Trophic differentiation between the endemic Cypriot mouse and the house mouse: a study coupling stable isotopes and morphometrics

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Abstract

Two cryptic species of mice coexist on Cyprus: the introduced house mouse and the endemic Cypriot mouse, that remained unnoticed until the beginning of the 21st century. Their trophic position was investigated using isotopic ecology. The shape and biomechanics of the mandible provided a complementary insight into their respective diets. The Cypriot mouse exhibits generalist habits relying on various natural food resources, including invertebrates, while the house mouse exploits a broad spectrum of anthropic food resources. The Cypriot mouse has a large mandible optimized for chewing at the molars that facilitates consumption of large and hard food items presumably abundant in the natural vegetation of Cyprus. The small mandible size of the house mouse is compensated by a large masseter area and an optimization for incisor biting, making it an all-rounder tool for foraging on diverse non-natural items. This fine-tuning of generalist feeding behavior ensures efficient niche partitioning between the two species on Cyprus.

Keywords. Biomechanics, geometric morphometrics, isotopic ecology, mandible morphology, *Mus cypriacus, Mus musculus domesticus*.

Author contribution. SR, EAH and LS designed the study. SR, MB and EF acquired and analyzed the morphometric data; LS, MB, EF and FF performed the isotopic analyses. EAH and GPM supervised the trapping campaign and ElH collected samples of basal resources and participated to the trapping. SR, EAH and LS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Introduction

During the Pleistocene, Mediterranean islands hosted a low taxonomic diversity but included many endemic species. These species featured emblematic species such as dwarf elephants (*Palaeoloxodon cypriotes* and *Palaeoloxodon xylophagou*) and hippopotami (*Phanourios minor*) (Hadjisterkotis et al. 2000; Hadjisterkotis 2012) but also several less emblematic large-sized rodents (Sondaar 1977; Marra 2005). Almost all these endemic faunas disappeared after the arrival of humans, due to a range of anthropogenic effects including land clearance and the introduction of allochthonous animals to only name a few (Hadjisterkotis and Masala 1995). Only a few shrew and a single rodent species have survived up to modern times (Masseti 2009). The rodent species is the Cypriot mouse *Mus cypriacus*, endemic to Cyprus. Its resemblance with the house mouse *Mus musculus domesticus* made it inconspicuous, leading to its late description at the beginning of the 21st century (Bonhomme et al. 2004; Cucchi et al. 2006). As a consequence, little is known about the ecology of this species. It is found in cultivated areas at moderate altitudes, and niche modelling suggests that potentially its favorable habitats cover more than 80% of Cyprus (Hardouin et al. 2024). Described as a semi-commensal rodent due to its presence in cultivated area (Cucchi et al. 2006), the Cypriot mouse is nevertheless almost absent from areas with high anthropogenic pressure (Cucchi et al. 2006; Kryštufek and Vohralík 2009). This might be due to a competitive exclusion with the house mouse (Cucchi et al. 2006). The latter species was introduced on Cyprus as early as c. 8300 BC by the very first agropastoral societies coming from the mainland (Cucchi et al. 2002; Cucchi et al. 2023). Known to be commensal since the first Natufian wave of proto-sedentism, c. 12,500 BC (Cucchi et al. 2020), the house mouse occasionally thrives outdoor in mild climates (Auffray et al. 1990), especially in insular environments devoid of rodent competitors (Ganem 2012). Both the Cypriot mouse and the house mouse could thus coexist in agricultural fields in Cyprus, but they are seldom found in syntopy (Cucchi et al. 2006; Macholan et al. 2007). This exclusion seems to date back to the introduction of the house mouse on the island (Cucchi et al. 2002).

The aim of the present study is to further explore the ecology of the Cypriot mouse and its niche partitioning with the domestic mouse, in order to better understand how this species survived the anthropic pressure and the competition with its commensal relative, as the sole rodent among the Pleistocene endemics from the Mediterranean islands. Two methods were combined to study and compare Cypriot and domestic mice from Cyprus. Carbon and nitrogen stable isotope analyses were used to quantify the trophic structure of the two species from Cyprus, and to determine their degree of specialization and trophic level (Layman et al. 2007). On the same individuals, a morpho-functional analysis of the mandible was performed. The mandible constitutes the moving tool bringing teeth into occlusion; in rodents, due to the large diastema, incisors and molars cannot be occluded at the same time (Cox et al. 2012) and they are involved in different functions, namely biting and chewing, respectively. The mandible morphology thus varies depending on the diet, among species (Monteiro and Reis 2005; Michaux et al. 2007) but also within species (Monteiro et al. 2003; Renaud and Michaux 2003). Geometric morphometric was used to quantify the morphology of the mandible while biomechanical ratios were used to assess the potential functional consequences of shape differences (Anderson et al. 2014). The comparison of morphological variables with isotopic proxies of diet further allowed for a test of the ecological relevance of the morphometric and biomechanical measurements.

Hypotheses were that the house mouse, as a commensal rodent strongly relying on highly diverse and transformed anthropogenic food resources, would display a higher trophic level and a wider niche than the Cypriot mouse (Dammhahn et al. 2017; Guiry and Buckley 2018; Anders et al. 2022). Both species are expected to exhibit a generalist mandible morphology. However, compared to the Cypriot mouse, the mandible of the house mouse may be further optimized to process a broader array of items, including non-natural ones, potentially requiring powerful molar chewing as well as efficient biting at the incisor.

Material

Mice were trapped on Cyprus during a field mission in 2015 (García-Rodríguez et al. 2018; Hardouin et al. 2024) (Fig. 1). Among them, 14 belonged to the endemic Cypriot mouse *Mus cypriacus* (CYP) while 17 were domestic mice of the Western European subspecies *Mus musculus domesticus* (DOM) (see Online Resource 1: Table S1). Cypriot mice *M. cypriacus* were trapped outdoor, in cultivated or natural environments. Domestic mice were trapped inside buildings of various farms (García-Rodríguez et al. 2018), except for one specimen found in an abandoned quarry (Xylophagou) where it was trapped in syntopy with one specimen of *M. cypriacus*.

Mice were collected following local regulations for field collection of small mammals and sacrificed following procedures approved by the American Society of Mammalogists (Sikes et al. 2011). Species identification was achieved using mitochondrial D-loop (García-Rodríguez et al. 2018; Hardouin et al. 2024). Nuclear microsatellites were genotyped as well but no evidence of hybridization has been found so far. External measurements (body weight, head-body length, tail length, hindfoot length, ear length) were taken for each specimen (see Supp. Table 1). A piece of cheek muscle was collected from each mouse and frozen at -20°C until further processing. The rest of the head was stored in alcohol.

To estimate the variability of the carbon and nitrogen stable isotope composition of the potential food sources of the mice, and to establish isotopic baselines, samples documenting the local vegetation were collected throughout Cyprus in 2021, along with samples of crops and of livestock feed (Fig. 1; Online Resource 1: Table S2).

Methods

Stable isotope analyses

Mouse muscle samples were freeze-dried and ground, and approximately 500 µg were weighed into 5 x 3 mm tin capsules (Elemental Microanalysis Uk ltd). Potential food sources (vegetation and livestock feed) were dried in an oven at 70 °C during 48 h, ground using a ball mill grinder (Retsch MM-200) and 2.5 mg were weighed into 9 x 5 mm tin capsules (Elemental Microanalysis Uk ltd). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotope ratios were measured using an isotope ratio mass spectrometer (Isoprime 100, Elementar UK Ltd) coupled in continuous flow to an elemental analyzer (Vario PyroCube, Elementar GmbH). Working standards calibrated against IAEA-N1, IAEA-N2, IAEA-CH6 and IAEA-C3 reference materials (Coplen et al. 2006; Brand et al. 2014) were analyzed with the samples, and the standard deviations of the replicate analyses were lower than 0.20‰. Although the SI unit to report isotopic ratios is the Urey (Ur), for practical reasons the carbon and nitrogen stable isotope compositions were expressed as δ ‰ unit relative to Vienna PeeDee Belemnite (VPDB) and atmospheric nitrogen (AIR) as reference for $\delta^{13}C$ and $\delta^{15}N$, respectively, with 1δ ‰ = 1 mUr.

The stable isotope compositions of vegetation and livestock feed were compared among sites using Analyses of Variance (ANOVA). The normality and the equality of variance were tested using Shapiro-Wilk and Levene tests. The isotopic space area was used to quantify the trophic diversity of the two species (Layman et al. 2007). To evaluate the diversity of the basal resources consumed by the two species, the individual diversity of the trophic level and the total trophic diversity, we calculated the $\delta^{13}C$ range, the $\delta^{15}N$ range, and the total convex hull area (TA), respectively (Layman et al. 2007). As the convex hull emphasizes the importance of extreme individuals on the $\delta^{13}C$ - $\delta^{15}N$ space, standard ellipse areas (SEA) were calculated with the R package SIBER (Jackson et al. 2011), using a Bayesian framework with 2×10^4 iterations, 10^3 burning-in and 10% of thinning.

The $\delta^{13}C$ and $\delta^{15}N$ values provide information about the trophic position (TP) of the mice, because there is an increase in the $\delta^{15}N$ value between a consumer and its food source. The dual isotopic baseline approach was used, that includes a mixing model between the two distinct sources of C and

N (natural vegetation and livestock feed). In this mixing model, the parameter α is the proportion of C and N derived from the baseline 1 (natural vegetation) (Post 2002; Quezada-Romegialli et al. 2018). The commonly employed values of 3.4 ‰ ± 0.1 for N and of 0.4 ‰ ± 1.3 for C (Post 2002) were used as trophic discrimination factors.

All statistical analyses were performed using R (R Core Team 2020) with the *car* (Fox and Weisberg 2019), *SIBER* (Jackson et al. 2011) and *tRophicPosition* (Quezada-Romegialli et al. 2018) packages.

<u>3D geometric morphometrics and biomechanics</u>

Mouse heads were scanned at a cubic voxel resolution of 12 µm on the General Electric (GE) Nanotom microtomograph (µCT) of the AniRA-ImmOs platform of the SFR Biosciences, Ecole Normale Supérieure (Lyon, France). The right mandible (or occasionally the left mandible) was manually segmented, including the molar row and the incisor, using the software Avizo. Twenty-one landmarks were digitized on the model of the right mandible (or mirrored left mandibles) using MorphoDig (Lebrun 2018) (Fig. 2).

Landmark coordinates were superimposed with a Generalized Procrustes Analysis (GPA). Several GPAs were performed on subsets of landmarks in order to study different parts of the mandible: 1) the 21 landmarks describing the mandible including the molar and incisor teeth; 2) the 17 landmarks describing the mandibular bone only; 3) the five landmarks describing the incisor, including the top of the incisor and the basis of the bevel; and 4) the four landmarks describing the masseteric region. The GPAs were performed using the R package geomorph (Adams and Otarola-Castillo 2013). The aligned coordinates (Procrustes residuals) were thereafter considered as shape variables. The shape variance was summarized using a principal component analysis (PCA) applied to the variance-covariance matrix of the aligned coordinates. Procrustes ANOVAs on the aligned coordinates were used to investigate the effect of species, size, and isotopic variables on mandible geometry.

Morphological disparity was estimated as the Procrustes variance within each species, and differences between disparity levels were tested using permutations (Adams and Otarola-Castillo 2013). Permutation-based Procrustes ANOVA and tests of morphological disparity were performed using the R package geomorph (Adams and Otarola-Castillo 2013). Topologies of the specimens on different multivariate spaces were compared with Protests (Peres-Neto and Jackson 2001) using the package vegan (Oksanen et al. 2013). These tests are based on a Procrustes superimposition of the two configurations, providing a Procrustes coefficient of correlation R and a permutation-based assessment of the significance of the correlation. The centroid size (Csize) of the right (or mirrored-left) configuration was calculated as the square root of the sum of the squared distances of all landmarks from their centroid (Slice et al. 1996) and provided an estimate of size for the mandible, mandibular bone, erupted incisor, and masseteric region.

A measure of the efficiency of the mandible to transmit force from the muscles to the bite point can be assessed by the mechanical advantage, defined as the ratio of the in-lever (distance from the condyle to the point of muscle attachment) and the out-lever (distance from the condyle to the bite point) (Hiiemae 1971). In- and out-levers were calculated as distances between the relevant landmarks (Fig. 2). Out-levers were estimated as the distance from the basis of the condylar articulation (playing here the role of fulcrum) to the incisor tip, and to the tip of the first molar anterior cusp. Three in-levers were measured. The effect of the superficial masseter was approximated by considering the distance from the condyle to the posterior tip of the angular process. The deep masseter action was described by the distance from the condyle to the anterior termination of the masseteric ridge. The distance from the condyle to the posterior tip of the coronoid was used to describe the action of the temporalis (Anderson et al. 2014).

Six mechanical advantages were investigated: temporalis / incisor, superficial master / incisor, deep masseter/incisor, temporalis / molar, superficial masseter / molar, and deep masseter / molar, knowing that the temporalis is mostly used in the movement of closing the jaw and thus moving the incisors for gnawing, whereas the masseter and molars are dominantly involved in the action of mastication.

Differences between univariate estimates (size and mechanical advantages) were tested with non-parametric Kruskal–Wallis (KW) tests. Linear regressions and Pearson correlations were used to test for covariation between univariate variables and linear models for investigating the effect of multiple factors and their interaction.

All permutation tests were performed with 9999 permutations. All analyses were conducted in R (R Core Team 2020). The 3D surfaces of ten mice, illustrating the intra- and inter-specific variation of *M. cypriacus* and *M. m. domesticus*, are deposited in MorphoMuseum (Renaud et al. 2024). Isotopic and morphometric data can be found in Online Resource 1: Table S1; isotopic data on basal resources are available in Online Resource 1: Table S2.

Results

<u>Isotope analysis</u>

The C and N isotope composition of the different basal resources, *i.e.* vegetation and livestock feed, differed among sampling sites in $\delta^{13}C$ (ANOVA, $P < 0.0001$), but not in $\delta^{15}N$ (ANOVA, $P = 0.571$). The difference in $\delta^{13}C$ was explained by higher $\delta^{13}C$ values of the livestock feed, up to -15.0‰ (Fig. 3). The isotopic composition of the muscles differs between two species. The $\delta^{13}C$ values for *M. cypriacus* range between -26.9‰ and -23.2‰, whereas $\delta^{13}C$ for *M. m. domesticus* ranges between -25.6‰ and -18.5‰ (Fig. 4a). The $\delta^{13}C$ range (CR) that provides an estimate of the diversity of the basal resources used by the species (Layman et al. 2007), was twofold higher for *M. m. domesticus* (CR = 7.1) than for *M. cypriacus* (CR = 3.7). The large $\delta^{13}C$ range, shifted towards higher values, of *M. m. domesticus*, is due to the incorporation of carbon derived from livestock feed characterized by high $\delta^{13}C$ values (Fig. 3).

M. m. domesticus tends to exhibit higher $\delta^{15}N$ values than *M. cypriacus*. This suggests that house mice forage on livestock feed, characterized by high $\delta^{15}N$ values, and likely include animal preys in their diet. Nevertheless, *M. cypriacus* exhibits a wider $\delta^{15}N$ range (NR = 6.6) than that of *M. m. domesticus* (NR = 4.0), indicating a higher inter-individual diversity in the trophic levels of the consumed food. Individuals with low $\delta^{15}N$ values probably feed on plant matter while those with high $\delta^{15}N$ values include animal preys in their diet.

The isotopic niche quantified by the standard ellipse area (SEAb) does not differ between the two species (Table 1.; Fig. 4b), but the convex hull area, giving more weight to extreme individuals, is higher for *M. m. domesticus* (Table 1), indicating a larger trophic niche, with some individuals feeding on different resources than the majority of the population. Moreover, the small overlap between standard ellipses and convex hull in the $\delta^{13}C$ - $\delta^{15}N$ space (Fig. 4a) indicated different trophic niches for the two species.

The trophic position of *M. m. domesticus* (TP = 2.7) was significantly higher than that of *M. cypriacus* (TP = 2.3; Fig. 5a) (pairwise comparison, $P = 0.025$). This higher trophic position is at least partly due to a greater contribution of carbon and nitrogen derived from anthropogenic food to the diet of *M. m. domesticus*. Calculation of trophic positions from isotope compositions showed that the food web of *M. m. domesticus* relies significantly less on natural vegetation (36%, Fig. 5b) than the food web of *M. cypriacus* (77%, Fig. 5b) (pairwise comparison, $P = 0.030$).

<u>Morphometrics</u>

<u>Size measurements</u>

The two species did not differ in their body weight ($P_{KW} = 1.000$; Fig. 6a) nor tail length ($P_{KW} = 0.9167$). *M. cypriacus* (CYP) displayed a larger mandible ($P_{KW} = 0.0062$; Fig. 6b) and longer hind foot ($P_{KW} = 0.0424$) but a shorter body ($P_{KW} = 0.0002$) than *M. musculus domesticus* (DOM).

Mandible size slightly increased with body weight, the two species sharing a similar allometric relationship as indicated by the non-significant effects of the species and interaction terms in the model (Mandible Centroid Size ~ Weight * Species, $P_{weight} = 0.0325$, $P_{species} = 0.9687$, $P_{interaction} = 0.4882$).

Mandible shape

Patterns of mandible shape variation were investigated using a PCA (Fig. 7a). The first axis (31.0% of total variance) opposed *M. m. domesticus* and *M. cypriacus* with almost no overlap. This highly significant difference (Procrustes ANOVA P = 0.0001) involved all parts of the mandible (Fig. 7b). *M. cypriacus* is characterized by a longer incisor, longer molar row, elongated coronoid process but reduced angular process compared to *M. m. domesticus*. *M. cypriacus* tended to display a lower level of morphological disparity than *M. m. domesticus* (Procrustes variance CYP = 0.0013, DOM = 0.0017) but this difference did not reach the significance threshold (P = 0.0818), possibly due to the low sample size.

Despite the importance of the signal related to the incisor, similar results were obtained when focusing on the landmarks describing the mandibular bone only (Protest comparing the scores on the first five PC axes on the landmarks describing the mandible including the teeth vs mandibular bone only: Procrustes R = 0.8594, P = 0.0001).

Incisor region

M. cypriacus displayed a longer incisor length relative to mandibular bone centroid size than *M. m. domesticus* (Incisor Csize / Md_Bone Csize, $P_{KW} = 0.0029$) (Fig. 6c). Accordingly, *M. cypriacus* and *M. m. domesticus* differed in the shape of their incisor region (P = 0.0011), with *M. m. domesticus* occasionally displaying short incisors with truncated bevels (Fig. 8). The incisor region was twice as variable in *M. m. domesticus* than in *M. cypriacus* (Procrustes variance CYP = 0.0041, DOM = 0.0095), but this difference did not reach the significance threshold (P = 0.0621), possibly due to reduced sample size.

Masseter region

Relatively to the mandibular bone, the masseteric region was larger in *M. m. domesticus* than in *M. cypriacus* (Masseter Csize / Md Bone Csize, $P_{KW} = 0.0011$) (Fig. 6d), leading to a similar absolute size of the masseteric region in the two species (Masseter Csize, $P_{KW} = 0.3825$). The shape of the masseteric region was different as well (Procrustes ANOVA P = 0.0298), but displayed a similar level of disparity in the two species (Procrustes variance CYP = 0.0048, DOM = 0.0055, P = 0.6872).

Biomechanics

Regarding the mechanical advantages, *M. m. musculus* presented higher values than *M. cypriacus* in the ratios involving the temporalis muscle (MA temporalis / incisor P_{KW} = 0.0033; MA temporalis / molar P_{KW} = 0.0042) (Fig. 9). The two species did not differ in the mechanical advantages involving the superficial masseter (MA superficial masseter / incisor P_{KW} = 0.3210; MA superficial masseter / molar P_{KW} = 0.2497). *M. cypriacus* exhibited slightly higher mechanical advantages involving the deep masseter (MA deep masseter / incisor P_{KW} = 0.0237; MA deep masseter / molar P_{KW} = 0.0172).

Relationship between trophic niche and mandible morphology

The relationship between the two proxies of the trophic niche ($\delta^{13}C$ and trophic position [TP]) with morphometric variables were then investigated among all specimens, with species as covariate. Mandibular bone shape was only slightly correlated with $\delta^{13}C$. Trophic position (TP) was related with mandible shape, mandibular bone shape, and the temporalis / molar mechanical advantage. The relationship with the temporalis / incisor was close to the significance threshold (Table 2).

The shape change involved with increasing trophic position was visualized using regression score (mandible shape ~ TP + Species) and the associated shape predictions for minimum and maximum values (Fig. 10a, b). The shape change is distributed all over the mandible, with high TP values being associated with longer incisor, expanded angular region and shortened coronoid process compared to low TP values.

These changes in mandible shape had consequences on some mechanical advantages. *M. m. domesticus* exhibited overall temporal-related larger mechanical advantages than *M. cypriacus*, and a higher trophic position. As a consequence, the regressions on the total sample (CYP+DOM) were significant; furthermore, the within-CYP regressions were significant as well (Fig. 10c, d). The total and within-CYP relationships linking the temporal-related mechanical advantages and the individual trophic position were very close. The same relationships were not significant within *M. m. domesticus*, with two not mutually exclusive explanations: (1) the range of trophic position was limited in DOM, precluding the detection of covariation; (2) one specimen (SOT3; see also Fig. 9a, d) strongly departed from the general trend. This specimen displays a very pronounced coronoid process decreasing the temporalis in-lever; it is among the heaviest mice and its molar teeth show an advanced wear, suggesting that it may correspond to a senescent individual.

Discussion

Trophic niche segregation between Cypriot and domestic mice

The carbon and nitrogen stable isotope analyses provided evidence of trophic niche segregation between the two *Mus* species present on Cyprus. Compared to the endemic Cypriot mouse, the domestic mouse relies on a wider range of basal resources and feeds at a higher trophic level. This is a typical signature for commensal rodents, that can find protein-rich, high-quality food resources in anthropogenic habitats (Guiry and Buckley 2018; Anders et al. 2022). Despite frequently foraging in cultivated areas, the Cypriot mouse displays an isotopic profile pointing to the preferential use of wild vegetation items and a relatively wide range of trophic levels, indicating that some individuals incorporate animal prey, such as invertebrates, in their diet. This profile matches the generalist habitat use of the Cypriot mouse (Hardouin et al. 2024). Accordingly, little evidence of genetic structure across the island has been found, suggesting regular gene flow between populations of *M. cypriacus* (Hardouin et al. 2024).

Contrary to other endemic Mediterranean rodents, the Cypriot mouse has been consistently subjected to terrestrial predation pressure, evolving in a context where an endemic genet was present on the island (Hadjisterkotis et al. 2000; Masseti 2009). This may have enabled the Cypriot mouse to cope with the early arrival of domestic cats, introduced on the island to mitigate the plague of domestic mice as early as the 9th millennium BC (Cucchi et al. 2023). Its small body size, comparable to that of the house mouse, should favor escape in shelters such as holes in rocky habitats. Additionally, its long hindfoot length may further point to a greater locomotory agility compared to the domestic mouse. Altogether, its generalist habits that allow the exploitation of various natural food resources as well as its adaptation to predation pressure may explain the success of this endemic species surviving the arrival of humans and their associated animals on the island, notably the house mouse and the domestic cat.

The Cypriot mouse: signature of a generalist rodent

The Cypriot mouse diverged from its mainland sister species *M. macedonicus* ~0.67 million years ago (mya), and from the domestic mouse more than 2 mya (Hardouin et al. 2024). Given its endemic evolution, morphological divergence may have accumulated due to drift in isolation, as well as adaptation to its unique environment (Renaud et al. 2018). Accordingly, the mandible morphology of the Cypriot mouse differs markedly from that of sympatric house mice. Several functionally relevant traits are involved, foremost the mandible size, which is significantly larger than in domestic mice despite a similar body size. The size of the craniofacial apparatus is a good predictor of bite force in rodents (Ginot et al. 2018) and it is known to favor niche segregation by allowing the foraging of food items of different size (Dayan et al. 1990). Accordingly, the Cypriot mouse can probably feed on larger items than the domestic mouse. It further exhibits a slight increase in the mechanical advantage involving the deep masseter, presumably adaptive for stronger chewing with the molars, but without showing an expansion of the angular process typical for herbivorous rodents (Michaux et al. 2007; Firmat et al. 2010). Finally, the Cypriot mouse displays an elongated incisor with a long bevel. Incisor morphology is the result of a complex interplay between abrasion (caused by contact with food items), attrition (caused by tooth-to-tooth contact), and eruption rate, which is continuously adjusted to wear (Taylor and Butcher 1951). As a consequence, incisor morphology can vary depending on the diet of mice, with sharper bevels associated with predatory behavior, due to reduced abrasion (Renaud et al. 2019). The long and sharp incisor of the Cypriot mouse may be the result of lower eruption rate, lower abrasion, and/or active attrition behavior, contributing to maintaining the incisor at an adequate length for occlusion while allowing for the sculpture of a sharp bevel (Taylor and Butcher 1951). An unequivocal functional interpretation of the long and sharp incisor in Cypriot mice is however difficult, since it may be advantageous for seizing prey but also for piercing of seeds and other food items. Overall, the mandible of the Cypriot mouse shows a typical morphology for a generalist murine rodent, without a clear optimization either for chewing or gnawing (Michaux et al. 2007).

The domestic mouse: an opportunistic diet accommodated by an “allrounder” mandible morphology

Compared to the Cypriot mouse, the house mouse from Cyprus displays several traits that are comparatively optimized. The large masseteric region allows for the insertion of powerful masticatory muscles, compensating for the small mandible size. The house mouse further presents high mechanical advantages involving the temporalis muscle. A high temporalis / incisor mechanical advantage is interpreted as favoring biting at the incisor, and it is characteristics of house mice displaying a shift towards increased predatory behavior on Sub-Antarctic islands (Renaud et al. 2018). This is in line with the higher trophic level observed for the house mice from Cyprus, compared to Cypriot mice. Supporting an adaptive interpretation of this biomechanical difference, the mechanical advantages involving the temporalis muscle are the only biomechanical variables correlated with the trophic position at the individual level. This indicates that the exploitation of higher trophic levels is associated with an optimization of the temporalis muscle action. However, contrary to the Sub-Antarctic mice, which show an optimization of incisor biting to the detriment of mastication at the molar (Renaud et al. 2018), house mice from Cyprus seem to display a compromise between high performance in biting at the incisor, while maintaining powerful mastication by expanding the zone of insertion of the masseter muscles. This should allow them to forage on dry seeds and cereals occurring on Cyprus but not on Sub-Antarctic islands.

The house mice from Cyprus further tend to display higher levels of morphological disparity compared to Cypriot mice, especially regarding the incisor that can present very short and apparently abraded bevels (Fig. 8). This echoes the isotopic signals showing an expanded trophic niche for the domestic mice. In anthropic environments, house mice are confronted with the challenge of very diverse food items, and possibly also gnaw on very hard non-food items, leading to unusual and variable patterns of incisor morphology, and an "allrounder" mandible morphology that insures both powerful chewing and gnawing. The comparison between the Cypriot and the house mice, in that respect, shows that a "generalist" feeding behavior can be manifested differently in natural and anthropic environments, with various consequences on the masticatory apparatus.

Complex coupling between trophic signature and morphological differences

Specialized dietary habits promote the evolution of morphological differences in the mandible (Renaud et al. 2007). An increased predatory behavior in rodents is associated with an elongated mandible favoring the capture of prey items (Michaux et al. 2007; Rowe et al. 2016; Fabre et al. 2017) whereas herbivorous diets requires expanded angular processes allowing for the attachment of powerful masticatory muscles (Firmat et al. 2010). Such trends are well expressed among species, but they can also be observed within species, according to geographic gradients (Renaud and Michaux 2003) or as the result of diet shift in unique insular environments (Renaud et al. 2018). Morphological divergence can also occur due to neutral genetic divergence, and the coupling between diet preferences and mandible morphology may not always be straightforward (Ginot et al. 2020). Relying on external data, such as isotopic ecology or molar microwear, to support the functional interpretation of morphological differences between taxa is thus very important to avoid *ad hoc* interpretations (Firmat et al. 2010; Kerr et al. 2017; Premate et al. 2021). Good biomechanical estimates of bite force can be derived from dissections (Becerra et al. 2011; Ginot et al. 2018) but such analyses cannot be performed on museum or fossil specimens. Furthermore, bite force may not be directly related to trophic position when resources of different consistencies are involved. Morpho-functional analyses performing a coupling with proxies of trophic levels and niche are rare, especially down to the individual level. In the present study, the range of basal resources exploited, estimated by $\delta^{13}C$ isotopic values, did not correlate with any morphological parameters. Functional requirements during food processing are related to mechanical properties of the food items that are most probably not fully captured by their isotopic composition. More convincing results were found regarding trophic position, estimated by $\delta^{15}N$ values, that was related to overall mandible morphology and to the mechanical advantages involving the temporalis muscle, both between species and at the individual level within the Cypriot mouse. This strongly supports the interpretation of this biomechanical proxy as related to increased consumption of prey items, even as a modulation of overall generalist, omnivorous diets.

Conclusion

The present study provides insight into the dietary ecology of the inconspicuous Cypriot mouse *Mus cypriacus*. Its survival into modern times as the sole rodent among the past endemics of the Mediterranean islands was favored by its generalist habits allowing the exploitation of various natural food resources, including invertebrates. In contrast, house mice from Cyprus forage mostly on anthropic food resources, ensuring an efficient niche partitioning between the two species. The morphology of their mandible suggested fine tuning around shared common features characteristics of omnivorous diets. *Mus cypriacus* displays a large mandible, optimized for chewing at the molars, facilitating the consumption of large and hard food items presumably abundant in the natural vegetation of Cyprus. In *Mus m. domesticus*, the small mandible size is compensated by a large masseter area and an optimization for incisor biting, making it an all-rounder tool for foraging on diverse non-natural items. Such items may be more abrasive than natural items, occasionally causing unusual abrasion patterns on the incisor, with very short and blunt bevel pointing to a high abrasion / high eruption balance.

The seldom achieved comparison, down to the individual level, of isotopic and morphometric data allowed validation of the relationship between temporalis-related biomechanical indicators and trophic position. This strengthens the importance of morphological estimators in assessing diet of museum and fossil specimens.

Data Availability Statement

Isotopic and morphometric data can be found in Online Resource 1: Table S1; isotopic data on basal resources are available in Online Resource 1: Table S2. The 3D surfaces of ten mice, illustrating the intra- and inter-specific variation of *M. cypriacus* and *M. m. domesticus*, are deposited in MorphoMuseum (Renaud et al. 2024).

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Tables

	CR (‰)	NR (‰)	TA (‰²)	SEA_b (‰²)
M. cypriacus	3.72	6.56	14.14	5.77
M. m. domesticus	7.11	4.02	17.04	6.02

Table 1 Niche metrics based on isotopic analyses for the two species. Abbreviations: CR: $\delta^{13}C$ range, index of basal resource use; NR: $\delta^{15}N$ range, index of trophic level diversity; TA: convex hull area, total isotopic niche; SEA_b: standard ellipse area, isotopic niche.

	$\delta\ ^{13}C$ + Species		TP + Species	
	P $\delta^{13}C$	P species	P TP	P species
Md Csize	0.9103	*0.0119*	0.3921	*0.0258*
Md Bone Csize	0.9533	*0.0177*	0.3734	*0.0417*
Md Shape	0.0651	**0.0001**	*0.0035*	**0.0001**
Md Bone Shape	*0.0484*	**0.0001**	*0.0019*	**0.0001**
Inc / Md Bone Csize	0.5288	**0.0002**	0.3728	*0.0014*
Masseter / Md Bone Cize	0.0544	**0.0001**	0.1967	*0.0117*
MA Temp / Inc	0.5312	*0.0084*	0.0573	0.1199
MA Sup Mass / Inc	0.5790	0.1800	0.2770	0.6830
MA Deep Mass / Inc	0.7831	0.0503	0.7207	0.0962
MA Temp / Mol	0.6110	*0.0081*	*0.0223*	0.1326
MA Sup Mass / Mol	0.5160	0.2020	0.3640	0.6430
MA Deep Mass / Mol	0.5598	*0.0351*	0.6030	*0.0370*

Table 2. Relationship between the two proxies of the trophic niche, $\delta^{13}C$ and trophic position, with the morphometric variables, with species as covariate. All specimens (*M. cypriacus* and *M. m. domesticus*) are included. In bold P-values ≤ 0.001 are in bold, P-values ≤ 0.05 are italicized. Abbreviations: Csize: centroid size; Inc: incisor; MA: mechanical advantage; Mass: masseter; Md: mandible; Temp: temporalis; TP: trophic position.

Figure Captions

Fig. 1 Map of Cyprus with locations of trapping and collection of basal resources. Both *M. cypriacus* and M*. m. domesticus* were found in the abandoned quarry of Xylophagou (XYL).

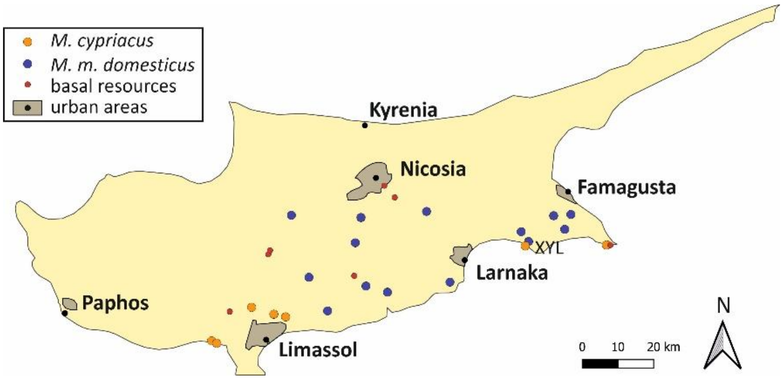

Fig. 2 Position of the landmarks and definition of the in- and out-levers defining the mechanical advantages, illustrated on a right mandible of *Mus cypriacus* (specimen # Cypriacus 5GE). **a-b.** Location of the landmarks (green dots) on the mandible in lingual (**a**) and labial (**b**) view; **c**. In-levers (in red), as distance from the condyle to the point of muscle attachment; **d**. Out-levers (in blue), corresponding to the distance of the condyle to the bite point. Abbreviations: Deep Mass: deep masseter; Inc: Incisor; Mol: First lower molar; Sup Mass: superficial masseter; Temp: temporalis.

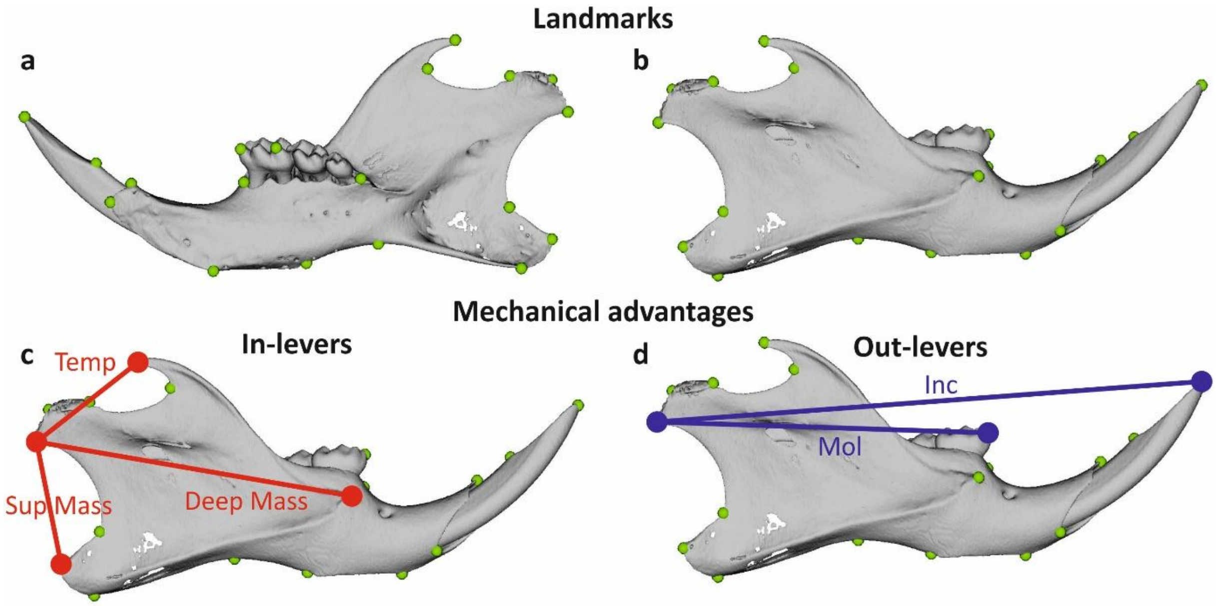

Fig. 3 Carbon and nitrogen stable isotope compositions of the basal resources, separated into vegetation and livestock food. Dots represent individual measurements of resource items, open circles represent the mean of each group ± standard deviation.

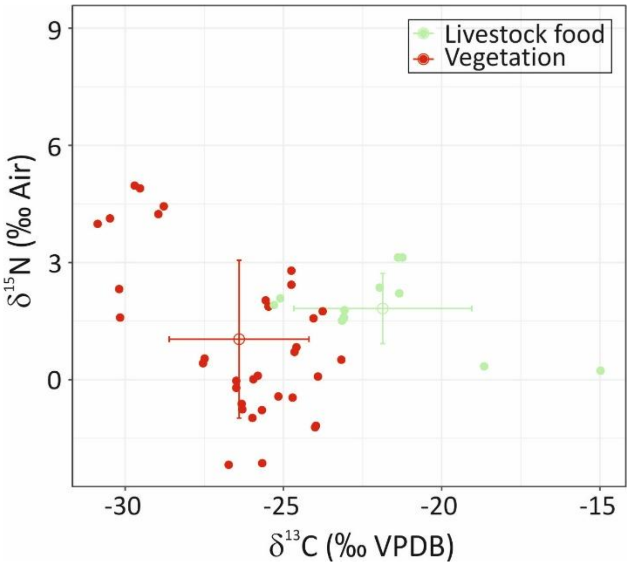

Fig. 4 a. Carbon and nitrogen stable isotope compositions of the cheek muscle of *M. cypriacus* and *M. musculus domesticus*. The ellipse and the dashed lines correspond to the SIBER ellipse and to the convex hull area, respectively; **b**. Box plot of the standard ellipse area calculated within a Bayesian framework for *M. cypriacus* and *M. musculus domesticus*. Black dots represent their mode, and the shaded boxes represent the 50%, 75% and 95% credible intervals from dark to light color.

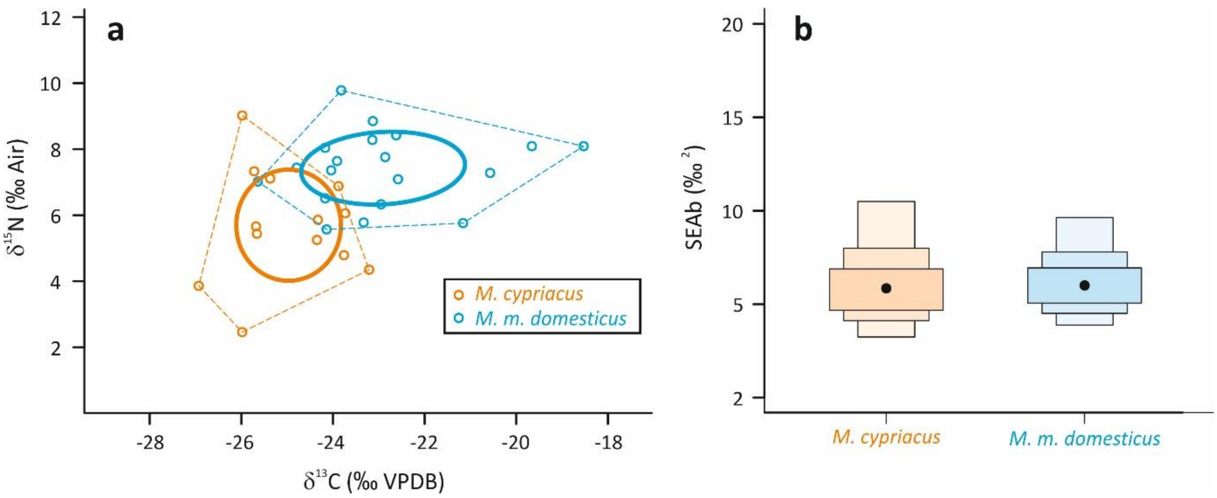

Fig. 5 a. Trophic position of the two species, calculated by tRophicPosition; **b**. α value, representing the contribution of the natural vegetation versus livestock food (see. Fig. 3) in the diet. An α value of 1 indicates that basal resources come at 100% from baseline 1 (natural vegetation), and α = 0 indicates basal resources only from baseline 2 (livestock food). The red dotted line corresponds to 50% - 50% contribution of baseline 1 and baseline 2.

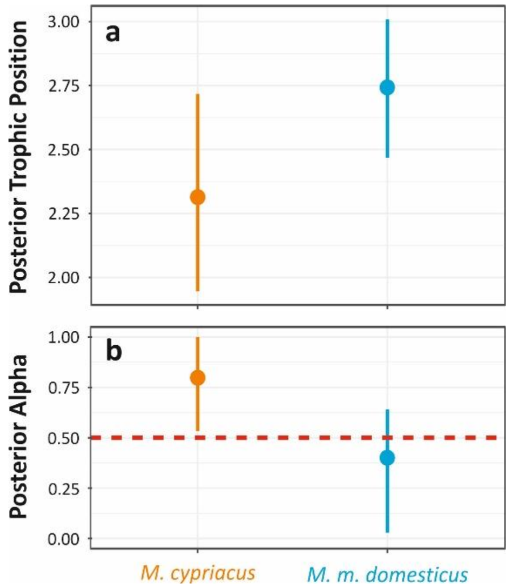

Fig. 6 Size differences in body weight (**a**) and mandible size (**b**) between *M. cypriacus* (CYP) and *M. m. domesticus* (DOM). Differences in relative size of the incisor (**c**) and masseter (**d**) region, relative to the size of the mandibular bone. Size is estimated as the centroid size (Csize) for mandible, mandibular bone (Md Bone), incisor and masseter region.

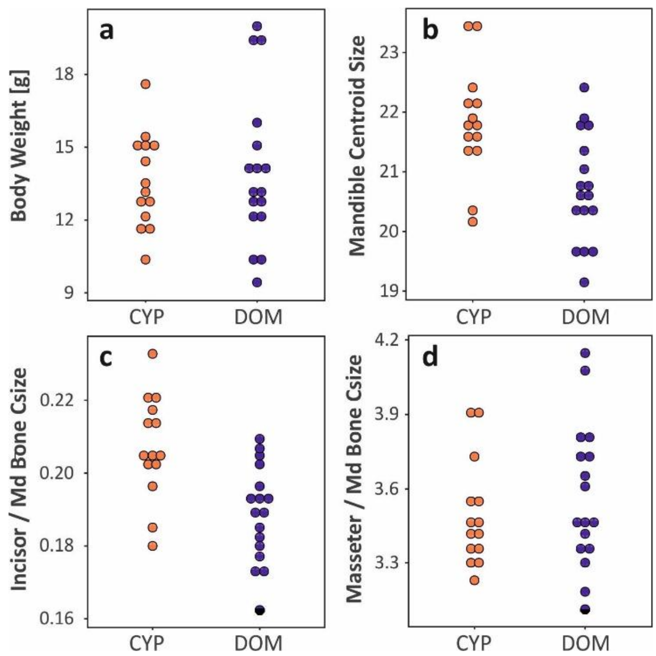

Fig. 7 Mandible shape of *M. cypriacus* and *M. m. domesticus*. **a**. Morphospace figuring the first two axes of a PCA on the aligned coordinates describing the shape of the mandible, including the incisor; **b**. Superimposed mean shapes of the two species.

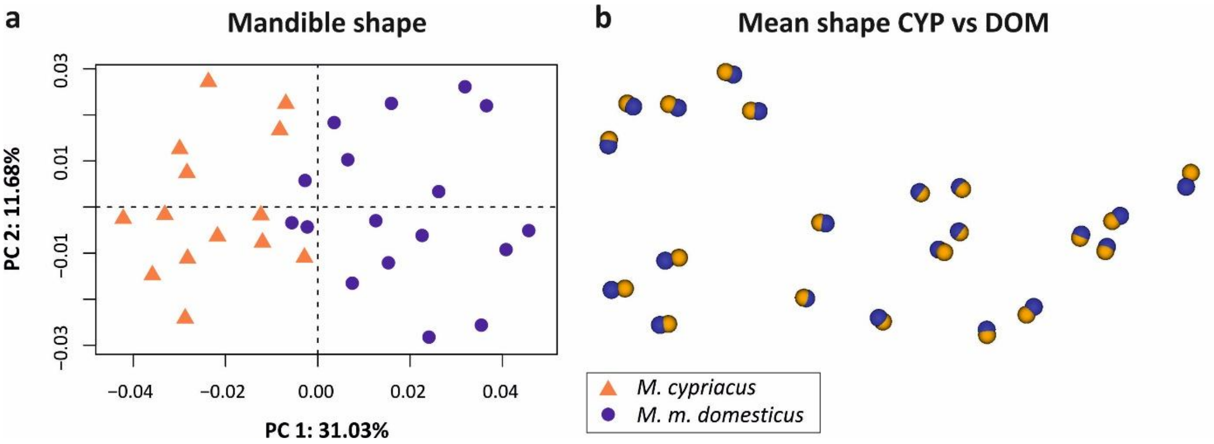

Fig. 8 Variation of the incisor region in *Mus cypriacus* and *M. m. domesticus*. The morphospace corresponds to the first two axes of a PCA on the aligned coordinates describing the incisor region of the mandible, with examples illustrating the variation in incisor shape and bevel morphology.

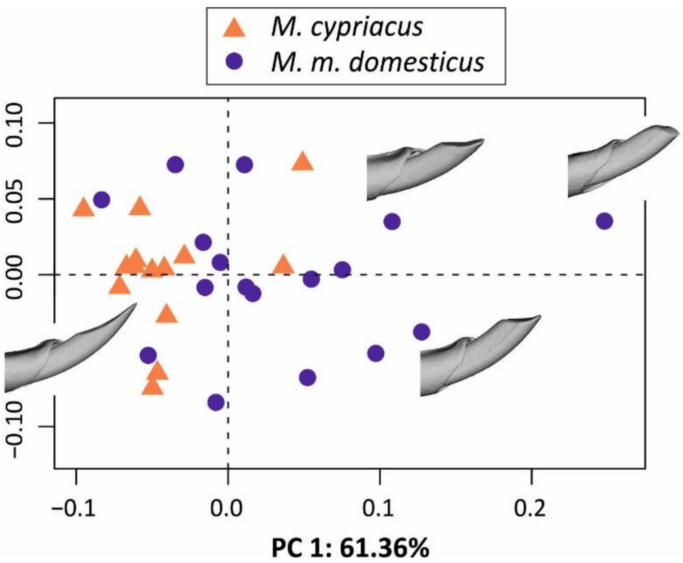

Fig. 9 Biomechanical properties of the mandible in *Mus cypriacus* (CYP) and *M. m. domesticus* (DOM), assessed by mechanical advantages temporalis / incisor (**a**), superficial masseter / incisor (**b**), deep masseter / incisor (**c**), temporalis / molar (**d**), superficial masseter / molar (**e**), deep masseter / molar (**f**).

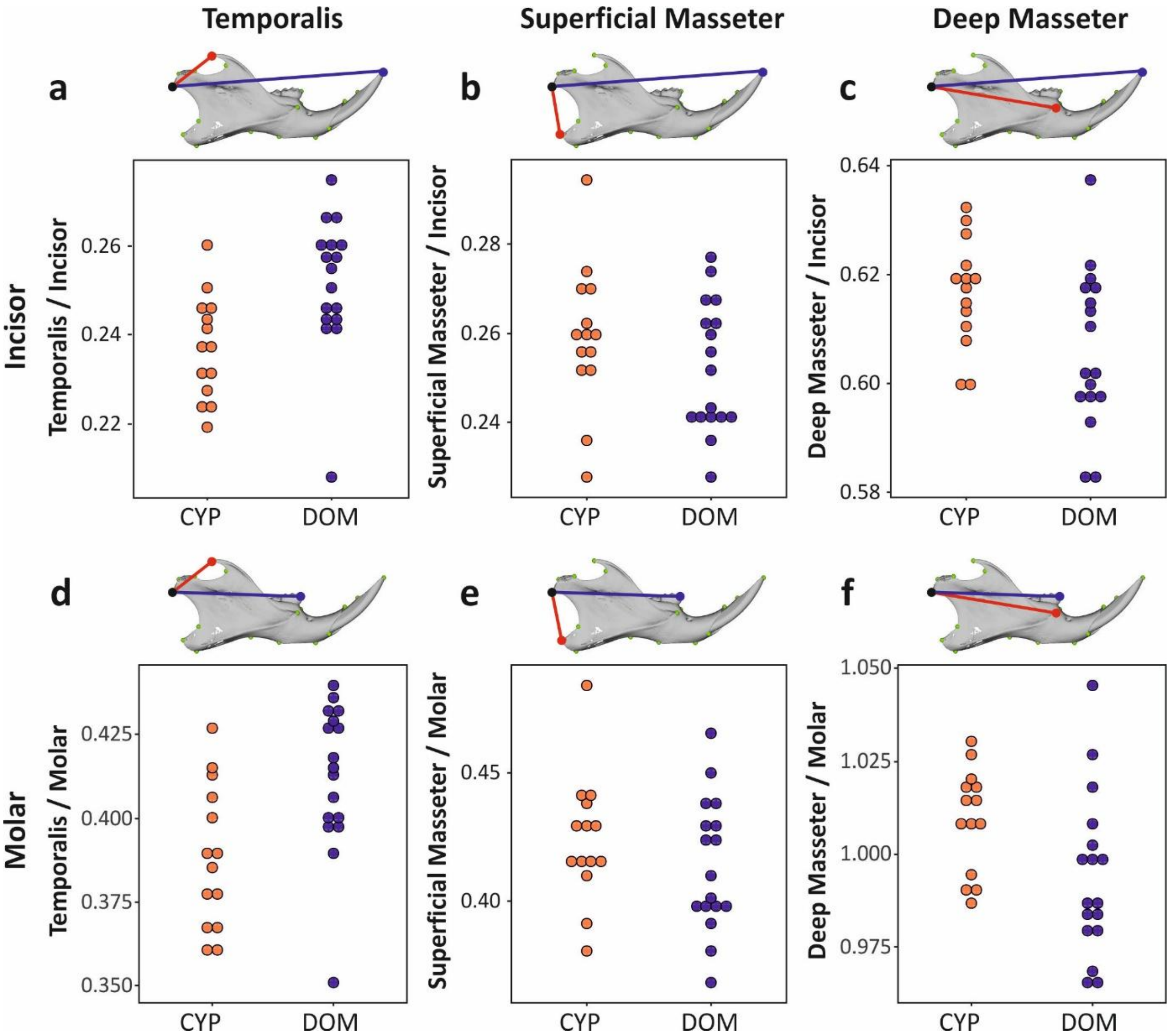

Fig. 10 Relationship between trophic position and mandible morphology. **a**. Relationship between TP and mandible shape, represented by the regression score based on the model Shape ~ TP + Species. **b**. Visualization of the shape change from minimum (min) to maximum (max) RegScore (low to high TP). **c-d**. Relationships between TP and mechanical advantages involving the temporalis action with incisor (**c**) and with the molar (**d**). For each graph, the lines represent the correlation between regression score and TP (full lines, Pearson correlation P-value < 0.05, dotted line, 0.10 < P-value ≤ 0.05). Black line, total; color line, regression per species. P-values of the Pearson correlations are mentioned on the graphs (in bold P < 0.001, in italics P < 0.05). Abbreviation: TP, trophic position.