

UDC599.742.4:591:575.86(1-021.21)

## FUNCTIONAL AND PHYLOGENETIC ASPECT IN MODULARITY OF PALEARCTIC MUSTELIDS (CARNIVORA, MUSTELIDAE) MANDIBLE

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### **Functional and Phylogenetic Aspect in Modularity of Palearctic Mustelids (Carnivora, Mustelidae) Mandible.**

**Romaniuk, A.** — Geometric morphometrics was used to investigate morphological integration and modularity in mustelid mandible. A set of 16 two-dimensional landmarks was digitized on the mandibles of 14 extant species of Palearctic Mustelidae (genera *Enhydra*, *Gulo*, *Meles*, *Lutra*, *Martes*, *Mustela*). The original data size-corrected data and phylogenetically independent contrasts (PICs) were analyzed. Several hypotheses were tested: two and three-modules with the masseteric fossa included in corpus or ramus of the mandible. As a result, the two-module hypothesis (subdivision into the alveolar region and the ascending ramus) with masseteric fossa included in corpus for all sets of data was supported. A clear modularity in mustelid mandible is seen at interspecific level, whereas there was large within species covariation between mandibular corpus and ramus. The allometry correction reduces estimates of covariation for the mustelid mandibles. Due to the analysis of PICs the shape changes with maximum evolutionary covariation were found: elongated, sloped backward ramus and thick corpus.

Key words: allometry, geometric morphometrics, integration, mandible, masseteric fossa, Mustelidae, partial least squares, phylogenetic correction.

### **Introduction**

An important task for morphometric research is to determine whether a structure is a single integrated unit or consists of several distinct modules and to evaluate hypotheses about their boundaries (Klingenberg, 2009). The geometric morphometric methods give good possibilities for the study of morphological integration and modularity in a different comparative context. In this sense, morphological modules are groups of landmarks that are minimally correlated with other such groups (Klingenberg, 2009).

The mustelid jaw apparatus is a convenient model for studying complex morphological structures. Extant mustelids display extensive ecomorphological diversity, reflecting the adaptation to different habits and habitats (Koepfli et al., 2008). Among mustelids there are carnivorous, omnivorous, piscivorous and durophagous. In my previous paper I showed that mandibular corpus and ramus respond in different ways to trophic specializations in Palearctic mustelids (Romaniuk, 2018): corpus shape correlates with the diet, whereas ramus shape can be

radically different in various species of the same trophic group. In addition, Meloro et al. (2011) showed that, in Carnivora, predators and non-predators are significantly distinct in corpus shape, while ramus shape data does not support such an evident distinction between these functional groups.

The division of mandible into the alveolar region and ascending ramus was investigated on mouse jaw by several authors using various approaches (e. g., Mezey, Cheverud & Wagner, 2000; Ehrich et al., 2003; Klingenberg, Leamy & Cheverud, 2004; Klingenberg, 2009). An array of authors (Monteiro, Bonato & dos Reis, 2005; Márquez, 2008; Zelditch et al., 2008) discussed the possibility of subdivision of lower jaw into more than two modules.

The aim of my work was to check whether the alveolar region (mandibular corpus) and ascending ramus are separate modules in mustelid. Besides, I tested whether there is an additional set of modules in the alveolar region. I also clarified whether the anterior edge of masseteric fossa belongs to the mandibular corpus or to the mandibular ramus.

The effects of size can produce global integration throughout the whole landmark configuration and may obscure a possible modular structure (Klingenberg, 2009). I verified the effect of size correction in mustelid mandible. Finally, I tried to estimate amount of evolutionary integration in the mustelid mandible.

## Material and methods

Mandibles of 290 specimens, representing 14 species of extant mustelids, have been used in geometric morphometric analysis (table 1). I selected only wild caught specimens (both males and females) with fully erupted dentition from different localities. The previous study showed that the effect of sex differences on mandible shape is not noticeably compared to species and diet differences. The specimens are from the National Museum of Natural History at the National Academy of Sciences of Ukraine (NMNH, Kyiv) and the Zoological Museum of Lomonosov State University (ZM, Moscow).

The mandibles were photographed with a Canon Power Shot SX200IS digital camera in left lateral view, oriented with the longest axis of the mandible parallel to the photographic plane. If it was incomplete or unavailable (8 % of the overall sample), I used samples of the right side. On each mandible photo, I identified and digitized a subset of 16 landmarks using the software tpsDig2 (Rohlf, 2010). Landmarks 1 to 6 and 13 to 16 describe the mandibular corpus (the alveolar region), whereas landmarks 7 to 12 describe the mandibular ramus (the ascending ramus) (fig. 1, A): 1, intersection of canine anterior edge and dental bone; 2, intersection of canine posterior edge and dental bone; 3, intersection of carnassial anterior edge and dental bone; 4, projection of the protocone cusp on the m1 baseline; 5, intersection of carnassial posterior edge and dental bone; 6, posterior edge of dentition; 7, tip of the coronoid process; 8, the dorsal extreme between coronoid and condylar process; 9, the most lateral extreme point of the condylar process; 10, the posterior extreme between condylar and angular process; 11, the most lateral extreme point of angular process; 12, the most anterior point of masseteric fossa; 13–16, intersection of the perpendicular to line 1–6 through landmark 6, 4, 3, 2, respectively, with ventral edge of mandible.

A generalized full Procrustes fit was performed on two-dimensional landmark coordinates to extract shape information. To visualize the phylogenetic history of shape change, I display the phylogeny tree with the average shapes for species.

Two main hypotheses of landmarks partitioning of the lower jaw of mustelid are considered. The first one is dividing the mandible into two modules — the alveolar region (mandibular corpus) and the ascending

**Table 1. The studied species of Mustelidae**

Species	Number of studied specimens
Sea otter, <i>Enhydra lutris</i> (Linnaeus, 1758)	18
Wolverine, <i>Gulo gulo</i> (Linnaeus, 1758)	19
River otter, <i>Lutra lutra</i> (Linnaeus, 1758)	20
Badger, <i>Meles meles</i> (Linnaeus, 1758)	18
Pine marten, <i>Martes martes</i> (Linnaeus, 1758)	24
Stone marten, <i>Martes foina</i> (Erxleben, 1777)	23
Sable, <i>Martes zibellina</i> (Linnaeus, 1758)	22
Yellow-throated marten, <i>Martes flavigula</i> (Boddaert, 1785)	19
White polecat, <i>Mustela eversmani</i> Lesson, 1827	23
Black polecat, <i>Mustela putorius</i> Linnaeus, 1758	24
European mink, <i>Mustela lutreola</i> (Linnaeus, 1761)	23
Siberian weasel, <i>Mustela sibirica</i> Pallas, 1773	19
Ermine, <i>Mustela erminea</i> Linnaeus, 1758	20
Weasel, <i>Mustela nivalis</i> Linnaeus, 1766	18

ramus (mandibular ramus). The second one is dividing the mandible into three modules — anterior region, molar region and the ascending ramus (fig. 1, B, C). Besides, two additional hypotheses were tested:  $H_1$  is that the anterior edge of masseteric fossa belongs to the mandibular corpus (or to the molar region), and  $H_2$  — belongs to the mandibular ramus. The RV coefficient (or multi-set RV coefficient for three modules) was used to quantify the magnitude of covariation between the coordinates of sets of landmarks. Hypotheses concerning the boundaries of jaw modules were tested by comparing the RV coefficients among alternative partitions of the configuration into subsets of landmarks. The proportion of partitions for which the RV coefficient is less than or equal to the RV value for the partition of interest was interpreted as the analog of a p-value (Klingenberg, 2009). Only spatially contiguous alternative partitions were considered.

To characterize the patterns of integration between the mandibular corpus and ramus, I use Partial least squares analysis (PLS) (Bookstein, 1991; Klingenberg & Marugán-Lobón, 2013). Since this study aims to consider all the pair-wise covariations between blocks, particularly the effects of the relative arrangement and sizes of lower jaw parts, I use the analysis based on a joint Procrustes fit of the whole

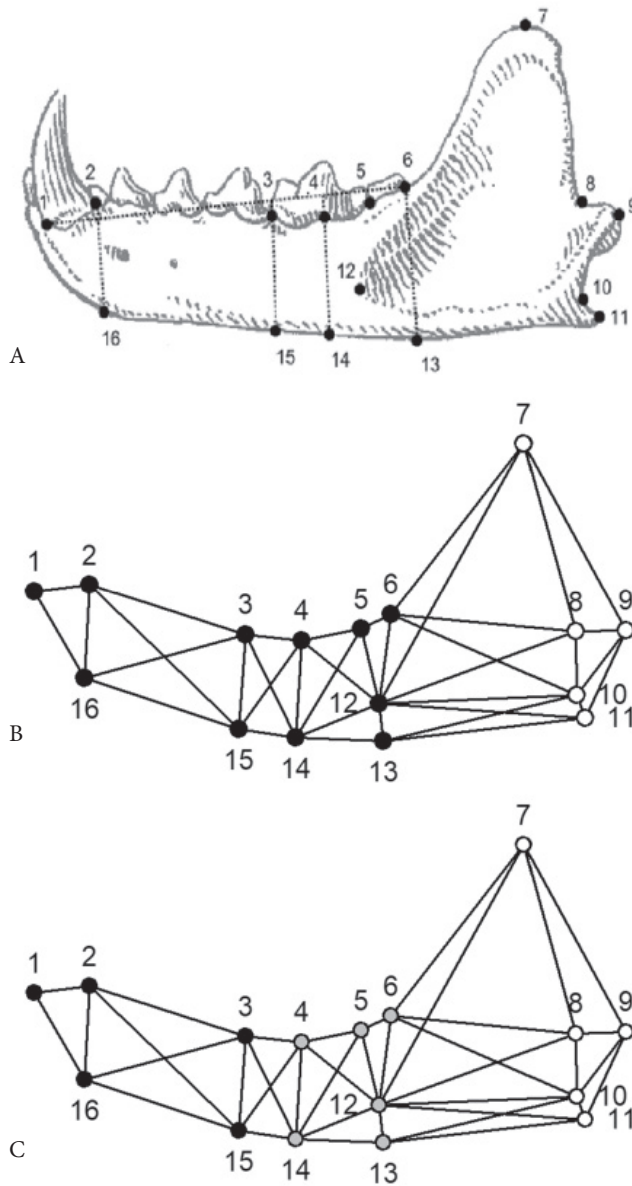


Fig. 1. The position of landmarks on a mandible outline of *Mustela lutreola*. A — scheme of landmarks from Romaniuk (2018); the picture of mandible is adapted from Novikov (1956). And the subdivisions into two (B) and three modules (C) with the lowest RV coefficients.

landmark configuration. To test the covariation between corpus and ramus, I use a permutation test against the null hypothesis of total independence and the correlation coefficient of PLS scores between blocks as the test statistic (Klingenberg, 2009).

The effect of allometry was verified using the multivariate regression of shape (Procrustes coordinates) on size ( $\log_{10}$ -transformed mandibular length) (Monteiro, 1999). The mandible length (the distance from the anterior edge of the incisors alveoli to the posterior edge of the condylar process) is treated here as a proxy for general mandible size. The covariance matrix of the residuals from the multivariate regression was performed to analyze modularity after removing the influence of allometry (Klingenberg, 2009). The relationship between shape and size was tested using a generalized Goodall's F test.

The phylogenetic study was based on the phylogenetic tree of Koepfli et al. (2008) with a nearly complete generic-level phylogeny of the Mustelidae based on a data matrix comprising 22 gene segments. To investigate whether the morphometric data contain a phylogenetic signal, I used a permutation approach (Klingenberg & Gidaszewski, 2010) that confirmed the presence of the phylogenetic signal in the Procrustes coordinates ( $p = 0.0186$ , after 10,000 permutations). Phylogenetically independent contrasts (PICs) of shape data were used to analyze the covariation of evolutionary changes between the corpus and ramus. PLS axes computed from independent contrasts therefore identify shape features with maximal evolutionary covariation (Klingenberg & Marugán-Lobón, 2013). I used angular comparisons between the vectors from evolutionary PLS and size-corrected PLS to assess its similarity quantitatively (Klingenberg, 2011; Klingenberg & Marugán-Lobón, 2013).

To estimate evolutionary allometry, I use multivariate regression of phylogenetically independent contrasts of Procrustes coordinates, as the shape variables, on independent contrasts of  $\log_{10}$ -transformed jaw length, as the size measure.

Morphometric analyses was performed with MorphoJ 1.06d (Klingenberg, 2011), datasets for different methods were exported and visualized with R v. 3.4.2. (R Core Team, 2017) and R package geomorph v.3.0.5 (Adams et al., 2017). Within-species RV coefficients were estimated with geomorph v. 1.1.0 (Adams & Otárola-Castillo, 2013).

## Results

### Size correction and evolutionary allometry

The relationship between lower jaw shape and size is quite clear. The multivariate regression of the Procrustes coordinates on  $\log_{10}$ -transformed mandible length shows that allometry is statistically significant ( $p < 0.0001$ , permutation test with 10,000 random permutations).  $\log_{10}$ -transformed mandible length accounts for 11.05 % of the total shape variance. Shape changes associated with allometry are shown in figure 2: the coronoid process is lowering, the mandibular corpus under m2 is getting more massive, the angular process becomes longer, and the masseteric fossa shifts to the ramus with increasing of mandible size.

The multivariate regression of independent contrasts (after mapping the Procrustes coordinates onto phylogenetic tree) of mandible shape on independent contrasts of  $\log_{10}$ -transformed mandible length accounts for 9.02 % of the shape variance, and is not significant statistically ( $p = 0.30$ ). Further, I will use PICs without correction for the length of mandible.

### Modularity of lower jaw

The RV coefficients of two-modules subdivision both for the uncorrected and size-corrected data are the lowest of any possible partitions of the configuration (table 2). The two-modules RV coefficient is lower for the size-corrected than for uncorrected shape data assuming modularity is more apparent when the effects of allometry are removed from the shape data. RV coefficients are lower for configuration when masseteric fossa belongs to the mandibular corpus. Thus, two modules (alveolar region and ascending ramus) hypothesis with masseteric fossa belonging to the corpus best explains general variation of lower jaw shape in mustelids.

However, within species RV coefficients of two-module configurations are large, and for some species masseteric fossa tends to be the part of the mandibular ramus (table 3). Paired t-test does not reveal significant difference in within-species RV coefficients for two configurations ( $p = 0.57$ ).

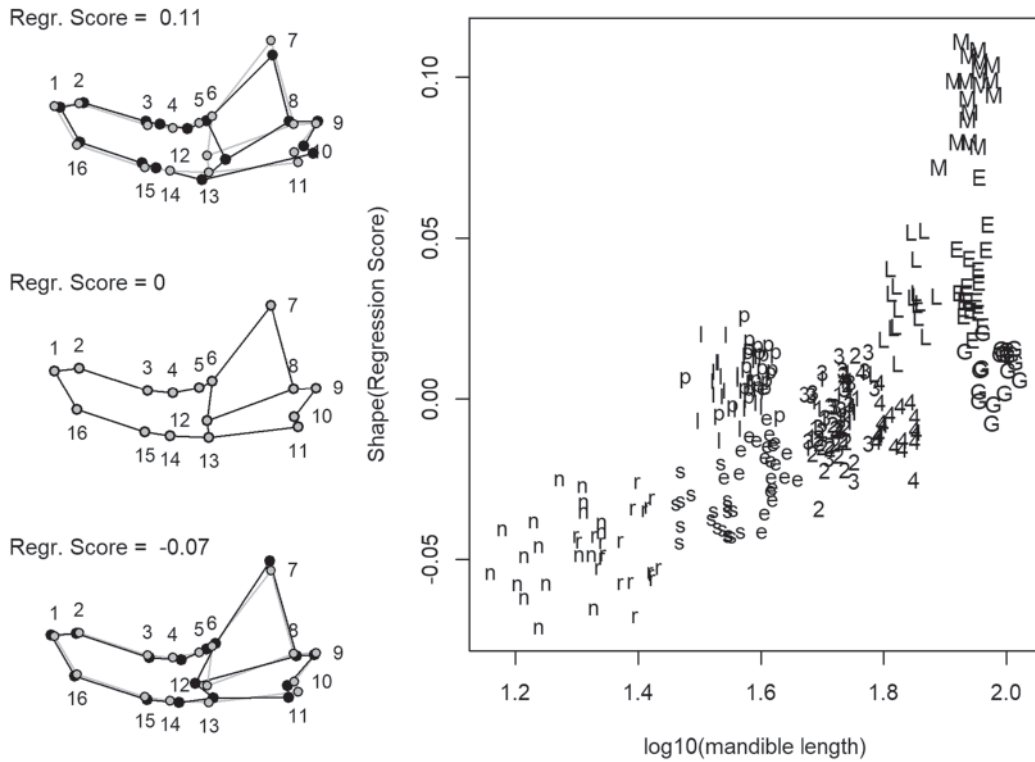


Fig. 2. Distribution of mustelid specimens in the scatterplot of the allometric shape component (Regression Score 1) vs  $\log_{10}$ -transformed mandible length. Mandible shape changes associated with allometry are shown for zero (average shape, grey outline), minimal and maximal values of the regression scores (with magnification factor 1). Masseteric fossa (landmark 12) is linked with landmarks 6, 8, 13 for the ease of visualization. Species are abbreviated: *E. lutris* = E; *G. gulo* = G; *M. meles* = M; *L. lutra* = L; *Martes*: *M. martes* = 1; *M. foinea* = 2; *M. zibellina* = 3; *M. flavigula* = 4; *Mustela*: *M. eversmani* = e; *M. putorius* = p; *M. lutreola* = l; *M. sibirica* = s; *M. erminea* = r; *M. nivalis* = n.

I considered subdivisions of mustelid mandibles into more than two parts (fig. 1, C) and found that the multi-RV coefficient for a subdivision of mandible into three parts namely the anterior region, the molar complex and the ascending ramus is the lowest of any other partitions of the configuration (into more than two parts). In contradiction to the subdivision into two modules, the size correction increases the integration between three modules. Three-modules multi-set RV coefficients are greater than two-modules one (table 2). Inclusion of the masseteric fossa in the mandibular corpus is also the partition with minimum covariation. The multi-set RV coefficients of size-corrected shape data are

**Table 2. RV coefficients and their permutation p-values for subdivisions of mandible into two and three modules for the uncorrected, size-corrected and phylogeny-corrected shape data**

Shape data	Two modules				Three modules			
	$H_1$ hypothesis		$H_2$ hypothesis		$H_1$ hypothesis		$H_2$ hypothesis	
	RV	p	RV	p	Multi-set RV	p	Multi-set RV	p
Uncorrected	0.231	0.003	0.284	0.005	0.249	0.001	0.318	0.026
Regr. resid.	0.201	0.003	0.231	0.002	0.278	0.003	0.336	0.054
PICs	0.382	0.006	0.482	<b>0.133</b>	0.424	0.024	0.520	<b>0.239</b>

Note. The variants are considered: (i) when masseteric fossa belongs to the mandible corpus ( $H_1$  hypothesis) and (ii) when masseteric fossa belongs to the mandible ramus ( $H_2$  hypothesis). Insignificant p-values are marked in bold.

**Table 3. Within-species RV coefficients and their permutation p-values for subdivisions of mandible into two modules for size-corrected shape data**

Species	H <sub>1</sub> hypothesis		H <sub>2</sub> hypothesis	
	RV	p	RV	p
<i>E. lutris</i>	0.431	0.139	0.316	<b>0.004</b>
<i>G. gulo</i>	0.639	0.309	0.558	0.059
<i>M. meles</i>	0.594	0.630	0.481	0.051
<i>L. lutra</i>	0.491	0.190	0.545	0.295
<i>M. martes</i>	0.361	<b>0.012</b>	0.406	<b>0.044</b>
<i>M. foina</i>	0.454	0.209	0.425	0.053
<i>M. zibellina</i>	0.247	< <b>0.001</b>	0.322	<b>0.016</b>
<i>M. flavigula</i>	0.332	<b>0.022</b>	0.378	0.065
<i>M. eversmani</i>	0.432	0.102	0.409	<b>0.028</b>
<i>M. putorius</i>	0.462	0.095	0.436	<b>0.012</b>
<i>M. lutreola</i>	0.561	0.094	0.556	<b>0.049</b>
<i>M. sibirica</i>	0.556	0.087	0.551	<b>0.040</b>
<i>M. erminea</i>	0.403	<b>0.020</b>	0.460	0.099
<i>M. nivalis</i>	0.520	0.181	0.508	0.083

Note. The variants are considered: (i) when masseteric fossa belongs to the mandible corpus (H<sub>1</sub> hypothesis) and (ii) when masseteric fossa belongs to the mandible ramus (H<sub>2</sub> hypothesis). Significant p-values are marked in bold.

increased comparing to raw data due to the larger integration within the alveolar region (between the anterior region and molar complex) remaining low between the ascending ramus and other parts of the mandible. For example, when H<sub>1</sub> is considered (similar pattern is seen within H<sub>2</sub>) RV between anterior and molar regions increased from 0.381 to 0.492, but RV between molar region and ramus decreased from 0.240 to 0.212 after correction for allometry. Thus, the size-corrected two-modules configuration with masseteric fossa belonging to mandibular corpus (the configuration with minimal RV) is used for further analysis.

### Integration of mandibular corpus and ramus

PLS-within-configuration was made for size-adjusted dataset and considering two-modules with masseteric fossa belonging to the mandibular corpus landmark partition. First PLS axes (PLS1) accounts for 49.3 % of the total squared covariance between the mandibular corpus and ramus; singular value = 0.00047,  $p < 0.001$  (hypothesis of no covariation is rejected). The most of integration in mustelid mandible is between the slope of ramus and length of corpus, emplacement of masseteric fossa: maximum scores of PLS1 associate with high coronoid process, short angular process (straight ramus) and elongated corpus with masseteric fossa displaced forward (fig. 3, B, C). Correlation between mandibular corpus and ramus is high ( $r = 0.58$ ), and does not deviate from the correlation expected for random two-module partition of landmarks ( $p = 0.37$ ).

*E. lutris* is away from the general trend (fig. 3).

### Evolutionary integration

Using phylogenetic independent contrasts on average shapes of the species increases RV coefficient in two-modules, H<sub>1</sub> hypothesis (RV = 0.382,  $p = 0.006$ ; table 2). Evolutionary PLS1 quite differs from the standard size-corrected PLS1 (angle 40.7°,  $p < 0.00001$ ). The most of integration is observed between slope of ramus and depth of corpus. For the maximal scores of PLS1: coronoid process becomes higher and directs backward, angular process shifts forward, and this shape changes in ramus are associated with thickened corpus and reduced m2 in mandibular corpus (fig. 3, D, E).



## Discussion

### The number of modules in mustelid mandible

To evaluate hypotheses of modularity I analyzed covariation of the two parts of mustelid mandible — the alveolar region (mandibular corpus) and ascending ramus — and found the lowest degree of multivariate correlation between them. The fact that the mandibular corpus and ramus are the separate modules confirms the results of an earlier study on mandible shape of representatives of different trophic groups in Palearctic mustelids. There is a functional subdivision between these two parts. Different killing and food-processing forces directly act on the mandibular corpus, and the ascending ramus is a place of attachment of the muscles necessary for effective mastication (Weijs, 1994) and articulating with the skull. Also, such a subdivision can be explained as the result of developmental processes: because the cells that form the mandible, while universally deriving from the neural crest, originate from several different progenitor cell condensations (Ehrich et al., 2003). Differences in mandibular corpus shape occur at the family level in large fissiped carnivores, suggesting early morphological differentiation (Meloro et al., 2008).

The hypothesis of modularity is consistent with the results of PCA in my previous study (Romaniuk, 2018), which also points to strong modularity of the mustelid mandible. In PCA the mustelid mandible does not seem as the whole structure. The shape changes are associated with the separate (discrete) parts of mandible. Traits of shape variation that are specific to these parts make up a considerable proportion of the total variation and are reflected by the principal components. Mandibular corpus shapes are different with the same shape of mandibular ramus. And the ratio of parts of molar complex co-varies with increase of premolar region length. The shape changes associated with PCs and PLS axes are quite similar. It implies that features of covariation between the mandibular corpus and ramus are among the dominant features of shape variation (Klingenberg & Marugán-Lobón, 2013). Shape changes of the first PCs are associated mainly with length and height of premolar region, length ratio of different area of molars and with the position of ramus processes. This is probably the result of different applying of these mandible parts during the capture and processing of prey. It suggests the possibility of subdivision into more than two modules. The results of the analysis showed that subdivision into the anterior region, the molar complex and the ascending ramus has minimal covariation between all possible subdivisions into 3 parts. But still, the covariation between the anterior region and molar complex is high.

However, within species covariation between mandibular corpus and ramus is large. The mandible is highly integrated at intraspecific level, and a clear modularity in mustelid mandible is seen at interspecific level. This confirms that modularity is a matter of the relative degrees (Klingenberg et al., 2003, 2004).

### To what module does the masseteric fossa belong?

The mandibular ramus (ascending ramus) includes coronoid and angular processes for the attachment of the masticatory muscles and glenoid process for articulation with the skull. Given the importance of the masseteric fossa for the attachment of masseter muscle (Ewer, 1973), it might also be expected that it is a part of the mandibular ramus. The results indicate that attributing the anterior edge of masseteric fossa to the corpus reduces covariation between parts of lower jaw, increasing modularity in mustelid mandible. It seems that the masseteric fossa is a part of mandibular corpus or, in more detail, of molar regions of corpus. It may be due to a possible integration the jaw coupling forces generated by muscle contraction with those experienced at teeth (Zelditch et al., 2008). Though, within species masseteric fossa tends to be the part of the mandibular ramus. The question as to which part (module) of mouse jaw include



the place of masseter muscle attachment was studied by several authors (Monteiro et al., 2005; Márquez, 2008; Zelditch et al., 2008). The results were ambiguous. In most rodent species there is a statistical association between the incisor alveolus and the part of the masseter attachment that spans the posterior portion of the incisor alveolus (Márquez, 2008). However, reorganization of the integrated groups of jaw depending on functional demands during evolutionary shape changes is possible (Monteiro et al., 2005). Possibly the ascending ramus is also divided into several modules since each muscle attachment may be an integrated unit as do jaw parts developmentally dependent on certain teeth (Zelditch et al., 2008).

#### The effects of allometry

Removing the effects of allometry, by using residuals from the regression of shape on size, affects the patterns of overall variation of lower jaw shapes, patterns of integration between mandibular corpus and ramus, and the strength of covariation. For the subdivisions into two and three modules used in this article, this allometric correction had different effects. Removing the effects of size reduces the RV coefficients between the alveolar region and ascending ramus. Using the residuals from the regression makes the assignment of the masseteric fossa to the mandibular ramus (hypothesis H<sub>2</sub>) the case of subdivision with minimum covariation as well as the assignment of the masseteric fossa to the mandibular corpus (hypothesis H<sub>1</sub>) (table 2). On the contrary, the size correction increases the multi-set RV coefficients for subdivision into three modules.

Modularity is more apparent when the effects of allometry are removed for the alveolar region and ascending ramus subdivision. However, subdivision into the anterior region, the molar complex and the ascending ramus is more dependent on the influence of size since the covariation between these jaw parts is stronger when removing the effects of size. Allometry is a contributing factor to overall integration, but is not accounting for all the integration in the mustelid mandible.

#### Integration and diet

The most of integration in mustelid mandible is observed between the slope of ramus, length of corpus and emplacement of masseteric fossa. It should be noted that the sea otter (*E. lutris*) is away from the general trend in PLS1. Apparently, it is associated with the influence on the jaw apparatus of specific diet. Feeding on hard objects probably leads to somewhat different from the other mustelids integration patterns. Carnivorous *Mustela* and omnivorous *Martes* are located in separate groups (fig. 3). At that, species of *Mustela* differ from each other more along the mandibular ramus. Being hypercarnivorans, *G. gulo* and *M. erminea* with *M. nivalis* are the closest to *E. lutris* in mandible integration patterns.

#### The evolutionary integration

The variation of lower jaw shape contains a clear phylogenetic signal. Despite this phylogenetic signal, however, there is considerable divergence among closely related taxa such as *E. lutris* and *L. lutra* or *G. gulo* and *Martes*, or species among *Mustela* (fig. 4), and convergence in features of mandible shape associated with the diet, for example, among *E. lutris* and *M. meles* or among *Gulo* and *Mustela* (Romaniuk, 2018). Some mustelid species retain the ancestral ecological niche during evolution, while others within the same group can change the direction of their ecological specialization indicating that the group retains its plasticity. That reflects the flexibility of the mandibular corpus and ramus to evolve, for instance, in response to functional aspects of jaw morphology.

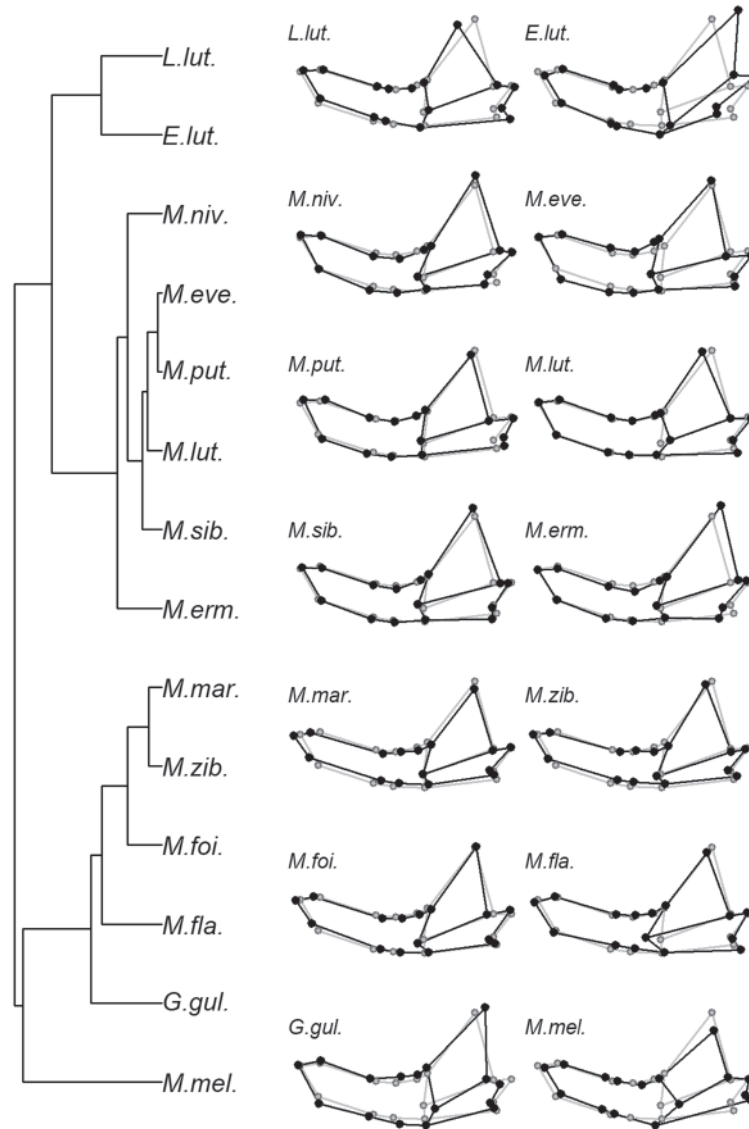


Fig. 4. Phylogeny tree with the average shapes for species. Raw Procrustes coordinates are used for visualization. Reference (grey outline) is an overall average shape. Magnification scale for shapes is 1.5.

The hypothesis of modularity in mustelid mandible holds for evolutionary divergence of shape. Though, the RV coefficients in this analysis are the highest among the other data sets, and when the masseteric fossa is considered as the mandibular ramus, are not significant (table 2). The phylogenetic correction reduces the modularity in mustelid mandible. The analyses showed that there is a certain degree of integration of evolutionary changes throughout the mustelid mandible, and evolutionary trajectory is different from size-corrected one. The most of evolutionary integration is observed between sloped backward ramus and thick corpus with reduced m2. Modularity of mustelid mandible is more apparent when phylogeny is not considered. Confirming that phylogeny does contribute to the overall level of integration. The multivariate regression indicates that there is low not significant

statistically evolutionary allometry. Despite contributing to integration throughout the jaw, therefore, allometry is not the main determinant of evolutionary integration in mustelid mandible.

I am grateful to Vladimir S. Lebedev (Zoological Museum of Moscow Lomonosov State University), Pavel V. Putshkov and Dmitry V. Ivanoff (National Museum of Natural History, Kyiv) for kindly providing access to museum collections and help during the visits. I thank Igor Dzeverin and Pavel Gol'din for discussion, help and valuable advice. I especially grateful to Maria Ghazali, whose comments and suggestions greatly improved the manuscript.

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Received 8 December 2017

Accepted 9 February 2018