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Ancient DNA Contradicts the Presence of Social Voles (Genus *Microtus*, Subgenus *Sumeriomys*) in the Late Pleistocene of Western Europe

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Abstract: Taxonomic decisions made by palaeontologists are often based on a few morphological features preserved in the fossil material. This practice may sometimes lead to the description of new species based on single specimens, which are, in fact, extreme or aberrant morphological variants of known taxa. Ancient DNA (aDNA) analysis of the Late Pleistocene specimens from the archaeological site Petits Guinards (Creuzier-la-Vieux, Allier, France), described as a new vole *Microtus* (*Sumeriomys*) *bifrons*, did not confirm the species distinctness of the studied population. The genetically examined specimens belonged to *Stenocranius anglicus* and/or *Microtus arvalis*, the dominant species at the site. Our findings show that it is risky to describe new fossil taxa on the basis of phenotypic outliers or morphologically aberrant, rare specimens that do not fall within the previously known population variability. We also highlight the importance of ancient DNA in resolving taxonomic and nomenclature problems and classifying fossil mammals of the Late Pleistocene age.

Keywords: arvicolines; taxonomy and systematics; dental morphology; ancient DNA; *Cytb*; last glacial period

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1. Introduction

Rodents belonging to the subfamily Arvicolinae (voles and lemmings) are the second most diverse subfamily of cricetids (Cricetidae) [1], with species distributed in the Palearctic and Nearctic and a rapid rate of speciation [2]. Therefore, there is ongoing progress in the systematics and taxonomy of extant fauna of this group of mammals, resulting in the description of new species or verification of the taxonomic status of some of them [3]. The central and most speciose genus of Arvicolinae is *Microtus* Schrank, 1798 with ca. 60 species when one narrowly defined this taxon (excluding *Lasiopodomys* Lataste, 1887, *Neodon* Horsfield, 1951 and *Alexandromys* Ognev, 1914) [1]. The genus was also common and diversified in Europe during the Late Pleistocene.

The Late Pleistocene species of *Microtus* are relatively well known as most of them represent extant taxa. However, still new species are occasionally described based on morphological evidence. An example is a new species of pine vole *Microtus* (*Terricola*) *grafi* Brunet-Lecomte, Nadachowski et Chaline, 1992 from Bacho Kiro in Bulgaria. It has been distinguished on the basis of the morphometric features of the first lower molar (m1), which show intermediate metric parameters between *Microtus* (*Terricola*) *subterraneus* (Sélys, 1836) and *Microtus* (*Terricola*) *multiplex* (Fatio, 1905) [4]. A second example is a new species from the group of social voles, *Microtus* (*Sumeriomys*) *bifrons* Jeannet et Fontana, 2015 described

from the Les Petits Guinards site in southern France, which was distinguished on the basis of m1 measurements and morphology [5]. Morphological analyses have recently been supplemented with studies of ancient mitochondrial DNA (aDNA), which significantly expanded the interpretation possibilities of the paleontological materials. Molecular tools have been successfully used to study the demographic history of small mammals from the Late Pleistocene and both morphological and molecular data should be coupled whenever possible. Recent studies of ancient mitochondrial DNA (mtDNA) allowed revision of the taxonomic status of the Late Pleistocene European narrow-headed vole and ranking it as *Stenocranius anglicus* (Hinton, 1910), a species distinct from the Asiatic *Stenocranius gregalis* (Pallas, 1779) [6,7]. In another case, among Late Pleistocene grey voles (subgenus *Microtus*) from southeastern Europe, a new cryptic species was discovered representing a divergent lineage of the Tien Shan vole (*Microtus ilaeus* Thomas, 1912) [8].

The scope of this study Is to review the taxonomic status of *Microtus bifrons* based on ancient DNA and explore its taxonomic relationship with other species of *Microtus*.

2. Material and Methods

2.1. Ancient Sample and Morphological Methods

A new vole species (*Microtus bifrons*) was described from Petits Guinards (Creuzier-la-Vieux, Allier, France), a rockshelter discovered in 1981 and excavated in subsequent years [9]. The site yielded numerous lithic and bone industries and fauna of large and small mammals (altogether about 125,000 remains), dated to the period between ca. 19,500 and 13,230 uncal. years BP [5,10,11]. Isolated first lower molars (m1) of five specimens from Petits Guinards classified as *Microtus bifrons* nov. sp. were chosen for DNA analyses. Voles of the genus *Microtus* can be recognised in the fossil record based on morphology of the m1. Tooth morphologies were studied following Nadachowski [12]. The nomenclature of dental pattern followed guidelines in Hibbard [13] and van der Meulen [14]. The total length of the tooth was measured following guidelines in van der Maulen [14]. We investigated teeth of various morphology of the anteroconid complex (ACC) and different degrees of development of BSA 4, BSA 5, LSA 5, and LSA6, as well as T6–T9 (explanations of acronyms are shown in Figure 1A) to preserve intraspecific morphological variation. Among the examined specimens there occurred paratype (MI2869), distinguished by Jeannet and Fontana [5] (Figures 4/14 and 16b, CVPG 47-957). Taxonomy and nomenclature followed Kryštufek and Shenbrot [3] with some modifications. For example, narrow-headed voles have been ranked as a genus *Stenocranius* Kashchenko, 1901 and not as a subgenus of *Lasiopodomys*. The names of the morphotypes (e.g., "*extratriangulatus*") were assumed after Nadachowski [15,16].

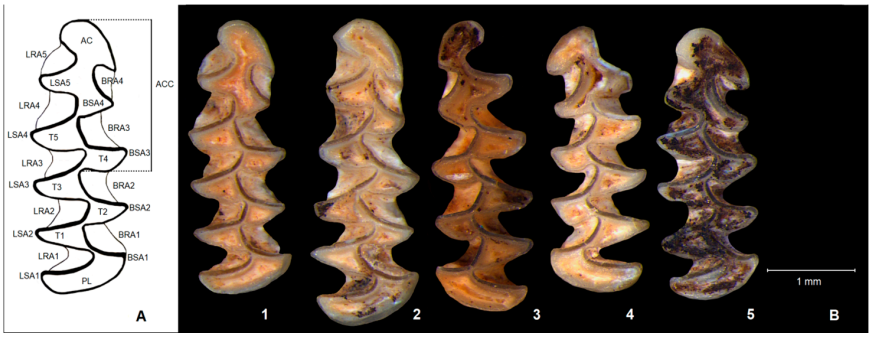

Figure 1. (**A**) dental nomenclature of Arvicolinae first lower molars (m1) (after Hibbard [13] and van der Meulen [14]); AC—anterior cap, T—dental triangle, ACC—anteroconid complex, LRA–lingual re-entrant angle, LSA—lingual salient angle, BRA—buccal re-entrant angle, BSA—buccal salient angle, PL—posterior lobe; (**B**) 1–5—*Microtus bifrons* from Les Petits Guinards, occlusal surface of first lower molars (m1); 1—MI2868 (original collection number: CVPG 18-357, Figure 4/15 in [5]), 2—MI2869 (CVPG 47-957, Figure 4/14), 3—MI2870 (CVPG 59-1309, Figure 4/2), 4—MI2871 (CVPG 56-1213, Figure 4/8), 5—MI2872 (CVPG 51-1065, Figure 4/13).

2.2. DNA Extraction, Enrichment and Sequencing

DNA extraction and pre-PCR library preparation steps were performed in the dedicated ancient DNA laboratory at the Centre of New Technologies at the University of Warsaw. Each tooth was washed with 500 µL of ultrapure water in a 2 mL tube and crushed with a pipette tip. DNA was extracted following the protocol optimised for short DNA molecules [17]. To control possible contamination, a negative control without biological material was processed alongside each batch of 15 samples. For each specimen we prepared two libraries: a double-stranded one following Meyer and Kircher [18] and a single-stranded one following Gansuage et al. [19], each from 20 µL of DNA extract.

The libraries were enriched for vole mtDNA using the in-solution target enrichment protocol described by Horn [20]. A hybridisation bait set was produced from amplified mtDNA of various arvicoline species including the common vole *Microtus arvalis* (Pallas, 1779), field vole *Microtus agrestis* (Linnaeus, 1761), root vole *Alexandromys oeconomus* (Pallas, 1776), bank vole *Clethrionomys glareolus* (Schreber, 1780), and narrow-headed vole *Stenocranius gregalis* (Pallas, 1779). This bait set was efficient in enriching libraries in mtDNA molecules of species divergent from the ones used to build this set, such as the collared lemming *Dicrostonyx torquatus* (Pallas, 1779) [21]. Up to five libraries were pooled for enrichment reaction. Two rounds of hybridisation were performed at 65 °C for 22–24 h each. After each round enriched libraries were amplified in tree replicates for 10–15 cycles. The enriched pools were combined, quantified using Qubit 4 Fluorometer (ThermoFisher, Waltham, MA, USA), and sequenced on the Illumina NextSeq550 platform (San Diego, CA, USA) using MID output, 2 × 75 bp kit (see Supplementary Information for more details).

2.3. Sequencing Data Processing

The raw sequencing reads were demultiplexed using bcl2fastq v. 2.19 (Illumina). Overlapping reads were collapsed, and adaptor and quality trimmed using AdapterRemoval v. 2.2.2 [22]. The reads were mapped using the MEM algorithm in BWA v. 0.7.17 [23] to the mtDNA genomes of three species: *Microtus guentheri* (Danford et Alston, 1880), a representative of the subgenus *Sumeriomys* Argyropulo, 1933; *M. arvalis*; and *S. anglicus*. The two latter are the most common species identified at Les Petits Guinards. Each reference was combined with a human mtDNA sequence. The competitive mapping approach eliminates possible contamination with human DNA [24]. In each case, duplicates, as well as short (<30 bp) and low mapping quality (MAPQ < 30) reads, were filtered out using *samtools v. 1.7* [25]. Variants and consensus sequences were called using *bcftools* v. 1.7 and *ivar* v. 1.3 [26]. The bam alignment files were inspected in Tablet v. 1.21 [27]. Positions with a coverage lower than 3 were masked, and bases supported by <75% of the reads were coded with the appropriate IUPAC symbol. MapDamage v.2.08 [28] was used to assess the damage patterns and length distribution of the DNA molecules. In the case of specimens identified as *S. anglicus* we called the entire mtDNA genome, while in the case of *M. arvalis* we called only 4.2 kb fragment as in Baca et al. [29] (see Supplementary Information for more details).

2.4. Phylogenetic Analyses

We preliminarily assigned specimens to species based on the number of reads mapping to specific mtDNA genome and further verified based on the percentage of divergence of mapped reads from the reference calculated by Tablet software. To further confirm taxonomic assignment of samples we reconstructed a phylogeny based on a dataset of 24 species representing main genera of Arvicolinae. We used IQ-Tree2 [30] to reconstruct Maximum Likelihood phylogeny based on 3 kb mtDNA fragment spanning nad5, nad6, and *Cytb* genes and some tRNAs. We used this fragment because it was the only one available for the specimen identified as *M. arvalis*. We used previously published intraspecific mitogenomic datasets on the common vole (*Microtus arvalis*) [29] and the European narrow-headed vole (*Stenocranius anglicus*) [7] to estimate the phylogenetic position and age of the samples using the tip-dating approach [31]. The common vole dataset consisted of

199 sequences of 4.2 kb fragment of mtDNA. Fifty-one sequences were from modern specimens whereas 148 were from ancient ones. The specimens originated from multiple localities across Europe and their ages ranged from ca. 55 ka to modern times. Twenty sequences came from directly radiocarbon-dated specimens. The *Stenocranius anglicus* dataset consisted of 145 partial mitogenome sequences of which six came from modern and 139 from ancient specimens originating from localities across Europe and Western Asia. Ten sequences came from directly radiocarbon-dated specimens. We performed phylogenetic analyses using BEAST 1.10.4 [32] and parameters established previously for each dataset. First the age of each specimen was estimated in separate analyses using sequences of modern and radiocarbon-dated specimens to calibrate the molecular clock before we ran joint analysis using the whole dataset. In this analysis we fixed ages of not dated specimens to the values estimated previously [7,29] and we set lognormal priors on the ages of the specimens from Les Petits Guinards based on the results of individual age estimations (see Supplementary Information for more details).

3. Results

3.1. Morphological Characteristics of M. bifrons

A new species was described due to the peculiar morphology of the anteroconid complex of m1 relative to that of other *Microtus* species found in Petits Guinards. Species of the genus *Microtus* s.l. (understood by the authors broadly, including *Stenocranius*, *Alexandromys*, and *Chionomys* Miller, 1908) were dominant in the assemblages of the small mammals at this site (Figure 2 in [5]). Among the 1776 m1 belonging to *Microtus arvalis* (687), *Microtus agrestis* (79), *Stenocranius gregalis* (919), *Alexandromys oeconomus* (87), and *Chionomys nivalis* (Martins, 1842) (4), Jeannet and Fontana [5] selected 16 m1 of unusual morphology (Figure 4 in [5]) and described them as *Microtus* (*Sumeriomys*) *bifrons* nov. sp. They defined the new species as a large-sized vole with six or seven dental triangles (T6–T7), sometimes five (T5), and a highly dissymmetrical anterior cap with voluminous spur-shaped BSA 5. In most specimens the anterior part of the tooth (AC + T7 + T8) is wider and flatter than that of *M. arvalis* and *M. agrestis*, and is characterised by a pronounced development of BSA 5 and LSA 6. However, if someone carefully looks at the specimens classified as *M. bifrons* (Figure 4 in [5]), each individual differs from the others in morphological details, and it is difficult to identify features common to all specimens. The holotype (CVPG 57-1248a, Figure 16a in [5]) and the paratype (CVPG 47-957, Figure 16b in [5]) are clearly larger than the other teeth (in both L = 3.45 mm), with the almost identical anteroconid complex, probably representing one individual.

The morphology of the five genetically studied individuals is characterized as follows (Figure 1):

1. MI2868 (CVPG 18-371) (right m1, L = 3.04 mm): five triangles (T1–T5); T6 and T7 broadly confluent; BSA 4 less pronounced in comparison with LSA 5; and anterior cap (AC) wide with spur-shaped BSA 5 (Figure 1/1);
2. MI2869 (CVPG 47-957) (right m1, L = 3.45 mm, paratype): seven dental triangles (T1–T7); T6 and T7 separated; BSA 4 and LSA 5 almost of the same width; and anterior cap (AC) wide, with spur-shaped BSA 5 (Figure 1/2);
3. MI2870 (CVPG 59-1309, left m1, L = 3.22 mm): five dental triangles (T1–T5); T6 and T7 confluent; BSA 4 much less pronounced in comparison with LSA 5; and anterior cap (AC) separated from T6–T7 with small BSA 5 (Figure 1/3);
4. MI2871 (CVPG 56-1213, left m1, L = 2.95 mm): five dental triangles (T1–T5); T6 and T7 broadly confluent; BSA 4 pronounced as much as LSA 5; and anterior cap (AC) not fully separated from T6–T7 with pronounced BSA 5 and very small LSA 6 (Figure 1/4);
5. MI2872 (CVPG 51-1065, right m1, L = 2.98 mm): five dental triangles (T1–T5); T6 and T7 broadly confluent; BSA 4 pronounced as much as LSA 5; and anterior cap (AC) wide with spur-shaped BSA 5 (Figure 1/5).

The remaining specimens presented in Figure 4 [5] are mostly smaller (L ranges from 2.80 to 3.22 mm), have a variable anteroconid complex, and, in most of them, the anterior cap is morphologically different from the holotype and paratype. Therefore, the opinion of homogeneity of the sample distinguished as a new species cannot be maintained. They might represent aberrant or phenotypic outliers of *M. arvalis*, *M. agrestis*, and/or *S. anglicus.*

3.2. Phylogenetic Analysis and Molecular Dating

We tentatively assigned the specimens from Les Petit Guinards to species based on the number of reads mapping to mtDNA genomes of three vole species: common vole, narrow-headed vole, and Gunther's vole (Table 1), and the divergence of mapped reads from the respective reference sequence calculated by the Tablet software.

Table 1. Genetic assignment of Les Petit Guinards samples. Lib: DNA library type; DS–double stranded; SS–single stranded. For each sequencing library the highest number of mapping reads is given in bold.

Sample	Lib	Reads	Unique Mapped Reads			Assigned Species	Mean mtDNA Coverage	Fraction of mtDNA Genome Recovered *	Estimated Age (Cal BP)	
			M. arvalis	*S. anglicus*	*M. guentheri*				Median	95% HPD
MI2868	SS	990,916	217	1439	345	*S. anglicus*	8.4	0.83	19,290	[22, 212, 16, 004]
	DS	593,827	403	1133	489					
MI2869	SS	4,776,656	475	2440	629	*S. anglicus*	17.5	0.95	20,819	[23, 474, 16, 858]
	DS	2,436,718	1048	2500	1102					
MI2870	SS	701,333	110	608	161	*S. anglicus*	5.1	0.69	19,735	[22, 575, 15, 943]
	DS	808,330	316	874	352					
MI2871	SS	86,906	238	75	123	*M. arvalis*	2.9	0.46	n.a.	n.a.
	DS	280,844	521	316	431					
MI2872	SS	3,510,503	8339	2837	4884	*M. arvalis*	27.8	0.96	23,410	[27, 369, 19, 290]
	DS	113,370	862	416	639					

*—in the case of *M. arvalis*, fraction of the analyzed 4.2 kb fragment is given.

Three of the specimens were assigned to *S. anglicus* and two to *M. arvalis*. The results from two libraries produced from the DNA extracted from each specimen were consistent. In each library the mapped DNA molecules exhibit the deamination pattern characteristic for ancient DNA (Supplementary Figures S1 and S2). For the three specimens identified as *S. anglicus* and one as *M. arvalis* the number of reads uniquely mapped to the respective mtDNA reference was sufficient to reconstruct partial mtDNA genomes (Table 1). We used the resulting mtDNA sequences to confirm the taxonomic assignment and to estimate the phylogenetic position and the age of these specimens.

The reconstructed Maximum Likelihood phylogeny of Arvicolinae showed topology identical to those obtained in previous studies [6,33] and confirmed taxonomic assignment of samples from Petit Guinards as *S. anglicus* and *M. arvalis* (Figure 2).

The reconstructed intraspecific phylogeny of narrow-headed voles was similar to the previous ones [7] and revealed three main lineages corresponding to the Asiatic *S. gregalis* and *S. raddei* (Polyakov, 1881) and the European *S. anglicus* (Figure 3A). Six lineages were distinguished (AA–AF) within the *S. anglicus* clade. The three *S. anglicus* specimens from Petit Guinards were placed in the lineage AF and yielded ages between 20.8 and 19.3 ka ago (Table 1, Figure 3A). The AF lineage was widespread in Europe until ca. 40 ka ago, but later it became confined to southwestern France [7].

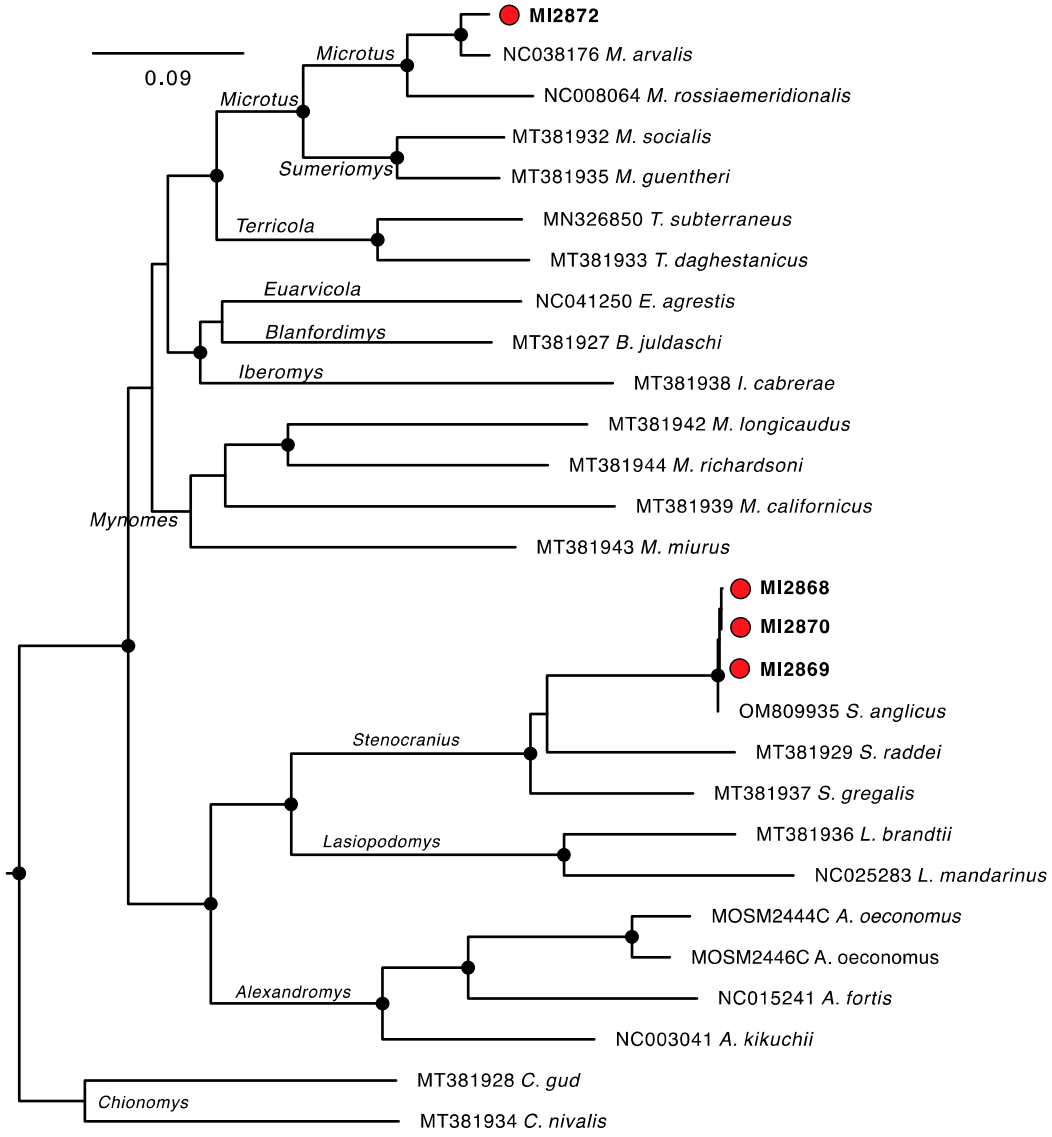

Figure 2. Maximum Likelihood phylogeny of Arvicolinae rodents based on 3 kb fragment of mtDNA. Black circles at nodes indicate ultrafast bootstrap support higher than 90. Tree is rooted with the sequence of *Arvicola amphibius* (not shown). Red circles denote samples analyzed in this study. Generic names are given above the branches and follow the nomenclature of Abramson et al. [33] and Kryštufek and Shenbrot [3].

The three investigated specimens were closely associated with specimens of similar age from the Taillis des Coteaux site in Central Western France.

The reconstructed phylogeny of the common vole revealed a topology similar to that obtained in a previous study [29]. Six main lineages were detected. The studied specimen belonged to the WN lineage which occurred, starting at least 30 ka ago, in Western Europe except for the Iberian Peninsula. It was the most closely related to the specimen from the Coulet des Roches site from southern France and yielded an age of 23.4 ka.

The estimated age and phylogenetic position of the four specimens are highly consistent with the dating and geographic location of the site. This further strengthens the reliability of molecular analyses.

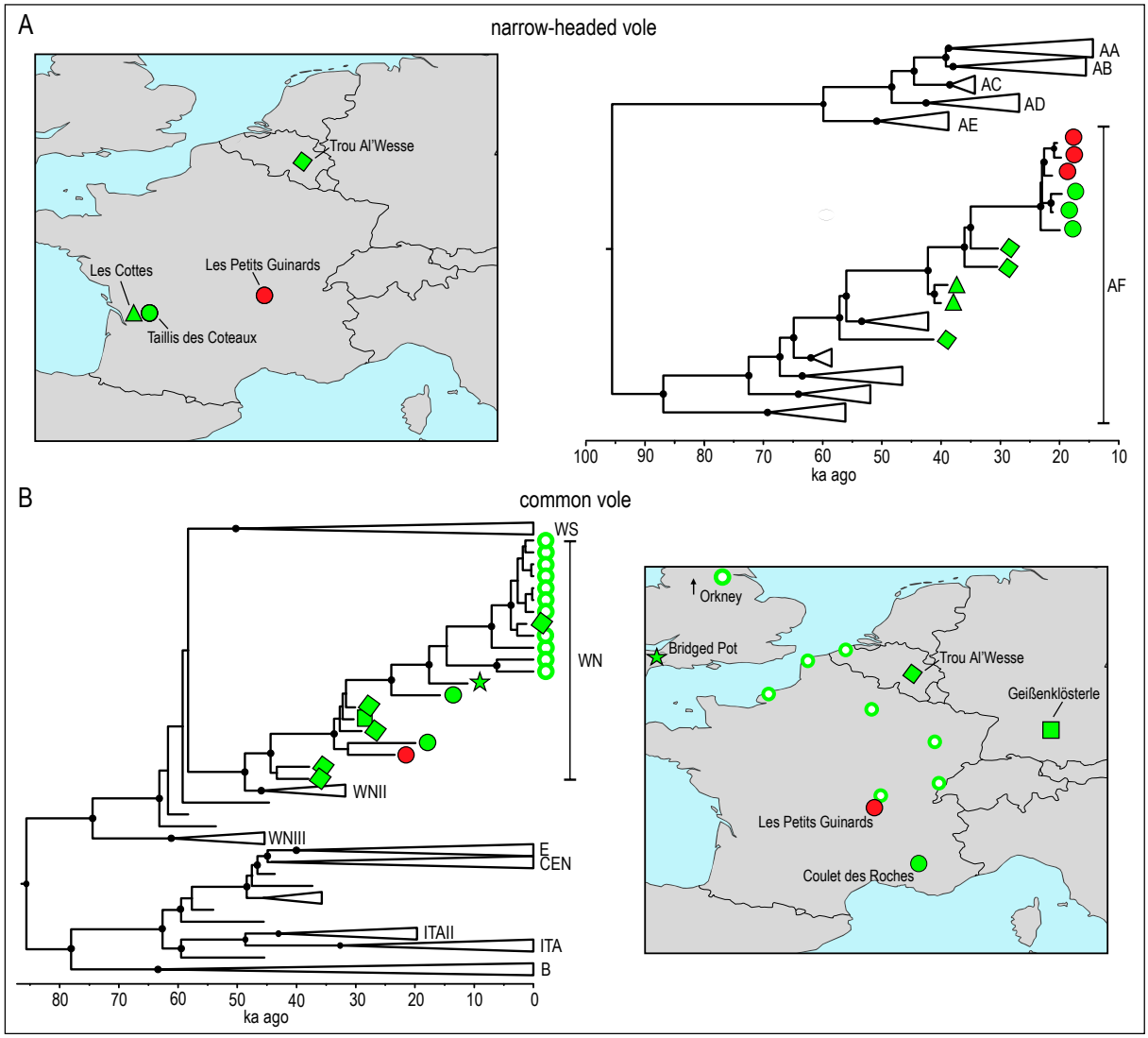

Figure 3. Genetic analyses of Les Petits Guinards specimens. (**A**) The Bayesian phylogeny of European narrow-headed voles (*Stenocranius anglicus*) based on mtDNA genome sequences. The Asiatic lineages (*S. gregalis* and *S. raddei*) are not shown. (**B**) The Bayesian phylogeny of European common voles (*Microtus arvalis*) based on 4.2 kb fragment of mtDNA. On both panels, the majority of the branches were collapsed and only a fragment containing sequences originating from Les Petits Guinards is presented. Symbols at tips denote localities, as presented on the accompanying maps. The filled symbols represent Late Pleistocene and empty symbols represent modern localities. Black dots indicate nodes with posterior probability above 0.95.

4. Discussion

4.1. Taxonomic Attribution Revealed from Morphological Characteristics and Genetic Research

From the whole set of available specimens Jeannet and Fontana [5] selected a few individuals of peculiar anteroconid complex, which did not fit well into the observed variation of *M. arvalis*, *M. agrestis* and/or *Stenocranius*. The specimens designated as *M. bifrons* have an additional triangle (T6) or two supernumerary (T6 and T7) triangles within the ACC of m1. Almost half of the Petits Guinards population (7 specimens, 43.7%) exhibits these morphological features (Figures 4/1, 3, 5, 9, 10, 14 and 16 in [5]). An equally important feature (10 specimens, 56.2%) is the very wide anterior cap (AC), highly dissymmetrical with voluminous spur-shaped T8 (Figures 4/1, 7–9 and 11–16 in [5]). In light of findings of previous studies [34,35], as well as morphometric analyzes (Figures 8 and 9 in [5]), Jeannet and Fontana [5] concluded that the aberrant teeth may belong to one of the representatives

of the social voles, subgenus *Sumeriomys.* Although most of the previous studies paid little attention to the detailed morphology of m1, some noted the presence of extra triangles and/or very wide anterior cap in the first lower molars of extant and subfossil *Sumeriomys* [35–37], which coincide with the features of *Microtus* (*Sumeriomys*) *bifrons* nov. sp. Currently, social voles consist of at least eight nominal, partly cryptic species, primarily diversified in Turkey and Western Iran with marginal occurrence from southern Balkans to Central Asia and North Africa (Libya) [3].

Genetic identification of the five specimens attributed to *M. bifrons* revealed that three belonged to *S. anglicus* and two to *M. arvalis*, but this identification was in some cases inconsistent with morphological examination results and required further discussion. Morphology of the two first specimens (MI2868, MI2869), which according to genetic data belong to the European narrow-headed vole (*Stenocranius anglicus*), contradict their attribution to this species (Figure 1/1–2). The genus *Stenocranius* is traditionally characterised by a complete absence or a strong reduction of T6 and BSA 5 in m1 and a relatively small anterior cap. These are the most important features distinguishing *Stenocranius* from *Microtus* species in the fossil record. In contrast, the two specimens have an extensively built anteroconid complex with a fully developed T6 and a wide anterior cap with pronounced BSA 5 and LSA 6. Such occlusal morphology of m1 has never been described earlier in both extant and fossil populations of *Stenocranius* [12,38–50].

The third specimen (MI2870) genetically assigned to *Stenocranius* has a reduced T6, has not pronounced BSA 5 and LSA 6 (Figure 1/3), seems to be a morphologically advanced *S. anglicus*, and is known as a most complex morphotype in extant populations of *S. gregalis* [40,47]. It has also been described from a number of sites in the Late Pleistocene of Eurasia [12,38,42,46,49,51].

The last two genetically studied specimens (MI2871 and MI2872) belong to *Microtus arvalis* (Figure 1/4–5). The first of them has an aberrantly shaped anterior cap, a not developed LSA 6, and a strongly marked BSA 5, which causes a large asymmetry of AC. Such a morphology is very rare in extant and subfossil populations of *M. arvalis* (Figure 15 D3 in [12], Figure 6 in [52]) and also occurs in some other species, e.g., in *M. transcaspicus* Satunin, 1905 (Figure 307b′ in [3]) and *M. irani* Thomas, 1921 (Figure 317a′ in [3]). The last genetically determined m1 is similar to that of the holotype and paratype of *M. bifrons* with a very wide anterior cap, but differs from them by having confluent T6 and T7.

4.2. Supernumerary Triangles

The genus *Microtus* s. l., as treated by Jeannet and Fontana [5], is characterised by the presence of five closed triangles (T1–T5) in m1 and the differently developed anteroconid part of the tooth where diagnostic features of individual species can be identified. The presence of additional closed triangles (T6 and T7) was the basis for the description of the new species [5], in agreement with Ognev (Figure 163 in [34]) who used the presence of extra triangle in m1 to distinguish one of the species of social voles—*Microtus* (*Sumeriomys*) *socialis* (Pallas, 1773).

However, the morphotype with one extra developed triangle (T6), sometimes called "*extratriangulatus*" (Figures 20 and 21 in [15]), is found with a low or very low frequency in almost all extant gray voles (genus *Microtus*) and narrow-headed voles (genus *Stenocranius*). In social voles (subgenus *Sumeriomys*) this morphotype is rarely illustrated in publications, e.g., in *M. socialis* and *M. dogramacii* Kefelioğlu et Kryštufek, 1999 (Figures 313a′ and 326d′,f′, respectively, in [3]), without specifying the frequency of its occurrence in various *Sumeriomys* species. The frequency of the "*extratriangulatus*" morphotype can vary greatly in *M. agrestis* and range from ca. 5–6% (Figure 1d,f,g, Table 1 in [53]), through 7% (Figure 4f in [54]) to ca. 17% (Figure 20/49–50 in [16] and Table 1, Abb. 1e,f in [55]). This morphotype is present in populations from the Urals and Western Siberia (Figure 43 in [46]) and was also recorded in *Microtus lavernedii* (Crespon, 1844) (Figure 253f′ in [3]), a close relative of *M. agrestis*. Specimens with an extra triangle have already been found in *Microtus nivaloides* Major, 1902, a putative direct ancestor of *M. agrestis* [16,56–58]. The

"*extratriangulatus*" morphotype is present in geographically distant sites dated to the end of the Early Pleistocene from the Kozarnika Cave in Bulgaria (Figure 6/10 in [59]) and Chigirin, Ukraine (Figure 12–13/13 in [60]), as well as to the early Middle Pleistocene (from MIS 17 to MIS 11), for example, in West Runton in the UK (Figure 65/3 in [61] and Figure 19/50–51 in [16]), Kozi Grzbiet in Poland (Figures 20–22 in [15]), Za Hájovnou Cave in Czechia (listed as *Microtus* aff. "*coronensis*") (Text-Figure 2/D in [62]), Semybalka 1 (Figure 13/11 in [57]) and Morozivka 2 (Figure 3/19 in [63]) in the Ukraine, and in Gornopravdinsk 2, West Siberia, Russian Federation (Figure 3 in [58]). This morphotype probably extended its distribution and gradually increased its frequency in the late Middle Pleistocene, as it is quite common, for instance, in *Microtus agrestis* from Saint-Estève-Janson in France (Figures 32/14, 15, 16 and 22 in [38]). The morphotype "*extratriangulatus*" was also recorded in the Late Pleistocene population of *M. agrestis* in Teixoneres Cave, Spain (Figure 3/5–6 in [64]), Porlyuk Cave and Istállóskő Cave, Hungary (Figures 4/M and 4/P, respectively, in [65]), as well as Baranica Cave, Serbia (Figure 5e in [66]). Nadachowski [12] estimates the frequency of "*extratriangulatus*" in the Late Pleistocene populations of Poland at 7–24% (Figure 19 in [12]), depending on the studied population and site.

In extant populations of *M. arvalis*, the "*extratriangulatus*" morphotype is much less common, although Rörig and Börner [67] present a drawing of such a tooth (Taf. V, Figure 106 in [67]). This morphotype does not occur in most of the studied extant populations; however, its frequency in some populations ranges from 0.8% (Figure 4b in [54]) to 4.0% (Figure 3/7, Table 5 in [68]). In fossil populations of *M. arvalis* from the Middle Pleistocene, teeth with an additional T6 triangle have been observed from Bulgarian locality Morovitsa Cave (Figure 5/17 in [69]), some Late Pleistocene samples from France (Figure 31/8 in [38]), and Holocene assemblages from Poland (Figure 15/J in [12]). Although the morphotype "*extratriangulatus*" was absent in extant species belonging to the *M. arvalis* group, subfossil specimens with an extra triangle have been described from Bacho Kiro Cave (Figure 9/I in [70]) and Cave 16 in Bulgaria (Figure 22/8 in [71]), which most likely belong not only to *M. arvalis* but also to *M. ilaeus* [8].

Publications describing the variability of m1 in *Stenocranius* consider the presence of the "*extratriangulatus*" morphotype, although its frequency is always very low (Figure 23/H_3 in [12], Figure 24/VI-P in [37], Figure 38/5 in [42], and Figure 38/5 in [46]). A tooth with an additional triangle was illustrated for *Stenocranius gregalis* from Zabaykalsky Krai, Russian Federation (Figure 239 a′ in [3]).

Whereas one supernumerary triangle (T6) is relatively common in some populations of *Microtus* species, two supernumerary triangles are reported in the literature only occasionally. In extant populations, such specimens have been documented in *Microtus arvalis* from Croatia (Figure 297c′ in [3]) and *M. agrestis* from Germany (Figure 1e in [53]), as well as in the subfossil population of *M. arvalis* from Bacho Kiro Cave in Bulgaria (Figure 9/J in [70]). A morphotype with two closed triangles in *M. agrestis* has also been published from Khotylevo 2, Ukraine (Figure 33/18 in [60]) and from Western Siberia (Figure 44/2 in [46]).

This study shows that the claim that additional triangles/prisms are present only in the *Sumeriomys* subgenus and cannot be further maintained.

4.3. Wide and Spur-Shaped Anterior Cap

Another distinguishing feature of *Microtus bifrons* is a wide anterior cap with a specific spur-shape morphology on the buccal side, developed independently of the number of closed triangles [5]. The wide or very wide anterior cap occurs primarily in species of the subgenus *Sumeriomys*, e.g., in *M. guentheri* (Figure 2 in [35] and Figure 5A,B in [37]) and *M. hartingi* Barrett-Hamilton, 1903 (Figure 5A in [72]) from Turkey and Bulgaria, as well as subfossil populations of *M. guentheri* from Israel (Figure 66a–c in [36]). This type of morphology also occurs in *Microtus arvalis* from Croatia (Figure 297c′ in [3]) and *M. mystacinus* (Filippi, 1865) from Iran (Figure 302c′ in [3]). A wide anterior cap is rare in subfossil populations of *M. arvalis* and *M. agrestis* from Poland (Figures 15/G4 and 18/H,

respectively, in [12]). However, in all these cases the spur-shape buccal triangle is not as distinct as in *M. bifrons*.

4.4. Reasons for Incorrect Species Determination Based on Morphological Criteria

In the evolution of Arvicolinae rodents, complication of molars by adding new elements to the crown is a general trend that reflects adaptation to the low-calorie diet of vegetative plant parts, especially grasses [73]. Voles and lemmings developed several structural adaptations in dentition such as high crown check teeth with cusps transformed to prisms and flat occlusal surfaces with enamel cutting edges to increase and improve the efficiency of grating [61]. The crown complexity and occlusal regularity of Arvicolinae teeth are characters closely associated with fitness [74,75]. The frequencies of extra elements of occlusal surface in natural populations is a variable trait that tends to exhibit high frequencies in small populations during the periods of relatively low species abundance [76]. Therefore, the contribution of naturally occurring bottlenecks to phenotypic evolution under natural selection is the most plausible explanation.

A comparison of laboratory colonies of *Dicrostonyx* and *Microtus* with wild populations suggests that dental aberrations typical of small and inbred populations are also present in the wild and are not eliminated by large-scale population dynamics [76,77]. The emergence of atypical, aberrant individuals in small and geographically isolated populations is probably a relatively common phenomenon. Aberrant specimens may increase when a population is spatially restricted, and inhabits an isolated niche in a mosaic landscape. It was hypothesise that the signs of the intensified genetic drift might be associated with the patchy distribution of the species in heterogeneous landscapes [75]. The occurrence of peculiar morphology in the relic population of *Dicrostonyx* from the Chaleux site (Belgium), probably shortly before the extinction of this species in Europe, might be an example of this phenomenon [78]. Extremely rare dental traits related to close breeding in extant species were documented in *M. arvalis* [79] and extant populations of *D. torquatus* [76]. For example, an unusual specimen with seven triangles was identified in the population of *M. arvalis* from Cetinsko polje, Croatia (Figure 297c′ in [3]). The population at this site is isolated and inhabits a karstic field surrounded by eroded and frequently barren slopes (B. Kryštufek–pers.info.). The probability of trapping individuals with a rare dental trait in extant populations is higher during the periods of low species abundance when the species is confined to source habitats or live in ephemeral populations formed after major outbreaks [76].

When the characters related to small population sizes occur simultaneously in different species in one fossil assemblage, we can attribute them to the impact of important changes in the environment [76]. The appearance of the extra wide anterior cap with spur-shaped buccal salient angle might be explained as an adaptation to local food resources in the vicinity of the Petits Guinads site. Ecomorphological adaptation is supported by the fact that almost identical anterior caps developed in the specimens genetically confirmed as *S. anglicus* (Figure 1/1–2) and *M. arvalis* (Figure 1/5).

It seems that the morphological variability of molar teeth within one species is unexpectedly high. The nature of this variability is complex and depends on many factors, which are sometimes difficult to identify [80]. Therefore, one should be very cautious with species identification based exclusively on extraordinary morphological criteria.

5. Conclusions

The most unexpected finding of this study was the discrepancy between the conclusions based on analyses of aDNA and those based on morphological characters. In light of this genetic study, the morphology-based taxonomic attribution of the studied specimens cannot be maintained. Literature on m1 morphological variation in extant species of *Microtus* and *Stenocranius*, as well as fossil populations of these genera, showed that the criteria proposed to distinguish a new taxon based solely on m1 variation are not sufficient for taxonomic decisions at the species level. Specimens described as *Microtus bifrons* are

just extreme, very rare morphological variants of the most common Petits Guinards species and should be treated as outliers.

The frequency of rare morphotypes keeps below 1% or almost zero, but their manifestation is sometimes spectacular. Such phenotypic outliers fall outside the expected species-specific range of variability, can also be found in related taxa, and their presence in subfossil populations depends on the size of the sample; the greater it is, the higher is the chance to find aberrant specimens.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15040538/s1, Figure S1: DNA damage patterns produced by mapDamage software for double-stranded libraries produced in this study. The double-stranded libraries were not USER treated; Figure S2: DNA damage patterns obtained using mapDamage software for single-stranded (SS) libraries produced in this study; Table S1: Primers used to generate mitogenomes from various vole species; Supplementary Information S1: Supplementary materials and methods. References [81–83] are cited in the supplementary materials.

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