). Glacial activities of the Greenland, Scandinavian and North American ice sheets increased between 2.8 and 2.6 Ma marking the beginning of a global trend towards reduced temperatures (Flesche Kleiven et al., 2002; Haug et al., 2005; Hill et al., 2017) which culminated in the initiation of the Quaternary glacial/interglacial alternations (Zubakov and Borzenkova, 1990). From 2.6 to 1.2 Ma

(Early Pleistocene), the intensity and durations of the glacial cycles were regulated by a 41 kyr periodicity (Lisiecki and Raymo, 2005). The onset of this cooling trend led to a gradual disappearance of sub-tropical plant taxa that were abundantly represented in the vegetation composition of the European continent during the Pliocene, with herbaceous species expanding in the now drier environments leading to the diffusion of more open habitats (Bertini, 2003, Fortelius et al., 2006; Bertini, 2010, 2013; Combourieu-Nebout et al., 2015). Following these gradual alterations of the plant biota, the composition of the terrestrial fauna witnessed important turnovers, as the disappearance of taxa strongly linked to the presence of humid and closed environments (such as the tapirid *Tapirus arvernensis*), and the arrival of large mammals adapted to dwell in more open landscapes which marked the so-called "wolf event" (i.e. the spread of large pack hunters canids of the genus *Canis*) (Azzaroli, 1983) and the "*Equus*-Elephant event" (i.e. the diffusion of large gregarious horses like *Equus stenonis* and of the very large-sized *Mammuthus meridionalis*) (Azzaroli, 1995).

The EMPT occurred around 1.2 Ma (or as far back as 1.4 Ma according to some authors see Head and Gibbard [2015]) with glacial-interglacial cycles changing from a 41 kyr to a 100 kyr periodicity (Lisiecki and Raymo, 2005, 2007). This abrupt variation in the climate system, whose causes are still a matter of debate (Head and Gibbard, 2015 and references therein), led to longer and more intense glacials which deeply influenced Northern Hemisphere ecosystems. During the onset, stabilization, and conclusion of the EMPT (from 1.4 to 0.4 Ma), severe cooling events were registered at MIS 22, 16 and 12, and pronounced interglacials occurred at MIS 31, 25, 21 and 11 (Clark et al., 2006; DeConto et al., 2012; Head and Gibbard 2015). The MIS 31 interglacial and MIS 22 glacial are of particular importance because of the extreme intensity of the recorded climatic episodes. MIS 31 is known as a "super-interglacial" due to the especially pronounced interglacials which occurred in this phase (DeConto et al., 2012), while one of the most intense glacials of the Quaternary was recorded in correspondence of MIS 22 around 0.9 Ma (the so called

"0.9 Ma event") (Clark et al., 2006). The consolidation of the EMPT is conventionally placed around the MIS 12 – 11 passage (~424 ka) during the Mid-Brunhes Event (MBE) after which the amplitude of the interglacials increased as evidenced by marine, terrestrial and ice-core records (Lang and Wolff, 2011; McClymont et al., 2013). All these climatic shifts had important repercussions on the ecosystems and the mammal fauna composition of the European continent, which resulted in multiple extinctions, dispersals and adaptations (Kahlke et al., 2011 and references therein). Fossil pollen records from European terrestrial localities dated around the EMPT show, however, no noticeable changes in the vegetation of the Mediterranean area. Between 1.2 and 0.7 Ma no strong perturbations of the flora composition are recorded and no new plant taxa are registered after 1.5 Ma (Suc and Popescu, 2005). This finding contrasts the striking large mammal faunal turnover observed in the same region during the EMPT, with the occurrence of two bioevents, *Praemegaceros verticornis – Bison menneri* FO and *Crocuta crocuta* FO, which mark the Epivillafranchian (Bellucci et al., 2015), as well as the arrival, dispersion and subsequent radiation of the genus *Homo* in the European continent, which persisted in environmental refugia even after the Mid-Brunhes Event (Manzi et al., 2010, 2011; Rubini et al., 2014). Consequently, the Mediterranean region represents an optimal laboratory to investigate the regional effects of these two major climatic events and the response of species and terrestrial ecosystems to them which was of crucial importance for the European Continent during the Early and Middle Pleistocene.

In palaeoecology, fossil mammals play an important role in palaeoenvironmental reconstructions as from the study of their adaptations and distribution it is possible to gather a great deal of information on past habitats (Rowan and Reed, 2015). Taxon-based methodologies have been historically used to investigate a number of palaeoecological aspects such as landscape openness degree and niche partitioning, but recently new taxon-free methods (i.e. not linked to phylogenetic information) have been developed in order to avoid taphonomic, collection or identification biases (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; DeGusta and

Vrba, 2003; Cerling et al., 2013; Solounias et al., 2014). Dental patterns analysis of fossil mammals is one well-established taxon-free method of investigation which allow to acquire information on dietary behaviour of extinct species and consequently of palaeoenvironmental conditions (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; Rivals and Athanassiou, 2008; Rivals et al., 2010; DeMiguel et al., 2011; Bernor et al., 2014; Danowitz et al., 2016).

For modern mammals food represents the main connection between the organism and its environment. As such animal feeding behaviours provide crucial ecological information (Ungar, 2010). During their evolutionary history mammals have developed a wide array of dietary adaptations to obtain, exploit and process the amount of food needed in order to sustain their high metabolism. Based on their dietary preferences, these animals can be roughly divided into three main categories: those who feed mostly on plants (primary consumers), animals who prey and feed mostly on other animals (secondary or tertiary consumers) and animals that feed on both plant and animal resources (omnivores) (Rozin and Rozin, 1981). Moreover mammals can either be specialists by feeding mostly on one kind of food resource (e.g. a single plant species) or generalists by feeding on diverse resources with no noticeable preferences (Ungar, 2010).

Herbivore mammals, as primary consumers, are highly susceptible to changes in vegetation as a consequence of climatic shifts and other environmental alterations (DeMiguel et al., 2010). Ungulates are the most diverse herbivore mammal group, with an evolutionary history that can be traced back to the Eocene (Rose, 1996; Métais and Vislobokova, 2007; Gordon and Prins, 2008) and which adapted to thrive in different environments ranging from tropical forests to cold tundras and even deserts and semi-deserts (Hofmann 1989; DeMiguel et al., 2014). Therefore, through the investigation of the feeding behaviours of ungulate taxa it is possible to obtain crucial data on type of vegetation and habitat, plant resource availability, niche occupation and other auto- and synecological parameters (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002;

Semprebon et al., 2004; Bernor et al., 2014; Solounias et al., 2014; Bignon-lau et al., 2016; Berlioz et al. 2017).

The study of dietary adaptations in modern species is mostly based on direct observation of animals in their habitats (Khan, 1994), investigation of the gut content (Jiang et al., 2009), and examination of anatomical adaptations such as skull morphology and teeth shape (Kubo et al., 2007). Investigating the feeding behaviour of fossil species is however more challenging: digestive tract is rarely fully preserved and only in extremely rare and exceptional cases is possible to infer information about ingested food from fossilized guts. Teeth, however, due to their remarkable resistance to diagenetic processes (Sakae et al., 1998; Dauphin and Williams 2004), are the most abundant skeleton element in the mammalian fossil record. For this reason and because they record dietary information, fossil teeth represent the primary source of information on the feeding behaviours of fossil mammals.

Teeth are efficient tools used to break down food items for ingestion and digestion, which form and structure have been selected to maximize nutrient acquisition. Dental shape and structure are therefore strongly linked to the type of preferred food resources to ingest (Ungar, 2010). Plant resources are extremely heterogeneous, ranging from soft fruits and leaves, to tougher twigs or hard seeds or more abrasive grasses, and consequently they pose different mechanical challenges to herbivorous ungulate dentition (Baker et al., 1959; Waldron et al., 2003; Lucas, 2004; Ungar, 2010). The wide array of dental morphologies that can be observed in both modern and fossil ungulate taxa mirrors the different adaptations that this group has developed in its evolutionary history to efficiently feed on plant parts with diverse mechanical properties. Specifically, ungulate dietary behaviours can range from browsing (i.e. feeding mostly on soft plant parts such as leaves and fruits), to grazing (i.e. feeding mostly on abrasive items such as grasses), with diferrent "mixed feeding behaviours" corresponding to an intermediate style between these two extremes (Hofmann abd Stewart, 1972; Hofmann, 1989; Gordon and Prins, 2008). For these reasons, teeth of ungulates

are a pivotal source of biological and environmental information in both ecology and palaeoecology.

In vertebrate paleontology and paleoecology and in other related fields such as paleoanthropology, a number of methods are employed to obtain dietary information from fossil dental material: the study of tooth mechanical properties (Fortelius, 1985; Fritz et al., 2009; DeMiguel et al., 2015), of enamel stable isotopes (Feranec et al., 2010; Wolf et al., 2010; Pushkina et al., 2014; Bocherens et al., 2017), hypsodonty (i.e. molar crown height) and dental morphology (Janis, 1988; Fortelius et al., 2002; DeMiguel et al., 2015) and dental wear patterns (Grine, 1986; Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; DeMiguel et al., 2011; Danowitz et al., 2016; DeSantis, 2016; Merceron et al., 2018) are the most common ones. Hypsodonty and dental wear patterns in particular yield information about both environmental conditions (e.g. aridity degree and habitat openness) and feeding behaviours of individual specimens (Janis 1988; Solounias and Semprebon, 2002, Eronen et al., 2010a, b; DeMiguel et al., 2018). Accordingly, and because these methods do not require researchers to damage or alter fossil samples, they are considered optimal tools to investigate palaeoecological settings.

Moreover, by comparing long-term (mesowear) and short-term (microwear) dental wear patterns, it is possible to obtain information about the evolution of the feeding behaviour of a taxon through its whole lifespan, thereby allowing the observation of possible cyclical variations in food resource availability due to abiotic factors (e.g. marked seasonality) (Rivals et al., 2009).

Given all the context presented above, the aim of the present thesis is twofold: (i) to provide new and updated information about the knowledge gap of the effects of the two major climatic events of the Pleistocene on the ecosystems of Southern Europe; (ii) to investigate the fossil ungulates dietary behaviours in order to understand their niche occupation and response to environmental changes during the Early and Middle Pleistocene. This was achieved (i) through a detailed palaeoenvironmental/palaeoclimatic reconstruction of selected Early and Middle

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Pleistocene localities of the Mediterranean Europe using taxon-free methods, and (ii) with an in-depth analysis of the hypsodonty and dental wear patterns variation of artiodactyls and perissodactyls fossil assemblages following the Early-Pleistocene transition and the onset of the EMPT, also testing a new tool to gather dental microwear data from 2D pictures of the tooth enamel surface.

A series of studies have been conducted in order to address these topics and open issues.

In Chapter 2, niche partitioning among the several sympatric fossil ungulates of the Early Pleistocene site of Coste San Giacomo (Anagni basin, Central Italian Peninsula) is investigated by examining their range of feeding behaviours. Two dental mesowear analyses, one which focuses on the wear degree of the outer molar enamel bands and the other on the inner ones, have been combined to get information about diet variation at two different time scales (years-to-months versus weeks). This allowed the gathering of more minute information about dietary behaviours of fossil taxa, especially regarding the dietary flexibility of mixed feeders to detect possible leanings towards a browse- or grass rich mixed diet. Animals body masses are also here estimated to investigate how fossil ungulates of different sizes partitioned the available resources.

In Chapter 3, the response of fossil ungulates to the palaeoenvironmental changes that followed the Early Pleistocene cooling trend is investigated. The dietary adaptations of artiodactyls and perissodactyls of the middle Villafranchian locality of Coste San Giacomo (Anagni basin, Central Italian Peninsula) and the late Villafranchian site of Olivola (Aulla, Central Italy) are compared to get insights on the habitat conditions of the Italian Peninsula before and after the Gelasian/Calabrian transition.

In Chapter 4, the effects of the extreme glacial known as the "0.9 Ma event" on terrestrial ecosystems of Southern Europe are examined based on hypsodonty and dental wear patterns (mesowear and microwear) variation of fossil ungulates of the Vallparadís Section (Vallès-Penedès

Basin, NE Iberian Peninsula). Three ungulate assemblages of layers dated before and after MIS 22 are studied and compared to investigate if this major climatic event deeply exacerbated and altered environmental conditions and vegetation composition in the Mediterranean area.

In Chapter 5, the structure of the environments after the Mid-Brunhes event are examined in order to understand the habitat conditions that occurred at ~400 ka, which favored the dispersion of Middle Pleistocene *Homo* populations in Southern Europe. Feeding behaviours of the fossil ungulates of the Middle Pleistocene site of Fontana Ranuccio (Anagni basin, Central Italian Peninsula) which are associated to a rich collection of lithic artifacts and bone tools are here examined to get insight over both palaeoenvironments and climatic conditions of the region and the relative permanence versus mobility of fossil human populations in the area.

In Chapter 6, the functionalities of a free and open access software designed to optimize the study of dental microwear patterns are described. This tool allows for an easy and rapid sampling, quantification and classification of dental microwear features left on the enamel by ingested items allowing for a more complete and comprehensive investigation of feeding behaviours of extinct taxa. The idea behind the software has been conceived following some limitations of 2D microwear analysis which were observed while acquiring and processing the microwear data collected for this PhD project.

Chapters 2, 3, 5 and 6 are presented as published papers while Chapter 4 as a submitted manuscript. In all these papers, the PhD candidate contributed as first author outlining the research, collecting and elaborating the dental morphology and wear patterns data, and writing the main text of the articles. In Chapter 6, the PhD candidate also helped Dr. Antonio Profico in the code writing process and tested pre-alpha, alpha and beta versions of the R script on pictures collected by the author herself and by Dr. Daniel DeMiguel.

In Chapter 7, findings are discussed in light of the current knowledge of palaeoenvironmental changes during the Pleistocene in the European continent and how they can be integrated in the wider context of global climatic changes and trends of dietary adaptations among fossil ungulate groups are reviewed.

In Chapter 8, the conclusions of the project are stated.

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Chapter 2

Resource and niche differentiation mechanisms by sympatric Early

Pleistocene ungulates: the case study of Coste San Giacomo

Resource and niche differentiation mechanisms by sympatric Early Pleistocene ungulates: the case study of Coste San Giacomo

Flavia Strani [a, b, *], Daniel DeMiguel [c, d e, **], Raffaele Sardella [a, b], Luca Bellucci [b, f]

[a] Dipartimento di Scienze della Terra, Sapienza - Università di Roma, P.le Aldo Moro 5, 00185, Roma, Italy
[b] Istituto Italiano di Paleontologia Umana, Piazza R. Bonghi 2, 03012, Anagni, Frosinone, Italy
[c] Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici Z, C/ de les Columnes s/n, Campus de la UAB, 08193 Cerdanyola del Vallès, Barcelona, Spain
[d] Departamento de Ciencias de la Tierra, Área de Paleontología, Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain
[e] Fundación ARAID, Zaragoza, Spain
[f] Polo museale Sapienza, Sapienza − Università di Roma, P.le Aldo Moro 5, 00185, Roma, Italy

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ABSTRACT

Resource competition and niche partitioning among the exceptionally high number of sympatric ungulates of the Early Pleistocene site of Coste San Giacomo (Central Italy) is here examined through the study of their dietary proclivities and body size. The main aim of this study is to investigate the niche differentiation mechanisms that let the fossil ungulates coexist in the same region. We also provide information about the complementarity of two different methodologies that observe diet variation at a different time scales (inner and outer mesowear) in the study of dental wear patterns of fossil ungulates. Results from analyses of dental wear degree and body masses predictions show that a wide range of feeding behaviours were adopted by the taxonomical groups (i.e., cervids, bovids and equids) in order to avoid competition. Among larger ungulates diet ranges from strict browsing (*Eucladoceros* sp., *Gazellospira torticornis*), to mixed feeding (*Gallogoral meneghinii*, *Leptobos* sp.) to pure grazing (*Equus stenonis*), whereas smaller taxa are more selective feeders (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus*) with only one exception (*Gazella borbonica*). When taxa with the same feeding behaviour occurred in the same habitat, competition was minimised by differences in body size.

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1. Introduction

Competition between species occurs when species occupy the same habitat, use the same resources and when said resources are limiting. Ungulates can directly compete for shared resources of food or space, or can interfere against resources used by another species (interference competition) (Latham, 1999). Niche differentiation mechanisms, such as habitat segregation, differences in body weights and specialized dietary adaptations as well as beneficial interactions (facilitation), can however minimize competition allowing the co-existence of different sympatric ungulate in a region, (De Boer and Prins, 1990; Klein and Bay, 1994; Latham, 1999; Prins et al., 2006; Sietses et al., 2009). Such ecological mechanisms in fossil assemblages are often difficult to investigate, but they are indeed important to understand interspecific relationship among taxa, especially in those localities where multiple fossil ungulates occurred and overlapped. In extant herbivore ungulates, diet quality and body size are strongly related (Henley and Ward, 2006; Codron et al., 2007), with the former decreasing when body size increases (Mysterud, 1998; references within; Codron et al., 2007) and with animals of similar body mass and digestive system foraging on similar foods (Henley and Ward, 2006) This is because larger herbivores require larger quantities of food, and the more abundant plants parts (e.g. stems or twigs) are generally of lower nutritional quality than less abundant, higher-quality parts (e.g. leaves, fruits or forbs). Contrarily, smaller herbivores require smaller quantities of food, and they are generally selective feeders adapting to use mostly high quality forage (Clauss et al., 2013). As a

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result, herbivores exhibiting different body size and different dietary preferences can coexist in the same ecosystem (Prins and Olff, 1998).

The Early Pleistocene large mammal assemblage of Coste San Giacomo (herein referred as CSG) in Anagni (Central Italy), recently dated around 2.1 Ma (Bellucci et al., 2014), is characterized by an unusual abundance of herbivore taxa, mostly represented by artiodactyls and perissodactyls (Bellucci et al., 2012; Strani et al., 2015). Among these, three fossil cervids (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus* and *Eucladoceros* sp.), four bovids (*Gallogoral meneghinii*, *Gazella borbonica*, *Gazellospira torticornis* and *Leptobos* sp.), and one equid (*Equus stenonis*) have been identified (Bellucci et al., 2012; Bellucci and Sardella, 2015). These taxa have been recently studied from a paleodietary point of view in order to gain new information about the palaeoclimate and palaeoenvironmental conditions that prevailed in Central Italy during the Gelasian (Early Pleistocene) through the analysis of their dental wear degree (mesowear) and molar crown height (hypsodonty) (Strani et al., 2015).

With this new updated information of their dietary preferences, this research relies on the working hypothesis that the extraordinary number of herbivore ungulates werw forced to exhibit different mechanisms in CSG in order to avoid strong direct competition, partition the niche space and optimize exploitation of the available vegetation. We consider indeed CSG as an exceptional case study to do this because of the wide spectrum of diets and body sizes exhibited, and the occurrence of different taxonomic groups in the same assemblage. To do so, we focus on new traits of ecological relevance (e.g., body size) and more precise dietary inferences also adopting the new inner mesowear approach (Solounias et al., 2014) to better understand how niche and resource partitioning work in fossil communities. Importantly, and given the abundant dietary data provided by the CSG mammal community, here we also aim to examine the complementary nature of two proxy methods based on the dental wear degree (inner and outer mesowear) comparing the obtained results and test their combined use in paleoecological studies.

2. Material and methods

The studied fossil material belongs to the Early Pleistocene (Gelasian) locality of Coste San Giacomo (CSG) near the town of Anagni (Frosinone, Central Italy). Extensive fieldwork has been carried out since 1978 by researchers of the Italian Institute of Human Palaeontology (IsIPU) (Segre Naldini et al., 2009; Bellucci et al., 2014 and references therein). The material is currently housed at the IsIPU laboratory in Anagni (Frosinone, Central Italy). The updated faunal list of CSG (Bellucci et al., 2014) comprises 19 large and 7 micromammal taxa. Among them, the fossil material here studied consists of 102 dental (molar) specimens of the following ungulate taxa: *Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus*, *Eucladoceros* sp., *Gazella borbonica*, *Gazellospira torticornis*, *Gallogoral meneghinii*, *Leptobos* sp. and *Equus stenonis*.

2.1. Body mass estimation

Herbivore body mass is an important ecological trait in niche repartitioning as it influences diet choices (Henley and Ward, 2006). Mammalian body size is usually predicted by proximal limb bones (Scott, 1983, 1990), cranial (MacFadden and Hulbert, 1990) or dental (Janis, 1990) measurements. Due to the lack of well represented post-cranial and cranial remains for most of the CSG ungulates, only dental measurements were taken. The occlusal lengths of upper second (M2) or lower first (m1) molars were used to estimate body size following the procedure described by Janis

(1990). The intercepts and slopes of prediction equations for the different cranio-dental measurements for 1) perissodactyls, 2) cervids only and 3) bovids only were taken from Janis (1990). That is, in order to predict the weight of the CSG taxa, we used the provided slopes and intercepts of the linear regressions based on log M2 and log m1 occlusal length, form the "Perissodactyls and hyracoids only" group (for *E. stenonis*), from the "Cervids only group" (for *Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus*, *Eucladoceros* sp.), and from the "Bovids only group" (for *Gazella borbonica*, *Gazellospira torticornis* and *Gallogoral meneghinii*). The inverse logarithm was calculated to obtain the body mass for each animal.

The results were compared with modern taxa listed in Janis (1990). Note that body size estimation for *Leptobos* sp. was not performed due to the lack of both M2 and m1 specimens for this taxon.

2.2. Dental mesowear

Mesowear is considered a good dietary indicator in herbivore species, as it represents the cumulative effects of the items ingested (both foods and exogenous particles such as dust and grit) on the dental morphology that are produced in a long period of time compared to the lifespan of the animal (Fortelius and Solounias, 2000).

Traditional mesowear (Fortelius and Solounias, 2000), referred as "outer mesowear" (Solounias et al., 2014; Danowitz et al., 2016), analyses the sharpness (i.e., morphology) of the cusps and the height of the occlusal relief of the most labial enamel bands of upper molars and the most lingual of the lower ones (Kaiser and Solounias, 2003; DeMiguel et al., 2012). In Strani et al. (2015), occlusal relief (high or low) and cusp shape (sharp, rounded or blunt) of the apex of the paracone and metacone of the M1-M3 and the metaconid and entoconid of the m1–m3 were examined and scored, and data compared with those of a database of extant ungulates with known diets (Fortelius and Solounias, 2000). The variables were also converted to a score following Rivals et al. (2009) as follows: (0) teeth showing a combination of high relief and sharp cusps; (1) teeth with high relief and rounded cusps; (2) teeth with low relief and rounded cusps; (2.5) teeth with low relief and sharp cusps; and (3) teeth with low relief and blunt cusps.

The inner mesowear method, recently developed by Solounias et al. (2014), analyses instead the second enamel band that forms the lingual margin of the metacone or paracone from an occlusal view, which is generally more frequently preserved in fossil specimens. Inner mesowear reflects dietary preferences that are intermediate in time (days-weeks; Danowitz et al., 2016) between outer mesowear (months-years; Fortelius and Solounias, 2000; Mihlbachler et al., 2011; Sánchez-Hernández et al., 2016) and dental microwear (days-hours; Solounias and Semprebon, 2002). For inner mesowear, the enamel band is scored on the mesial and distal sides of the metacone using a 4 point scaling system described by Solounias et al. (2014) and Danowitz et al. (2016): (1) flat and planar with no gouges or indentations on the surface of the enamel; (2) nearly flat with several gouges that traverse the surface from either edge—the labial and lingual edges of the enamel band are somewhat rounded; (3) similar to score 2, but more rounded with less defined edges and more gouges; and (4) rounded—the surface is smooth without gouges, and there are no well-defined edges. The junction of the mesial and distal sides termed J is similarly scored: (1) it joins at a sharp, well-defined junction; (2) it is somewhat sharp, and often contains a gouge; (3) it is rounded, but the mesial and distal sides appear as distinct, separate surfaces; and (4) the J point lacks a discrete apex, and the mesial and distal sides of the enamel band form one continuous surface. The method was originally applied by Solounias et al. (2014) only on upper

second molars (M2), and here it has been extended to M1 and M3 molars of different individuals in order to widen the sample and allow for more complete comparisons among mesowear databases (Table 1). Whenever possible, we used the enamel band of the metacone of the upper molars. If this region was taphonomically damaged, the band of the paracone was examined. We compared our results of the average inner mesowear scores of the mesial, J point and distal surfaces with those of a comprehensive database (Danowitz et al., 2016) of 8 extant ungulates with well-studied diets (browsing, mixed feeding and grazing).

2.3. Statistical methods

Discriminant analyses were performed to analyse the resolution of both outer and inner mesowear variables applied to the fossil taxa. For the outer mesowear, the percentage of high relief, rounded and blunt cusps were used as independent variables and two dietary (conservative and radical; Fortelius and Solounias, 2000) classifications were used alternately as a grouping variable in order to recognize those taxa that although showing mixed-feeders adaptations had diets engaged in either browse or grass consumption. For the inner mesowear, we used the diet of extant species (Danowitz et al., 2016) as a grouping variable, and the mesial, distal and J point scores as variables to infer the dietary preferences of species. All analyses were performed using SPSS Statistics 23.

3. Results

3.1. Body mass estimation

For the cervids, the body mass has been estimated around 84 kg for *Axis* cf. *lyra* (comparable to the extant mule deer *Odocoileus hemionus*; 91/57 kg; Janis, 1990), 63 kg for *Croizetoceros* cf. *ramosus* (similar to that of the modern sika deer *Cervus nippon* [64/41 kg; (Janis, 1990)] and the fallow deer *Dama dama* [67/44 kg; (Janis, 1990)], and around 239 kg for *Eucladoceros* sp. (comparable to the wapiti *Cervus canadensis*; 400/250 kg; Janis, 1990). With regard to the bovids, the body mass of *Gallogoral meneghinii* is around 217 kg (comparable to the extant takin *Budorcas taxicolor*; 250 kg; Janis, 1990), 30 kg for *Gazella borbonica* (similar to the dibatag *Ammodorcas clarkei*) and 214 kg for *Gazellospira torticornis* (comparable to the mountain nyala *Tragelaphus buxtoni*; 216/150 kg; Janis, 1990). Finally, the body mass of the equid *Equus stenonis* is around 386 kg (thus being similar to that of the Grévy's zebra *Equus grevyi*; 400 kg; Janis, 1990).

3.2. Outer (traditional) mesowear

Analyses of outer mesowear of ungulates from CSG (Strani et al., 2015) show different patterns of wear and a wide spectrum of feeding behaviours, with cervids (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus* and *Eucladoceros* sp.) and the bovid *G. torticornis* having a predominance of sharp cusps and high occlusal relief and browser diets; the bovids *G. borbonica*, *G. meneghinii* and *Leptobos* sp. showing sharp and rounded cusps and high relief and intermediate diets; and the equid *E. stenonis* showing blunt and rounded cusps and low relief and a strict grazer behaviour (Table 1).

3.3. Inner mesowear

The distribution of the inner mesowear scores (mesial, J point and distal) of the ungulate community of CSG includes mostly intermediate values (from 1 to 3) (Table 2). The highest ones (>3) are observed in *E. stenonis*. When plotting data from CSG with those of modern ungulates grouped into browsers, mixed feeders and grazers (Fig. 1), the scores distribution suggests a spectrum of dietary preferences that extend from browsing to mixed feeding, with the exception of *E. stenonis*. The scores of *A.* cf. *lyra*, *C.* cf. *ramosus*, *Eucladoceros* sp., and *G. torticornis* are comparable to those of modern browsers and a high variability of the mesial and distal scores are observed (though the J point score tends to have similar values). *G. meneghinii* scores are similar to those of a browser as well, while higher scores are observed in *Leptobos* sp. and *G. borbonica* which are placed close to mixed feeders. These results should however be taken as tentative due to the small sample available for these taxa (N = 2). *E. stenonis* differs by being the only species whose average inner mesowear scores are comparable to those of modern grazers.

3.4. Discriminant analyses

Discriminant analysis performed with the outer mesowear variables provides a satisfactory dietary discrimination with 74.1% of extant taxa (68.5% in cross-validation, p < 0.001 for Function 1, and p = 0.38 for Function 2 with canonical correlation of 0.80 and 0.35) correctly classified according to a conservative classification and 74.1% (74.1% in cross-validation, p < 0.001 for Function 1, and p = 0.50 for Function 2 with canonical correlation of 0.80 and 0.16) according to the radical one. Bivariate diagrams based on the discriminant analysis (Fig. 2A–B) show that all the fossil deer and the antelope *G. torticornis* are classified as browser, *G. meneghinii*, *G. borbonica* as mixed feeders, and *E. stenonis* as a grazer in both the conservative (Fig. 2A) and radical (Fig. 2B) classifications. *Leptobos* sp. is classified as a mixed feeder in the conservative classification (Fig. 2A), and considered as a grazer in the radical (Fig. 2B).

Discriminant analyses for the inner mesowear predicted the diet of the individuals with a high accuracy rate (73,6% of cases correctly classified; 71,3% in cross-validation) resulted in two significant

Table 1

Summary of body mass estimation, outer and inner mesowear. Abbreviations: Number of specimens (N); size estimation based on M2 (M2 L), m1 (m1 L) length and average size (AS); percentage of specimens with high (%H) and low (%L) occlusal relief; percentage of specimens with sharp (%S), rounded (%R) and blunt (%B) cusps; Mesowear Score (MS); mesial (Mesial), distal (Distal) and J point (J) scores; and diet classification (Diet). Outer mesowear data from Strani et al. (2015).

Species	Body Mass (kg)			Outer Mesowear							Inner Mesowear			
	M2 L (N)	M1 L (N)	AS	N	%H	%L	%S	%R	%B	MS	N	Mesial	Distal	J
Axis cf. *lyra*	75.87 (2)	92.89 (5)	84.38	26	100	0	76.9	23.1	0	0.19	7	1.5	1.8	1.43
Croizetoceros cf. *ramosus*	/	63.39 (1)	63.39	5	100	0	80	20	0	0.2	3	1	1.5	1.67
Eucladoceros sp.	214.70 (3)	263.13 (1)	239.9	26	100	0	88.5	11.5	0	0.12	12	1.3	1.5	1.83
Gazella borbonica	31.08 (1)	28.74 (3)	29.91	8	87.5	12.5	50	50	0	0.63	2	2.5	2.5	3
Gazellospira torticornis	214.44 (4)	/	214.4	8	100	0	87.5	12.5	0	0.13	4	1.5	1	1.75
Gallogoral meneghini	217.27 (1)	/	217.3	2	100	0	50	50	0	0.5	2	1.5	1	2
Leptobos sp.	/	/	/	2	100	0	0	100	0	1	2	2.5	2.5	2.5
Equus stenoni	303.17 (5)	468.77 (4)	387	21	4.8	95.2	28.6	38.1	28.6	2.33	11	3.33	3.29	3.63

Table 2
Dietary predictions from discriminant analysis using inner mesowear variables.

Species	Frequency Browser	Frequency Mixed Feeder	Frequency Grazer	Undetermined
Axis cf. lyra	4			3
Croizetoceros cf. ramosu	2			1
Eucladoceros sp.	8			4
Gazella borbonica		2		
Gazellospira torticornis	2			
Gallogoral meneghinii	1			1
Leptobos sp.	1	1		
Equus stenonis		1	5	5
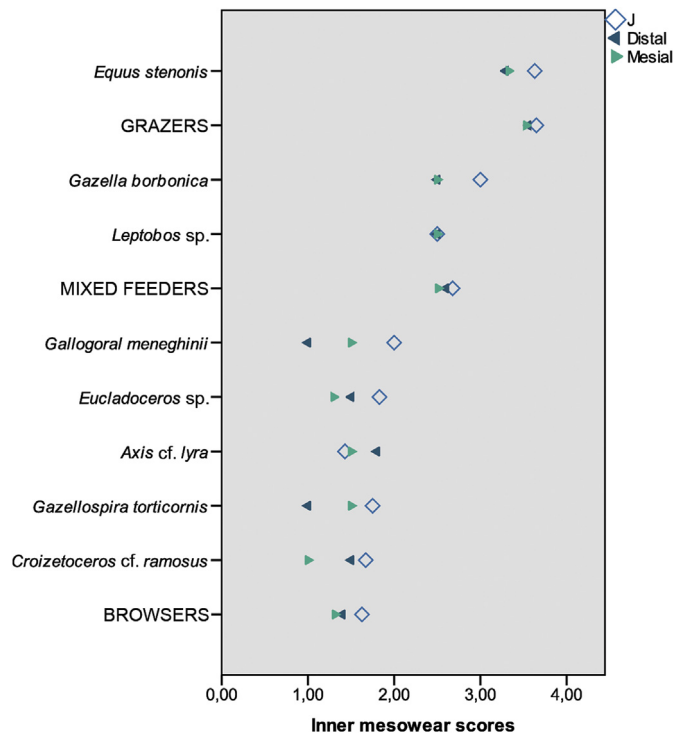
Fig. 1. Mean inner mesowear scores for fossil taxa and modern browsers, mixed feeders and grazers (data for extant species from Danowitz et al., 2016).

discriminant functions (p < 0.001 for Function 1, and p = 0.74 for Function 2) with canonical correlation of 0.80 and 0.08. Note that individuals lacking mesial/distal bands or J scores were not classified (Undetermined). Table 2 depicts individual results of the dental specimens and their variability thus showing that all the suitable individuals of *Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus*, *Eucladoceros* sp., *Gazellospira torticornis* and *Gallogoral meneghinii* are browsers, *Gazella borbonica* individuals are mixed feeders, one specimen of *Leptobos* sp. is considered to be a browser and other a mixed feeder, and most of the individuals of *E. stenonis* are grazers (with only one classified as a mixed feeder).

Globally, inner mesowear results (Table 3) considering both the individual results (Table 2) and the mean distribution of the inner mesowear scores (Fig. 1) show a diversity of dietary preferences. A browsing feeding behaviour can be observed for the cervids as well as for the bovids *G. torticornis* and *G. meneghinii*. Results also indicate a mixed feeding behaviour for *G. borbonica* and *Leptobos* sp., whereas *E. stenonis* appears to be a grazer.

4. Discussion

4.1. Comparison between outer and inner mesowear

Both outer and inner mesowear methods show similar dietary predictions for most of the cases (Table 3), and only one discrepancy can be observed according to the primary (conservative classification) diet of the species. Thus, *G. meneghinii* is classified as a mixed feeder and a browser according to the outer and inner mesowear, respectively. Given that the inner mesowear occurs at a smaller time scale than the outer, this can indicate a mixed feeding-
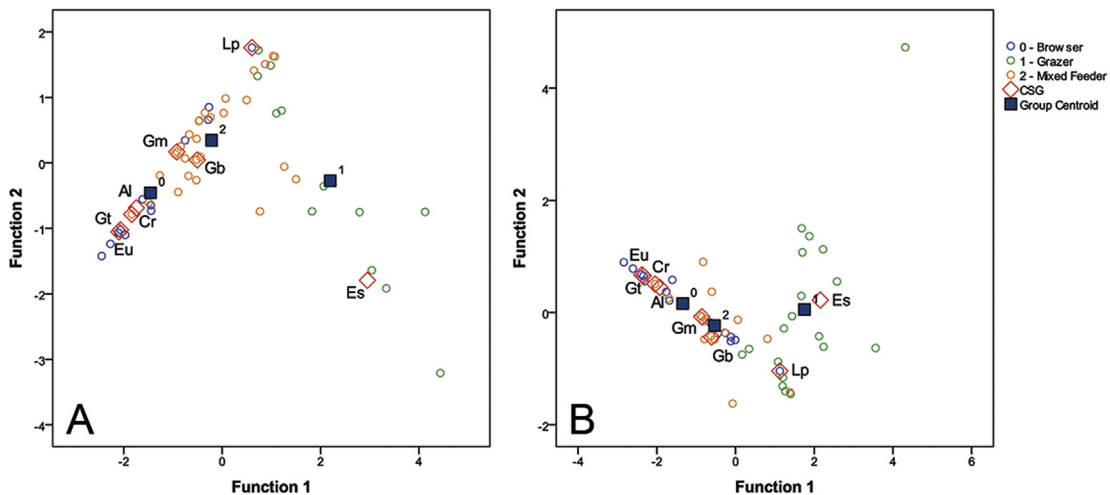

Fig. 2. Bivariate diagrams based on discriminant analysis using conservative (A) and radical (B) classifications. Fossil taxa abbreviations: Al, *Axis* cf. *lyra*; Cr, *Croizetoceros* cf. *ramosus*; Eu, *Eucladoceros* sp.; Gb, *Gazella borbonica*; Gt, *Gazellospira torticornis*; Gm, *Gallogoral meneghinii*; Lp, *Leptobos* sp.; Es, *Equus stenonis* (data from Strani et al., 2015).

Table 3
Dietary inference from outer and inner mesowear analyses.

Species	Outer mesowear		Inner mesowear
	Diet prediction (conservative classification)	Diet prediction (radical classification)	Diet prediction
Axis cf. *lyra*	Browser	Browser	Browser
Croizetoceros cf. *ramosus*	Browser	Browser	Browser
Eucladoceros sp.	Browser	Browser	Browser
Gazella borbonica	Mixed Feeder	Mixed Feeder	Mixed Feeder
Gazellospira torticornis	Browser	Browser	Browser
Gallogoral meneghinii	Mixed Feeder	Mixed Feeder	Browser
Leptobos sp.	Mixed Feeder	Grazer	Mixed Feeder
Equus stenonis	Grazer	Grazer	Grazer

towards-browsing diet type or a temporary shift towards a more browsing feeding behaviour. When the secondary (or more radical) diet obtained from the outer mesowear is compared with results from the inner mesowear, there is a discrepancy for *Leptobos* sp. Inner mesowear scores indicate a mixed feeding behaviour (Table 3, Fig. 1) thus differing from the grazing diet inferred from the radical classification (Table 3, Fig. 2A–B). This discrepancy may suggest that this taxon leaned generally towards a diet richer in abrasive foods (months-to-years according to the outer mesowear time scale), and that it assumed a more mixed feeding behaviour during short periods of time (days-to-weeks inner mesowear time scale). The small available samples for both these taxa may impact the robusticity of these interpretations, but it must be noted that the discrepancies were obtained between the mixed feeder and the browser/grazer style and, importantly, no species were classified as a grazer while being a browser, and vice versa.

Overall, both methods are here shown to provide very similar (almost identical) results, and it should be noted that the inner mesowear is most effective with specimens where all the three variables can be scored (when all the three parts of the inner second enamel band are not damaged or ruined), as highlighted by the number of undetermined individuals (Table 2). Nevertheless using both methods is highly advantageous and recommendable to obtain information about diet variation at different time scales.

4.2. Niche and resource partitioning

All the cervids (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus* and *Eucladoceros* sp.) share similar dental wear pattern to those of extant browsers. However, the size of the two former differs greatly from that of *Eucladoceros* sp. by being significantly smaller (84 and 63 kg vs. 239 kg, respectively). It is therefore possible that, despite exhibiting the same type of diet, differences in foraging in terms of height (vertical stratification) and the incorporation of different browse and ligneous foods, may explain the coexistence of the two small deer with *Eucladoceros* sp. Thus, *Eucladoceros* due to its larger body size may have exhibited a generally poorer quality diet (such as twigs of ligneous plants) if compared to that of the other cervids, and this could have been more evident during (short) periods of drier conditions and resource limitation. In this adverse periods small browsers would have foraged on most of the scarce high quality foods (see Prins et al., 2006 for an explanation of modern African species). Alcalde (2013) reports post-cranial adaptations for *Axis lyra* (=*Metacervoceros rhenanus*) similar to those of some modern browser species (such as the Eurasian elk *Alces alces*) that dwell and feed on humid areas. Although *A. alces* is significantly larger than all the CSG deer (450/318 kg; Janis, 1990), it has outer and inner mesowear comparable to those of *Axis* cf. *lyra*. Both species display high percentage of sharp cusps coupled with mesial, distal and J scores close to 1.5 (Table 2) (Fortelius and Solounias, 2000; Solounias et al., 2014; Danowitz et al., 2016). This suggests

that CSG *Axis* cf. *lyra* may had preferably browsed in wetlands areas. *G. torticornis* is the other taxon whose wear patterns reflect a selective (browsing) feeding behaviour and is comparable in size to *Eucladoceros* sp. (Fig. 3). Because post-cranial morphologies similar to those of modern gazelles have been reported for this fossil antelope (Alcalde, 2013), an adaptation to arid habitats as well as some features typical of woodlands may have been possible. Thus, *G. torticornis* might have avoided competition with both the medium-sized browser deer *Eucladoceros* sp. and larger open-land taxa such as the mixed feeders *Leptobos* sp. and *G. meneghinii* by browsing on brushes of transitional ecotones (e.g., transition zone between closed woodland and open grassland) or by partitioning the available vegetation in terms of height above ground level (Hulbert and Andersen, 2001) foraging on the same plant species but eating different parts as observed in African Wildebeest and Zebra (Owaga, 1975).

Alcalde (2013) also reports post-cranial adaptations for steep terrains and rather open landscapes for *G. meneghinii*, thus habitat segregation (i.e. a preference for mountainous regions) combined with a more flexible diet may had been key factors for niche partitioning in *G. meneghinii* populations. Wear patterns for *G. borbonica* indicate a mixed feeding (with a probably tendency towards grazing) composed by a higher amount of abrasives in diet if compared to all the cervids and the other bovids from CSG. This seems to be related to an occurrence in more open grasslands habitats (Strani et al., 2015). *G. borbonica* is also the smallest ungulate of the mammal assemblage (Fig. 3) and its broader diet fits
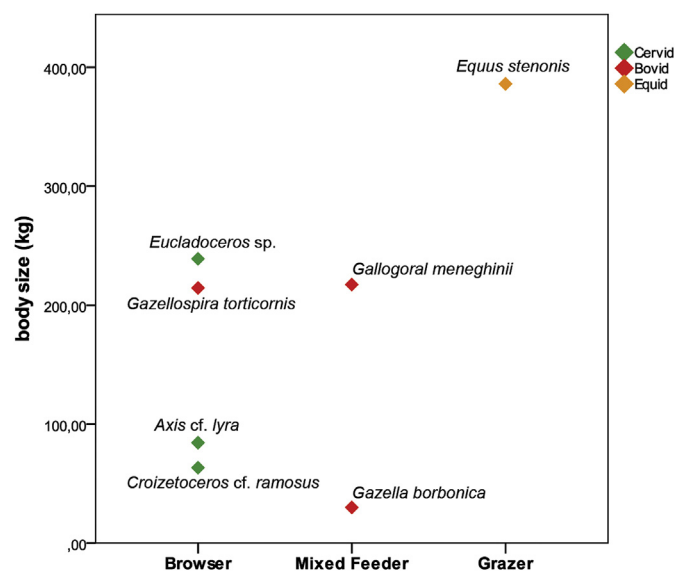

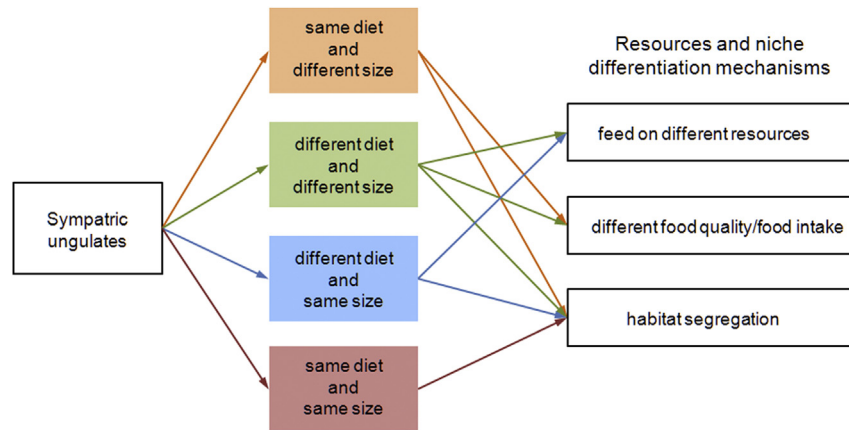**Fig. 3.** Average body size (kg) of the CSG ungulates plotted against feeding behaviour.

Fig. 4. Flow diagram showing niche partitioning mechanisms in sympatric ungulates based on dietary preferences and body size.

well with the coexistence with the other larger mixed feeders *Leptobos* sp. and *G. meneghinii* and the strict grazer *E. stenonis* in open environments. Overall, larger ungulates tend to display different diet ranging from strict browsing to grazing, whereas small taxa, with the exception of the bovid *G. borbonica*, appear to be more selective feeders (Fig. 3). It should also be noted that *A.* cf. *lyra*, *Eucladoceros* sp. and *E. stenonis* are the most common and well represented ungulates of the CSG assemblage (Bellucci et al., 2014), and the relative abundance of these species in the fossil deposit most probably influenced niche partitioning among the CSG herbivores as well. According to niche breadth concepts modern assemblage composed of abundant and relatively rare ungulates, uncommon species may persist by either consuming preferably few sparsely but widely available resource types, or by foraging in areas where their favoured resource is concentrated and aren't heavily exploited by the most common species (Brown, 1984; Macandza et al., 2012 and references therein). Following this it can be hypothesize that less common CSG ungulate taxa (*C.* cf *ramosus*, *G. torticornis*, *G. borbonica*, *Leptobos* sp., *G. meneghinii*) had a narrower niche breadth, restricting their diet to selected resources or smaller patches, while the most common species (*A.* cf. *lyra*, *Eucladoceros* sp., *E. stenonis*) would exploit a broader landscape.

According to all this, we propose a schematic model for explaining the major mechanisms of niche differentiation among sympatric herbivore ungulates based on diet and body masses (Fig. 4).

5. Conclusions

Data obtained from this study inform about how niche differentiation occurs in fossil assemblages, which is especially relevant in the study of species coexistence under limiting resources. Using the Early Pleistocene assemblage of Coste San Giacomo as a case study, we see that ungulates sharing the same feeding behaviour (browsing, mixed feeding, grazing) generally have different body size indicating different food intake demands and thus different diet quality. For similar-sized ungulates, a difference in the feeding behaviour is usually observed, and by consuming different type of resources they can share same habitats. For ungulates that share dietary type and body size, differences in habitat preference play a key role in niche partitioning with generalist species feeding in grasslands and woodlands (or in transitional areas), and more specialised taxa foraging mostly in specific environments (e.g., wetlands, rocky regions).

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Chapter 3

Dietary response of early Pleistocene ungulate communities to the climate oscillations of the Gelasian/Calabrian transition in Central Italy
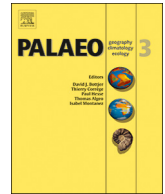
Dietary response of early Pleistocene ungulate communities to the climate oscillations of the Gelasian/Calabrian transition in Central Italy

Flavia Strani[a,b,*], Daniel DeMiguel[c,d,e], Luca Bellucci[b,f], Raffaele Sardella[a,b]

[a] *Dipartimento di Scienze della Terra, Sapienza - Università di Roma, P.le Aldo Moro 5, 00185 Roma, Italy*
[b] *Istituto Italiano di Paleontologia Umana, via U. Aldrovandi 18, I-00197 Roma, Italy*
[c] *Fundación ARAID, Zaragoza, Spain*
[d] *Departamento de Ciencias de la Tierra, Área de Paleontología, Universidad de Zaragoza, C/ Pedro Cerbuna 12, 50009 Zaragoza, Spain*
[e] *Institut Català de Paleontologia Miquel Crusafont (ICP), Universitat Autònoma de Barcelona, Edifici Z, C/ de les Columnes s/n, Campus de la UAB, 08193 Cerdanyola del Vallès, Barcelona, Spain*
[f] *Polo museale, Sapienza - Università di Roma, P.le Aldo Moro 5, 00185 Roma, Italy*

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ABSTRACT

Climatic oscillations at the Gelasian/Calabrian transition modified terrestrial palaeoenvironmental settings in the European region. A gradual drop in global temperatures beginning about 2.7 Ma led to drier conditions and to a reduction in, and subsequent disappearance of, sub-tropical vegetation in the central Mediterranean area by ca 1.2 Ma. Large ungulates are sensitive to vegetation changes and faced with harsher environmental settings may shift their feeding strategies to exploit available food resources in different ecosystems. In fossil assemblages such dietary adaptations are reflected by tooth morphology (a phylogenetic signal) and tooth wear degree (a direct signal of the species' diet). In this paper, we investigate how large herbivores responded to palaeoenvironmental changes that occurred at the passage between the Gelasian and Calabrian ages in the Italian Peninsula, analysing the dental wear patterns and hypsodonty of the early Pleistocene fossil ungulates assemblage of Olivola (Aulla, Central Italy). We found that while ungulate feeding behaviours during the Gelasian spanned from browsers to grazers, in the locality of Olivola this group of herbivores display a narrower range of diet types with many taxa adopting a mixed feeding behaviour. Cervids in particular, whose fossils are often associated only with wooded environments, as a response to the reduction of covered sub-tropical vegetation, shifted from a strict browsing diet in mostly closed habitats to a more abrasive one taking advantage of the spread of open landscapes. We also provide new data on the feeding behaviour of the rare fossil caprines, *Procamptoceras* and *Gallogoral meneghinii*, suggesting a grass-rich mixed diet for the former and a certain degree of dietary plasticity for the latter. Our research thus contributes to the better understanding of how ungulates adapted in the past to exploit different resource types during pivotal climatic changes and how environments changed in Central Italy on the onset of colder and more arid conditions.

1. Introduction

A gradual drop of the global temperatures characterized the late Pliocene, with seasonality and glacial activities increasing in the Northern Hemisphere (Flesche Kleiven et al., 2002). From this, the beginning of the Quaternary (around 2.6 Ma) corresponds to the initiation of the glacial/interglacial cycles marked by a 41 ka periodicity (Zubakov and Borzenkova, 1990; Lourens et al., 1996; Lisiecki and Raymo, 2005). These alternations exacerbated the climatic conditions with successive drop in temperature and humidity from the Gelasian (Kahlke et al., 2011; Head and Gibbard, 2015). In the Italian Peninsula, several cold and arid phases led to a type of vegetation (such as

herbaceous plants and shrubs; mostly *Artemisia* and *Ephedra*) typical of open landscapes, while deciduous forests were developed during the more humid interglacial phases with a steady decline of subtropical taxa (Bertini, 2003; Fortelius et al., 2006; Kahlke et al., 2011; Bertini, 2013; Combourieu-Nebout et al., 2015).

Landscape alteration deeply influences mammal communities both in terms of composition (e.g., diversity) and ecology, having to develop new resource exploitation strategies to avoid extinction. This is especially true for large herbivores that, while having the ability to tolerate a range of unfavourable climate oscillations and palaeoenvironmental changes (e.g., from open landscapes to forests, from forests to wooded steppes), are highly susceptible to vegetation modification and their
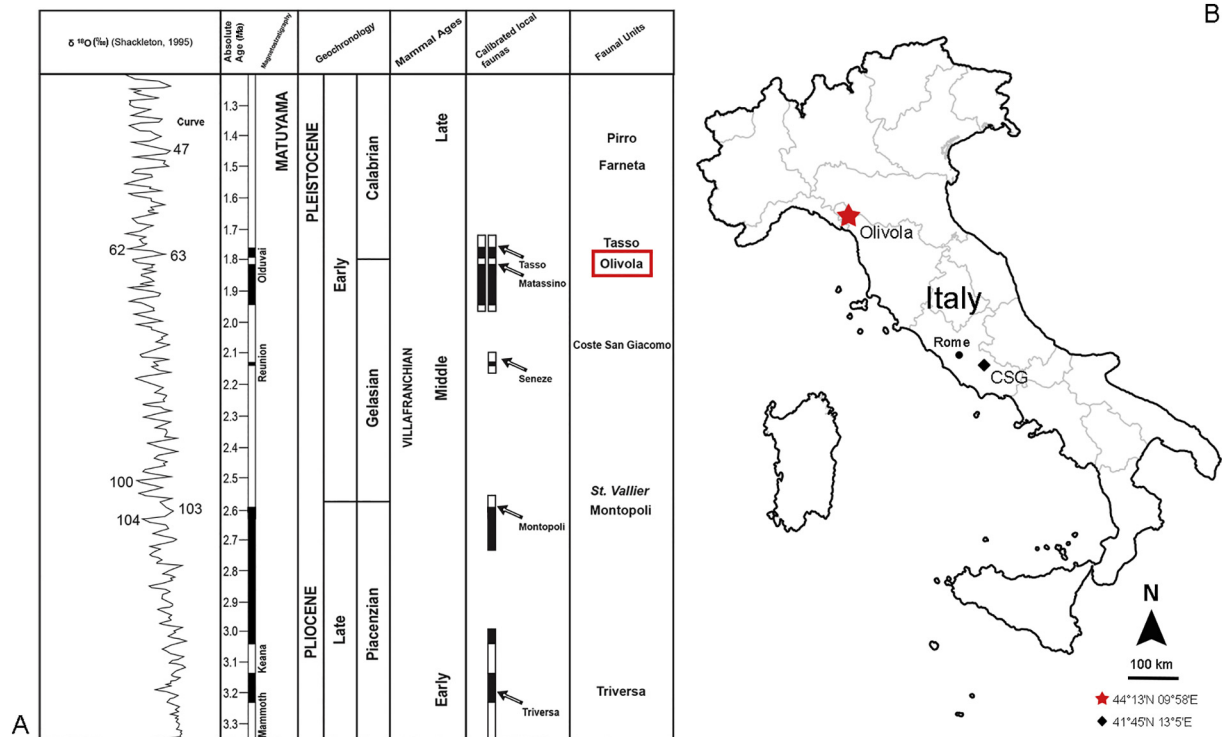
Fig. 1. A, Integrate chronological scheme for the Fontana Ranuccio Faunal Unit (modified from Gliozzi et al., 1997); B, geographical location of the early Pleistocene sites of Olivola and Coste San Giacomo.

diets mirror the availability of plant resources (DeMiguel et al., 2010, 2011). By investigating the feeding behaviour of large herbivores, it is thus possible to collect valuable information on how they adapted to, and cope with, alterations in habitat (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; Rivals and Athanassiou, 2008; Pushkina et al., 2014; DeMiguel, 2016; Bernor et al., 2017; Rivals et al., 2018).

According to the original definition of Azzaroli (1977), the Olivola Faunal Unit (FU) is the first one of the late Villafranchian placed at Gelasian/Calabrian boundary (at 1.8 Ma) (Torre et al., 1992) (Fig. 1A). As a result, this FU records the important changes in the large mammal faunal composition and diversity that occurred at the Gelasian/Calabrian transition and the passage from the middle to the late Villafranchian: the spread of large ungulates living in herds such as the leptobovine *Leptobos etruscus*, of two derived deer (*Eucladoceros dicranios* and *Pseudodama nestii*) and the appearance of the rare Caprinae *Procamptoceras brivatense* whose presence is registered only in the Olivola FU (Gliozzi et al., 1997; Rook and Martínez-Navarro, 2010). Among the carnivores, the first occurrence of the large hyena, *Pachycrocuta brevirostris* and the modern dog, *Canis etruscus* marks the spread of pack-hunter carnivores known as the "Wolf-event" (Azzaroli, 1983) or the "*Pachycrocuta brevirostris* event" due to the high-impact of this carrion eater on the early Pleistocene faunal assemblages (Sardella and Palombo, 2007; Palombo et al., 2008; Martínez-Navarro, 2010). Moreover, the early late Villafranchian also marks the arrival of the first *Homo* populations in the European continent, as testified by the exceptional hominid findings at Dmanisi (Georgia) (Gabunia and Vekua, 1995; Lordkipanidze et al., 2006 and references therein). The Olivola local fauna from northwest Tuscany (Aulla) is the most important site of the Olivola FU and, according to biochronological comparison with the nearby palaeomagnetically calibrated Matassino site, is probably located at the Olduvai Subchron (Gliozzi et al., 1997). Olivola faunal assemblage thus represents an excellent case study to investigate the effects of palaeoenvironmental alterations on large herbivores. By studying the dietary adaptations of the fossil ungulates of this site and

by comparing the results with those obtained from the similar faunal assemblage of the middle Villafranchian locality of Coste San Giacomo (Anagni, Central Italy) (Strani et al., 2015, 2017), pivotal information can be gathered on the response of large ungulates in terms of resource exploitation to the climatic changes that occurred during the Gelasian/Calabrian transition.

2. Material and methods

The rich fossil fauna of Olivola comes from fluvial pelitic sandstone and conglomerate deposit ("Olivola Conglomerates") underlying the Olivola village (Aulla, Tuscany) (Fig. 1B). The site is known from the 18th century and palaeontologists as Igino Cocchi and Giovanni Capellini, collected some specimens now stored in the palaeontological museums of the Universities of Pisa and Bologna. At the end of the 18th century the Olivola site was excavated by Forsyth Major (Forsyth Major, 1890) obtaining a large mammal collection now housed at the Natural History Museum of the University of Florence with some fossils stored in the Natural History Museum of London. The site yielded several ungulate remains belonging to 7 taxa: a large bush antlered (*Eucladoceros dicranios olivolanus*) and a medium-sized (*Pseudodama nestii*) deer, a leptobovine (*Leptobos etruscus*), two caprines (*Gallogoral meneghinii* and *Procamptoceras* cf. *brivatense*), one rhinoceros (*Stephanorhinus etruscus*) and one equid (*Equus stenonis*) (Azzaroli, 1950; Napoleone et al., 2003). The material studied consists of 84 teeth belonging to 84 individuals housed at the Natural History Museum of the University of Florence (Florence, Italy).

2.1. Hypsodonty index

Molar crown height (hypsodonty) has been examined as it yields some information about both feeding ecology and habitat openness (especially aridity degree) (Janis, 1988). Crown height in fossil taxa has been measured according to the index defined by Janis (1988) and a hypsodonty index (HI) was determined for both unworn (or if not

available minimally worn) lower third molars (HIm3) and either upper or lower second molars (HIMm2). The former was determined as m3 height divided by m3 width according to Janis (1988), and teeth classified as brachydont (HIm3 < 1.5), mesodont (1.5 < HIm3 < 2.5) and hypsodont (HIm3 > 2.5). We then calculated an average HIm3 (n = 5) for 3 taxa. The latter was determined as the ratio of height to length for unworn either upper or lower second molars (HIMm2) (n = 11) following Fortelius et al. (2002) and teeth classified as brachydont (HIMm2 < 0.8), mesodont (0.8 < HIMm2 < 1.2), and hypsodont (HIMm2 > 1.2). An average HIMm2 was also calculated for 4 taxa.

2.2. Dental mesowear

Mesowear is considered a good dietary indicator in herbivore species, as it represents the cumulative effects of the items ingested (both foods and exogenous particles such as dust and grit) on the dental morphology that are produced in a long period of time compared to the lifespan of the animal (Fortelius and Solounias, 2000).

Traditional mesowear (Fortelius and Solounias, 2000), referred as "outer mesowear" (Solounias et al., 2014; Danowitz et al., 2016), analyses the sharpness (i.e., morphology) of the cusps and the height of the occlusal relief of the most labial enamel bands of upper molars and the most lingual of the lower ones (Kaiser and Solounias, 2003; DeMiguel et al., 2012). Occlusal relief (high or low) and cusp shape (sharp, rounded, or blunt) of the apex of the paracone and metacone of upper molars (M1-M3) and the metaconid and entoconid of lower molars (m1–m3) were examined and scored. In the case of the lower molars of the equid E. stenonis the protoconid and hypoconid were scored (following Kaiser and Fortelius, 2003). Only upper molars (particularly M1 and M2) of S. etruscus were analysed as the effect of attrition and abrasion produce different kind of morphologies in rhinoceros lower teeth (Hernesniemi et al., 2011a, 2011b) making comparison with other ungulates mesowear results complex. In all cases, if available, M2 and m2 were preferably selected. The obtained data was then compared with those of a database of extant ungulates with known diets (Fortelius and Solounias, 2000). Occlusal relief and cusp shape scores were also converted to a single mesowear score (MWS) following "mesowear ruler" introduced by Mihlbachler et al. (2011). The method is based on seven cusp types (numbered from 0 to 6), ranging in shape from high and sharp (stage 0) to completely blunt with no relief (stage 6). A total of 80 specimens were scored using this method.

The inner mesowear method, recently developed by Solounias et al. (2014), analyses instead the second enamel band that forms the lingual margin of the metacone or paracone from an occlusal view, which is generally more frequently preserved in fossil specimens. Inner mesowear reflects dietary preferences that are intermediate in time (days-weeks; Solounias et al., 2014; Danowitz et al., 2016) between outer mesowear (months-years; Fortelius and Solounias, 2000) and dental microwear (days-hours; Solounias and Semprebon, 2002). For inner mesowear, the enamel band is scored on the mesial and distal sides of

the metacone using a 4 point scaling system described by Solounias et al. (2014) and Danowitz et al. (2016): (1) flat and planar with no gouges or indentations on the surface of the enamel; (2) nearly flat with several gouges that traverse the surface from either edge—the labial and lingual edges of the enamel band are somewhat rounded; (3) similar to score 2, but more rounded with less defined edges and more gouges; and (4) rounded—the surface is smooth without gouges, and there are no well-defined edges. The junction of the mesial and distal sides termed J is similarly scored: (1) it joins at a sharp, well-defined junction; (2) it is somewhat sharp, and often contains a gouge; (3) it is rounded, but the mesial and distal sides appear as distinct, separate surfaces; and (4) the J point lacks a discrete apex, and the mesial and distal sides of the enamel band form one continuous surface. The method was originally applied by Solounias et al. (2014) only on upper second molars (M2), and here it has been extended to M1 and M3 molars of different individuals (following Strani et al., 2017, 2018) to widen the sample and allow for more complete comparisons among mesowear databases (Table 2). Whenever possible, we used the enamel band of the metacone of the upper molars. If this region was taphonomically damaged, the band of the paracone was examined. We compared our results of the average inner mesowear scores of the mesial, J point and distal surfaces with those of a comprehensive database (Danowitz et al., 2016) of 8 extant ungulates with well-studied diets (browsing, mixed feeding and grazing). A total of 41 specimens were examined using this method.

The obtained mesowear scores were then compared to those of the ungulate taxa of the middle Villafranchian site of Coste San Giacomo previously published in Strani et al. (2015, 2017), which has a similar faunal composition (Bellucci et al., 2012, 2014).

2.3. Statistical analysis

We use hierarchical cluster analysis to distinguish herbivorous taxa groups based on dental wear patterns with percentage of high relief, rounded and blunt cusps of fossil and extant species (data from Fortelius and Solounias, 2000) as variables. The analysis was executed using Euclidean distance and Ward's method. We incorporated also to the study discriminant analyses to examine the resolution of outer mesowear variables applied to the fossil taxa. The percentage of high relief, rounded and blunt cusps were used as independent variables and two dietary (conservative and radical) classifications were used as grouping variables (Fortelius and Solounias, 2000). All analyses were performed using SPSS Statistics 24.

3. Results

3.1. Hypsodonty inference

The deer E. d. olivolanus display generally low molar crowns (Table 1) and has a brachydont dentition according to its HI for the lower third molar (HIm3 = 1.3) and a mesodont one for the upper or

Table 1
Summary of hypsodonty results. Abbreviations: number of specimens measured (N); hypsodonty index calculated as in Janis (1988) (HIm3); hypsodonty index calculated as in Fortelius et al. (2002) (HIMm2).

Taxa	Hypsodonty index							
	N	HIm3	HIm3 min/max	Category	N	HIMm2	HIMm2 min/max	Category
Eucladoceros dicranios olivolanus	1	1.3	1.3	Brachydont	3	0.8	0,83/0,86	Mesodont
Pseudodama nestii	3	1.6	1,39/1,71	Mesodont	6	0.8	0,74/0,86	Mesodont
Leptobos etruscus	/	/	/	/	1	1.0	1.0	Mesodont
Gallogoral meneghinii	/	/	/	/	/	/	/	/
Procamptoceras cf. brivatense	/	/	/	/	/	/	/	/
Equus stenonis	/	/	/	/	/	/	/	/
Stephanorhinus etruscus	1	0.9	0.9	Brachydont	2	0.7	0,63/0,75	Brachydont

lower second molars (HIMm2 = 0.8). The other cervid of the assemblage, *P. nestii*, displays higher crowns and exhibits a mesodont dentition according to both indices (HIm3 = 1.6; HIMm2 = 0.8). The bovid *L. etruscus* also displays mesodont teeth (HIMm2 = 1.0) and *S. etruscus* has brachydont ones (HIm3 = 0.9; HIMm2 = 0.7) (Table 1). No HI was calculated for *E. stenonis* because all the dental specimens are either enclosed in the mandible/maxilla or are worn. Nonetheless, this equid is characterized by a highly hypsodont dentition, and HI have been reported for this species and other early Pleistocene equids in other localities (Rivals and Athanassiou, 2008; Strani et al., 2015).

3.2. Dental mesowear

3.2.1. Outer mesowear

The predominant mesowear pattern for the cervids *E. d. olivolanus* and *P. nestii* is to display high occlusal relief and both sharp and rounded cusps with the former having more rounded apices (61.1%) and the latter showing an equal percentage of sharp and rounded cusps (50%). The lack of incidence of blunt cusps for both cervids and the small percentage of low relief observed in *P. nestii* (2.8%) indicate low abrasive diets for these two taxa. The bovids *L. etruscus* and *P.* cf *brivatense* show a predominance of high relief (100%) and rounded cusps (100%), which is indicative of intermediate levels of abrasion. The caprine *G. meneghinii* displays equal percentage of high and low occlusal relief and rounded cusps, which point to a slightly more abrasive diet. Among the perissodactyls, *E. stenonis* exhibits a mesowear comprised of a high percentage of low relief (100%) and blunt apices (60%) (this is the only taxon of the assemblage with this pattern), thereby suggesting a diet with a high degree of abrasion. *S. etruscus* individuals have all high relief (100%) and with mostly rounded cusps (66.7%), indicating relatively low level of abrasion. Olivola fossil ungulates display outer mesowear scores that range from 1.1 (*P. nestii*) to 5.2 (*E. stenonis*) (Table 2). Cervids have score values comparable to those of modern herbivores with a low or intermediate degree of abrasion in their diets (Fig. 2A, B, D). Bovids and the rhinocerotids *S. etruscus* display outer mesowear score values comprised between 1.7 (*S. etruscus*) and 3.0 (*G. meneghinii*), which are similar to those of extant ungulates with an intermediate-to-high abrasive feeding behaviour (Fig. 2A, B, D). The equid *E. stenonis* has a score value (5.2) that is close to those of modern grazing herbivores (Fig. 2A, B, D).

Two main clusters can be observed which separate ungulates with a low-to-medium abrasive diet (cluster A)—composed by browsers (subcluster A.1) and mixed feeders (subcluster A.2)—from those with a highly abrasive feeding (cluster B)—typical of grazers (Fig. 3). *E. stenonis* falls in the grazer cluster grouped with other extant equids such as *Equus burchelii* and *Equus grevyi*, and with the bovid *Bison bison* (cluster B). *G. meneghinii* is grouped in the grazer cluster as well. *L. etruscus* and *P.* cf. *brivatense* are both grouped within the mixed feeder cluster with large- and medium sized bovids showing heterogeneous diets (the browser *Tragelaphus strapticeros*, the mixed feeders *Syncerus caffer* and *Redunca redunca*, and the grazers *Kobus ellipsiprymnus* and *Hippotragus equinus*) (subcluster A.2.1). *S. etruscus* also falls into this group close to modern mixed feeders such as *Camelus dromedarius* and *Ovibos moschatus*, among others. Both *E. d. olivolanus* and *P. nestii* are grouped in the mixed feeder cluster with the latter being close to the cervid *Cervus canadensis* (subcluster A.2.2). None of the Olivola fossils fall in the browser cluster.

Discriminant analysis performed with the outer mesowear variables provides a satisfactory dietary discrimination with 74.1% of extant taxa (68.5% in cross-validation) correctly classified according to a conservative classification and 74.1% (74.1% in cross-validation) according to the radical one. The cervids *E. d. olivolanus* and *P. nestii* and the rhinoceros *S. etruscus* are classified as mixed feeders in both the conservative and radical classification (Fig. 4A–B). *L. etruscus* and *P.* cf. *brivatense* are mixed feeders according to the conservative classification (Fig. 4A), but grazers according to the radical one (Fig. 4B), thereby suggesting a more abrasive, grass-dominated mixed feeding diet for these taxa. *G. meneghinii* and *E. stenonis* are classified as grazers in both conservative and radical classification (Fig. 4A–B). In both classifications no fossil browsers can be observed.

3.2.2. Inner mesowear

The distribution of the inner mesowear scores (mesial, J point and distal) of the ungulate community of Olivola mostly includes values ≥ 2.0 (Table 2) with the only exception of *P. nestii* which shows scores that ranges from 1.5 to 1.9. *Leptobos* sp., *G. meneghinii* and *E. stenonis* display the highest values, with the equid having the highest scores (≥ 3.5 in all the three variables). When the inner mesowear scores are plotted with those of extant ungulates (Fig. 5A), data distribution suggests a dietary diversity comprised by mostly mixed-feeding and grazing traits. *P. nestii* scores are close to those of the modern ungulates *Okapia johnstoni*, *Giraffa camelopardalis* (browsers) and *Cervus*

Table 2

Summary of outer and inner mesowear. Abbreviations: number of specimens (N); percentage of specimens with high (%High) and low (%Low) occlusal relief; percentage of specimens with sharp (PerSharp), rounded (PerRound) and blunt (PerBlunt) cusps; outer mesowear score (MWS); mesial (Mesial), distal (Distal) and J point (J) scores. CSG outer mesowear scores from Strani et al. (2015) with the 4 points method proposed by Rivals et al. (2007) converted to the 7 points method proposed by Mihlbachler et al. (2011).

| Locality	Taxa	Outer mesowear							Inner mesowear			
		N	%High	%Low	PerSharp	PerRound	PerBlunt	MWS	N	Mesial	Distal	J
Olivola												
	Eucladoceros dicranios olivolanus	18	100	0	38.9	61.1	0	1.2	10	2.1	2.1	2.3
	Pseudodama nestii	36	97	2.8	50	50	0	1.1	18	1.5	1.5	1.9
	Leptobos etruscus	14	100	0	0	100	0	2.0	8	3	3.3	3.3
	Gallogoral meneghinii	2	50	50	0	100	0	3.0	2	2.5	4	3
	Procamptoceras cf. *brivatense*	2	100	0	0	100	0	2.0	/	/	/	/
	Equus stenonis	5	0	100	0	40	60	5.2	3	3.7	3.7	3.3
	Stephanorhinus etruscus	3	100	0	33.3	66.7	0	1.7	/	/	/	/
Coste San Giacomo												
	Axis cf. *lyra*	26	100	0	76.9	23.1	0	0.4	7	1.5	1.8	1.4
	Croizetoceros cf. *ramosus*	5	100	0	80	20	0	0.4	3	1.0	1.5	1.7
	Eucladoceros sp.	26	100	0	88.5	11.5	0	0.2	12	1.3	1.5	1.8
	Gazella borbonica	8	87.5	12.5	50	50	0	1.3	2	2.5	2.5	3.0
	Gazellospira torticornis	8	100	0	87.5	12.5	0	0.3	4	1.5	1.0	1.8
	Gallogoral meneghinii	2	100	0	50	50	0	1.0	2	1.5	1.0	2.0
	Leptobos sp.	2	100	0	0	100	0	2.0	2	2.5	2.5	2.5
	E. senezensis aff. *E. sen. stehlini*	21	4.8	95.2	28.6	38.1	28.6	4.7	11	3.3	3.3	3.6
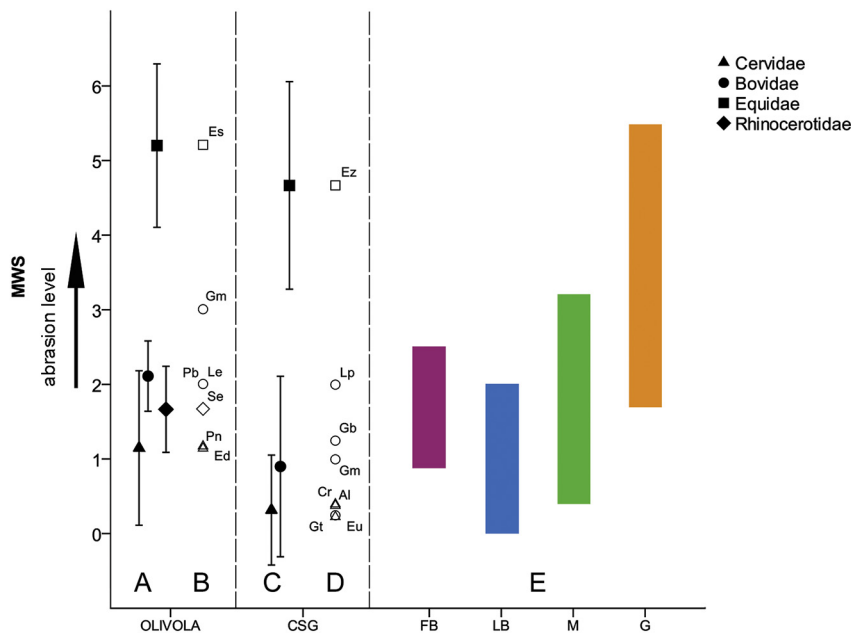
Fig. 2. Outer mesowear results of fossil (from Olivola and Coste San Giacomo, CSG) and extant ungulates. CSG data converted from Strani et al. (2015); modern ungulates data from Fortelius and Solounias (2000) and Rivals et al. (2010, 2014, 2016). A, Mean mesowear score (MWS) of fossil families from Olivola; B, mean MWS of single taxa from Olivola; C, mean MWS of fossil families from CSG; D, mean MWS of single taxa from CSG; E, extant ungulates and dietary categories. Fossil taxa abbreviations: Al, *Axis* cf. *lyra*; Cr, *Croizetoceros* cf. *ramosus*; Ed, *Eucladoceros dicranios olivolanus*; Es, *Equus stenonis*; Ez, *E. senezensis* aff. *E. sen. stehlini*; Eu, *Eucladoceros* sp.; Gb, *Gazella borbonica*; Gt, *Gazellospira torticornis*; Gm, *Gallogoral meneghinii*; Pn, *Pseudodama nestii*; Le, *Leptobos etruscus*; Lp, *Leptobos* sp.; Pb, *Procamptoceras* cf. *brivatense*; Se, *Stephanorhinus etruscus*. Other abbreviations: FB, extant fruit browsers; LB, extant leaf browsers; M, extant mixed feeders; G, extant grazers. Error bars correspond to standard deviation (± 1 SD).

canadensis (mixed feeder), with values that range from 1.3 to 1.8 (data from Solounias et al., 2014). Higher scores are observed in the other cervid *E. d. olivolanus* with values ranging from 2.1 to 2.3 and being close to those of the modern mixed feeder *G. granti*, which displays values from 2.3 to 2.8 (data from Solounias et al., 2014). *L. etruscus* has scores with values (2.6 to 3.1) that are comparable to those of the modern mixed feeder *N. granti* and the modern grazer *Kobus ellipsiprymnus* (3.2 to 3.6; data from Solounias et al., 2014). Both *G. meneghinii* and *E. stenonis* have scores (2.5 to 4.0 for the bovid and 3.3 to 3.7 for the equid) that are comparable to those of modern grazers *Kobus ellipsiprymnus* and *Connochaetes taurinus* (3.2 to 3.9; data from Solounias et al., 2014) and grass dominated-mixed feeder *Ourebia ourebi* (3.6 to 3.8) (Fortelius and Solounias, 2000; Solounias et al., 2014).

3.3. Comparison with the ungulate community of Coste San Giacomo

When mesowear scores of ungulates from Olivola are compared to those from CSG, late Villafranchian taxa display overall higher outer and inner mesowear values (Figs. 2; 5). When outer mesowear scores are compared (Fig. 2A–D), Olivola cervids display MWS close to 1.0 (1.2 for *E. d. olivolanus* and of 1.1 for *P. nestii*) whereas the same group in CSG shows very low values, below 0.5 (Table 2), indicating a diet based on the consumption of softer food in this locality. Similarly, bovids from Olivola display a generally higher degree of abrasion in their diet with MWS values comprised between 2 (*Leptobos etruscus*, *P.* cf. *brivatense*) and 3 (*G. meneghinii*), while their scores range in CSG from 0.2 (*G. torticornis*) to 2 (*Leptobos* sp.). The case of *G. meneghinii* is worth noting, since it is recorded in both sites. According to our results, it displays a mesowear score of 3.0 and 1.0 in Olivola and CSG, respectively. Such clear difference in its mesowear seems to indicate that this species could have successfully fed on a variety of resource types depending on their availability (i.e., seasonally). However, this information should be taken as tentative, as *G. meneghinii* is represented in both Olivola and in CSG by very few (< 5) individuals. The leptobovines have the same outer mesowear scores (2.0) in both localities, while *E. stenonis* has in Olivola a MWS of 5.2 (the highest recorded in this locality), and *E. senezensis* aff. *E. sen. stehlini* (referred as *E. stenonis* in Strani et al., 2017 and in works prior to Palombo et al., 2017) a MWS of 4.7 in CSG. Mean inner mesowear trends display similar discrepancies between the diets of the Olivola and CSG taxa (Fig. 5A, B). Olivola inner mesowear score values are mostly above 2 with three species (*L.*

etruscus, *G. meneghinii* and *E. stenonis*) displaying very high values (ranging from 2 to 4) (Fig. 5A) while most of the taxa in CSG display low or intermediate inner mesowear (from 1 to 3) scores, with only one species (*E. senezensis* aff. *E. sen. stehlini*) having values above 3, and with *G. meneghinii* having much lower values than in Olivola (Fig. 5B). The medium-small deer *P. nestii* from Olivola and *A.* cf. *lyra* from CSG show, however, similar inner mesowear low values, around 1.5 (Fig. 5A, B).

4. Discussion

Hypsodonty is mostly associated to wear resistance with higher crowns indicating a higher level of abrasion in dry and open habitats (e.g. grasslands) (Janis, 1988; Eronen et al., 2010a, 2010b), though it appears to be also linked to biomechanical effectiveness on consuming resistant items (DeMiguel et al., 2015). Considering these factors, the presence of one hypsodont, two mesodont, one brachydont and one brachy-mesodont ungulate seems to suggest a certain degree of aridity and habitat openness. When the dental wear patterns are taken into account, the absence of strict browsers suggests that soft, ligneous plant resources were not abundant in the region, while the occurrence of two strict grazers (*G. meneghinii* and *E. stenonis*) and of mixed feeders with a strong tendency towards grazing (*L. etruscus* and *P.* cf. *brivatense*) indicates diffused open and dry grasslands. The presence of ungulates with a mixed feeding behaviour (*E. d. olivolanus* and *S. etruscus*) and of a mixed feeder with a diet richer in browse (*P. nestii*) on the other hand points out to incidence of less opened areas possibly represented by patchy thickets or open woodlands.

This setting differs from that recently reconstructed (Strani et al., 2015, 2017) for the middle Villafranchian (Gelasian) site of CSG, where a mosaic of habitats, from wetlands to closed forests to grasslands, was inferred based on hypsodonty and dental mesowear of the ungulate taxa. In strong contrast to the ungulate community of Olivola, the CSG herbivores display diversified dietary adaptations (Table 3) with several browsers (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus*, *Eucladoceros* sp., *Gazellospira torticornis*) and mixed feeders (*Gazella borbonica*, *Gallogoral meneghinii*, *Leptobos* sp.), and a strict grazing feeding behaviour represented by the equid *E. senezensis* aff. *E. sen. stehlini*.

In the Italian Peninsula, palynological data from the Poggio Rosso section (Montevarchi Synthem Depositional Unit) from the geographically close Upper Valdarno Basin (Tuscany, Central Italy), reveal a significant increase (up to 89%) in herbs, mostly Asteraceae and
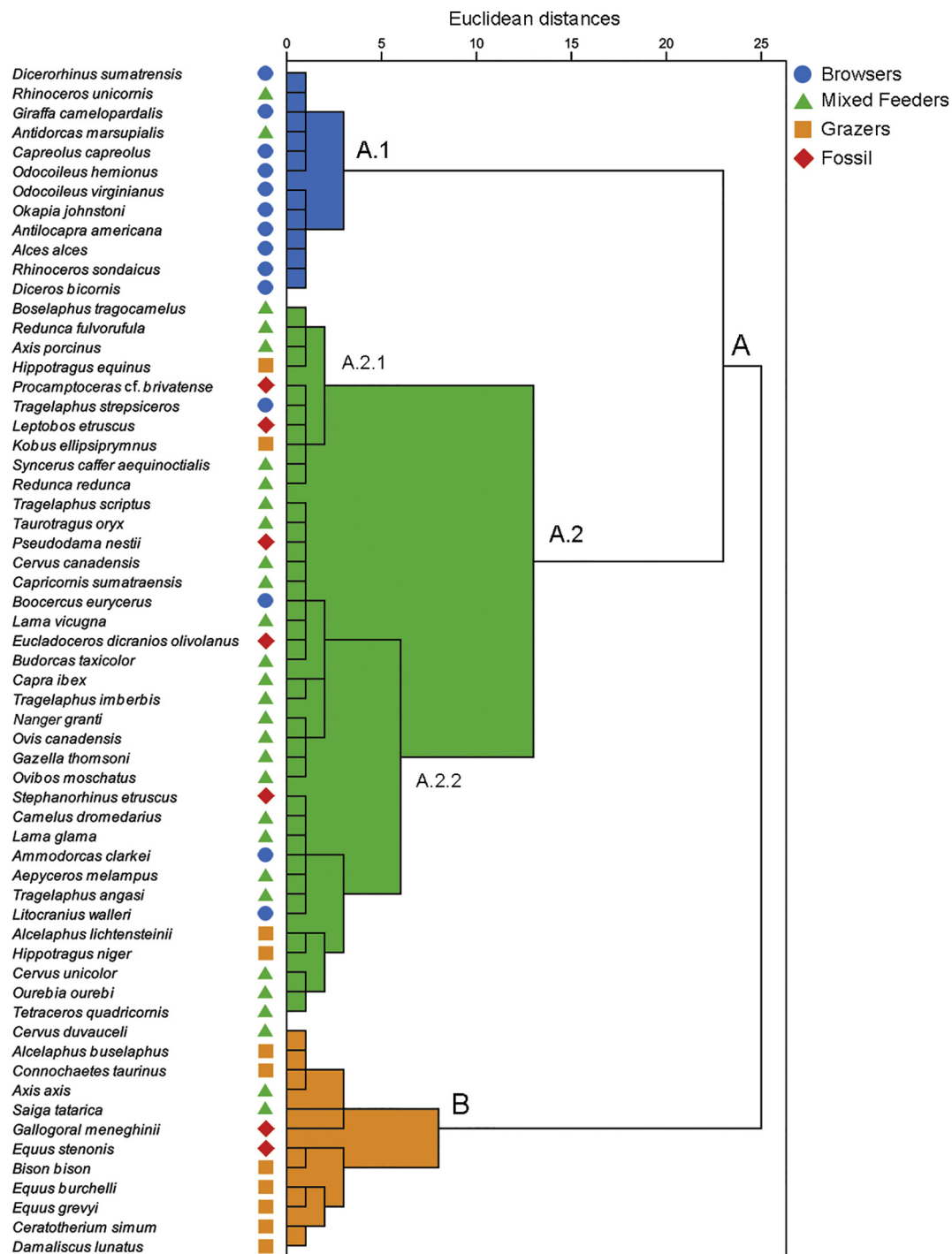
Fig. 3. Mesowear hierarchical cluster diagram based on the percentage of high occlusal relief, round and blunt cusps of fossil populations with extant species. (Data for extant species from Fortelius and Solounias, 2000.)

Cichorioideae, before the top of the Olduvai subchron marking a cold and dry phase possibly corresponding to the MIS 64 (Bertini et al., 2010; Bertini, 2013). In the same section, in deposits between 2.2 and 1.7 Ma, a general decrease of plant species diversity is recorded and a loss of subtropical taxa is attested (Bertini, 2013), a trend that characterizes the whole vegetation of the Italian Peninsula at the beginning of the Pleistocene (Bertini, 2003; Bellucci et al., 2014; Combourieu-Nebout et al., 2015).

According to all these data, it is possible to increase our understanding about how large herbivores adapted to the overall direction towards colder and drier conditions of the early Pleistocene in the

Italian Peninsula. First, our data show that bovids and cervids most prominently shifted their feeding behaviours towards a diet richer in abrasive plants, thereby taking advantage of the expansion of herbaceous and steppe vegetation (Bertini, 2003, 2013) (Table 2). Considering the different degree of hypsodonty observed in these groups, bovids may have fed on plants with abundant phytolith production (e.g. grasses) while brachy- or mesodont deer on herbs with a lower silica content (e.g. forbs) (Piperno, 1988, 2006; Morris et al., 2009). This shift towards a more abrasive diet is particularly evident in the fossil deer: the large sized bush-antlered deer *Eucladoceros* and the medium-to-small sized deer (*Pseudodama nestii*, *Axis* cf. *lyra*) which clearly adopted
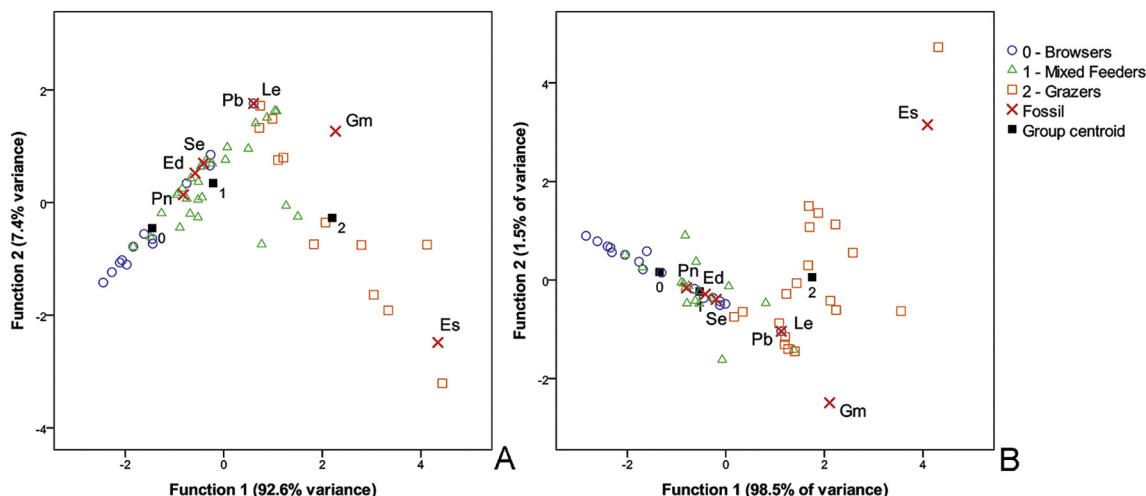
Fig. 4. Bivariate diagrams based on discriminant analysis: A, conservative classification; B, radical classification. Fossil taxa abbreviations: Ed, *Eucladoceros dicranios olivolanus*, Pn, *Pseudodama nestii*; Le, *Leptobos etruscus*; Gm, *Gallogoral meneghinii*; Pb, *Procamptoceras* cf. *brivatense*; Es, *Equus stenonis*; Se, *Stephanorhinus etruscus*.

a strict browsing behaviour in CSG, display a mixed diet in the more recent site of Olivola. Few studies are available on the diet of medium or small sized middle/early late Villafranchian fossil deer (such as *Pseudodama* and *Croizetoceros*), but in other similarly dated localities in Europe (e.g., Greece, France and Britain), these taxa usually display a browsing or mixed feeding behaviour (Rivals and Athanassiou, 2008; Valli and Palombo, 2008; Rivals and Lister, 2016). Contrary a recent study based on the dental microwear texture analysis reported a greater diversity of feeding behaviours (from browser to grazer) for the large bush-antlered deer *E. ctenoides*, suggesting a significant dietary plasticity for this early Pleistocene cervid (Berlioz et al., 2017). Dental microwear however records information about the last few meals (hours and days) of an animal before its death (whereas dental mesowear reflects dietary preferences over a longer period; weeks and months) (Sánchez-Hernández et al., 2016; Mihlbachler et al., 2018), thus the observed discrepancy could reflect seasonal differences. A more abrasive diet is also recorded for the Olivola specimens of *E. d. olivolanus* (mixed feeder) and for *P. nestii* (browse-dominated mixed feeder), suggesting that in the Italian Peninsula, both *Eucladoceros* lineage and the "*Axis*-like" deer group (which include fossil *Axis*, *Pseudodama* and *Metacervoceros*), adapted to exploit a wider spectrum of food resources

Table 3
Summary table of dietary behaviours of the ungulates of Olivola and CSG (CSG data from Strani et al., 2015, 2017).

Group	Dietary behaviour	
	Olivola	CSG
Cervids	Mixed feeders/browse-dominated mixed feeders	Strict browsers
Bovids		
Antilopines	–	Browser/mixed feeders
Caprines	Grass-dominated mixed feeders/grazers	Mixed feeders
Leptobovines	Grass-dominated mixed feeders	Grass-dominated mixed feeders
Rhinocerotids	Mixed feeders	–
Equids	Grazers	Grazers

during the late Villafranchian, probably driven by the steady reduction of subtropical-like environments, especially during glacial periods (Bertini et al., 2010; Combourieu-Nebout et al., 2015). The development of this dietary plasticity may have been a winning strategy for the
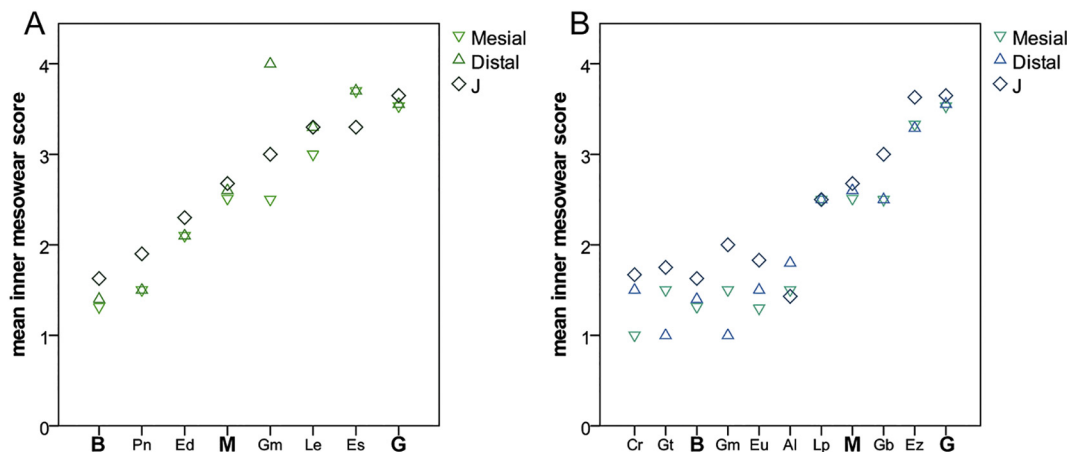

Fig. 5. Comparison between inner mesowear scores of fossil ungulates from Olivola and CSG. A, Mean inner mesowear scores for Olivola fossil taxa and modern browsers, mixed feeders and grazers (data for extant species from Danowitz et al., 2016); B, mean inner mesowear scores for CSG fossil taxa (data from Strani et al., 2017) and modern browsers, mixed feeders and grazers (data for extant species from Danowitz et al., 2016). Fossil taxa abbreviations: Al, *Axis* cf. *lyra*; Cr, *Croizetoceros* cf. *ramosus*; Ed, *Eucladoceros dicranios olivolanus*; Es, *Equus stenonis*; Ez, *E. senezensis* aff. *E. sen. stehlini*; Eu, *Eucladoceros* sp.; Gb, *Gazella borbonica*; Gt, *Gazellospira torticornis*; Gm, *Gallogoral meneghinii*; Pn, *Pseudodama nestii*; Le, *Leptobos etruscus*; Lp, *Leptobos* sp.; Pb, *Procamptoceras* cf. *brivatense*; Se, *Stephanorhinus etruscus*. Other abbreviations: B, modern browsers; M, modern mixed feeders; G, modern grazers.

spread of both groups through the European continent which occurrences are reported up to the Epivillafranchian and early Galerian (Rook and Martínez-Navarro, 2010; Bellucci et al., 2015). The same flexibility in food exploitation is also observable in extant cervids such as the red deer (*Cervus elaphus*) (Gebert and Verheyden-Tixier, 2001), the sika deer (*Cervus nippon)* (Takatsuki, 2009; Kubo and Yamada, 2014) or in the modern chital (*Axis axis*) (Sankar and Acharya, 2004). Dietary flexibility has been also demonstrated in a large variety of extinct herbivorous mammals (including deer, giraffids, dromomerycids, proboscideans, antilocaprids, etc.) across their range (Solounias et al., 1988, 2000; Solounias and Moelleken, 1994; Semprebon et al., 2004; Rivals et al., 2009, 2012; DeMiguel et al., 2008, 2010; Kahlke and Kaiser, 2011; Rivals and Semprebon, 2011; Haiduc et al., 2018; Strani et al., 2018), probably as a response to environmental shifts and changing environments (DeMiguel et al., 2010). The leptobovine lineage assumes consistently a grass-dominated mixed feeding behaviour in both localities, whereas *G. meneghinii* displays a more abrasive diet in Olivola than in CSG. *E. stenonis* and *E. senezensis* aff. *E. sen. stehlini* exhibits dental wear patterns that are typical of extant grazer species in both sites, with the equid from Olivola having blunter cusps suggesting a stricter grazing behaviour in this locality. Although no mesowear data of *S. etruscus* from the CSG are available, tooth meso- and microwear data from East Runton (Britain) (Rivals and Lister, 2016) suggest a browsing behaviour for this species. The mixed diet inferred for this species in Olivola can be considered, therefore, as an adaptation to the drier conditions, which is in well accordance with the case of the remaining cervids.

Overall the mesowear signatures of the cervids *P. nestii* and *E. d. olivolanus* appear to be comparable to that of the modern mixed feeder wapiti (*Cervus canadensis*), a large deer that shows high occlusal reliefs, no blunt cusps, and similar percentages of sharp and rounded cusps (Fortelius and Solounias, 2000). This large deer feeds mostly on shrubs, tree shoots, sedges and grasses in both meadows and forested areas (Mattioli, 2011; Jung et al., 2015). Accordingly, both fossil deer could have exhibited similar dietary preferences, thus feeding in both open and closed areas and partitioning the available resources through body size differences (*Eucladoceros* is a large deer with a body mass of over 200 kg similar to that of modern red deer; Kaiser and Croitor, 2004; Baygusheva and Titov, 2013; Strani et al., 2017), with *P. nestii* displaying a more selective browsing behaviour. The large *E. stenonis*, *S. etruscus* and *L. etruscus* most probably foraged in grasslands, with the bovid and the rhinocerotid adopting a more generalist diet. *P.* cf. *brivatense*, a small caprine quite rare and often poorly represented in the fossil record (Cregut-Bonnoure, 2007), may have occupied open areas as well, and exhibited a mixed diet somewhat rich in abrasive items. Alcalde (2013) reports postcranial morphologies typical of ungulate adapted to rocky and steep areas for *G. meneghinii* suggesting that this species in Olivola like in the middle Villafranchian site of CSG may have preferred mountainous habitats. Unlike in CSG, the only individual of *G. meneghinii* suggests a diet based on high consumption of abrasive items (e.g., grasses, dust, and grit), but the small sample available in both localities does not allow for more precise inferences.

Other late Villafranchian European and Western Asian localities, as Barranco de los Conejos (Guadix-Baza Basin, Spain) and Dmanisi (Caucasus, Georgia), have also been object of climatic and palaeoenvironmental reconstructions. In Barranco de los Conejos, a trend towards drier and colder conditions has been documented for the lowermost levels in the upper Matuyama Chron, as indicated by the evolution of the arvicolid local lineages towards developing ever-growing molars, and evidences from other vertebrate assemblages (Agustí et al., 2013). Different proxies (pollen, phytoliths, fossil fruits, herpetofauna, insectivorans, micro- and large mammals) suggest the presence of arid environments at Dmanisi as well, ranging from steppe or semi-desert to open Mediterranean forests in a warm and dry Mediterranean climate (Gabunia et al., 2000; Blain et al., 2014 and references therein).

The Gelasian/Calabrian transition (middle/late Villafranchian) in Southern Europe seems to have been characterized by an increase of the aridity level (though some areas such as Sésklo in Greece exhibited mostly arid conditions even during the middle Villafranchian, as reported by Rivals and Athanassiou, 2008 or experienced an expansion of steppes restricted in time and space such as in the Padania plain, as reported by Bertini, 2010 and references therein) and in the Italian Peninsula this change seems to have led ungulates, browsers in particular, to adapt to consume more abrasive foods in response to the expansion of grasslands and steppe-like vegetation.

5. Conclusions

Analysis of the dental morphology and mesowear patterns of the Olivola ungulates allowed us to gather new information about the response of fossil ungulates to the Gelasian/Calabrian climatic changes, and on the habitats that characterized the region during this phase of significant environmental modifications. The occurrence of taxa with, mostly, medium-to-high molar crowns coupled with the lack of strict browsers, the abundance of mixed feeders (*E. d. olivolanus*, *P. nestii*, *L. etruscus*, *P.* cf. *brivatense*, *S. etruscus*) and grazers (*G. meneghinii*, *E. stenonis*) reflects a mostly dry landscape characterized by open grasslands with subordinate thickets and open woodlands. Overall when compared to the middle Villafranchian (Gelasian) fossil ungulate assemblage of Coste San Giacomo, the late Villafranchian herbivores of Olivola display less diversified dietary behaviours. Cervids and *G. meneghinii* shifted to a more abrasive diet exploiting the now more open landscapes, suggesting a high dietary plasticity for these groups.

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Chapter 4

The effects of the "0.9 Ma event" on the Mediterranean ecosystems during

the Early-Middle Pleistocene Transition as revealed by

dental wear patterns of fossil ungulates

The effects of the "0.9 Ma event" on the Mediterranean ecosystems during the Early-Middle Pleistocene Transition as revealed by dental wear patterns of fossil ungulates

Flavia Strani[a,b,*], Daniel DeMiguel[c,d,e], David M. Alba[e], Salvador Moyà-Solà[e,f,g], Luca Bellucci[b,h], Raffaele Sardella[a,b], Joan Madurell-Malapeira[e]

[a] *Dipartimento di Scienze della Terra, "Sapienza – Università di Roma", P.le Aldo Moro 5, 00185 Roma, Italy*

[b] *PaleoFactory, "Sapienza – Università di Roma", P.le Aldo Moro 5, 00185 Rome, Italy.*

[c] *Fundación ARAID, Zaragoza, Spain*

[d] *Departamento de Ciencias de la Tierra, Área de Paleontología. Universidad de Zaragoza, C/ Pedro Cerbuna 12, 50009 Zaragoza, Spain*

[e] *Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, c/ Columnes s/n, Campus de la UAB, 08193 Cerdanyola del Vallès, Barcelona, Spain*

[f] *Institució Catalana de Recerca i Estudis Avançats, Pg. Lluís Companys 23, 08010 Barcelona, Spain*

[g] *Unitat d'Antropologia (Departament de Biologia Animal, Biologia Vegetal i Ecologia), Universitat Autònoma de Barcelona, Campus de la UAB, 08193 Cerdanyola del Vallès, Barcelona, Spain*

[h] *Polo museale, "Sapienza - Università di Roma" , P.le Aldo Moro 5, 00185 Roma, Italy*

*Corresponding author.

E-mail address: flavia.strani@uniroma1.it (F. Strani).

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Abstract

The Early-Middle Pleistocene Transition (EMPT) (ca. 1.4-0.4 Ma) represents a fundamental transformation in the Earth's climate state, starting at 1.4 Ma with a progressive increase in the amplitude of climatic oscillations and the establishment of strong asymmetry in global ice volume cycles. The progressive shift from a 41kyr to 100kyr orbital rhythm was followed by the first major build-up of global ice volume during MIS 24-22, the so-called "0.9 Ma event". The Vallparadís Section (Vallès-Penedès Basin, NE Iberian Peninsula) is one of the few Pleistocene series in Europe that spans the onset of the transition (from 1.2 to 0.6 Ma), thus representing a pivotal array of localities to investigate the effect of glacial dynamics on environmental conditions in Southern Europe. Here we inspect the effects of the EMPT on terrestrial ecosystems by examining the dietary adaptations (through dental meso- and microwear patterns) of fossil ungulates from the Vallparadís Section dated before and after the "0.9 Ma event". Results show a steady presence of open grasslands before MIS 22 and more humid conditions at MIS 21. Both before and after MIS 22, a consistent presence of ungulates with long-term patterns that point to a grazing or grass-rich mixed feeding behaviour is observed, while noticeably, short-term patterns point to increased seasonality right after the "0.9 Ma event" glacial period. This increment of seasonality may have had an important effect on the Mediterranean habitats leading to recurring changes in the quality of plant resources available to large herbivores, which in response periodically adopted more mixed feeding behaviours widening their dietary breadth to consume also sub-optimal food items during adverse seasons.

1. Introduction

The latest Early Pleistocene (Epivillafranchian; Kahlke, 2000, 2007; Bellucci et al., 2015) and the beginning of the Middle Pleistocene (ca 1.4-0.4 Ma) were characterised by a progressive increase in the amplitude of climate oscillations, the shift from a 41-kyr to quasi-100 kyr orbital rhythm, the increasing of the long-term average global ice volume, and the establishment of strong asymmetry in global ice volume cycles; the former time interval is known as the Early-Middle Pleistocene Transition (EMPT; Lisiecki and Raymo, 2005; Maslin and Ridgwell, 2005; Clark et al., 2006). It started around 1.4 Ma and was complete at 0.4 Ma, being characterised by a series of important environmental alterations with long and severe glacial periods, a steep decrease of sea surface temperatures, and an increase of both seasonality and aridity in the Northern Hemisphere (Clark et al., 2006; Kahlke et al., 2011; Head and Gibbard, 2015). A long-term (ca. 80 kyr) and extreme glaciation, known as the "0.9 Ma event", occurred in correspondence to MIS 24 and MIS 22, with global ice volumes increasing substantially and surface sea temperatures reaching the lowest values of the EMPT in the North Atlantic (Clark et al., 2006; Maslin and Brierley, 2015; Head and Gibbard, 2015). All these climatic shifts had important repercussions on the ecosystems and the mammal fauna composition of the European continent, though the effects were probably milder in the Mediterranean region than in Central Europe (Madurell-Malapeira et al., 2010; Kahlke et al., 2011).

The Vallparadís Section in Terrassa (Vallès-Penedès Basin, NE Iberian Peninsula) is one of the few chronologically well-constrained (1.2–0.6 Ma; Madurell-Malapeira et al., 2010, 2014) sets of localities in Europe that record the EMPT and the corresponding faunal changes associated with climatic instability. In particular, this composite section includes two different sites (Cal Guardiola and Vallparadís Estació) with multiple fossiliferous layers that have yielded abundant and well-preserved vertebrate remains, among which artiodactyls and perissodactyls constitute the most abundant taxa (Berástegui et al., 2000; Alba et al., 2008; Madurell-Malapeira et al., 2009, 2010,

2017). The Vallparadís Section thus represents an exceptional opportunity to investigate the environmental context that characterised the Mediterranean region at the onset and during the stabilization of the high-amplitude 100 kyr glacial cycles, as well as the magnitude and the aftermath of the "0.9 Ma event" on habitat composition at lower latitudes on the European continent.

The exacerbation of climatic conditions is well known to deeply influence mammal communities, in terms of composition and adaptations to exploit the available plant resources (DeMiguel et al., 2010). This is particularly true for herbivorous ungulates, which are highly susceptible to alteration of vegetal communities and for which feeding strategies accurately reflect plant resource availability, as well as preferred environmental settings. By reconstructing the dietary adaptations of fossil ungulate assemblages, it is possible to obtain pivotal palaeoecological information, such as niche occupation and habitat settings (i.e., abundance of grasslands versus forests, type of specific vegetation, habitat openness, degree of aridity, etc.; Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; Rivals and Athanassiou, 2008; DeMiguel at al., 2010, 2011, 2018; Strani et al., 2015, 2018a, 2018b, 2018c).

Accordingly, here we aim to reconstruct changes in the terrestrial ecosystems of the Mediterranean region at the onset of the EMPT and specially after the "0.9 Ma event" by inspecting for the first time the long- and short-term dental wear patterns (to infer diet) and other ecologically relevant traits (such as molar crown height) of the fossil ungulates of the Vallparadís Section.

1.1. Regional and geological setting

The Vallparadís composite section includes two different sites (Madurell-Malapeira et al., 2010): Cal Guardiola and Vallparadís Estació, respectively located on the western and eastern banks of the Torrent de Vallparadís, in the heart of the town of Terrassa (Catalonia, NE Spain; Fig. 1). The

whole area belongs to the Vallès-Penedès Basin, a NE-SW narrow half-graben, parallel to the

Catalan margin (Cabrera and Calvet, 1996). The Vallès-Penedès narrow depression (about 15 km

wide) is a basin filled by Neogene and Quaternary clastic sediments: the transported materials are

linked with alluvial-colluvial depositions, which are perfectly represented in the Quaternary alluvial

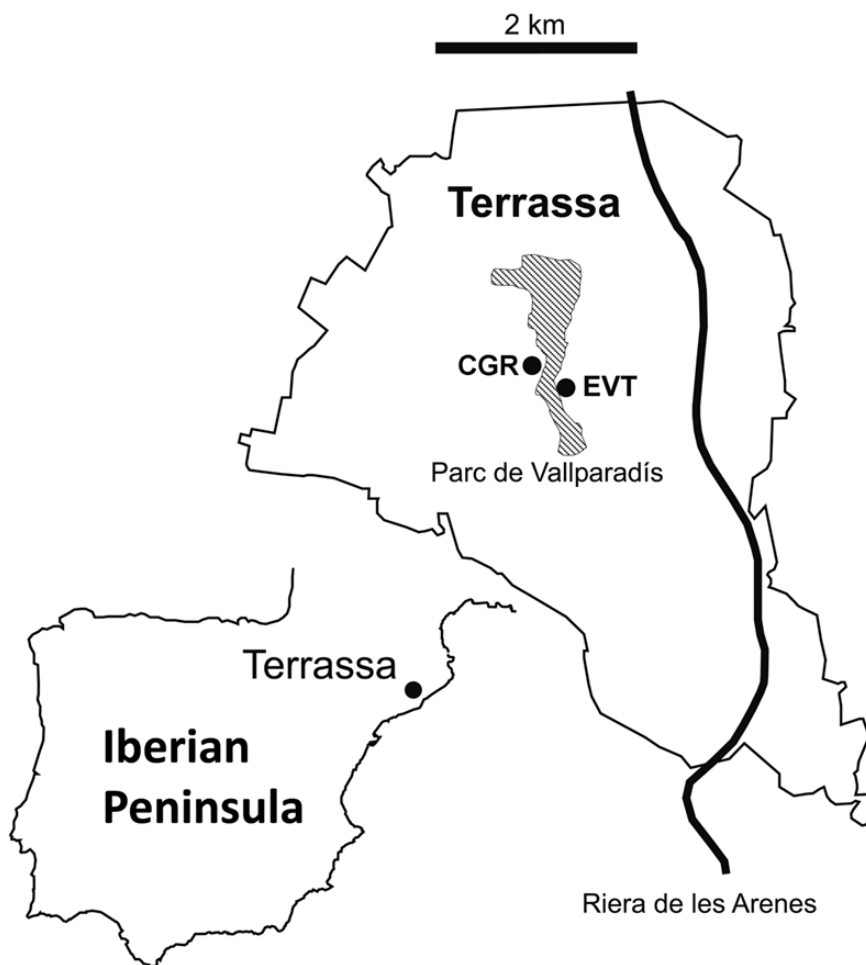fan of Terrassa (Madurell-Malapeira et al., 2010, 2017).

Figure 1. Geographical location of the Vallparadís composite section.

Vallparadís Estació consists of a 14 m-thick Quaternary sequence that lays on the Miocene

palaeorelief, which presented at least a 12 m drop, deeply modeling the sedimentation (Madurell-

Malapeira et al., 2010, 2017). Three sections were studied, correlated to each other and with the Geomagnetic Polarity Time Scale, so that it is possible to reliably determine the chronology (Fig. 1): late Early Pleistocene to early Middle Pleistocene, ca. 1.2–0.6 Ma (Madurell-Malapeira et al., 2010, 2014, 2017). Within this local section, three layers provided more than 90% of the totally recovered large mammal fossils: EVT12, EVT7 and EVT3 (see Madurell-Malapeira et al., 2010, 2017 for further details) (Table 1).

Layer EVT12 is correlated with the Jaramillo subchron (1.07–0.99 Ma), roughly corresponding to the interglacial stage MIS 31. This layer yielded more than 2400 vertebrate remains, of which less than 600 (ca. 25%) are prepared and available for study.

Layer EVT7 is correlated with the post-Jaramillo interval of the Matuyama chron (0.99–0.78 Ma). An absolute dating of 0.858 ± 0.087 Ma was obtained for this layer based on the ESR method (Duval et al., 2015), which probably corresponds to the interglacial stage MIS21 (Madurell-Malapeira et al., 2010, 2017). This layer yielded 20,286 vertebrate remains, of which 3234 (16.0%) are currently prepared for study (Number of Identified Specimens 1/4 2,508, 12.4% of all recovered fossils).

Finally, layer EVT3 is correlated with the Brunhes magnetostratigraphic chron, with a maximum age of 0.6 Ma according to the identified small-mammal assemblage (Madurell-Malapeira et al., 2010; Minwer-Barakat et al., 2011). This layer yielded 2121 vertebrate remains, of which 397 (18.7%) are currently available for study.

	EVT12	EVT7	EVT3
Large mammals			
Mammuthus sp.	x	x	x
Hippopotamus antiquus	x	x	
Sus sp.	x	x	
Bison sp.	x	x	x
Bovidae indet.	x	x	
Caprini indet.	x	x	
Dama vallonnentensis	x	x	
Megaloceros savini	x	x	
Cervus elaphus			x
Equus altidens	x	x	
Equus cf. *ferus*			**X**
Equus cf. *hydruntinus*			**X**
Stephanorhinus hundsheimensis	x	x	
Stephanorhinus sp.			x
Megantereon whitei	x		
Lynx pardinus	x	x	
Felidae indet. (large form)		x	
Panthera gombaszoegensis		x	
Puma pardoides		x	
Pachycrocuta brevirostris	x	x	
Lycaon lycaonoides	x	x	
Canis mosbachensis	x	x	
Vulpes praeglacialis	x	x	
Vulpes sp.			x
Meles meles atavus	x	x	
Ursus deningeri	x	x	
Macaca sylvanus cf. *florentina*	x	x	
Small mammals			
Mimomys savini	x	x	
Iberomys huescarensis	x	x	
Eliomys quericinus	x	x	
Hystrix refossa	x	x	
Stenocranius gregaloides		x	
Apodemus cf. *sylvaticus*		x	
Microtus aff. *arvalidens*			x
Arvicola mosbachensis			x

Table 1. Large and small mammals from Vallparadís Section based on findings described in Alba et al. (2008), Madurell-Malapeira et al. (2010, 2014, 2017), Minwer-Barakat et al. (2011), Boscaini et al. (2016) and Bartolini-Lucenti et al. (2017).

2. Materials and methods

2.1. Hypsodonty

We examined molar crown height (or hypsodonty), as it yields information about feeding ecology (Janis, 1988; DeMiguel et al., 2008) and habitat openness (especially aridity degree; Janis, 1988). Traditionally, hypsodonty is believed to be linked to wear resistance and grazing in open and dry environments (Janis, 1988; Eronen et al., 2010; Semprebon and Rivals, 2010), as grasses are often rich in abrasive phytoliths, are covered in grit, and have a high fiber content, which require longer and repeated chewing cycles to fully process them (Healy and Ludwig, 1965; Ungar, 2010 and references therein). It has been also recently proposed that biomechanical effectiveness when feeding on resistant foods could be a factor as well (DeMiguel et al., 2015).

Crown height in the fossil specimens has been measured according to the index defined by Janis (1988) and Fortelius et al. (2002); this hypsodonty index (HI) was determined for both unworn (or, if not available, minimally worn) lower third molars (HIm3) and either upper or lower second molars (HIMm2; Fortelius et al., 2002). Him3 was decomputed as m3 height divided by m3 width following Janis (1988), and teeth classified as either brachydont (HIm3 < 1.5), mesodont (1.5 < HIm3 < 2.5) or hypsodont (HIm3 > 2.5). We calculated an average HIm3 (n = 12) for 5 taxa (*D. vallonnentensis*, *E. altidens*, *M. savini*, *Bison* sp., *E.* cf. *ferus*). HIMm2 was determined as height to length ratio for unworn upper or lower second molars (HIMm2; n = 32) for 6 taxa (*D. vallonnentensis*, *E. altidens*, *Bison* sp., *E.* cf. *ferus* and *S. hundsheimensis*) following Fortelius et al. (2002). Teeth were classified as either brachydont (HIMm2 < 0.8), mesodont (0.8 < HIMm2 < 1.2), or hypsodont (HIMm2 > 1.2), and an average HIMm2 was calculated for each taxon.

2.2. Dental mesowear

Mesowear is a direct signal of herbivorous species' diets and represents the cumulative effects of the items ingested (both foods and exogenous particles such as dust and grit) on the dental morphology that are produced over a long period of time compared to the lifespan of the animal (Fortelius and Solounias, 2000). The outer mesowear method is based on the relief of the worn occlusal surface and on the shape of the cusps, which are heavily influenced by attrition (tooth-to-tooth contact) and abrasion (tooth-to-food contact). High levels of attrition produce sharper cusps and higher tooth relief, whereas high levels of abrasion produce blunter cusps and lower tooth relief (Fortelius and Solounias, 2000; DeMiguel et al., 2008). Mesowear analysis, originally limited to the second upper molar (M2), was here extended as well to upper molars (M1–M3; following Kaiser and Solounias, 2003) and lower molars (m1–m3; following DeMiguel et al., 2010, 2012) to obtain a reasonably accurate classification of the samples. However, in *S. hundsheimensis* only upper molars (particularly M1 and M2) were examined, as attrition and abrasion produce a different kind of morphologies in rhinoceros lower teeth (Hernesniemi et al., 2011a, b), complicating the comparisons with the results for other ungulates. Regardless, if available, second upper molars were preferably selected. Occlusal relief (high or low) and cusp shape (sharp, rounded, or blunt) of the apex of the paracone and metacone of the M1–M3 and the metaconid and entoconid of the m1–m3 were examined in a total of 136 dental specimens (really worn teeth, teeth belonging to juvenile specimens and molars with damaged cusps were excluded from this analysis) by either the naked eye or using a 6× magnifying portable glass and qualitatively scored. The resulting mesowear data were compared to those of 54 modern ungulate taxa with well-defined diets (original data from Fortelius and Solounias, 2000).

Occlusal relief and cusp shape scores were also converted to a single mesowear score (MWS) following Rivals et al. (2007): a score of 0 is given to teeth showing a combination of high relief and sharp cusps; 1 to the teeth with high relief and rounded cusps; 2 to teeth with low relief

and rounded cusps; 2.5 to teeth with low relief and sharp cusps; and 3 to teeth with low relief and blunt cusps.

2.3. Dental microwear

Dental microwear represents the abrasion of teeth produced by food and other items consumed during the last few days prior to the death of an animal, an event known as the "last supper effect" (Grine, 1986). Microwear features in enamel were examined using high-resolution epoxy casts following cleaning, moulding, casting, and examination protocol by Solounias and Semprebon (2002) and Semprebon et al. (2004). Occlusal surfaces of upper or lower molars were cleaned using acetone and alcohol. The surfaces were moulded using high-resolution silicone (vinylpolysiloxane) and casts were made using clear epoxy resin. All the moulded specimens (n = 56) available for study (belonging to *D. vallonnentensis*, *E. altidens*, *Bison* sp. and *S. hundsheimensis*) were carefully screened under a stereomicroscope (Leica M205 C) at 35× magnification. Those with badly preserved enamel or taphonomic alterations (specimen with unusual morphology and size, or fresh features made during the collecting process or during storage) were removed from the analysis. We selected mostly the M2 anterior lingual blade of the paracone and the m2 posterior buccal blade of the protoconid as areas of study for artiodactyls, as these facets are in occlusion during mastication and on second molars an intermediate wear stage between first and third molars can be observed (Gordon, 1982). For the equid *E. altidens*, the lingual enamel band of the paracone of upper molars and the labial enamel band of the hypoconid of lower molars were preferably sampled. For the rhinocerotid *S. hundsheimensis*, the labial enamel band of the paracone or the lingual edge of the protocone of upper molars and the labial edge of the protoconid were preferably selected.

We used the classification of features defined by Solounias and Semprebon (2002) and quantified all categories of microwear features in a standard square area of 0.16 mm^2. Features were analyzed using an early version of MicroWeaR software (Strani et al., 2018d) and automatically divided into four categories: small pits, large pits, fine scratches, and coarse scratches. The presence of cross-scratches and gouges was also reported. Average number of scratches and pits discriminate between browsers (i.e., animals feeding on ligneous forage such as bushes, leaves and fruits), mixed feeders (i.e., species feeding on both ligneous and herbaceous foods) and grazers (i.e., animals feeding on grass). The percentage of individuals with scratch numbers falling into a low scratch range (0–17%) defined in the 0.16 mm^2 area can also separate mixed feeders from browsers and grazers as follows (Semprebon and Rivals, 2007): grazers have 0.0–22.2% of individuals with scratches between 0 and 17; mixed feeders have 20.9–70.0%; and leaf-dominated browsers have 72.7–100.0%. Following Rivals (2012), scratch textures were converted into a scratch width score (SWS) to simplify representation of the data by giving a score of '0' to teeth with predominantly fine scratches per tooth surface, '1' to those with a mixture of fine and coarse types of textures, and '2' to those with predominantly coarse scratches. Individual scores for a sample were then averaged to get the SWS of the taxa.

2.4. Statistical analysis

Discriminant analyses were performed to examine the resolution of both mesowear and microwear variables applied to the fossil taxa. For the mesowear analysis, the percentages of high relief, rounded and blunt cusps were used as independent variables and two dietary (conservative and radical) classifications were used as grouping variables (Fortelius and Solounias, 2000). For the microwear study, the number of pits and the number of scratches were selected as criterion variables using modern taxa data from Solounias and Semprebon (2002). All analyses were performed using IBM SPSS Statistics 24.

3. Results

3.1. Hypsodonty

Fossil ungulates display generally high-crowned molars (Table 2), with HIm3 ranging from 2.0 (*M. savini* from EVT12 and *D. vallonnetensis* from EVT7) to 5.3 (*E.* cf. *ferus* from EVT3) and HIMm2 from 0.8 (*D. vallonnetensis* and *S. hundsheimensis* from EVT12) to 2.7 (*E. altidens* from EVT7). More specifically, the cervids *D. vallonnetensis* and *M. savini* display a mesodont dentition in both EVT12 and EVT7, and the horses *E. altidens* and *E.* cf. *ferus* are consistently hypsodont taxa through all the examined layers and have the highest HI values. *Bison* sp. shows a hypsodont dentition in EVT7, but no data are available from EVT12 due to the lack of well-preserved unworn molars. The rhinoceros *S. hundsheimensis* is mesodont in both EVT12 and EVT7, with a slightly higher hypsodonty index observed in layer EVT7 (value of 1.1). No brachydont taxa are observed in the succession, though the HIMm2 values (0.8) of *D. vallonnetensis* from EVT7 and *S. hundsheimensis* from EVT12 are at the threshold between brachydont and mesodont dentitions. There are no available data for the cervid specimen of the EVT3 layer, as the sample is represented only by a worn m2 enclosed in a mandible.

EVT3	Hypsodonty					
Taxa	N (HIm3)	HIm3	Classification	N (HIMm2)	HIMm2	Classification
Cervus elaphus		/	/	/	/	/
Equus cf. *ferus*	1	5.3	Hypsodont	4	2.5	Hypsodont
EVT7	Hypsodonty					
Taxa	N (HIm3)	HIm3	Classification	N (HIMm2)	HIMm2	Classification
Dama vallonnetensis	4	2.0	Mesodont	6	0.9	Mesodont
Equus altidens	2	4.9	Hypsodont	2	2.7	Hypsodont
Bison sp.	1	3.7	Hypsodont	3	1.7	Hypsodont
Stephanorhinus hundsheimensis	/	/	/	9	1.1	Mesodont
EVT12	Hypsodonty					
Taxa	N (HIm3)	HIm3	Classification	N (HIMm2)	HIMm2	Classification
Dama vallonnetensis	/	/	/	2	0.8	Mesodont
Megaloceros savini	2	2.0	Mesodont	3	1.0	Mesodont
Equus altidens	2	4.7	Hypsodont	1	2.1	Hypsodont
Bison sp.	/	/	/	/	/	/
Stephanorhinus hundsheimensis	/	/	/	2	0.8	Mesodont

Table 2. Summary of hypsodonty results. Abbreviations: number of specimens measured (N); hypsodonty index (HIm3) calculated as in Janis (1988); hypsodonty index (HIMm2) as in Fortelius et al. (2002).

3.2. Dental wear patterns before and after the "0.9 Ma event"

3.2.1. EVT12 (MIS 31; ca. 1.0 Ma)

In the oldest layer, the fallow deer *D. vallonnetensis* displays a mesowear pattern characterised by an equal distribution of sharp and rounded cusps (50%) and a high occlusal relief (100%; see Table 3). This species displays a MWS of 0.5, pointing to a relative predominance of attrition over abrasion in the wear process (Fig. 2). A similar condition is observed for the giant deer *M. savini*, with a slight predominance of sharp apices (57.1%) over rounded ones (42.9%) and a MWS of 0.4, the lowest recorded in this layer. *Bison* sp. shows rounded apices (100%), either high or low occlusal relief (50%) and a MWS of 1.5 which is indicative of intermediate-to-high levels of dietary abrasion. *Equus altidens* exhibits a mesowear pattern mostly comprised of round cusps (75%) and low occlusal relief (75%) that, coupled with a lack of sharp cusps and a MWS of 1.7, suggests a high level of abrasion. *Equus altidens* is also the only species from the EVT12 layer to display blunt cusps (25%). The single specimen of *S. hundsheimensis* shows rounded cusps and high occlusal relief, and a MWS of 1.0.

Although there are few specimens from the EVT12 layer suitable for microwear analysis, the available sample yields peculiar information. All the taxa display a high average number of scratches and share the same low scratch range value of 0 (Table 3), which points to abrasive feeding behaviours. *Dama vallonnetensis* has the highest average number of scratches (NS = 26.5) and, according to the bivariate plot (Fig. 3A), it is grouped with modern ungulates with a highly abrasive diet (such as grass-dominated mixed feeders or grazers) and with non-seasonal mixed feeders, a specific group of ungulates which vary their diet daily and display a highly scratched microwear pattern (Solounias and Semprebon, 2002). The giant deer *M. savini* also displays microwear patterns comprised of a high amount of fine scratches (NS = 22.2; SWS = 0) and is

plotted (Fig. 3A) with modern grass-dominated mixed feeders and grazers. These results contrast highly to those obtained from mesowear, which points to a browsing feeding behaviour. Such a discrepancy may reflect a temporary shift towards a more abrasive diet due to strong seasonality. The fossil horse *E. altidens* from the EVT12 layer displays a high incidence of fine scratches (NS = 22; SWS = 0.2), which suggests a somewhat abrasive diet (Fig. 3A). The single individuals of both *Bison* sp. and the rhino *S. hundsheimensis* also show a high number of fine scratches (23 and 22, respectively) and few small pits (11 and 8, respectively), which seems to suggest a highly abrasive diet, in accordance with results from mesowear.

3.2.2. EVT7 (MIS 21; ca. 0.86 Ma)

The fallow deer *D. vallonnetensis* displays a mesowear pattern characterised by the predominance of sharp cusps (67.6%) and high relief (97.1%) and a MWS of 0.4 which points to a low abrasive diet. Almost all individuals of *Bison* sp. show rounded apices (94.4%) and most of them display high occlusal relief (72.2%; Table 3). This finding, coupled with a MWS of 1.3, is indicative of intermediate levels of abrasion. Among the perissodactyls, the equid *E. altidens* exhibits a mesowear pattern mostly comprised of round cusps (77.3%), low occlusal relief (77.3%) and the highest MWS value recorded in this layer (1.9), thus suggesting a medium-to-high level of abrasion. The most diversified mesowear pattern can be observed in the rhinoceros *S. hundsheimensis*, which displays a similar frequency of sharp (42.1%) and round (36,8%) apices, a certain amount of blunt cusps (21.1%), and mostly high occlusal relief (68.4%). This heterogeneity of cusp shape and an intermediate MWS (1.4) suggest a variable level of dietary abrasion for this species.

Microwear features for *D. vallonnetensis* (Table 3) are concordant with those of modern mixed feeders according to the average number of scratches and pits (Fig. 3B). The relative

abundance of fine scratches (%0–17 = 57.1; SWS = 0.1) and the presence of a certain amount of large pits (50%) also points to a medium degree of abrasion in its diet, as observed in modern mixed feeders. Similarly, both the bivariate plot of average number of scratches and average number of pits (Fig. 3B) and the low scratch range (%0–17 = 42.9) indicate a medium degree of abrasion for the horse *E. altidens.* This species is also characterised by a marked predominance of fine textured scratches (SWS = 0), which is an atypical feature for modern grazers, as they usually display abundant coarser scratches (Solounias and Semprebon, 2002). Microwear results for *Bison* sp. are concordant with those from mesowear, as the high average number of scratches (NS = 21.3; %0–17 = 22.2), and the relatively low number of pits (NP = 13.7) point to a grass-dominated feeding behaviour (Fig. 3B). However, the predominance of finely textured scratches (SWS = 0.1) and a high incidence of large pits (%Lp = 66.7) is an unusual feature in modern ungulates that feed mostly on grasses, only observed in the extant bovid *Tetracerus quadricornis* according to Solounias and Semprebon (2002). Microwear patterns of the rhino *S. hundsheimensis* point to a mixed diet according to both the bivariate plot (Fig. 3B) and the low scratch range (50.0%), which is also in well accordance with mesowear patterns. This taxon also displays the lowest average number of pits (NP = 7.3) and lowest incidence of large pits (%Lp = 12.5).

3.2.3. EVT3 (ca. 0.6 Ma)

The single individual of *C. elaphus* displays high occlusal relief and rounded cusps (and a MWS of 1.0), while the horse *E.* cf. *ferus* exhibits a mesowear pattern mostly comprised of round apices (66.7%) and low occlusal relief (75.0%). The high MWS value (1.8) of the latter suggests a medium-to-high level of abrasion. It is important to note that it also displays the highest percentage of sharp cusps (8.3%) when compared to the remaining horses from EVT12 and EVT7 (Table 3).

Because only few specimens from EVT3 were suitable for microwear analysis (*C. elaphus*, N = 1; *E.* cf. *ferus*, N = 2), results for this layer must be considered tentative. The low amount of both pits and scratches observed in the fossil deer and the horse (*C. elaphus*, NP = 4, NS = 15, 0–17% = 100; *E.* cf. *ferus*, NP = 8, NS = 16.5, 0–17% = 50; Fig. 3C) seems to indicate a medium-to-low level of abrasion in their diets.

3.2.4. Dietary classifications based on mesowear variables

According to the discriminant analysis performed with mesowear variables, *E. altidens* and *Bison* sp. from layer EVT12 are classified as grazers in both conservative and radical classifications, while *S. hundsheimensis* is classified as a mixed feeder according to the conservative classification and as a grazer according to the radical (Table 3). The cervids *D. vallonnetensis* and *M. savini* from the same layer are determined as a mixed feeder and a browser, respectively, in both classifications (Table 3). *Equus altidens*, *Bison* sp. and *S. hundsheimensis* from layer EVT7 maintain the same classifications as those for layer EVT12 (Table 3). The discrepancy between conservative and radical results for *S. hundsheimensis* obtained in both layers seems to point to a mixed feeding long-term behaviour, albeit with some leaning towards grazing. *Dama vallonnetensis* is classified as a browser in this layer (Table 3). *Equus* cf. *ferus* from layer EVT3 emerges as a grazer according to both classifications, while the single individual of *C. elaphus* is considered a mixed feeder according to the conservative classification and a grazer according to the radical.

EVT3		Mesowear						Predicted diet		Microwear							
Taxa	**N**	**%High**	**%Low**	**%Sharp**	**%Round**	**%Blunt**	**MWS**	**Cons**	**Rad**	**N**	**NP**	**NS**	**%Lp**	**%G**	**%XS**	**SWS**	**%0-17**
Cervus elaphus	1	100.0	0.0	0.0	100.0	0.0	1.0	M	G	1	4	15	0	0	0	0	100
Equus cf. *ferus*	12	25.0	75.0	8.3	66.7	25.0	1.8	G	G	2	8	16.5	0	0	0	0	50
EVT7		**Mesowear**						**Predicted diet**		**Microwear**							
Taxa	**N**	**%High**	**%Low**	**%Sharp**	**%Round**	**%Blunt**	**MWS**	**Cons**	**Rad**	**N**	**NP**	**NS**	**%Lp**	**%G**	**%XS**	**SWS**	**%0-17**
Dama vallonnetensis	34	97.1	2.9	67.6	32.4	0.0	0.4	B	B	14	14.5	17.8	50	21.4	14.3	0.1	57.1
Equus altidens	22	22.7	77.3	4.5	77.3	18.2	1.9	G	G	7	12.6	18.7	28.6	0	42.9	0	42.9
Bison sp.	18	72.2	27.8	0.0	94.4	5.6	1.3	G	G	9	13.7	21.3	66.7	22.2	33.3	0.1	22.2
Stephanorhinus hundsheimensis	19	68.4	31.6	42.1	36.8	21.1	1.4	M	G	8	7.3	17.9	12.5	12.5	50	0.4	50.0
EVT12		**Mesowear**						**Predicted diet**		**Microwear**							
Taxa	**N**	**%High**	**%Low**	**%Sharp**	**%Round**	**%Blunt**	**MWS**	**Cons**	**Rad**	**N**	**NP**	**NS**	**%Lp**	**%G**	**%XS**	**SWS**	**%0-17**
Dama vallonnetensis	10	100.0	0.0	50.0	50.0	0.0	0.5	M	M	3	20	26.5	33.3	66.7	0	0	0
Megaloceros savini	7	100.0	0.0	57.1	42.9	0.0	0.4	B	B	5	11	22.2	20	0	60	0	0
Equus altidens	8	25.0	75.0	0.0	75.0	25.0	1.7	G	G	5	8.6	22	40	20	40	0.2	0
Bison sp.	4	50.0	50.0	0.0	100.0	0.0	1.5	G	G	1	11	23	0	0	0	0	0
Stephanorhinus hundsheimensis	1	100.0	0.0	0.0	100.0	0.0	1.0	M	G	1	8	22	0	0	100	0	0

Table 3. Summary of dental mesowear and microwear analysis. Abbreviations: number of specimens measured (N); percentage of specimens with high (%High) and low (%Low) occlusal relief; percentage of specimens with sharp (PerSharp), rounded (PerRounded) and blunt (PerBlunt) cusps; average number of pits (AP); average number of scratches (AS); percentage of individuals with more than 4 large pits (%Lp); percentage of individuals with gouges (%G); percentage of individuals with more than 4 cross scratches (%XS); scratches width score (SWS); percentage of specimens with between 0 and 17 scratches (%0–17). Mesowear discriminant anlaysis provides a satisfactory dietary discrimination with 74.1% of extant taxa (68.5% with cross-validation) correctly classified according to a conservative classification (Cons) and 74.1% (74.1% with cross-validation) according to the radical one (Rad).
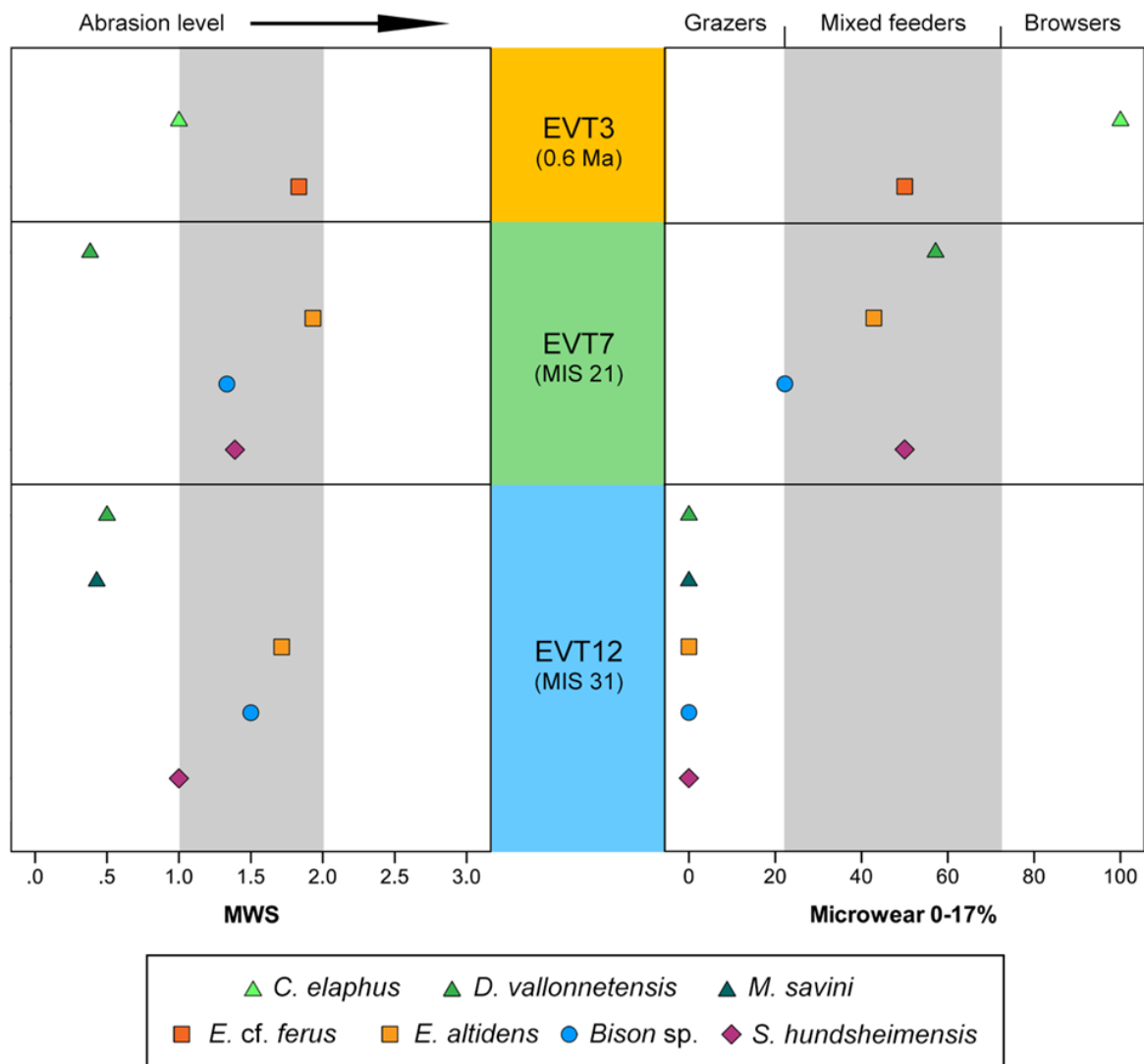
Figure 2. Mesowear scores (MWS; A) and low scratch percentages (%0–17; B) of fossil samples from Vallparadís layers EVT12, EVT7 and EVT3.
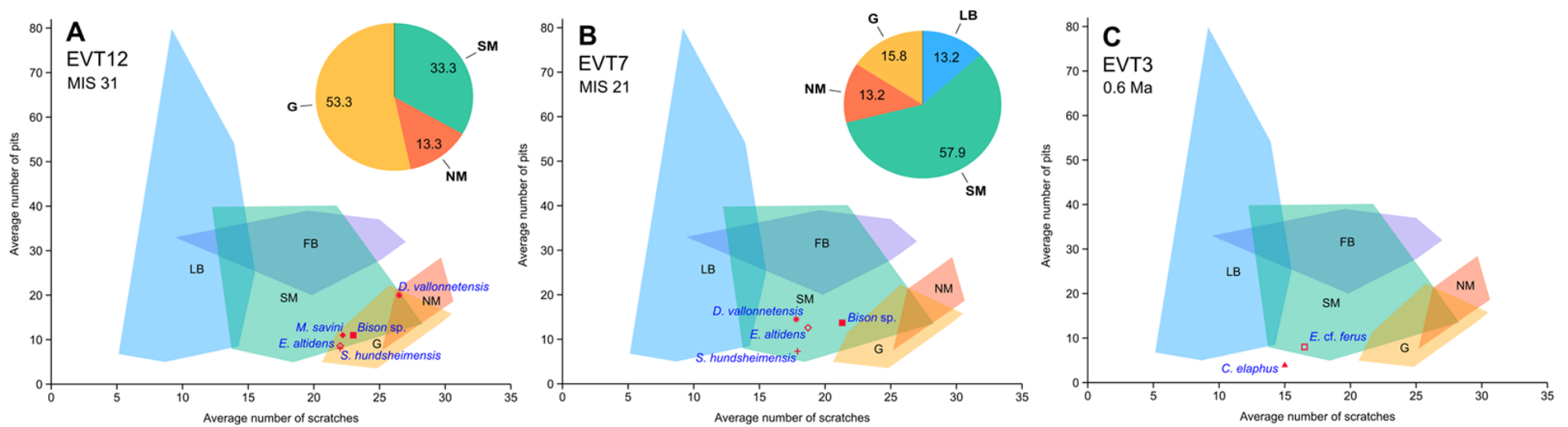
Figure 3. Bivariate plot of the average number of pits versus average number of scratches in extant ungulates (data from Solounias and S3mprebon, 2002) and fossil samples from Vallparadís Estació layers EVT12 (A), EVT7 (B) and EVT3 (C). Pie-diagrams showing relative abundance (%) of diets in layers EVT12 and EVT7. Abbreviations: fruit browsers (FB); leaf browsers (LB); seasonal mixed feeders (SM); non-seasonal mixed feeders (NM); grazers (G).

3.2.5. Dental microwear variability

We further investigated the microwear signal by analysing the dispersion of microwear variables (number of pits and number of scratches) and performing a discriminant analysis to force the classification of fossil cervids and equids represented in all the examined layers (*D. vallonnetensis* and *E. altidens* from EVT12 and EVT7; *E.* cf. *ferus* and *C. elaphus* from EVT3) into established dietary categories (Table 4).

Most individuals (N = 7) of *D. vallonnetensis* from EVT7 are classified as seasonal mixed feeders, while the remaining are almost equally plotted among the leaf browsers (N = 3), non-seasonal mixed feeders (N = 2), and grazers (N = 2). On the contrary, only a single specimen from the EVT12 layer (EVT25460) is classified as a seasonal mixed feeder and falls into the morphospace of variability of the EVT7 larger sample (Fig. 4). The other two individuals of *D. vallonnetensis* (EVT23798 and EVT26007) are classified as non-seasonal mixed feeders falling outside the morphospace of the EVT7 variability by displaying a larger number of pits (Fig. 4). The single *C. elaphus* from EVT3 is considered a seasonal mixed feeder.

Specimens of *E. altidens* from EVT12 are classified as either grazers (N = 3) or seasonal mixed feeders (N = 2), while individuals of the same species from EVT7 are mostly considered seasonal mixed feeders (N = 5). This fact, together with the low overlap in morphospaces of EVT12 and EVT7 sample variability (Fig. 4), seems to confirm that *E. altidens* from EVT7 had a more variable diet. The two individuals of *E.* cf. *ferus* from EVT3 are classified as seasonal mixed feeders, with the specimen EVT-16717 displaying overall few microwear features.

A relative abundance of individuals classified as seasonal mixed feeders can be observed also in the EVT7 populations of *Bison* sp. (N = 6) and *S. hundsheimensis* (N = 4).

Taxon	Layer	Predicted group based on microwear variables				
		FB	**LB**	**SM**	**NM**	**G**
Cervus elaphus	EVT3	0	0	1	0	0
Dama vallonnetensis	EVT7	0	3	7	2	2
Dama vallonnetensis	EVT12	0	0	1	2	0
E. cf. *ferus*	EVT3	0	0	2	0	0
Equus altidens	EVT7	0	0	5	0	2
Equus altidens	EVT12	0	0	2	0	3
Megaloceros savini	EVT12	0	0	2	0	3
Bison sp.	EVT7	0	0	6	2	1
Bison sp.	EVT12	0	0	0	0	1
Stephanorhinus hundsheimensis	EVT7	0	2	4	1	1
Stephanorhinus hundsheimensis	EVT12	0	0	0	0	1

Table 4. Dietary inferences from discriminant analysis using dental microwear variables.

Abbreviations: fruit browsers (FB); leaf browsers (LB); seasonal mixed feeders (SM); nonseasonal

mixed feeders (NM); grazers (G). Discriminant anlaysis provides a satisfactory dietary

discrimination with 63.6% of extant taxa (61.4% with cross-validation) correctly classified.
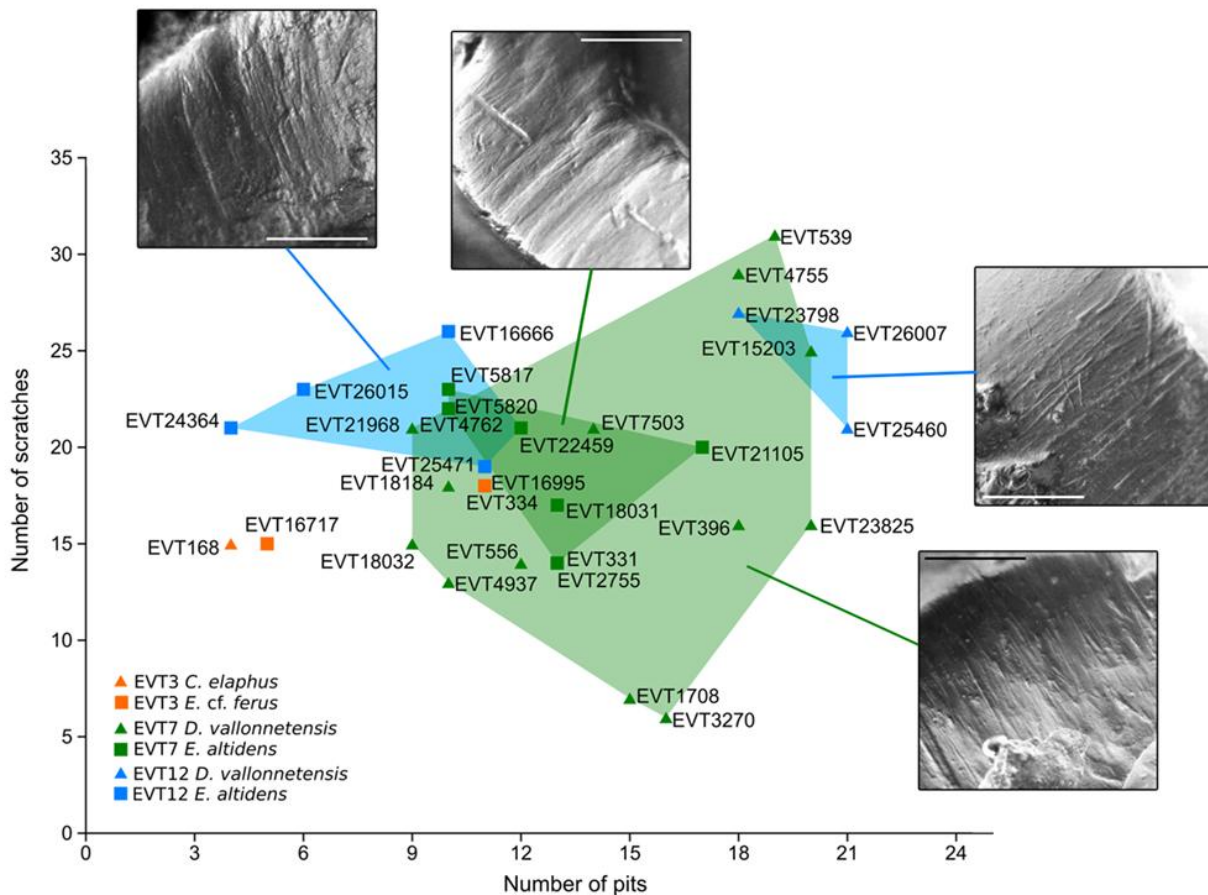
Figure 4. Variability morphospace of dental microwear features of fossil cervids and equids from Vallparadís Estació layers EVT12, EVT7 and EVT3, and photomicrographs of enamel surfaces at 35× magnification of selected fossil molars (scale bars = 500 μm).

4. Discussion

4.1. Dietary traits and ecology of fossil herbivore communities

Increased hypsodonty is generally considered a response to abrasive diets in regularly or seasonally dry and open environmental conditions (Janis, 1988; MacFadden, 1997; Strömberg, 2006). The occurrence of fossil ungulates displaying relatively high dental crown height, either as mesodont or hypsodont dentitions, in all examined layers and the lack of strict brachydont taxa

strongly attest a consistent presence of open and dry landscapes through the EVT12–EVT3 succession. This is in accordance with the steep increase in both seasonality and aridity following the onset of the EMPT, which led to some strong glacial periods such as MIS 24, MIS 22 and MIS 12 (Lisiecki and Raymo, 2005; Maslin and Ridgwell, 2005; Clark et al., 2006, Kahlke et al., 2011; Head and Gibbard, 2015).

Dental mesowear patterns show a persistence of ungulates with a medium-to-high degree of abrasion in their feeding behaviour ($1 < MWS < 2$; Fig. 2) in all examined layers, with only two taxa exhibiting a pattern comparable to those of modern browsers in EVT12 (*M. savini*) and EVT7 (*D. vallonnetensis*). Cervids display the greatest variation in long-term dietary adaptations, ranging from strict browsers (*M. savini* from EVT12; *D. vallonnetensis* in EVT7) to mixed feeders (*D. vallonnetensis* in EVT12) to species with a more marked abrasive mixed diet (*C. elaphus* in EVT3). Equids and the bovid *Bison* sp. consistently display mesowear features associated with a mostly grazing behaviour throughout all the layers, while the discrepancy between discriminant mesowear results for the rhinocerotid *S. hundsheimensis* points to a grass-dominated mixed feeding behaviour in both EVT12 and EVT7. Nevertheless, when dental microwear patterns are taken into consideration, a marked reduction of the grazing dietary adaptation can be observed from layer EVT12 to layer EVT7 (Figs. 2 and 3), implying a reduction of open dry habitats. As observed for the long-term timescale, fossil cervids show the widest range of adopted feeding behaviours from grazing to more selective diets.

A trend towards less abrasive diets in also observed in the dental mesowear patterns of fossil bisons from other late Early Pleistocene and early Middle Pleistocene Southern and Central European localities. During the late Early Pleistocene (ca.1.2 Ma) this group displays a grazing behaviour as reported from the Greece locality of Apollonia (Maniakas and Kostopoulos, 2017) with a high MWS value of 1.8. On the other hand, during the latest Early Pleistocene and early Middle Pleistocene, bison from Untermassfeld (MIS31; 1.05 Ma; Germany); Süssenborn (ca. 0.65

Ma; Germany); Isernia la Pineta (0.62 Ma; Italian peninsula); and Petralona Cave (0.6 – 0.4 Ma; Greek peninsula) display less abrasive diets with MWS values closer to 1 (Maniakas and Kostopoulos, 2017; van Asperen and Kahlke, 2017).

The case of the fallow deer *D. vallonnetensis* and the giant deer *M. savini*, from EVT7 and EVT12, respectively, requires further inspection as their meso- and microwear patterns indicate different feeding behaviours. According to the short-term (i.e., microwear) timescale, they display a signal which points to an abrasive diet, while according to the long-term one (i.e., mesowear), they show attrition-dominated dental wear patterns compatible to a selective feeding behaviour. The giant deer *M. savini* displays the more drastic discrepancy, with some individuals exhibiting microwear features that are commonly observed in modern grazers (Table 4). Some extant browsing ungulates that dwell in arid and desert environments can display dental wear patterns unusual for typical browsers due to the ingestion of grit-infested foliage (William and Kay, 2001; Solounias and Semprebon, 2002; Kaiser and Rössner, 2007; Kaiser, 2009). These "dirty browsers" have microwear characterised by heavy pitting, abundant gouges and coarser scratches if compared to normal "clean browsers". Neither *D. vallonnetensis* from EVT7 or *M. savini* display heavily pitted or gouged enamel bands and both are characterised by a relative abundance of fine textured scratches (Table 3), which excludes dietary abrasiveness due to a "dirty browsing" behaviour. Considering this, both *D. vallonnetensis* from EVT7 and *M. savini* from EVT12 probably had a diet mostly based on the consumption of browse material with a periodic intake of more abrasive plants.

4.2. Environmental changes through time

Overall dental wear patterns in EVT12 (MIS31 that is, before the 0.9 Ma cooling), point to a generally abrasive diet for the species and suggest an environment with a significant predominance of open dry grasslands. Given that mesowear and microwear record diet information on two

different time scales (Grine, 1986, Fortelius and Solounias, 2000), the pronounced difference in dental meso- and microwear pattern results, especially for *M. savini*, seems to suggest that seasonality may have occurred during this interval. Thus, it may have influenced the dietary behaviour of this giant deer and other species, which were possibly forced to expand their diet breadth during harsher periods. A predominance of open habitats is also observed in the late Early Pleistocene Greek locality of Apollonia (Kostopoulos and Koufos, 2000; Croitor and Brugal, 2007; Maniakas and Kostopoulos, 2017) and in the Italian locality of Colle Curti (MIS31, ca. 1.0 Ma), where pollen records from the base of the section (990 ka) indicate a prevalence of non arboreal taxa (Bertini, 2000).

After the "0.9 Ma event", Vallparadís Section ungulates display long-term dental wear patterns that point to a medium-to-high abrasion degree in their feeding behaviour, but some important differences can be observed in the short-term timescale. The reduction of grazing ungulates and the browse specialization inferred for *D. vallonnetensis* in the EVT7 layer, combined with the abundant remains of *Hippopotamus antiquus* found in this layer (Madurell-Malapeira et al., 2010, 2014, 2017), point to less dry conditions and closer habitats compared to those that were apparently present before (in EVT12). The analysis of the small mammal assemblage of EVT7 also suggests the presence of more humid habitats at this interval (Lozano-Fernández et al., 2015). Moreover, the seasonal mixed diet recorded by the short-term dental wear pattern in most of the specimens from the EVT7 layer (Fig. 3 and Table 4) indicates a periodically high availability of heterogeneous plant resources. This increment of humidity and relative increase of closed habitats is in accordance with the environmental conditions recorded in other Southern European localities dated after MIS 22. Pollen and vertebrate (herpetofauna, mammals and birds) fossil records from Gran Dolina (Iberian peninsula) TD6 level dated between 0,8 and 0.88 Ma, points to a patchy landscape with humid habitats and well represented woodlands (Burjachs, 2001; Cuenca-Bescós et al., 2017; Blain et al., 2018 and references therein). Abundant deciduous forest taxa (particularly

Quercus) are also recorded in the fossil pollen register of the Tenaghi Philippon (Greek peninsula) sequences correlated to the post-MIS 22 interglacial phases (Van der Wiel and Wijmstra, 1987a,b; Tzedakis et al., 2006).

Finally, the scarce remains from the EVT3 layer seem to point to environmental conditions characterised by heterogeneous landscapes, possibly including the predominance of open habitats—as further attested by the relatively high abundance of horse specimens (with two different species recorded)—though the limited sample does not allow a more in-depth comparison with the other examined units. This spread of open grasslands around 0.6 Ma is also attested at the Middle Pleistocene site of Isernia La Pineta (Italian peninsula) where herbaceous plants are the most represented in the pollen record (Arobba et al., 2004).

4.3. The effect of the "0.9 Ma event"

The analysis of the whole succession from 1.0 to 0.6 Ma points to the presence of open environments such as savannah-like grasslands before MIS 22 (EVT12; ca. 1.0 Ma) with a transition towards more humid conditions (and probably closer habitats) around 0.8 Ma (EVT7). The fossil remains from EVT3 suggest that open landscapes may had spread around 0.6 Ma, as attested by the relative abundance of highly cursorial-locomotion species (like horses) exhibiting a generally grazing behaviour. The relative high amount of ungulates showing microwear typical of seasonal mixed feeders in all the examined layers indicates that seasonality may have played a key role in shaping habitat settings of the region through the late Early Pleistocene and the early Middle Pleistocene. The increase of the relative number of seasonal mixed feeders from the assemblage dated after the "0.9 Ma event" (EVT7) suggests that seasonality became indeed more marked after the MIS 22. Late Early Pleistocene pollen records from South West Europe indicate that the effects of the EMPT on the plant biota were not especially pronounced in the Mediterranean region (Magri

and Palombo, 2013). Pollen records from Catalonia (NE Iberian Peninsula) and the Po Valley (Italian Peninsula) do not reflect any important change in vegetation cover following the 0.9 Ma event, with no greater expansions of open vegetation during the glacials and no marked reduction of forests during interglacials (Suc and Popescu, 2005). The increment of seasonal mixed feeders that evolved during MIS21 can be thus an indication of a periodic change, not in terms of plant resource type and composition (which were not significantly altered by the change in climate cycles), but in terms of food quality. In this scenario, large-sized ungulates like horses, bisons and rhinos—which need a larger absolute food intake to survive (Clauss et al., 2013) —may had to feed on suboptimal plant parts during the adverse season.

5. Conclusions

The dietary behaviours of the fossil ungulates from the Vallparadís Section provide new pivotal information on how the onset of the EMPT influenced terrestrial ecosystems in the Mediterranean region, especially at the end of the Early Pleistocene. While vegetation type and diversity were not as affected by climate shifts as at higher latitudes, a clear increase in seasonality is detected, which deeply influenced the ecosystems and, hereby, the dietary behaviours of the large ungulate community in the short-term timescale. Overall, our data show that environments before 0.9 Ma were characterised by a consistent presence of open grasslands with ungulates displaying mostly mixed or grazing dietary behaviours. A reduction of grazer taxa points to less dry conditions in the Vallparadís layer correlated to MIS 21 (EVT7), while the increment of seasonal mixed feeders suggests that the main consequence of the "0.9 Ma event" at lower latitudes was an even more intensified seasonality with periodic important changes in plant resource quality. The relative abundance of cursorial grazing horses in the EVT3 layer suggests a new spread of open environments around 0.6 Ma.

Acknowledgments

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Chapter 5

Ungulate dietary adaptations and palaeoecology of the Middle Pleistocene site of Fontana Ranuccio (Anagni, Central Italy)
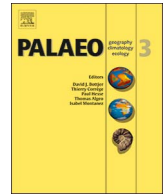
PALAEO 3

Ungulate dietary adaptations and palaeoecology of the Middle Pleistocene site of Fontana Ranuccio (Anagni, Central Italy)

Flavia Strani[a,b,*], Daniel DeMiguel[c,d,e], Fabio Bona[f], Raffaele Sardella[a,b], Italo Biddittu[b], Luciano Bruni[b], Adelaide De Castro[b], Francesco Guadagnoli[b], Luca Bellucci[b,g]

[a] Dipartimento di Scienze della Terra, Sapienza Università di Roma, P.le A. Moro 5, I-00185 Roma, Italy
[b] Istituto Italiano di Paleontologia Umana, via U. Aldrovandi 18, I-00197 Roma, Italy
[c] Departamento de Ciencias de la Tierra, Área de Paleontología, Universidad de Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain
[d] Fundación ARAID, Zaragoza, Spain
[e] Institut Català de Paleontologia Miquel Crusafont (ICP), Barcelona, Spain
[f] Dipartimento di Scienze della Terra "A. Desio", Università degli Studi di Milano, via L. Mangiagalli 34, I-20133 Milano, Italy
[g] Polo museale, Sapienza Università di Roma, P.le A. Moro 5, I-00185 Roma, Italy

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ABSTRACT

The Middle Pleistocene site of Fontana Ranuccio (Anagni Basin, Central Italy) has yielded nearly 25,000 remains belonging to large and small vertebrates, including four isolated teeth of *Homo* sp., and a rich lithic assemblage containing bone tools. Here we provide new dental wear analyses of the ungulate community to improve the palaeoenvironmental reconstruction for the site. Analyses indicate a mostly browsing diet for the cervids and a grazing diet for the perissodactyls *Stephanorhinus* sp. and *Equus* cf. *E. mosbachensis*, suggesting a range of habitats that spanned from forests to open grasslands. A discrepancy between mesowear and microwear results is observed in the case of the bovid, *Bos primigenius*; this can be tentatively explained as resulting from a temporal switch towards a sub-optimal diet, possibly reflecting the effects of marked seasonality. Findings shed new light on the structure of the 400 kyr ecosystems that existed during the early occupation of *Homo* in Europe.

1. Introduction

The Early–Middle Pleistocene Transition (EMPT) (Head and Gibbard, 2015 and references therein), marked a fundamental change in the Earth's climate system (Maslin and Ridgwell, 2005) with intensified glacial cycles (deMenocal and Bloemendal, 1995; Shackleton, 1995; Lisiecki and Raymo, 2005, Tzedakis et al., 2012) that led to a major episode in mammalian reorganization (e.g., van den Bergh et al., 2001; Markova, 2005; O'Regan et al., 2005; Raia et al., 2009). Compared to earlier times, during the 0.6–0.4 Ma interval, environmental conditions were relatively stable, with longer climatic cycles, resulting in alternating open and forested landscapes (Russo Ermolli et al., 2010; Kahlke et al., 2011; Magri and Palombo, 2013; Orain et al., 2013; Combourieu-Nebout et al., 2015). During this phase humans spread successfully into Europe as indicated by a number of sites where fossils or tools are reported (Carbonell et al., 2008; Manzi et al., 2011; Lordkipanidze et al., 2013; Carotenuto et al., 2016).

The palaeoenvironmental context that promoted the hominin dispersal in Eurasia is one of the most studied issues in palaeontology. Findings in Spain (Carbonell et al., 2008; Toro-Moyano et al., 2009),

southern France (Crochet et al., 2009; Bourguignon et al., 2016) and Italy (Biddittu and Segre, 1982a, 1982b; Biddittu, 1984; Arzarello et al., 2009) indicate that humans, as well as other large mammals of African origin, dispersed into Western Europe during the late Early Pleistocene, with a more consistent presence following the major faunal turnover linked to the Early–Middle Pleistocene Transition (EMPT).

Most of the human remains from the Italian Peninsula referable to the Middle Pleistocene have been found in Central Italy (Manzi et al., 2011) and, within these sites, Fontana Ranuccio (Anagni, Frosinone) is one of the most important (Segre and Ascenzi, 1984; Rubini et al., 2014). The Fontana Ranuccio site is located in the Latina Valley about 90 km southeast of Rome (Fig. 1A, B). The Latina Valley consists of several extensional tectonic basins, including the Anagni basin that hosts the Fontana Ranuccio site. A large number of archaeological tools (> 100), faunal remains (> 25,000) and four human teeth (recently revised by Rubini et al., 2014) were unearthed (Cassoli and Naldini, 1984; Segre and Ascenzi, 1984; Ascenzi et al., 1993). The archaeo-palaeontological layer that yielded *Homo* sp. teeth, Acheulean tool assemblages and mammal remains was dated by K–Ar on leucites at 0.458 ± 0.006 Ma (Biddittu et al., 1979; Segre and Ascenzi, 1984)
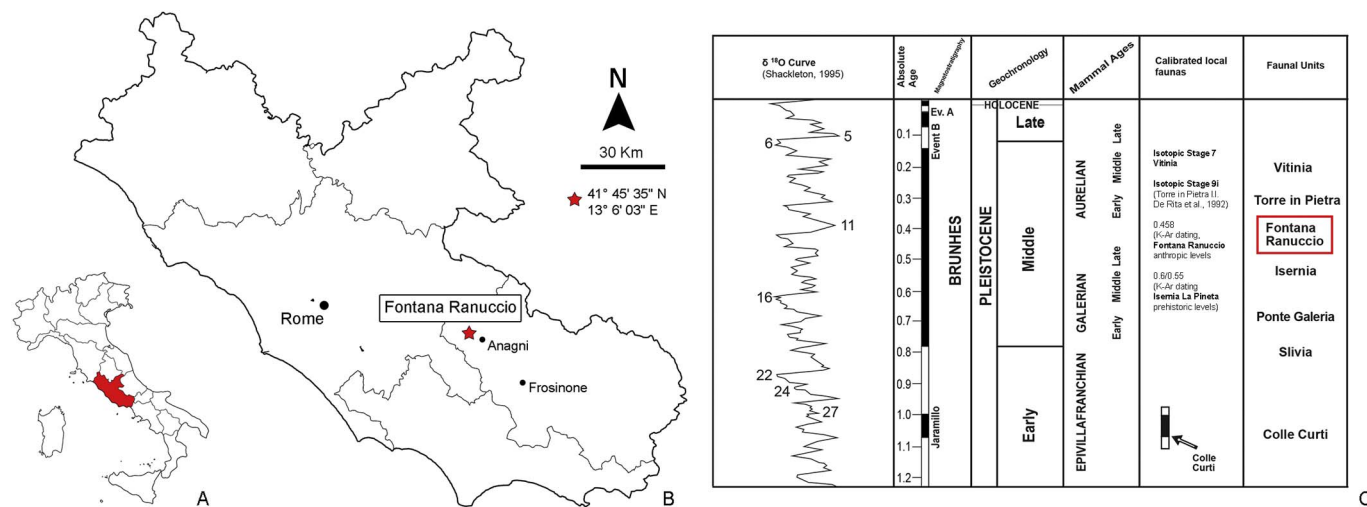
Fig. 1. A, Central Italy; B, geographical location of the Middle Pleistocene site of Fontana Ranuccio; C, integrate chronological scheme for the Fontana Ranuccio Faunal Unit. (Modified from Gliozzi et al. (1997).)

(Fig. 1C).

In order to better understand the environmental and climatic context of Central Italy during the epoch of dispersal of Middle Pleistocene *Homo*, we investigate the composition of the mammal assemblage of Fontana Ranuccio and explore the feeding behaviours of the fossil ungulates. Herbivore ungulates are highly susceptible to changes in vegetation and their diets provide precise information about niche occupation and habitat conditions (i.e. abundance of grasslands versus forests, type of vegetation, habitat openness, degree of aridity, etc.) (Fortelius and Solounias, 2000; Solounias and Semprebon, 2002; Rivals and Athanassiou, 2008; Valli and Palombo, 2008; Rivals et al., 2009b; DeMiguel et al., 2010, 2011; Solounias et al., 2014; Strani et al., 2015).

2. Materials and methods

The Fontana Ranuccio collection includes > 25,000 vertebrate specimens stored in the Italian Institute of Human Palaeontology (IsIPU) depository (Anagni, Frosinone). Among the large mammals, 1360 specimens have been attributed to 15 taxa: *Palaeoloxodon antiquus*, *Stephanorhinus* sp., *Equus* cf. *E. mosbachensis*, *Hippopotamus amphibius*, *Dama clactoniana*, *Cervus elaphus eostephanoceros*, *Praemegaceros* sp., *Bos primigenius*, *Sus scrofa ferus*, *Ursus deningeri*, *Panthera* sp., *Crocuta crocuta*, *Canis mosbachensis*, *Macaca sylvanus* and *Homo* sp. (Segre and Ascenzi, 1984; Ascenzi et al., 1993; Rubini et al., 2014).

The Artiodactyla is the best-represented order, comprising 68% of faunal remains (Table 1) of which 69% belong to cervids (n = 623) and 25% are bovids (n = 231). Remains of *Hippopotamus* and the wild boar,

Table 1
Fontana Ranuccio number and percentage of artiodactyls and carnivores fossil remains.

	Number	Percentage
Artiodactyla		
Hippopotamidae	11	1.2%
Bovidae	231	25.5%
Cervidae	623	68.7%
Suidae	5	0.6%
Artiodactyla indet.	37	4.1%
Tot	907	
Carnivora		
Ursidae	13	39.4%
Hyaenidae	3	9.1%
Felidae	1	3.0%
Canidae	16	48.5%
Tot	33	

Sus scrofa ferus are exceptionally rare and represented only by isolated teeth including a fragment of a canine belonging to *Hippopotamus amphibius* and a third lower molar attributed to *Sus scrofa ferus*. Carnivore remains are uncommon (n = 34) and fragmentary, with Canidae and Ursidae being the best represented families (Table 1).

2.1. Dietary assessment methodologies

To investigate climatic and environmental conditions in Fontana Ranuccio, we selected dental (upper and lower molars) fossil remains of six ungulates: *Cervus elaphus eostephanoceros*, *Dama clactoniana*, *Bos primigenius*, *Stephanorhinus* sp., *Praemegaceros* sp. and *Equus* cf. *E. mosbachensis* (Fig. 2A–F). Details on the examined specimen are reported in Table S1. We used the following techniques for the dietary assessment of the ungulate community.

2.1.1. Hypsodonty

Molar crown height (or hypsodonty) yields information about feeding ecology (Janis, 1988; Rivals and Semprebon, 2006; DeMiguel et al., 2008) and habitat openness (especially aridity degree) (Janis, 1988). We measured crown height in the fossil taxa according to the index defined by Janis (1988). Moreover Hypsodonty also carries phylogenetic information as it summarizes evolutionary adaptation of a species (Janis, 1988). The hypsodonty index (HI) was determined for both unworn lower third molars (m3) and either upper or lower second molars (Mm2).

In this study, HIm3 was determined as m3 height divided by m3 width (after Janis, 1988), and teeth were classified as brachydont (HIm3 < 1.5), mesodont (1.5 < HIm3 < 2.5) or hypsodont (HIm3 > 2.5). We calculated an average HIm3 for 7 specimens representing 3 taxa (*Cervus elaphus eostephanoceros*, *Dama clactoniana* and *Bos primigenius*). HIMm2 was determined as height to length ratio for unworn upper or lower second molars (after Fortelius et al., 2002) and teeth classified as brachydont (HIMm2 < 0.8), mesodont (0.8 < HIMm2 < 1.2), or hypsodont (HIMm2 > 1.2). We calculated an average HIm3 for 7 specimens representing 5 taxa (*Cervus elaphus eostephanoceros*, *Dama clactoniana*, *Praemegaceros* sp., *Bos primigenius* and *Equus* cf. *E. mosbachensis*).

2.1.2. Dental mesowear

Mesowear is considered a good dietary indicator in herbivore species, as it represents the cumulative effects of the items ingested (foods and exogenous particles such as dust and grit) on the dental morphology over a significant portion of the lifespan of the animal
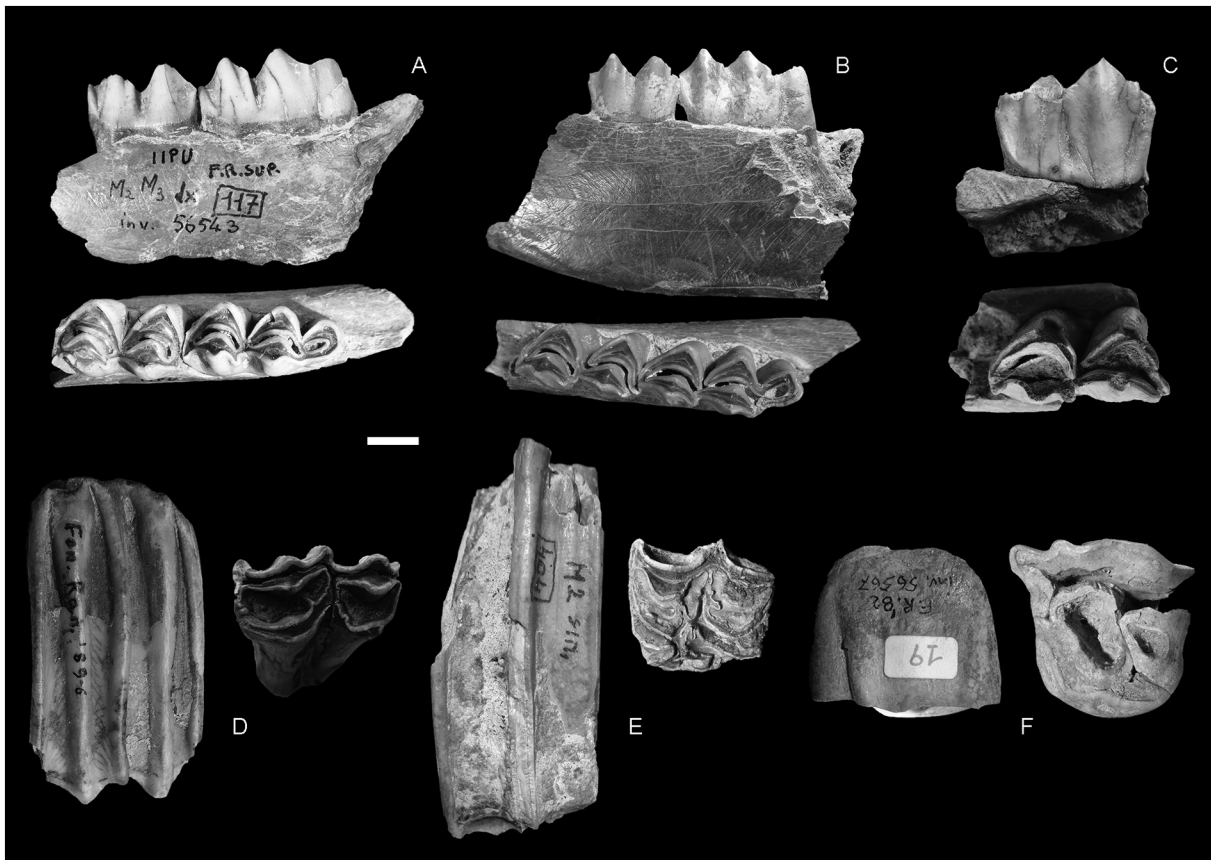
Fig. 2. Fontana Ranuccio selected ungulate fossil dental material. A, FR 56543 - *Cervus elaphus eostephanoceros* right hemi-mandible portion with m2 and m3 (top: lingual view; bottom: occlusal view); B, FR 25621 - *Dama clactoniana* right hemi-mandible portion with m2 and m3 (top: lingual view; bottom: occlusal view); C, FR 56556 - *Praemegaceros* sp. right m2 (top: lingual view; bottom: occlusal view); D, FR 89-6 *Bos primigenius* upper molar (left: labial view; right: occlusal view); E, FR 8/78-404 - *E.* cf. *E. mosbachensis* left M2 (left: labial view; right: occlusal view); F, FR 56567 – *Stephanorhinus* sp. left P4/M1 (left: labial view; right: occlusal view). Scale bar 10 mm.

(Fortelius and Solounias, 2000). Importantly, mesowear is, in contrast to hypsodonty, a direct indicator of diet. Two methodologies are used: the Outer Mesowear Method and the Inner Mesowear Method.

The Outer Mesowear Method is based on the relief of the worn occlusal surface and on the shape of the cusps. These factors are heavily influenced by attrition (tooth-to-tooth contact) and abrasion (tooth-to-food contact). High levels of attrition produce sharper cusps and higher tooth relief whereas high levels of abrasion produce blunter cusps and lower tooth relief (Fortelius and Solounias, 2000; Merceron et al., 2005; DeMiguel et al., 2008). Mesowear analysis, originally was conducted only on second upper molars (M2), but was later extended to upper (M1–M3) (after Kaiser and Solounias, 2003) and lower (m1–m3) molars (after DeMiguel et al., 2010, 2012). Although some studies (Franz-Odendaal and Kaiser, 2003; Fraser et al., 2014) have shown that mesowear scores differ between upper and lower molars, others show that there is no significant difference in the mesowear signal between tooth types (DeMiguel et al., 2010, 2012; Hernesniemi et al., 2011a, 2011b).

The Inner Mesowear Method (Solounias et al., 2014), focuses on the second enamel band that forms the lingual margin of the metacone or paracone from an occlusal view, which is generally more frequently preserved in fossil specimens. Inner Mesowear reflects dietary preferences that are intermediate (days-weeks) in time between outer mesowear (months-years) and dental microwear (days-hours) (Danowitz et al., 2016; Sánchez-Hernández et al., 2016). For inner mesowear, the enamel band is scored on the mesial and distal sides of the metacone using a 4 point scaling system described by Solounias et al. (2014) and Danowitz et al. (2016): (1) flat and planar with no gouges or indentations on the surface of the enamel; (2) nearly flat with several gouges that traverse the surface from either edge—the labial

and lingual edges of the enamel band are somewhat rounded; (3) similar to score 2, but more rounded with less defined edges and more gouges; and (4) rounded—the surface is smooth without gouges, and there are no well-defined edges. The junction of the mesial and distal sides termed J is similarly scored: (1) it joins at a sharp, well-defined junction; (2) it is somewhat sharp, and often contains a gouge; (3) it is rounded, but the mesial and distal sides appear as distinct, separate surfaces; and (4) the J point lacks a discrete apex, and the mesial and distal sides of the enamel band form one continuous surface.

In our Outer Mesowear study, occlusal relief (high or low) and cusp shape (sharp, rounded or blunt) of the apex of the paracone and metacone of the M1–M3 and the metaconid and entoconid of the m1–m3 were examined in a total of 113 dental specimens. Specimens were examined with the naked eye or using a $6\times$ magnifying portable glass and qualitatively scored following the method delineated by Fortelius and Solounias (2000). Mesowear data were compared to a published database of 54 modern ungulate taxa with well-defined diets (Fortelius and Solounias, 2000). In the case of *Stephanorhinus* sp. (a single P4/M1; Fig. 2E), only upper teeth were examined as the effect of attrition and abrasion produce different kind of morphologies in rhinoceros lower teeth where usually only cusp sharpness or facets development can be scored (Hernesniemi et al., 2011a, 2011b) making comparison with other ungulates mesowear results complex. In this analysis, really worn teeth, those belonging to juveniles, and molars with damaged cusps were excluded.

In our Inner Mesowear study, wherever possible, we used the enamel band of the metacone of the upper molars. If this region was taphonomically damaged, the band of the paracone was examined. We compared our results of the average inner mesowear scores of the

mesial, J point and distal surfaces with those of a comprehensive database (Danowitz et al., 2016) of 8 extant ungulates with well-studied diets (browsing, mixed feeding and grazing). 83 specimens were examined with this method. The method was originally applied by Solounias et al. (2014) only on upper second molars (M2), but in this study it has been extended to M1 and M3 molars of different individuals in order to widen the sample and allow meaningful comparison among mesowear databases.

2.1.3. Dental microwear

Dental microwear represents the abrasion of teeth produced by food and other items consumed during the last few days prior to the death of an animal known as the "Last Supper Effect" (Grine, 1986). Microwear features of dental enamel were examined using a stereomicroscope on high-resolution epoxy casts of teeth following the cleansing, molding, casting, and examination protocol developed by Solounias and Semprebon (2002) and Semprebon et al. (2004). The occlusal surface of the upper or lower molars of each taxon was cleaned using acetone and then alcohol. The surface was molded using high-resolution silicone (vinylpolysiloxane) and casts were created using clear epoxy resin.

In this study, 39 specimen molds were screened under incident light with a Leica M205 C stereomicroscope at $35\times$ magnification, using the refractive properties of the transparent cast to reveal microfeatures on the enamel. Those with badly preserved enamel or taphonomic distortions (specimens with unusual morphology and size, or fresh features made during the collecting process or during storage) were excluded from the analysis following King et al. (1999). Fossil molars belonging to *Equus* cf. *E. mosbachensis*, *Praemegaceros* sp. and *Stephanorhinus* sp. have deeply altered enamel and were also excluded. We selected mostly the M2 anterior lingual blade of the paracone and the m2 posterior buccal blade of the protoconid as areas of study, as these facets are in occlusion during the mastication and on second molars an intermediate wear stage between first and third molars can be observed (Gordon, 1982). Where these facets were absent or badly preserved, other facets were selected, as it has been observed that in ruminant microwear features sampled from any region of the tooth adequately reflect the diet of the individual (Mihlbachler et al., 2016).

Following Solounias and Semprebon (2002), we quantified all categories of microwear features in a standard square area of $0.16 \, \text{mm}^2$ using the open source image processing program ImageJ (Schneider et al., 2012). Features were divided into five categories: small pits, large pits, fine scratches, coarse scratches and gouges. The presence of cross scratches was also recorded.

Mean number of scratches and mean number of pits can be used to discriminate between browsers (i.e., animals feeding on ligneous forage such as bushes, leaves and fruits), mixed feeders (i.e., species feeding on both ligneous and herbaceous foods) and grazers (i.e., animals feeding on grass). The percentage of individuals with scratch numbers falling in a low scratch range (%0–17) defined in the $0,16 \, \text{mm}^2$ area (as described in Semprebon and Rivals, 2007) can also separate mixed feeders by browsers and grazers. For extant ungulates, the percentages of individuals in the low scratch range are as follows: grazers have 0.0–22.2% of individuals with scratches between 0 and 17; mixed feeders have 20.9–70.0% of individuals with scratches between 0 and 17; and leaf-dominated browsers have 72.7–100.0% of individuals with scratches between 0 and 17 (Semprebon and Rivals, 2007). Scratch textures were converted into a Scratch Width Score (SWS) to simplify representation of the data by giving a score of '0' to teeth with predominantly fine scratches per tooth surface, '1' to those with a mixture of fine and coarse types of textures, and '2' to those with predominantly coarse scratches. Individual scores for a sample were then averaged to get the SWS and compared to data of extant taxa (data from Rivals, 2012 and references there in). Coarse scratches are mostly observed in modern C4 grazers, bark eaters and fruit browsers (Solounias and Semprebon, 2002).

2.2. Statistical methods

Discriminant analyses were performed to examine the resolution of both outer and inner mesowear variables applied to the fossil taxa. For the outer mesowear, the percentage of high relief, rounded and blunt cusps were used as independent variables and two dietary (conservative and radical) classifications were used as grouping variables (Fortelius and Solounias, 2000). For the inner mesowear, we used the diet of extant species (Danowitz et al., 2016) as a grouping variable, and mesial, distal and J point scores as variables to infer the dietary preferences of species. All analyses were performed using SPSS Statistics 24.

3. Results

3.1. Hypsodonty inference

The cervids, *C. e. eostephanoceros* and *D. clactoniana* are mesodont sharing similar HI values for both the m3 (HIm3 = 1.6) and the M2/m2 (HIMm2 = 0.9 for *C. e. eostephanoceros* and 0.8 for *D. clactoniana*). HI results for *Praemegaceros* sp. slightly worn m2 are consistent with a mesodont condition as well (HIMm2 = 0.8). The bovid *Bos primigenius* and the equid *E.* cf. *E. mosbachensis* are both hypsodont displaying the highest HI value (*B. primigenius* HIm3 = 3.0; *E.* cf. *E. mosbachensis* HIMm2 = 2.4). It is generally accepted that hypsodonty is mostly linked to wear resistance, although it has been noted that biomechanical effectiveness in feeding on resistant food could factor in as well (DeMiguel et al., 2015), with high hypsodonty indices usually indicating higher dietary abrasion (i.e., grass-dominated feeders) in arid and more open environments (Janis, 1988; Eronen et al., 2010; Semprebon and Rivals, 2010). Given the lack of brachydont taxa, the ungulates indicate an increased dominance of dietary abrasion in more dry areas. HI values for individual species are reported in Table 2.

3.2. Dental mesowear

The predominant mesowear patterns for the deer *C. e. eostephanoceros* and *D. clactoniana* are high occlusal relief and similar distributions of both sharp and rounded cusps (45.2% of sharp and 54.8% of rounded cusps for *C. e. eostephanoceros*; 57.5% of sharp and 42.5% of rounded cusps for *D. clactoniana*), with *D. clactoniana* having more sharp apices than *C. e. eostephanoceros* (Table 2). The single specimen of *Praemegaceros* sp. appears to have a combination of high relief and sharp cusp (Fig. 2C). None of the cervids show incidence of blunt cusps and only one upper molar of *D. clactoniana* displays a low occlusal relief, hereby indicating low degree of abrasion in its diet. The bovid *Bos primigenius* shows a predominance of high relief (87.5%) and rounded cusps (95.8%), which is indicative of intermediate levels of abrasion. Among the perissodactyls, *Stephanorhinus* sp. upper tooth displays a low relief and a blunt cusp thereby suggesting a diet with a high degree of abrasion. *E.* cf. *E. mosbachensis* exhibits a mesowear comprised of low relief (100%) and mostly blunt cusps (50%), which is indicative of highly abrasive diet similar to those exhibited by extant grazers.

3.3. Inner mesowear

The distribution of the inner mesowear scores (mesial, J point and distal) of the ungulate community of FR mostly includes intermediate values (from 1 to 3) (Table 2). *B. primigenius* and *E.* cf. *E. mosbachensis* display the highest values, with the equid having the highest scores (≥ 3 in all the three variables). When the inner mesowear scores are plotted with those of extant ungulates (Fig. 3), data distribution suggests a wide dietary diversity that ranges from browsing to grazing. Scores of the deer *C. e. eostephanoceros* and *D. clactoniana* which range from 1.5 to 1.9 are close to those of modern browsers *Okapia johnstoni* and *Giraffa camelopardalis* and modern mixed feeder *Cervus canadensis*

Table 2
Summary of hypsodonty, dental mesowear and microwear results. Abbreviations: number of specimens measured (N); hypsodonty index calculated as in Janis (1988) (HIm3); hypsodonty index calculated as in Fortelius et al. (2002) (HIMm2); percentage of specimens with high (%High) and low (%Low) occlusal relief; percentage of specimens with sharp (PerSharp), rounded (PerRounded) and blunt (PerBlunt) cusps; mesial (Mesial), distal (Distal) and J point (J) scores; average number of pits (AP); average number of scratches (AS); percentage of individuals with > 4 large pits (%Lp); percentage of individuals with gouges (%G); percentage of individuals with > 4 cross scratches (%XS); scratches width score (SWS); percentage of specimens with between 0 and 17 scratches (%0–17).

Taxa	Hypsodonty						Outer mesowear						Inner mesowear				Microwear							
	N (HIm3)	Average HIm3	Classification	N (HIMm2)	Average HIMm2	Classification	N	%High	%Low	%Sharp	%Round	%Blunt	N	Mesial	Distal	J	N	AP	AS	%Lp	%G	%XS	SWS	%0–17
Cervus elaphus eostephanoceros	1	1.6	Mesodont	7	0.9	Mesodont	31	100.0	0.0	45.2	54.8	0.0	22	1.6	1.7	1.9	10	14.0	15.8	70.0	40.0	70.0	0.8	70.0
Dama clactoniana	3	1.6	Mesodont	4	0.8	Mesodont	40	97.5	2.5	57.5	42.5	0.0	22	1.7	1.7	1.5	16	16.1	13.7	62.5	56.3	25.0	0.8	75.0
Praemegaceros sp.	/	/	/	1	0.8	Mesodont	1	100.0	0.0	100.0	0.0	0.0	/	/	/	/	/	/	/	/	/	/	/	/
Bos primigenius	3	3.0	Hypsodont	4	1.5	Hypsodont	24	87.5	12.5	4.2	95.8	0.0	29	2.3	2.6	3.2	13	16.7	12.3	76.9	61.5	53.8	0.6	100.0
Equus cf. E. mosbachensis	/	/	/	3	2.4	Hypsodont	16	0.0	100.0	18.8	31.3	50.0	9	3.1	3.0	3.7	/	/	/	/	/	/	/	/
Stephanorhinus sp.	/	/	/	/	/	/	1	0.0	100.0	0.0	0.0	100.0	/	/	/	/	/	/	/	/	/	/	/	/
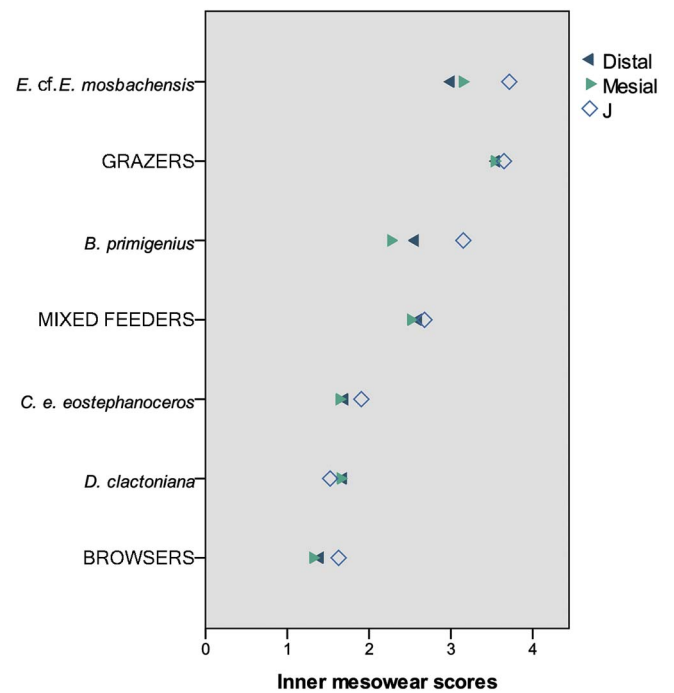
Fig. 3. Mean inner mesowear scores for fossil taxa and modern browsers, mixed feeders and grazers.
(Data for extant species from Danowitz et al. (2016).)

which display inner mesowear values from 1.3 to 1.6 (data from Solounias et al., 2014). Higher scores (2.3 to 3.2) are observed in *B. primigenius* which are close to those of mixed feeders like *Nanger granti* (2.3 to 2.9; data from Solounias et al., 2014). *E.* cf. *E. mosbachensis* is the only taxon with scores (3.1 to 3.7) comparable to those of extant ungulate grazers like *Kobus ellipsiprymnus* (3.1 to 3.5; data from Solounias et al., 2014). It must be noted that a somewhat high variability of the single bands scores is observed in both the bovid and the equid.

3.4. Discriminant analyses

Discriminant analysis performed with the outer mesowear variables provides a satisfactory dietary discrimination with 74.1% of extant taxa correctly classified according to the conservative and radical classifications (68.5% and 74.1%, respectively, in cross-validation). Bivariate diagrams based on the discriminant analysis (Fig. 4A, B) show that the deer *C. e. eostephanoceros* is classified as a mixed feeder while *D. clactoniana* is classified as a browser in both conservative (Fig. 4A) and radical (Fig. 4B) classification. *Praemegaceros* sp. is considered a browser and *Stephanorhinus* sp. a grazer according to both classifications, though these results should be tentatively interpreted due to the reduced sample size. The equid *E.* cf. *E. mosbachensis* is classified as a grazer in both the conservative (Fig. 4A) and radical (Fig. 4B) classifications. On the other hand *B. primigenius* is classified as a mixed feeder in the conservative classification (Fig. 4A), while it is considered to be a grazer in the radical one (Fig. 4B). This apparent discrepancy in *B. primigenius* suggests an intermediate diet with a strong leaning towards grazing.

Discriminant analyses for the inner mesowear predicted the diet of the individuals with a high accuracy rate of 73.6% (71.3% in cross-validation). Table 3 depicts individual results of the dental specimens. Individuals lacking mesial/distal bands or J scores were not classified (scored as Undetermined). The majority of *C. e. eostephanoceros* and *D. clactoniana* individuals are classified as browsers. Individuals of *E.* cf. *E. mosbachensis* are considered to be as grazers, with one of them being classified as a mixed feeder. The highest variability is observed for *B. primigenius*. The suitable individuals are almost equally split among the
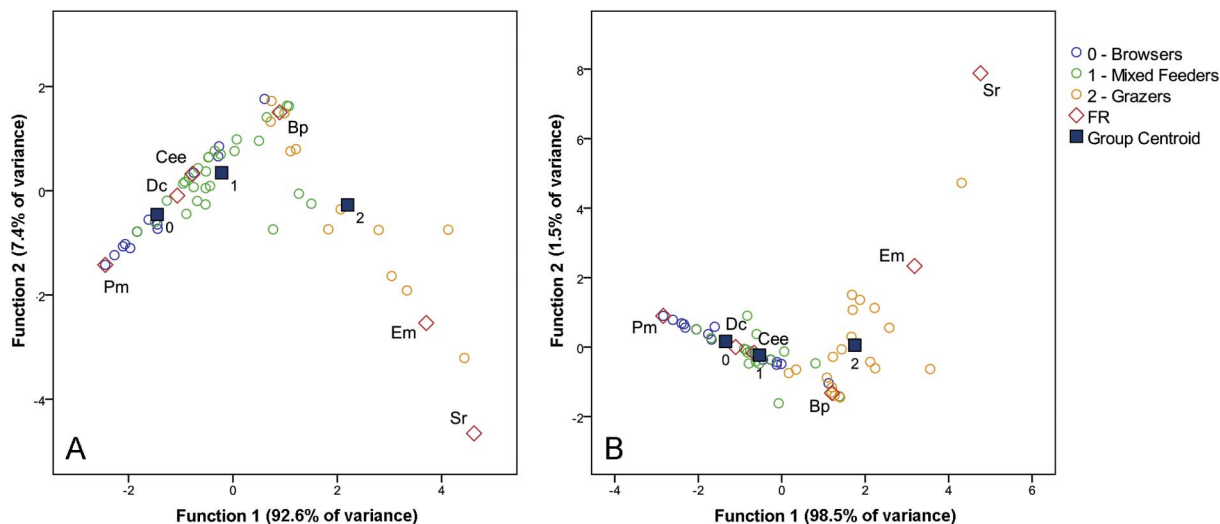
Fig. 4. Bivariate diagrams based on discriminant analysis: A, conservative classification; B, radical classification. Fossil taxa abbreviations: Cee, *Cervus elaphus eostephanoceros*; Dc, *Dama clactoniana*; Bp, *Bos primigenius*; Em, *Equus* cf. *E. mosbachensis*; Sr, *Stephanorhinus* sp.; Pm, *Praemegaceros* sp. Group centroids: browsers (0); mixed feeders (1); grazers (2).

Table 3
Dietary inferences from discriminant analysis using inner mesowear variables.

Taxa	Frequency browser	Frequency mixed feeder	Frequency grazer	Undetermined
Cervus elaphus eostephanoceros	16	2	1	3
Dama clactoniana	14	4	/	4
Bos primigenius	7	9	5	8
Equus cf. *E. mosbachensis*	/	1	4	4

three dietary classifications with a slight predominance of specimen being considered as mixed feeders.

3.5. Microwear

Microwear results for the two deer are mostly consistent with those from tooth mesowear. *D. clactoniana* microwear features are concordant with those of modern browsers such as *C. capreolus* with a low number of scratches (Average number of scratches = 13.7 and %0–17 = 75.0) and low SWS (0.8) (Figs. 5A, 6, 7A, B). *C. e. eostephanoceros* falls close to the browsing group when the average number of pits is plotted against average number of scratches (Fig. 6) displaying however a higher average number of scratches (15.8) compared to *D. clactoniana* (Fig. 5A, B). According to the percentage of individuals with a low scratch range

(%0–17 = 70.0), *C. e. eostephanoceros* is considered to be a mixed feeder close to the browsing group (Fig. 7A). *C. e. eostephanoceros* data are also close to those of modern European red deer rather than those of the north American elk, having a low number of scratches and an intermediate SWS value (0.8) (Fig. 7B). On the contrary, microwear results for *B. primigenius* are not in accordance with those from the mesowear analysis. The average number, percentage and type of microwear scars are concordant with a browsing feeding behaviour for this bovid (Figs. 6, 7A, B). The average number of scratches is low (AS = 12.3) and they are mostly fine (SWS = 0.6), a characteristic typical of most modern leaf browsers like the bovids *Litocranius walleri* and *Tragelaphus strepsiceros* (Solounias and Semprebon, 2002). *B. primigenius* also displays a relatively gouged enamel (%G = 61.5) and an abundance of large pits (%Lp = 76.9) (Fig. 5C). This wear pattern is not often observed in leaf browsers but is common in some modern fruit browsers, particularly in the duikers *Cephalophus dorsalis* and *Cephalophus niger* (Solounias and Semprebon, 2002). The extreme difference between outer mesowear (a grass dominated mixed feeder) and microwear (a browser) can be explained by the fact that microwear captures information of the last few meals of an animal before its death (Solounias and Semprebon, 2002), which in this case could represent only a portion of the mixed diet of the animal.

4. Discussion

Most of the ungulates from the Middle Pleistocene Fontana
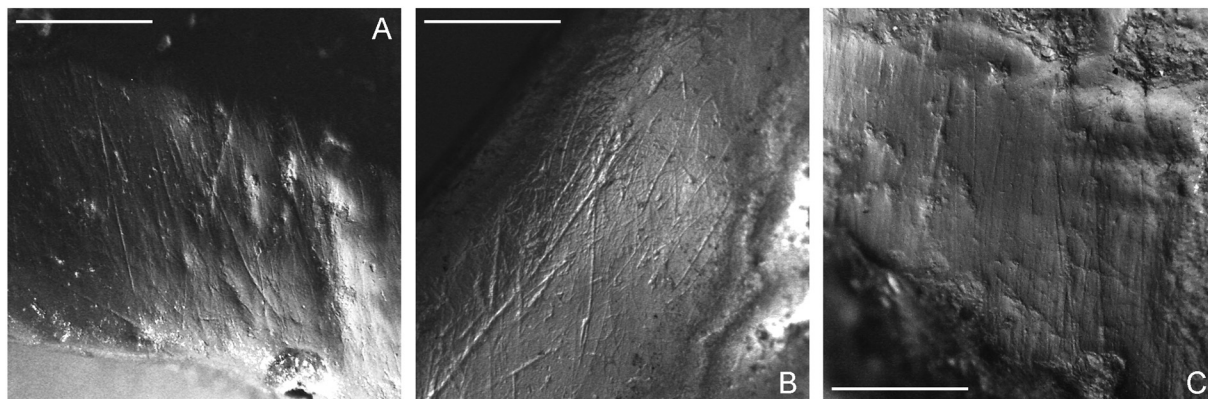

Fig. 5. Photomicrographs of enamel surfaces at 35× magnification of selected fossil artiodactyl molars. A, *Dama clactoniana* right m2 (FR 82-118); B, *Cervus elaphus eostephanoceros* right M1 (FR 78-142 C); C, *Bos primigenius* right m2 (FR 487). Scale bars 500 μm.
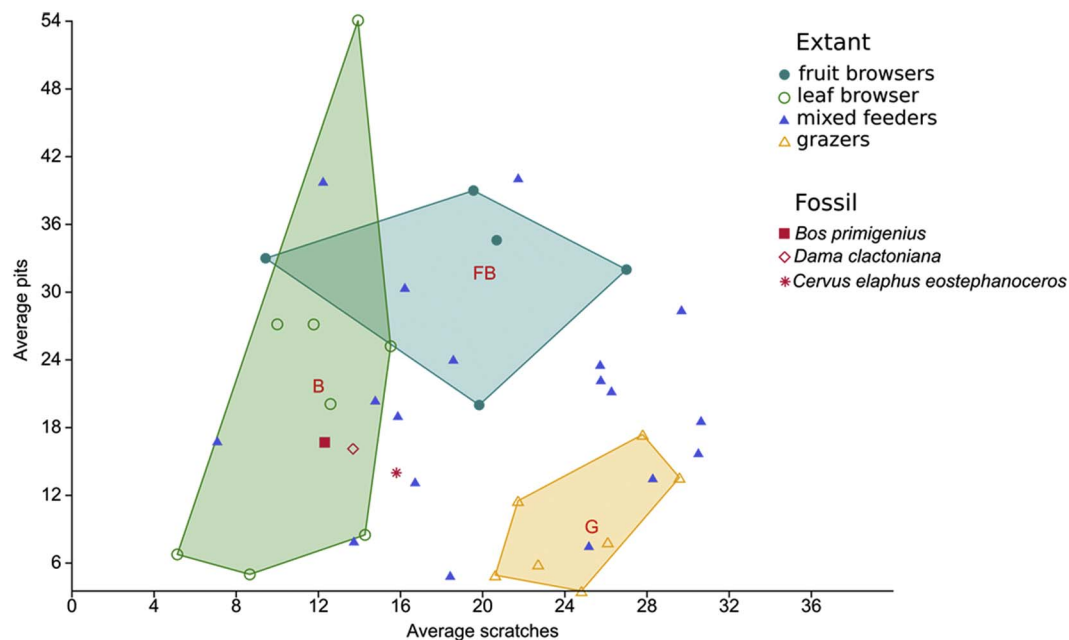
Fig. 6. Bivariate plot of the average number of pits versus average number of scratches in extant ungulates (data from Solounias and Semprebon, 2002) and fossil samples from Fontana Ranuccio. Extant fruit-browsers: *Cephalophus dorsalis, Cephalophus niger, Cephalophus natalensis, Cephalophus silvicultor, Okapia johnstoni*; extant leaf-browsers: *Alces alces, Antilocapra americana, Camelus dromedarius, Diceros bicornis, Tragelaphus eurycerus, Giraffa camelopardalis, Litocranius walleri, Tragelaphus strepsiceros*; extant grazers: *Alcelaphus buselaphus, Bison bison, Connochaetes taurinus, Equus quagga, Equus grevyi, Hippotragus niger, Kobus ellipsiprymnus*; extant mixed feeders: *Axis axis, Budorcas taxicolor, Capricornis sumatraensis, Cervus elaphus canadensis, Cervus duvauceli, Capra ibex, Cervus unicolor, Gazella granti, Gazella thomsonii, Lama glama, Lama vicugna, Ovis canadensis, Ovibos moschatus, Tragelaphus imberbis, Taurotragus oryx, Tetracerus quadricornis, Boselaphus tragocamelus, Tragelaphus scriptus*.

Ranuccio site display a relatively high molar crown height (from mesodont to hypsodont), a characteristic which is often considered a response to abrasive diets and that is linked to open dry (generally or seasonally) habitats (Janis, 1988; MacFadden, 1997; Strömberg, 2006). This can be interpreted as a response of taxa to the general increase in both aridity and seasonality that took place in the Northern Hemisphere following the climatic cycling switch from 41 kyr to 100 kyr periodicity during the early Middle Pleistocene (Lisiecki and Raymo, 2005; Kahlke et al., 2011), resulting in exacerbated glacial phases such as the MIS 12. Outer mesowear features are similar to those of extant ungulates with a diet characterised by a medium or high degree of abrasion with two mixed feeders, two grazers and only two ungulates (the cervids *D. clactoniana* and *Praemegaceros* sp.) having tooth mesowear patterns pointing to a browsing behaviour. Such traits are in accordance with the expanse of drier and generally more open environments. Similar results are obtained when inner mesowear and microwear analysis are taken into account with a slight difference observed in *C. e. eostephanoceros* (browse dominated mixed feeder according to outer mesowear and microwear, browser according to inner mesowear). A more drastic dissimilarity is observed in the dental wear patterns of *B. primigenius*. Outer and inner mesowear features point to a grass dominated mixed diet for this bovid, although microwear features indicate a lower degree of abrasion, with micro-scars typical of a browser (Figs. 6, 7A, B). This discrepancy in the results for *B. primigenius* is interpreted as microwear recording information about one part of the overall mixed diet of this taxon possibly reflecting the effects of marked seasonality. The combination of low number of scratches and high percentage of coarser features on the tooth surface (large pits and gouges), also may suggests that this species at times fed on both soft plant parts such as leaves or buds, and on hard items such as seeds that leave deep and coarse micro scars on the enamel. More generalist taxa can forage on different resources according to their availability, and during harsher seasons with a shortage of preferred food type they could feed on suboptimal resources, especially so large animals which need a larger absolute food intake to survive (Clauss et al., 2013). Middle Pleistocene pollen records from Italian Peninsula localities show that although most

interglacials were characterised by warm and humid conditions, some appear to have experienced cooler and drier winters than the glacials, especially during the MIS 11 as it has been registered in the site of Boiano (Campobasso, Central Italy) (Orain et al., 2013; Combourieu-Nebout et al., 2015) not far from the Fontana Ranuccio site. Fossil pollen based climate reconstructions from this locality indicate low winter precipitation (around 200 mm) during the MIS 11 and the lowest mean temperature of the coldest month of all the examined Middle Pleistocene interglacial stages (Combourieu-Nebout et al., 2015).

It has been observed that a high variation in herbivores dental microwear signal also indicates a long hominid occupation in archaeo-palaeontological sites (Rivals et al., 2009a), thus suggesting a low mobility and long-term settlement of the human groups in the Fontana Ranuccio area. The faunal assemblage therefore sheds new light on the habitats that characterised the region during the Middle Pleistocene. The lack of brachydont ungulates seems to suggest overall dry conditions and habitat openness. The presence of several cervids, the most represented mammal group in the Fontana Ranuccio site (Table 1), with either a browse rich mixed- or browsing diet indicates that these animals could have had access to abundant soft resources, such as leaves and buds, which are common in closed areas such as forests and thickets or in open woodlands. The well-attested presence of an intermediate feeder bovid (generally displaying a diet rich in abrasive food) coupled with the record of few large grazing perissodactyls, indicates however a significant presence of habitats with reduced tree cover such as shrublands and grasslands (Fig. 8). Similar palaeoenvironmental conditions are recorded also in the Middle Pleistocene (stratigraphic units referred to the MIS 13) locality of Caune de l'Arago (France), where fossil ungulates display a wide spectrum of dietary adaptations akin to the FR community (Rivals et al., 2008). Moreover, marked seasonality may have characterised the region influencing the diet patterns of some large ungulates such as the aurochs, who may had been forced to consume less optimal resources in adverse climatic conditions.
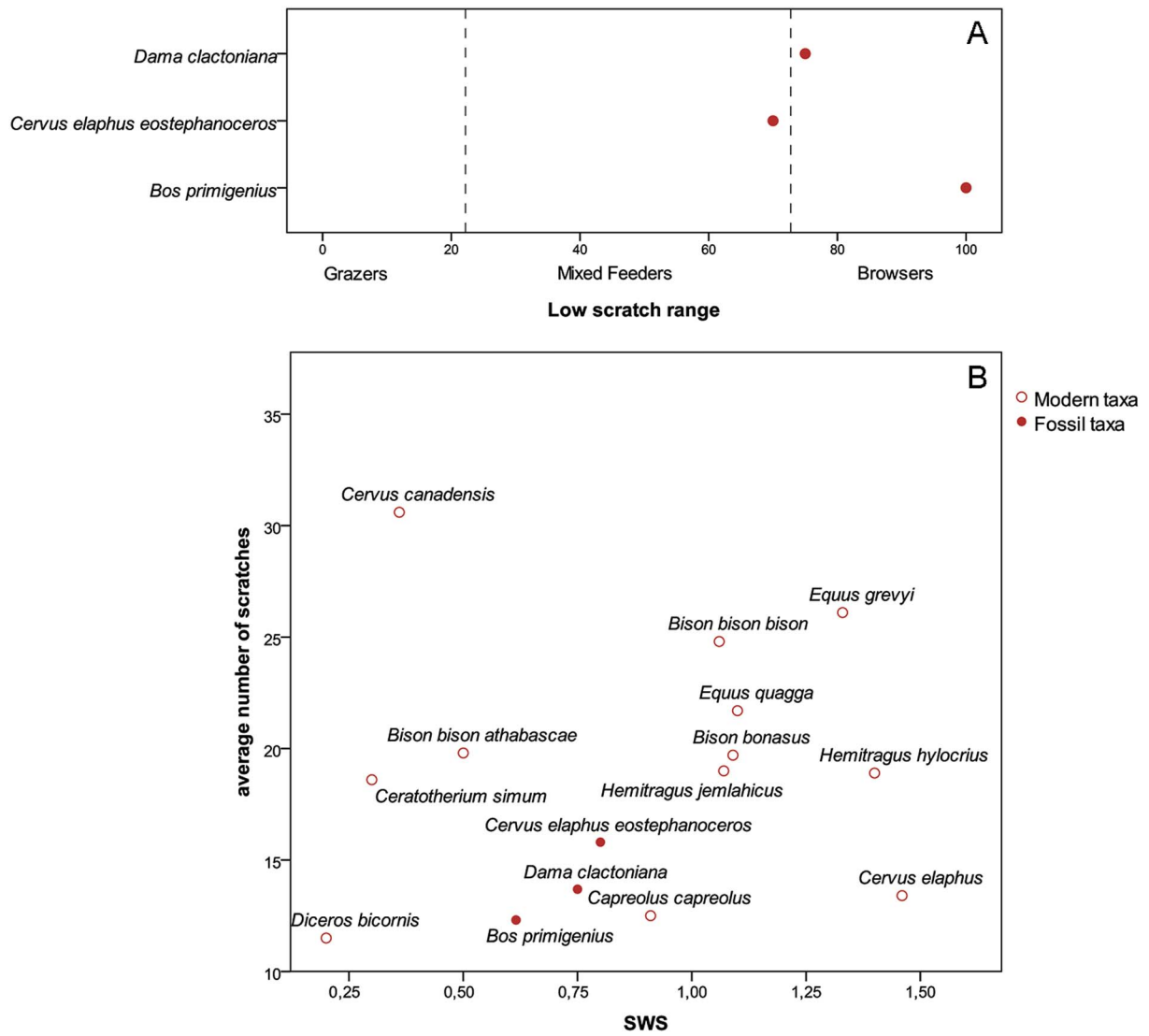
Fig. 7. A, Low scratch percentages (%0–17); B, average number of scratches vs. scratches width scores (SWS) of Fontana Ranuccio fossil ungulates and modern ungulates. (Modern taxa data from Rivals (2012) and references therein.)
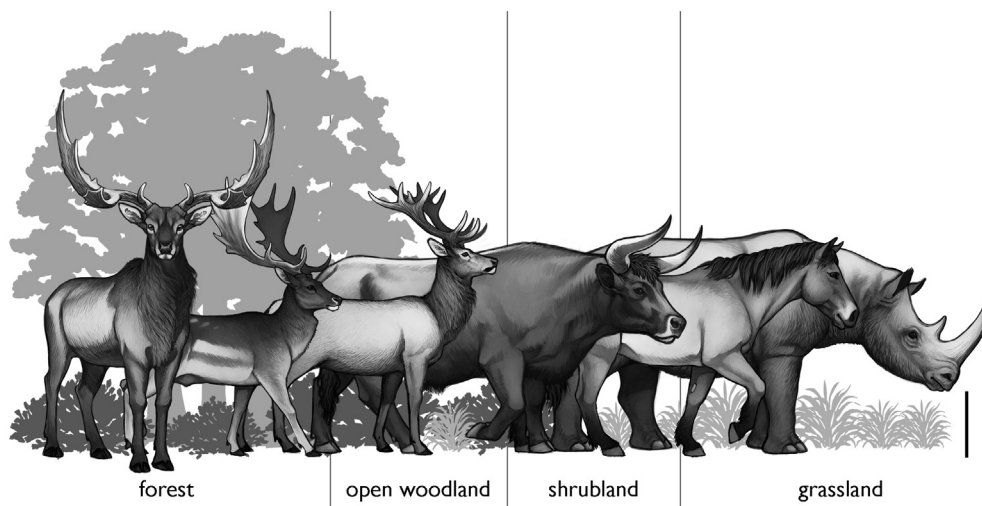

Fig. 8. Range of habitats occupied by the ungulates at the Fontana Ranuccio. From left to right: *Praemegaceros* sp., *Dama clactoniana*, *Cervus elaphus eostephanoceros*, *Bos primigenius*, *Equus* cf. *E. mosbachensis*, *Stephanorhinus* sp. Scale bar 50 cm.
(Artwork by Flavia Strani.)

5. Conclusions

Palaeoecological data from the Fontana Ranuccio site provide new information on the environments occupied by Middle-Pleistocene *Homo* in the Italian Peninsula. Dietary adaptations of the ungulate community and small mammal taxa suggest that *Homo* populations occupied a region characterised by grasslands inhabited mostly by groups of large bovids (*B. primigenius*) and grazing perissodactyls (*Stephanorhinus* sp., *Equus* cf. *E. mosbachensis*). The large number of cervids displaying a diet characterised by a low degree of abrasiveness attest the presence of wooded environments. Harsher climatic conditions during adverse seasons may had played a key role, driving ungulates that needed a large amount of food intake, such as *B. primigenius*, to feed on sub-optimal resources as well.

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Chapter 6

MicroWeaR: A new R package for dental microwear analysis

ORIGINAL RESEARCH

WILEY Ecology and Evolution
Open Access

MicroWeaR: A new R package for dental microwear analysis

Flavia Strani[1,2,7] (iD) | Antonio Profico[3] (iD) | Giorgio Manzi[3] | Diana Pushkina[4] |
Pasquale Raia[5] | Raffaele Sardella[1,2] | Daniel DeMiguel[6,7,8] (iD)

[1]Dipartimento di Scienze della Terra, Sapienza Università di Roma, Rome, Italy

[2]Istituto Italiano di Paleontologia Umana, Rome, Italy

[3]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Rome, Italy

[4]Department of Geosciences and Geography, University of Helsinki, Helsinki, Finland

[5]Dipartimento di Scienze della Terra, Università di Napoli, Federico II, Napoli, Italy

[6]Fundación ARAID/Universidad de Zaragoza, Zaragoza, Spain

[7]Departamento de Ciencias de la Tierra, Universidad de Zaragoza, Zaragoza, Spain

[8]Institut Català de Paleontologia Miquel Crusafont (ICP), Barcelona, Spain

Correspondence
Flavia Strani, Dipartimento di Scienze della Terra, Sapienza Università di Roma, Rome, Italy.
Email: flavia.strani@uniroma1.it
and
Daniel DeMiguel, Fundación ARAID/Universidad de Zaragoza, Zaragoza, Spain.
Email: demiguel@unizar.es
and
Antonio Profico, Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Rome, Italy
Email: antonio.profico@uniroma1.it

Abstract

Mastication of dietary items with different mechanical properties leaves distinctive microscopic marks on the surface of tooth enamel. The inspection of such marks (dental microwear analysis) is informative about the dietary habitus in fossil as well as in modern species. Dental microwear analysis relies on the morphology, abundance, direction, and distribution of these microscopic marks. We present a new freely available software implementation, *MicroWeaR*, that, compared to traditional dental microwear tools, allows more rapid, observer error free, and inexpensive quantification and classification of all the microscopic marks (also including for the first time different subtypes of scars). Classification parameters and graphical rendering of the output are fully settable by the user. *MicroWeaR* includes functions to (a) sample the marks, (b) classify features into categories as pits or scratches and then into their respective subcategories (large pits, coarse scratches, etc.), (c) generate an output table with summary information, and (d) obtain a visual surface-map where marks are highlighted. We provide a tutorial to reproduce the steps required to perform microwear analysis and to test tool functionalities. Then, we present two case studies to illustrate how *MicroWeaR* works. The first regards a Miocene great ape obtained from through environmental scanning electron microscope, and other a Pleistocene cervid acquired by a stereomicroscope.

KEYWORDS

diet reconstruction, open-source software, paleoecology, R package, tooth microwear

1 | INTRODUCTION

Dental microwear analysis studies microscopic wear patterns produced on the occlusal enamel surfaces of teeth during mastication.

It is one of the most valuable methods to assess dietary preferences in vertebrate taxa. Since the 1970s (see, among others, Gingerich, 1972; Grine, 1977; Puech, 1979; Walker, Hoeck, & Perez, 1978), microwear analysis has been successfully applied by anthropologists

and paleontologists to gain insights into the diet of several extinct groups, such as primates, including humans and hominins (DeSantis, 2016; Scott et al., 2005; Teaford & Walker, 1984), ungulates (DeMiguel, Fortelius, Azanza, & Morales, 2008; Kaiser & Brinkmann, 2006; Mihlbachler, Campbell, Ayoub, Chen, & Ghani, 2016; Semprebon & Rivals, 2007; Solounias & Hayek, 1993; Solounias & Semprebon, 2002), and carnivores (Schubert, Ungar, & DeSantis, 2010; Van Valkenburgh, Teaford, & Walker, 1990). Dental microwear analysis relies on the microscopic marks on the occlusal surfaces of tooth enamel (and/or dentin), left by the food chewed by an individual up to a few hours, days, or weeks before its death—a phenomenon referred to as the "Last Supper effect"—, depending on the rate of turnover in dental microwear of a particular consumer and food (Grine, 1986). The abundance, morphology, size, distribution, and orientation of marks are a consequence of the mechanic abrasion produced by mastication and are distinctive between different diets, depending on the fracture properties of the food items. In ungulates, a higher number of scratches over pits indicate tough-food (e.g., grasses) consumption. In contrast, a high number of pits indicate consumption of brittle, soft material such as leaves, fruits, and seeds (Solounias & Semprebon, 2002). In primates, a high occurrence of pits and coarse scratches is typical of hard-object feeders (which primarily feed on nuts and roots, and unripe fruits). Conversely, diet rich in leaves and soft fruits, which is typical of folivorous and frugivorous primates, is characterized by a low percentage of pits and narrower scratches (King, Aiello, & Andrews, 1999; Teaford, 1988).

The most common way to observe and study enamel marks is using high definition, two-dimensional pictures of a selected tooth crown region under either low or high magnification. The former, well-established approach, known as Low magnification microwear (LMM), employs high-precision casts of enamel surfaces observed by a standard stereomicroscope at 35× or 100× (for small mammals) magnification. Because it is fast and relatively low-cost, LMM is probably the most common dental microwear method today (Bastl, Semprebon, & Nagel, 2012; Rivals & Athanassiou, 2008; Rodrigues, Merceron, & Viriot, 2009; Semprebon, Taob, Hasjanova, & Solounias, 2016; Solounias & Semprebon, 2002). High magnification microwear (HMM) relies instead on pictures obtained through scanning electron microscope (SEM; DeMiguel et al., 2008; Galbany, Martínez, & Pérez-Pérez, 2004; King et al., 1999; Solounias, McGraw, Hayek, & Werdelin, 2000; Solounias & Moelleken, 1994), typically at 500× magnification. With environmental SEM (ESEM) devices, teeth can be observed directly without any damage, avoiding the risk of losing fine details during cast preparation. The downside of HMM is that it is more expensive and slower than LMM. Under both methods, enamel marks are classified, counted, and measured on a standard square area, whose size depends on the specific magnification adopted.

The recently introduced Dental microwear texture analysis (DMTA) (Merceron et al., 2009; Scott, Teaford, & Ungar, 2012; Scott et al., 2005; Ungar, Krueger, Blumenschine, Njau, & Scott, 2012) provides an alternative to both LMM and HMM. DMTA works with 3D surfaces and scale-sensitive fractal data. Unlike the traditional methods, DMTA does not require the identification of any individual feature, and the analysis is automated, thus being faster and less affected by observer error than more traditional methods (Scott et al., 2005). However, DMTA is an expensive method, as it requires the use of white-light scanning confocal microscopes (rather than simple 2D micrographs), and uses specific commercial software (Surfract®, ©2007; http://www.surfract.com/) and additional plugins (e.g., ToothFrax and SFrax) that increase the economic burden of the approach. Moreover, whereas traditional approaches record individual wear features to better understand individual morphologies and their orientations, DMTA focuses only on the overall pattern.

Both traditional (LMM and HMM) methods and DMTA require a software application to count and score enamel marks. Such software, except for *Microware* (Ungar, 1995), has never been specifically designed for microwear analysis and usually requires a costly license. In the case of *Microware*, one disadvantage is that it cannot discern between different subtypes of microscopic marks (e.g., large pits, coarse scratches). We therefore feel it is time to develop a freely available tool, specifically designed for microwear analysis, which allows for a more in-depth and complete investigation of the tooth occlusal features.

Here, we introduce *MicroWeaR*, a new free, open-access tool stored as an R package (Profico, Strani, Raia, & DeMiguel, 2018) that examines and scores microwear marks in a semiautomatic way. The method is designed to optimize sampling and classification of microscopic marks on high-resolution pictures of tooth surfaces, under different magnification levels. Using a picture of a dental surface (provided with a metric reference for the definition of the scale factor) as the input, the operator defines the size and position of a working area first, and then tracks the microwear features. Each mark is automatically classified into one of the two main categories, either "scratch" or "pit." It is important that, for each of these two categories, the tool recognizes two subcategories "small" and "large" pits, and "fine" and "coarse" scratches, and provides the user with summary statistics for each category and subcategory (count, mean, and standard deviation). We also provide *MicroWeaR* R code (R Development Core Team, 2009) along with the description of the application procedure. To illustrate the effectiveness of *MicroWeaR*, we further examined two case studies belonging to different taxonomic groups and different methodological procedures to obtain microwear information: a molar of the Miocene great ape *Anoiapithecus brevirostris* (see DeMiguel, Alba, & Moyà-Solà, 2014) and a molar of the Pleistocene cervid *Cervus elaphus eostephanoceros* (Strani et al., 2018).

2 | DESCRIPTION: *MICROWEAR* AS A TOOL FOR ESTIMATING MAMMAL DIETS

MicroWeaR has been developed to sample and semiautomatically classify multiple features from a picture at once. The tool functions (Table 1) support a variety of image file formats (i.e., "bmp," "png," "jpg," and "tif") and convert the input image into an .Ico object. The

Function	Description
class.Ico	Convert an image into an object of class Ico. At present, the formats "jpeg," "png," and "tiff" are supported. Limited to grayscale images
_plot_Ico_	Plot an image of class Ico. Setting the matrix that contains the coordinates of the microwear marks as set, the function returns to the image
_scale_Ico_	Scale an image of Ico class by an interactive plot selecting two points on the metric reference and defining the length of the latter
Warea.Ico	Select a working area of an image of class Ico through an interactive plot. The operator has to select the center of the working area and its dimensions
samp.traces	Record detectable microwear marks through the interactive plot. _samp. traces_ has an option to zoom in or out of the image of class Ico
_autom_class_	Classify the microwear marks in different subcategories as recorded by _samp.traces_ (object type). The output also provides a matrix (object Matrix), where the length and the width in micron are reported for each mark. In addition, the image with recorded marks is produced
cross.parallel	Detect pairs of scratches, which are "parallel" or "crisscross"
output.Ico	Print a summary statistics table reporting the number of pits and scratches (and the size of any subcategory)
mw.check	Check (via interactive multi-plot) the classification provided by the autom_class function. Before running _output.Ico_ using the a posteriori classification, the user must run again _cross.parallel_ using the updated microwear classification

R code provides the user with an interactive plot to scale the .Ico object to its original size using a metric reference that should be embedded in the picture. For each microscopic feature sampling is achieved by recording two distances using the left-click: the first one records the mark length, and the second its width. During the sampling procedure, the user may use the undo command to revert to a previous step and to zoom the picture in or out.

At the end of the sampling session, the function _autom_class_ provides an automatic classification of the marks as either pits or scratches. In turn, each pit is categorized as either "large" or "small" and each scratch is classified as either "fine" or "coarse." Automatic classification parameters can also be set manually to customize the sampling procedure. The tool provides an additional function of direction to detect pairs of "parallel" and "crisscross" scratches. The _autom_class_ function outputs a summary statistics table that can be exported in different format files (.txt, .sav for SPSS Statistics software, .csv for Excel spreadsheet), which includes the number of features of each type, the standard deviation and mean diameter of the pit, fine and coarse scratch lengths, and coarse scratch widths. Using the function _autom_class,_ the user is able to save the original picture overlaid by a transparent layer of the identified microscopic marks highlighted with a distinctive, user-defined color. The graphical rendering of the final output is itself fully customizable.

3 | APPLICATION OF THE _MICROWEAR_ PROCEDURE USING REAL CASE STUDIES

We provide two case studies as examples of the step-by-step application of _MicroWeaR_. These are the enamel occlusal surfaces of a lower left second molar (m2) ("Phase II" crushing/grinding facet 9) of the Miocene great ape _A. brevirostris_ (see DeMiguel et al., 2014) and an upper right first molar (M1) (antero-lingual enamel band of the paracone) of the Middle Pleistocene cervid _C. e. eostephanoceros_ (see Strani et al., 2018). The photomicrograph of the former was acquired through ESEM (at ×500 magnification) on the original specimen (Figure 1a), whereas the image of the latter was obtained using a stereomicroscope (×35 magnification) from a cast (Figure 1b). The mold and the cast of the molar tooth crown of _C. e. eostephanoceros_ were prepared following standard procedures (Semprebon, Godfrey, Solounias, Sutherland, & Jungers, 2004; Solounias & Semprebon, 2002). The impression was made using high-resolution Elite HD+ polysiloxane for the mold, and Araldite epoxy polymer for the cast. According to that, we provide microwear examples obtained from both high (×500) and low (×35) magnification and using either tooth originals or replicas. More comprehensive information on the taxa and the full description of the cleaning, molding/casting and examination procedures are available in DeMiguel et al. (2014) and Strani et al. (2018).

The _MicroWeaR_ package supports the file formats "bmp," "jpg," "tif," and "png." As the first step, the _MicroWeaR_ library is loaded into the R workspace. All the dependencies will be automatically installed or loaded as well. To begin the session, the user specifies the arguments _path_ and _image.type_ to import the image specifying where the file is located and its file format respectively.

```
require(devtools)
install _ github("MicroWeaR/MicroWeaR",local=FALSE)
library(MicroWeaR)
```
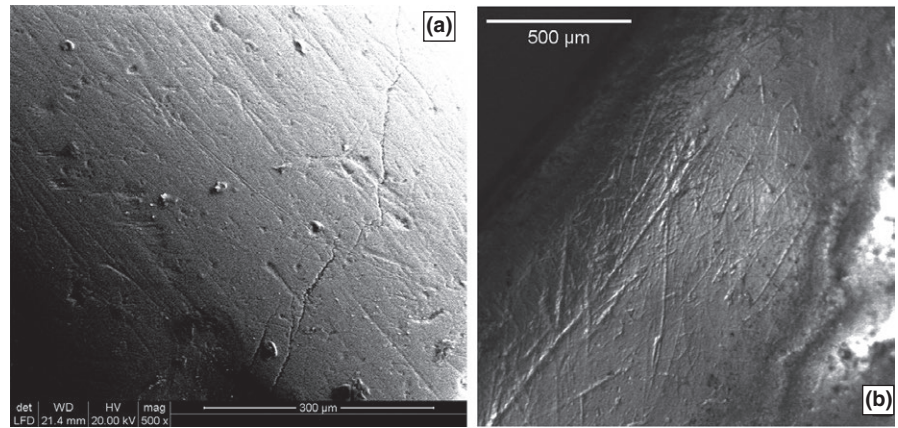
FIGURE 1 Enamel surface of the molars of *Anoiapithecus brevirostris* (a) and *Cervus elaphus eostephanoceros* (b)

```
library(zoom)
#load picture of C. e. eostephanoceros
data(C _ el _ pic)
#or load your picture typing:
#class.Ico(path, image.type = c("jpg", "png", "tiff"))
```

The function *scale_Ico* scales the picture to the real size in micron (μm). The scaling procedure requires the selection of two points on the image. In a successive way, the operator will specify the scale length on the console.

```
#load scaled picture of C. e. eostephanoceros
data(C _ el _ sca)
#or scale your picture typing:
#scale _ Ico(image.ico)
```

After loading and scaling the image, the operator defines a working area (e.g., 200 × 200 μm) and a magnification factor to be applied. The argument *sizes* of the function *area.param* allows setting the default square working area size to be displayed in the interactive 2D plot during the sampling session. By default, either 200 × 200 μm, 400 × 400 μm or 600 × 600 μm working areas are selected, yet the user can define a custom area by choosing the "select" option and typing the desired size (side length) on the console (Figure 2a).

```
#load the selected working area
data(C _ el _ war)
#or select the working area typing:
#Warea.Ico(image.ico)
```

Once the working area is defined, the sampling session begins (Figure 2b). The operator defines four points for each mark: the first two record the mark length, and the last two its width (Figure 2c).

```
#load the sampling session
data(C _ el _ sam)
#or start the sampling session typing:
#samp.traces(image.ico)
```

The arguments *cexp* and *lwdp* define the size and width of the points and lines of the marks, respectively. Considering that the

image is scaled in micron, we suggest setting these parameters in respect to the dimension of the scaled picture, or inserting any other reasonable number (e.g., *cexp* = 50; *lwdp* = 1). In any case, if the *cexp* and the *lwdp* parameters are set as *NULL* the *samp.traces* function will adjust the values of these parameters automatically.

After the manual sampling, the tool automatically classifies each mark within one of the two categories of features: "scratch" and "pit" (Figure 2d). The classification is based on the length/width ratio; by default, this is set to 4 μm (≤4 for Pit and >4 for Scratch as proposed by Ungar, 1995). For each of these two categories, the tool recognizes different subcategories based on the diameter (for pits) and width (for scratches): "small" and "large" for pits (by default diameter ≤8 and >8 μm, respectively), and "fine" and "coarse" for scratches (by default the width ≤3 and <3 μm, respectively). All default discriminating values can be changed by the user in the *autom_class* function by editing the *Pit_Scr*, *Sm.Lg_pit* and *Fi.Co_Scr* arguments.

```
# run type classification
class<-autom _ class(C _ el _ sam,C _ el _ war$image)
#or run the automatic classification typing:
#autom _ class(big _ matrix, image.ico, Pit _ Scr = 4,
Sm.Lg _ Pit = 8, Fi.Co _ Scr = 3)
```

The function *cross.parallel* calculates all the combinations of scratches and finds crossed and parallel scratch pairs. In detail, this function calculates the linear equation of the line passing through the two points that define the length of each mark. *MicroWeaR* uses the regression model parameters (intercepts and slopes) to classify scratch pairs as parallel (if the distance between the two scratches and their intersection point is greater than two-times the square of the working area), or crisscross (if otherwise). In the latter case, the angle between intersecting scratches is calculated and produced in the output.

```
scratches.ana<-cross.parallel(big _ matrix=  C _ el _
sam,image.ico= C _ el _ war$image,Type=class$Type)
```

In addition, *MicroWeaR* provides a summary statistics report for each category and subcategory (including count, mean, and standard deviation) and the input picture with the sampled marks that can be
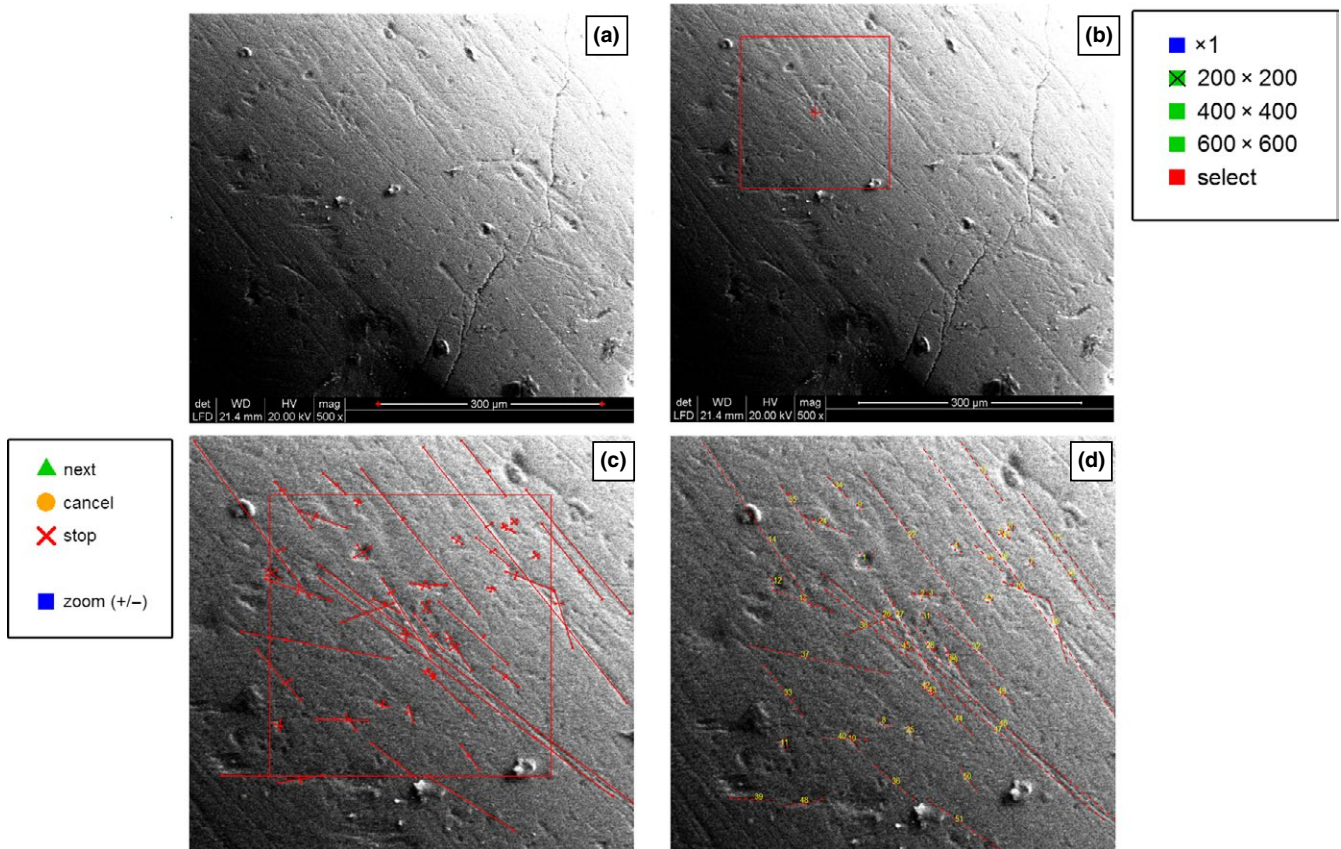
FIGURE 2 Step-by-step summary of semiautomatic enamel mark recognition performed using *MicroWeaR*. (a) Selection of two points on the reference metric scale to scale the image (top left). (b) Selection of the working area and size ("×1": the size of the working area corresponds to the size of the input image; "select": by selecting this option, the user can customize the size of the working area). (c) Sampling session (the "next" command allows to sample a new feature, the "cancel" command undoes the last sampling step, the "stop" command stops the sampling session, the "zoom" command allows to zoom in and out). (d) Sampled features displayed on the output image
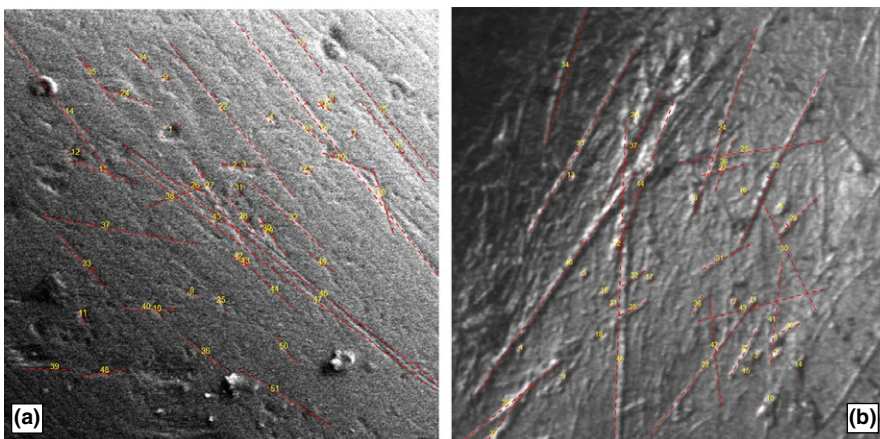
FIGURE 3 Final output images of *Anoiapithecus brevirostris* (a) and *Cervus elaphus eostephanoceros* (b). Microwear features were sampled on a 200 and a 400 μm² area, respectively

exported in different file formats. Automatic classification parameters can also be manually edited and set allowing customizing each sampling session.

At last, using the function *output.Ico* and specifying the matrix with the coordinates of the microwear marks, an image with the displayed marks is loaded as a plot (Figure 3).

```
output.Ico(C_el_sam,class$Type,scratches.
ana,C_el_war)
```

We provide a video tutorial as Supporting Information (Video S1) for the application of the tool in R environment.

3.1 | Case studies interpretation

Regarding the occurrence of pits (*N* = 17), *A. brevirostris* resembles extant frugivores/mixed feeders such as *Cebus nigrivittatus*. It further displays somewhat wide scratches (Mean_width = 2.77 μm), in the

range of *Pan troglodytes* (Mean_width = 2.6 µm) and *Pongo pygmaeus* (Mean_width = 2.8 µm), which suggests a certain degree of sclerocarpy. The results obtained by DeMiguel et al. (2014) show that, on average, *A. brevirostris* diet is somewhat intermediate in between *P. pygmaeus* and extant frugivores/mixed feeders such as *P. troglodytes* in terms of pitting incidence (N = 22), whereas it is similar to extant frugivores/mixed feeders in scratch width (Mean_width = 1.98 µm). These results confirm a soft-fruit diet (albeit with some sclerocarpic components) and are fully consistent with those obtained using *MicroWeaR* (Table 2).

The dental microwear pattern of the Pleistocene deer *C. e. eostephanoceros* has a similar amount of pits (N = 21) and scratches (N = 25) according to the *MicroWeaR* semiautomatic classification (Table 3). Most scratches are short and finely textured with a few long coarse scratches (Mean_length = 415.92 µm). Cross scratches are also detected (N = 15). Small pits are more abundant than larger ones (N = 13 and N = 8, respectively). A high number of pits and scratches with a prevalence of finely textured features indicates that *C. e. eostephanoceros* fed on a variety of plant types (both soft and abrasive), as commonly observed in modern mixed feeders (Solounias & Semprebon, 2002). The findings obtained using *MicroWeaR* are thus consistent with those obtained by Strani et al. (2018) where a larger, more indicative sample of *C. e. eostephanoceros* studied using both LMM and dental mesowear analysis, indicated a mixed feeder diet for this species.

4 | SIGNIFICANCE OF THE TOOL

Using traditional LMM and HMM methods, one key factor affects the validity of the results, that is how different operators count

and discriminate among microscopic marks (DeSantis et al., 2013; Mihlbachler, Beatty, Caldera-Siu, Chan, & Lee, 2012). The use of a semiautomatic approach minimizes the intraobserver error because the only manual step in the whole procedure is the definition of the initial and the end point of each enamel mark. The automatic differentiation between subcategories also helps to reduce interobserver error rates when it comes to detailed interpretation of microwear features, which are usually high with traditional semiautomatic approaches (Galbany et al., 2005; Grine, Ungar, & Teaford, 2002; Mihlbachler et al., 2012). Given that *MicroWeaR* can be used for the analysis of any 2D image containing scars, it is also useful for recording lineal striations (i.e., number, length and breadth of scratches) in micrographs taken on nonocclusal tooth surfaces and, therefore, extensible to buccal enamel microwear quantification (Galbany & Pérez-Pérez, 2004; Pérez-Pérez, Lalueza, & Turbón, 1994; Puech, 1981) as well.

Since the creation of the R platform, libraries addressing natural science applications have rapidly increased (R Core Team, 2000). The open-access nature of the R platform allows tools to be rapidly improved, by introducing new functionalities that are under immediate diffusion and testing through the R community. According to that, we designed *MicroWeaR* in order to work under different operating systems (i.e., Windows, OSX, Linux).

MicroWeaR allows the automatic classification of the marks left on the enamel surface by the last foods (Grine, 1986) processed. Such automaticity helps keeping inter- and intraobserver error low (categories automatically assigned to each mark can be nonetheless manually edited using the *mw.check* function; Figure 4) and makes

TABLE 2 Results of the microwear analysis applied to a tooth of *Anoiapithecus brevirostris*

	N.pits	N.sp	N.lp	%p	P	N.scratches	N.fs	N.cs	S	N.Ps	N.Xs	%Ps	%Xs
Count	17	9	8	33.3	425	34	20	14	850	62	9	85.3	26.5
Mean_length	7.64	5.29	9.73	/	/	20.94	22.38	18.87	/	/	/	/	/
Sd_length	3.75	1.06	4.08	/	/	19.24	23.14	12.21	/	/	/	/	/
Mean_width	2.86	2.54	3.14	/	/	2.77	1.13	5.12	/	/	/	/	/
Sd_width	1.95	1.54	2.31	/	/	2.41	1.41	1.35	/	/	/	/	/

Note. N.pits: number of pits; N.sp: number of small pits; N.lp: number of large pits; %p: percentage of pits; P: pits/mm^2; N. scratches: number of scratches; N.fs: number of fine scratches; N.cs: number of coarse scratches; S: scratches/mm^2; N.Ps: number of pairs of parallel scratches; N.Xs: number of scratches that cross each-other; %Ps: percentage of parallel scratches; %Xs: percentage of scratches that cross each-other.

TABLE 3 Results of the microwear analysis applied to a tooth of *Cervus elaphus eostephanoceros*

	N.pits	N.sp	N.lp	%p	P	N.scratches	N.fs	N.cs	S	N.Ps	N.Xs	%Ps	%Xs
Count	21	13	8	45.7	131	25	17	8	156	4	15	20.0	36.0
Mean_length	20.38	11.96	34.06	/	/	240.52	157.98	415.92	/	/	/	/	/
Sd_length	14.52	5.79	14.11	/	/	178.58	108	176	/	/	/	/	/
Mean_width	4.52	2.73	7.43	/	/	1.66	0.73	3.62	/	/	/	/	/
Sd_width	4.73	2.24	6.3	/	/	2.36	0.93	3.25	/	/	/	/	/

Note. N.pits: number of pits; N.sp: number of small pits; N.lp: number of large pits; %p: percentage of pits; P: pits/mm^2; N. scratches: number of scratches; N.fs: number of fine scratches; N.cs: number of coarse scratches; S: scratches/mm^2; N.Ps: number of pairs of parallel scratches; N.Xs: number of scratches that crosses each-other. %Ps: percentage of parallel scratches; %Xs: percentage of scratches that cross each-other.
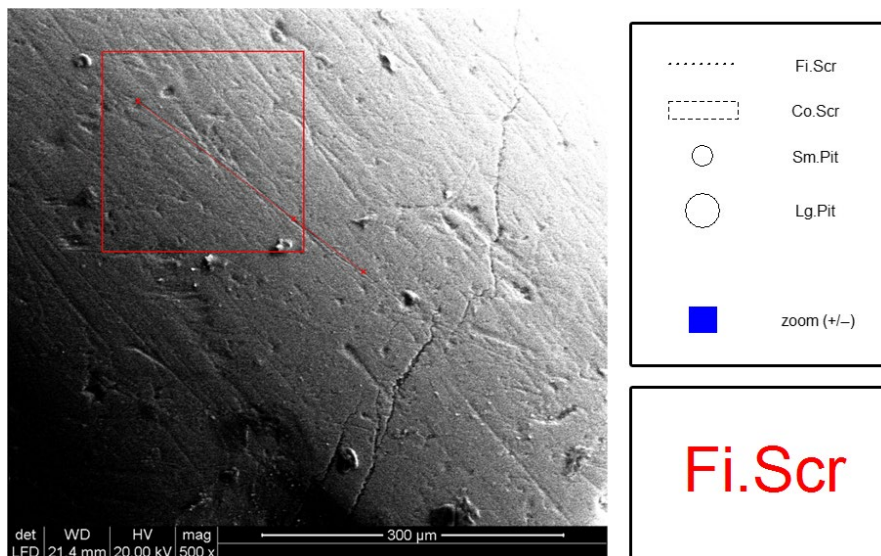
FIGURE 4 Additional *MicroWeaR* functionality: classification editing. The automatic classification of each mark can be manually edited at the end of the procedure using a multiplots interactive interface. Co.Scr: coarse scratch; Fi.Scr: fine scratch; Lg.Pit: large pit; Sm.Pit: small pit

the dental microwear analysis faster, more robust, and cheaper than with any other comparable application.

5 | CONCLUSIONS

A new software implementation for dental microwear analysis, *MicroWeaR*, offers a semiautomatic open-access tool for quantification and classification of the microscopic enamel marks, stored as an R package. *MicroWeaR* is less time-consuming and less prone to observer errors in comparison with the conventional microwear analysis with two-dimensional imaging methods (LMM, HMM), as it is inexpensive compared to a new three-dimensional method (DMTA). It works for any 2D image containing microwear scars. Thus, it is useful for the quantification of marks as observed under either high or low magnification, on both occlusal and nonocclusal (e.g., buccal) tooth surfaces (dentin or enamel), and from either tooth originals or replicas. *MicroWeaR* is designed to work in different operating systems (e.g., Windows, OSX, Linux) and due to its intrinsic characteristics, it is unique to be developed further.

CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

F.S., A.P., P.R., and D.DM. conceived the ideas and designed methodology; D.DM. and F.S. collected the data; F.S. and A.P. wrote the R code with the contribution of P.R. and D.DM.; F.S., A.P., P.R., and D.DM. led the writing of the manuscript and contributed to the implementation of example analyses. D.P., R.S., and G.M. contributed helpful comments and provided inputs for the manuscript. All authors revised the manuscript and gave final approval.

AVAILABILITY AND DATA ACCESSIBILITY

MicroWeaR can be downloaded from https://github.com/MicroWeaR (https://doi.org/10.5281/zenodo.1233505). We encourage authors to cite Strani et al. (this paper) if you use MicroWeaR for research, education, and outreach. As an application designed to be part of R, MicroWeaR is available as a package to run on different operating systems (Windows, Mac OS or Linux).

The results reported in this paper were obtained using *MicroWeaR* R package. The code and real examples of use are available in the *MicroWeaR* R package.

ORCID

Flavia Strani http://orcid.org/0000-0003-4566-3644
Antonio Profico http://orcid.org/0000-0003-2884-7118
Daniel DeMiguel http://orcid.org/0000-0001-6138-7227

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Chapter 7

Discussion

The results provide important insights into both the palaeoenvironmental settings of Southern European regions following the two major climatic events of the Pleistocene and the palaeoecological adaptations and responses to climatic shifts in term of dietary behaviour of fossil ungulate taxa. In particular, here is provided new specific data on niche partitioning mechanisms among Early Pleistocene ungulate communities following the onset of the Plio/Pleistocene (Chapter 2) and Gelasian/Calabrian transition (Chapter 3), the effects of the "0.9 Ma event" on biomes of the Iberian Peninsula (Chapter 4), and the habitats that characterised Central Italy after the Mid-Brunhes event (Chapter 5). Moreover, using dental microwear pattern data collected during the project or provided by Dr. Daniel DeMiguel), a new tool for the investigation of microwear features has been designed and developed, which will aid researchers in the challenging task of reconstructing the diets of fossil vertebrate taxa (Chapter 6).

Evolution of the ecosystems and environments during the Early and Middle Pleistocene

The findings from the analysis of the dietary behaviours of ungulates of Coste San Giacomo (Anagni, Central Italy) both shed light on the resource partitioning mechanisms among numerous sympatric fossil herbivores, and provided information on the palaeoenvironments that characterised the Italian Peninsula after the initiation of the Quaternary glacial cycles. Previous habitat reconstructions of the site were mostly based on faunal composition (Bellucci et al. 2012, 2014) with a taxon-free methodology applied only by Strani et al. (2015). Combining two different methods of long-term dental wear pattern analysis, as well as taking into account taxa body size variation, it has been possible to get a better understanding of the environments and ecological settings of the region. The diversity of feeding behaviours observed among the ungulate communities points to heterogeneous landscapes with a mosaic of biomes that spanned from wetlands to woodlands to open habitats. Specifically, large-sized ungulates display the widest range of diets (from strict browsers to strict grazers) observed, while small-sized species, with the notable

exception of the small *Gazella borbonica*, are more selective in their feeding behaviour. Taking into account also the relative abundance of some taxa (the cervids *Axis* cf. *lyra*, *Eucladoceros* sp. and the equid *E. senezensis* aff. *E. sen. stehlini*) (Bellucci et al., 2014; Palombo et al., 2017), and following the niche breadth concepts (Brown, 1984; Macandza et al., 2012) it is hypothesized that these more common ungulates would have exploited a broader landscape while less frequent species would have concentrated on fewer resources in probably more restricted areas.

The occurrence of species with a high degree of abrasion in their diets (such as the grazer equid *E. senezensis* aff. *E. sen. stehlini* and the grass-dominated mixed feeder bovid *Leptobos* sp.) as well as the species that may had grazed occasionally (such as the mixed feeder antelope *Gazella borbonica*), corroborates the presence of drier environments in accordance with the spread of steppe-like vegetation (with a particular abundance of plants belonging to the *Artemisia* genus) in Central and Southern Italian Peninsula (Bertini, 2003, 2010; Kahlke et al., 2011). Nevertheless when compared to other Early Pleistocene (middle Villafranchian) European regions it appears that Central Italian Peninsula environmental conditions were more humid than the ones recorded in South-Eastern Europe. The Greek Peninsula locality of Sésklo (dated around the MN17 biozone) had an ungulate community rich in grazers as well as mixed feeders with a strong leaning towards a grazing behaviour according to dental meso- and microwear analysis (Rivals and Athanassiou, 2008). This indicates drier and more open habitats with a predominance of grasslands compared to the more diverse environments of CSG. Environments of the Central Italian Peninsula seem to have shared stronger similarities with Central European localities during the middle Villafranchian, in particular with the French sites of Saint-Vallier (MN17) and Chilhac (MN17), where more humid conditions and a mosaic of habitats (with a predominance of forested areas in the case of Chilhac) is attested to according to mammal fauna composition, pollen record and dental wear patterns of fossil cervids (Boeuf and Gilbert, 1997; Argant, 2004; Guérin et al., 2004; Valli and Palombo, 2008; Berlioz et al., 2018). This would suggest that the trend towards more arid condition recorded during

the Early Pleistocene was a gradual process, with climatic conditions deteriorating earlier habitats from South-Eastern Europe than Central and Southern Europe; a hypothesis already proposed by Khalke et al. (2011) which is also in accordance with palynological data from the Italian Peninsula (Bertini, 2003, 2010, 2013).

This shift towards less humid conditions is more evident at the Gelasian/Calabrian (middle/late Villafranchian). In the late Villafranchian locality of Olivola (~1.8 Ma), which shares a similar faunal composition with Coste San Giacomo, fossil ungulates exhibit a narrower range of feeding behaviours with no incidence of taxa showing a selective diet. Bovids and cervids in particular display a marked shift towards the consumption of more abrasive food items, a result interpreted as an adaptive response to the spread of steppe-like vegetation. Specifically, large bush-antlered deer *Eucladoceros* and medium sized deer *Pseudodama* (=*Axis, "Metacervoceros"*), which adopted a strict browsing feeding behaviour mostly in the forested areas of the Italian Peninsula during the middle Villafranchian, display a mixed diet in the late Villafranchian. This development of drier habitats during the late Villafranchian is also recorded in the Iberian Peninsula at Barranco de los Conejos (Guadix-Baza Basin) and in the Caucasus at Dmanisi (Gabunia et al., 2000; Agustí et al., 2013; Blain et al., 2014).

Few mammal assemblages are dated around the onset and consolidation of the Early-Middle Pleistocene Transition (Madurell-Malapeira et al., 2010, 2014), making the results obtained about the Vallparadìs ecosystems evolution from 1.0 Ma to 0.6 Ma crucial to understanding how this major event influenced the habitats of the Mediterranean Europe. The establishment of climatic cycles ruled by a 100 kyr periodicity led to more intense glacial and interglacial phases in the Northern Hemisphere with the MIS 31 (known as a "super interglacial"; DeConto et al., 2012) and the MIS 22 glacial (also known as the "0.9 Ma event") being considered two of the most extreme climatic episodes ever recorded during the EMPT (Clark et al., 2006; Elderfield et al., 2012; Head and Gibbard, 2015). Dietary adaptations of fossil ungulates of layers EVT12 (corresponding to MIS

31) and EVT7 (corresponding to MIS 21) of the Vallparadìs section, allow to gather information on the effects of these extraordinary climate shifts in Southern Europe.

MIS 31 was a major Early Pleistocene interglacial characterized by a marked high-latitude insolation and an estimated eustatic rise of 20 m (Raymo et al., 2006). In the Iberian Peninsula, this extremely warm phase apparently led to drier conditions and to a further expansion of open grasslands, as testified by the occurrence of many ungulate taxa with a medium or high degree of abrasion in their diets from layer EVT12. Similar dry conditions are reported also from other Southern European Early Pleistocene localities such as Apollonia (Greek Peninsula) and Colle Curti (Italian Peninsula), with open habitats spreading earlier in Southern Eastern regions. Given the abrupt increase of marine and continental ice volume recorded during the "0.9 Ma event", a deep exacerbation of the habitat conditions in the whole European continent should be expected. Nevertheless, according to the palaeoecological results from Vallparadìs, no substantial changes in the terrestrial ecosystems is observed in Southern Europe after MIS 22. The same fossil taxa display similar feeding behaviours in both EVT12 and EVT7 implying that vegetation composition was not markedly affected by the glacial event. This is in accordance with the palynological data from other Southern European localities where no major reduction of forests and plant taxa turnover was observed in this phase of the Early-Middle Pleistocene Transition (Suc and Popescu, 2005). Moreover, the reduction of grazing individuals (see Fig. 3 of Chapter 4) coupled with the well attested presence of vertebrate taxa linked to humid habitats in layer EVT7 (e.g. *Hippopotamus antiquus*), suggest that an expected increase of the aridity degree after an extreme cooling event did not occur in the region. The presence of more humid conditions is also attested in Gran Dolina (Iberian Peninsula) (Burjachs, 2001; Cuenca-Bescós et al., 2017; Blain et al., 2018) and at Tenaghi Philippon (Greek Peninsula) (Van der Wiel and Wijmstra, 1987a,b; Tzedakis et al., 2006). After the "0.9 Ma event" however seasonality became more marked as denoted by the increment of seasonal mixed feeders from MIS 31 to MIS 21. After this humid phase an increase of aridity is observed

around 0.6 Ma as attested by the comparative predominance of grazing equids in layer EVT3 and in accordance with palinological results obtained from the Middle Pleistocene site of Isernia la Pineta in the Italian Peninsula (Arobba et al., 2004).

Seasonality seems to have played a key role in shaping past ecosystems also during the transition between MIS 12 and MIS 11 (the Mid-Brunhes Event), with the definitive consolidation of the glacial cycles ruled by a 100 kyr periodicity (Head and Gibbard, 2015). From MIS 11 onwards, interglacial are characterized by higher temperatures comparable to those registered in the Early Pleistocene before the onset of the EMPT (Head and Gibbard, 2015). During this transitional phase (around 450 ka), the palaeoeonvironments of the Italian Peninsula were characterised by heterogeneous habitats as suggested by the wide range of dietary behaviours of the fossil ungulates of the Middle Pleistocene archaeo-palaeontological locality of Fontana Ranuccio (Anagni basin, Central Italian Peninsula). Both habitats rich in soft plant resources and grasslands were available to be exploited by large herbivores, though an abundance of browsing cervids points to a relative dominance of woodlands. The discrepancy between the long- and short-term dental wear patterns of the aurochs *Bos primigenius* suggests that, similarly to that recorded during the EMPT, a marked seasonality influenced the quality and type of plant food items periodically. In this scenario, these large bovids, in order to guarantee the high amount of food intake required to sustain themselves, may had been forced to switch to sup-optimal plant resources during adverse periods. The occurrence of unfavorable seasons is also attested to by pollen records from the locality of Boiano (Campobasso, Central Italian Peninsula) (Orain et al., 2013; Combourieu-Nebout et al., 2015): even though after the Mid-Brunhes Event, interglacials were generally warmer than those predating this transitional phase; extremely cool and dry winters were recorded in the Italian Peninsula during the MIS 11 (Combourieu-Nebout et al., 2015).

In archaeo-palaeoentological localities where bones accumulation can be partially or fully linked by human activities, which is the case of Fontana Ranuccio where faunal remains are

associated to both human fossils and abundant artifacts (Cassoli and Naldini, 1984; Segre and Ascenzi, 1984; Ascenzi et al., 1993; Rubini et al., 2014), a high variation of ungulate dental wear signal can point to a long hominid occupation of the area (Rivals et al., 2009). The variability observed in the meso- and microwear patterns of the ungulates of Fontana Ranuccio would thus suggest a low mobility of *Homo* groups in this region, which would have inhabited the area even during adverse seasons. These results may be relevant to understand also the palaeoecological framework of the geographically and chronologically close locality of Ceprano, where an exceptional fossil human calvarium ascribed to an archaic form of *Homo heidelbergensis* (Manzi et al., 2010; Manzi, 2016; Di Vincenzo et al., 2017) has been unearthed.

When data from hypsodonty are taken into account, a noteworthy trend can be observed from the Early to the Middle Pleistocene. While in Coste San Giacomo a wide range of teeth with high molar crowns have been recorded (there are three brachydont, two mesodont and two hypsodont ungulates) (Strani et al., 2015), a reduction of brachydont taxa is noted from the late Villafranchian locality of Olivola onwards. Specifically, only one brachydont (*Stephanorhinus etruscus*) and one brachy/mesodont (*Eucladoceros dicranios olivolanus*) taxa are recorded in Olivola while in both the early Middle Pleistocene and Middle Pleistocene sites of Vallparadìs and Fontana Ranuccio ungulates display either a mesodont or hypsodont dentition.

Traditionally, the acquisition of high molar crowns are considered as an adaptive response to high rates of tooth wear due to the consumption of highly abrasive items in new habitats (Janis, 1988; Strömberg, 2002, 2006). Given that grasses tend to be rich in silica content (phytoliths) and that feeding in open lands usually leads to the ingestion of external abrasive items (such as grit or dust), it is believed that high molar crowns evolved for grazing in open dry habitats (Kovalevsky, 1873; Matthew,1926; Owen and Wiegert, 1981; Janis, 1988; Retallack, 2001; Strömberg, 2002, 2006). This traditional view is more complex than expected, as confirmed by a recent study that

suggests a biomechanical factor for the origin of hypsodonty to effectively feed on resistant foods (DeMiguel et al., 2015).

The marked reduction and eventual disappearance of brachydont ungulates appear to be in accordance with the gradual trend towards cooler and more arid environmental conditions that began in the Plio/Pleistocene transition and led to the reduction of sub-tropical forest canopies in favor of open grasslands (Fortelius et al., 2006; Bertini, 2010; Kahlke et al., 2011). Nevertheless, not all hypsodont fossil taxa display a strictly grazing diet; as fossil equids of Vallparadìs and the aurochs of Fontana Ranuccio show short-term dental wear patterns that are compatible with a mixed and browsing diet, respectively. This is not surprising, as while hypsodonty may have evolved with the spread of grasses, it is an adaptive trait that does not preclude the efficient consumption of other type of plant resources (Feranec, 2003; DeMiguel et al., 2014). As a matter of fact, there are additional records of both modern and fossil hypsodont ungulates adopting a mixed or even a browsing diet (MacFadden et al., 1999; Fortelius and Solounias, 2000; DeMiguel et al., 2008; Bernor et al., 2017). Conversely, not all the grazers or mixed feeding fossil taxa have high cheek tooth crowns. *Stephanorhinus etruscus* from Olivola has a brachydont dentition but a mixed diet according to mesowear patterns, while the mesodont giant deer *Megaloceros savini* from layer EVT12 of Vallparadìs displays microwear patterns compatible with a highly abrasive feeding behaviour. It should be noted however that none of the brachydont fossil taxa display a strict grazing behaviour in the long-term timescale.

Ecology of fossil herbivorous ungulates

The present project allowed for the improvement of the current knowledge of fossil ungulate niche occupation during the Pleistocene. Different dietary adaptations have been recorded in the examined herbivorous taxa which can display a certain degree of plasticity in their feeding

behaviours. The investigation of such plasticity also led to the development of a tool for the reconstruction of the diets of fossil herbivorous mammals.

Dietary flexibility

One important aspect that emerges from this project is the wide dietary flexibility of some specific groups of ungulates. Pleistocene deer are often considered as woodland dwellers and subsequently regarded as browsers despite that modern members of Cervidae can feed on several type of foods and occupy different habitats (as observed in the red deer *Cervus elpahus* that displays a significant dietary plasticity and can thrive in both closed and open environments; Groot Bruinderink and Hazebroek, 1995; Gebert and Verheyden-Tixier, 2001; Azorit et al., 2012; Berlioz et al. 2017). This occurs not only in modern species since even some of the earliest cervids (such as *Procervulus*) displayed diverse feeding behaviours (DeMiguel at al., 2008, 2010). This can result in an imprecise reconstruction of the palaeoenvironmental context of fossil mammal assemblages if it is taken only into account the relative abundance of deer remains in many Pleistocene fossil assemblages (e.g. Chapter 5, Madurell-Malapeira et al., 2010; Pavia et al., 2012). Here it is corroborated that fossil cervids displayed a flexible diet also during the Early and Middle Pleistocene, thereby ranging from browsing (*Axis* cf. *lyra*, *Croizetoceros* cf. *ramosus* and *Eucladoceros* sp. from Coste San Giacomo; *Dama vallonnetensis* and *Megaloceros savini* from layer EVT7 and EVT12 respectively of Vallparadìs; *Dama clactoniana* from Fontana Ranuccio) to mixed feeding (*Eucladoceros dicranios olivolanus* and *Pseudodama nestii* from Olivola; *Dama vallonnetensis* and *Cervus elaphus* from layer EVT12 and EVT3 respectively of Vallparadìs; *Cervus elaphus eostephanoceros* from Fontana Ranuccio) diets in the long-term timescale, and from browsing (*Dama clactoniana* from Fontana Ranuccio), to mixed feeders (*Dama vallonnetensis* from layer EVT7 of Vallparadìs; *Cervus elaphus* from layer EVT3 of Vallparadìs; *Cervus elaphus eostephanoceros* from Fontana Ranuccio), to grass-dominated mixed feeding/grazing (*Dama*

vallonnetensis and *Megaloceros savini* from layer EVT12 of Vallparadìs) in short-term timescale. Moreover, the whole mixed feeding behaviour continuum is recorded with cervids adopting browse-rich as well as grass-dominated mixed diets. This is in accordance with that reported for other Pleistocene fossil deer from different European regions, which can display both a selective and a somewhat grass rich diet (Rivals 2016; Berlioz et al., 2018). Furthermore, they also display a variation of molar crown height with Early Pleistocene (middle Villafranchian) taxa displaying a brachydont dentition and taxa from the late Villafranchian onwards a mesodont one.

The observed discrepancies between the long- and short-term dental wear patterns of the fallow deer *Dama vallonnetensis* (from EVT7) and the giant deer *Megaloceros savini* (from EVT12) of the Vallparadìs section have been also investigated to exclude the possibility that the abrasion-dominated microwear signal (see Fig. 3, 4 from Chapter 4) is indeed a fact masking a "dirty browsing" behaviour. Modern herbivores that browse in arid or desert habitats can exhibit dental microwear patterns unusual for typical browsers due to the fact that in extremely arid environments soft plant parts are usually covered by grit and soil (William and Kay, 2001; Solounias and Semprebon, 2002; Kaiser and Rössner, 2007; Kaiser, 2009). These "dirty browsers" display coarser scratches, heavy pitting and abundant gouges compared to browsers which feed in more humid environments. Neither *D. vallonnetensis* nor *M. savini* display this pattern, thus supporting the fact that these fossil deer could successfully feed on different types of plant resources.

Bovids display a certain degree of dietary plasticity as well, though they mostly tend to adopt a mixed or grass-rich diet and show higher molar crowns (mesodont or hypsodont) than cervids. One notable exception is the large Early Pleistocene antilope *Gazellospira torticornis* from CSG which displays mesowear patterns typical of a browser and possibly foraged in transitional ecotones (transitional zones between forest and open grassland) or selected different plant parts of the same plant species consumed by other ungulates. The case of the fossil aurochs *Bos primigenius*

from the Middle Pleistocene site of Fontana Ranuccio is also peculiar as this large bovid periodically shifted its feeding behaviour from a grass-rich mixed diet to a browsing one. A browse rich diet is also recorded in other Northern and Central European Middle and Late Pleistocene (MIS 7, MIS 9, MIS 11) (Rivals and Lister, 2016) and Holocene aurochs populations (Kerley et al., 2012; Hofman-Kamińska et al., 2018). In the case of the Holocene groups, however, this browsing diet may had been a consequence of the occupation of the few available open areas by human settlements, which drove large bovids (aurochs and bisons) to marginal less-optimal forested habitats (Kerley et al., 2012). At Fontana Ranuccio, this periodic change in diet may be linked to strong seasonal effects which would have driven the aurochs populations to feed also on less abrasive plant resources.

Equids display the highest crowned dentition and the lowest variation in dental wear patterns with all examined taxa exhibiting a mostly grazing diet. *Equus altidens* from layer EVT7 of Vallparadìs however, shows microwear patterns which indicates a mixed diet. This suggests that while large equids consistently occupied a similar niche (i.e. grazers in open habitats) through the Early and Middle Pleistocene, they could shift their diet to a certain degree to include also less abrasive food items. Both modern and fossil *Equus* are usually considered specialized grazers, but it has been observed that modern species can occupy different habitats and also include a noticeable amount of browse in their diet especially in the face of adverse conditions (e.g. *Equus zebra zebra* in Namibia during drought periods, Roeder, 1999). Moreover, mixed or browsing behaviours are also recorded in fossil hypsodont horses (Kaiser and Franz-Odendaal, 2004; Kuitems et al., 2015; Semprebon et al., 2016; Bernor et al., 2017) and even in Britain, *Equus altidens* displays microwear patterns that suggest browsing elements (Rivals and Lister, 2016). It is thus plausible that while Southern European fossil *Equus* feed preferably on grasses in open habitats, they were not always restricted to this niche during the Early-Middle Pleistocene Transition interglacials.

Fossil rhinocerotids display a brachydont dentition during the Early Pleistocene (Olivola) and a mesodont one during the late Early Pleistocene (Vallparadìs) adopting a mixed or grass-rich feeding behaviour. A trend towards an increasingly more abrasive diet can be observed in this group, from the mixed diet exhibited by *Stephanorhinus etruscus* (Olivola), to the grass-dominated mixed feeder *Stephanorhinus hundsheimensis* (Vallparadìs), to the single strict grazer individual of *Stephanorhinus* sp. at Fontana Ranuccio. A similar trend is also observed in fossil rhinoceros from Britain (Rivals and Lister, 2016), where Early Pleistocene *Stephanorhinus* mostly displays a mixed or browse-rich mixed diet and adopts a more abrasive feeding behaviour during the Middle Pleistocene, with some species (e.g. *Stephanorhinus hoemitechus*) showing a grass-rich mixed or grazing behaviour. During the early Middle Pleistocene, *Stephanorhinus* also adopted a more flexible diet in Central Europe (Kaiser and Kahlke, 2005; Kahlke and Kaiser, 2011; van Asperen and Kahlke, 2015), though Kaiser and Kahlke (2005) proposed that it fed preferably on browse if available (as at Voigtstedt in Germany).

In conclusion, results from this project highlight and confirm the overall dietary plasticity of fossil ungulates during the Early and Middle Pleistocene with the existence of taxa that are well-known to be highly specialised (such as stenonid and caballoids equids) showing a somewhat (albeit restricted in time) flexibility in their feeding behaviours.

Improving the investigation of fossil herbivores dietary behaviours

Dental microwear analysis plays a critical role in inspecting the degree of dietary flexibility in fossil herbivores and, as such, improving the current methods of investigation may be crucial to obtain more precise information about both resource exploitation mechanisms adopted by fossil species and the structure of past ecosystem.

When conducting this project, a number of limitations and difficulties were noted regarding the traditional dental microwear analysis (i.e. Low and High Magnification Microwear), particularly regarding the available methods to sample and score microscopic features on the enamel surface. For this reason, a new open (and free) access tool has been devised, developed and tested on some of the collected material. Both Low- and High Magnification Microwear require the observation and manual classification of the microwear features on 2D images (collected respectively with a standard stereomicroscope and a scanning electron microscope) (e.g. Solounias and Semprebon, 2002; DeMiguel et al. 2008). To do so, image editing software are usually employed and, with the exception of *Microware* designed by Ungar (1995), none of them are intended for dental microwear analysis and often require the payment of a license to be used. The software *MicroWeaR*, which has been developed during this project has been specifically conceived to provide a reliable tool for the investigation of microscopic scars. Compared to other existing programs, it offers several advantages and innovative features. The tool can count and classify (as pits or scratches) the enamel scars in a semi-automatic way with the user having only to select the initial points that define the length and width of the scar. More importantly, *MicroWeaR* can also differentiate four sub-categories (large pits, small pits, coarse scratches and fine scratches) based on a length/width ratio that can be set by user and all collected data can be exported in different formats (e.g. .txt, .sav or .csv).

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Chapter 8

Conclusions

Palaeoecological adaptations of fossil ungulates in response to environmental changes allowed new insights into the evolution of the terrestrial ecosystems of the Mediterranean Europe after the initiation of the Quaternary glacial/interglacial cycles and during the Early-Middle Pleistocene Transition (EMPT).

During the Early Pleistocene (middle Villafranchian) after the beginning of the cooling trend towards more arid climates that interested first South-East Europe and then West regions, a mosaic of biomes was present in the Italian Peninsula comprised of both forests and open grasslands as well as wetlands and rocky terrains. This heterogeneous landscape provided the conditions for the sympatric coexistence of numerous fossil herbivorous large mammals which were able to exploit a wide array of plant resources. Large bush-antlered deer (*Eucladoceros*), middle sized *Croizetoceros* and "*Axis*-like" deer exploited mostly wooded environments showing a strict browsing behaviour. Bovids (antelopines, leptobovines and caprines) and stenonid horses occupied more open landscapes with dietary adaptations that ranged from browsers, to mixed feeders to strict grazers. During the late Villafranchian, this same group of Early Pleistocene ungulates (with the exception of antelopines which disappear in the Italian Peninsula at the end of the middle Villafranchian) shift their diets towards more abrasive behaviours signaling expansion of open grasslands and reduction of covered canopies as the cooling trend progresses.

Comparison between long- and short-term dental wear patterns of Epivillafranchian ungulate taxa suggests that one effect of the EMPT in the Mediterranean region, particularly of one of the most extreme glacial periods that occurred in this phase (the so-called "0.9 Ma event"), was a steep increase in seasonality. An abundance of seasonal mixed feeders is recorded after MIS 22 and a reduction of grazing animals suggest that more humid conditions were present in the NE Iberian Peninsula similarly to what has been registered in other regions of Southern Europe. After this less dry phase, a new spread of open grasslands is recorded in the area during the early Middle

Pleistocene (around 0.6 Ma) with a relative high number of hypsodont strict grazing equids which is in accordance with what is reported from the Italian Peninsula.

The investigation of dietary proclivities of fossil artiodactyls and perissodactyls also provided information about the palaeoenvironmental conditions of habitats of the Central Italian Peninsula that may have acted as refugia for human populations during the Middle Pleistocene, which were characterised by both closed and open landscapes with a relative predominance of open woodlands. The notable difference between dental mesowear and microwear patterns of the fossil aurochs from Fontana Ranuccio points to long hominid occupation of the region even in adverse seasons.

With the implementation of dental wear analysis, a taxon-free method, on fossil mammal assemblages of the Italian and Iberian Peninsula, it has been possible to observe that some groups of ungulates did not always occupy the same niches during the Pleistocene, as in the case of Early Pleistocene cervids (i.e. *Eucladoceros*, "*Axis*-like" deer, *Dama vallonnetensis* and *Praemegaceros*) which are often considered only browsing forest dwellers, but could actually vary their dietary behaviours and take advantage of different habitats.

Some of the limitations of these taxon-free methodologies were addressed and the results obtained by testing *MicroWeaR* suggest that with future refinements, this open access and open source nature software can be implemented also in other fields where an in-depth investigation of microscopic patterns is required (e.g. palaeoanthropology, archeology and forensic sciences).

Findings from the analysis of ungulate dental wear patterns at different timescales highlight the pivotal role of seasonality in shaping the terrestrial ecosystems of Mediterranean Europe during and after the establishment of glacial cycles ruled by a 100 kyr periodicity. Future researches on long- and short-term dietary adaptations of fossil herbivores and a combined approach with other palaeoenvironmental proxies (e.g. tooth enamel stable isotope and palynological analysis) will also

help to understand how other major climatic events of the Pleistocene may have influenced

terrestrial ecosystems in Southern Europe.

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