

Variation and covariation of the molar crown elements in the genus *Ondatra* (Rodentia, Arvicolinae)

Igor Ya. Pavlinov

ABSTRACT. Variation and covariation of the molar crown elements of prismatic dentition in the genus *Ondatra* (Mammalia: Rodentia: Arvicolinae) was studied numerically by means of geometric morphometric and cluster analyses. 31 elements were identified in total, their shapes were described by semilandmarks, their covariation patterns were analyzed by cluster analysis of vectorized Procrustes distance matrices with bootstrap estimations of cluster supports. Within-tooth comparisons recognize the modules that combine crown elements corresponding to the opposing pairs of cusps characteristic of generalized cricetine dentition. Results of comparison of crown elements within each tooththrow indicate that integration effect, uniting homologous elements in adjacent teeth into dispersed modules, may be stronger than that uniting opposing elements in the same teeth. Comparison of crown elements of upper and lower tooththrows reveals covariation of anterior part of lower M1 and posterior part of upper M3, which underwent coherent complication in the arvicoline evolution. It is concluded that the approach, based on combination of elementaristic GM-description of dental crowns and cluster analysis of covariations of their elements, may become an important tool for exploration of integration patterns of specialized dentition in herbivorous mammals.

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KEY WORDS: dentition, levels of integration, geometric morphometrics, semilandmarks, cluster analysis, *Ondatra*.

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Изменчивость и ковариация элементов коронок коренных зубов в роде *Ondatra* (Rodentia, Arvicolinae)

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РЕЗЮМЕ. Вариации и ковариации элементов коронки коренных зубов призматического типа в роде *Ondatra* (Mammalia: Rodentia: Arvicolinae) изучены численно с помощью геометрического морфометрического и кластерного анализов. В общей сложности был идентифицирован 31 элемент, их конфигурация была описана полуметками, их ковариационные паттерны были проанализированы с помощью кластерного анализа векторизованных матриц Прокрустовых дистанций с бутстреп-оценками поддержки кластеров. Внутризубные сравнения выявили модули, которые объединяют элементы коронки, соответствующие противоположным парам вершин генерализованных бунодонтных коренных нижних хомякообразных. Результаты сравнения элементов коронок в пределах каждого зубного ряда показывают, что интеграционный эффект, объединяющий гомологичные элементы коронок соседних зубов в рассредоточенные модули, может быть сильнее, чем интеграционный эффект противоположащих элементов в одних и тех же зубах. Сравнение элементов коронок верхних и нижних зубных рядов выявило ковариацию передней части нижнего M1 и задней части верхнего M3, которые согласованно усложнялись в эволюции полёвок. Сделан вывод о том, что подход, основанный на сочетании элементаристского описания зубных коронок и кластерного анализа ковариаций их элементов, может стать важным инструментом для изучения уровней интеграции специализированных зубных рядов травоядных млекопитающих.

КЛЮЧЕВЫЕ СЛОВА: зубная система, уровни интеграции, геометрическая морфометрия, полуметки, кластерный анализ, *Ondatra*.

Introduction

Mammalian dentition is one of popular objects for studying variation and levels of integration of complex anatomical structures for its being conspicuously differentiated into particular elements clearly interpretable functionally, evolutionarily, and partly developmentally. Among different types of mammalian dentition, the prismatic one is peculiar in that its molars consist of the series of alternating prism-like elements. It is characteristic of some rodents, especially arvicolines and some cavioids, and its variation and covariation patterns are studied using standard methods of geometric morphometrics (hereafter GM) (Laffont *et al.*, 2009; Polly *et al.*, 2011; Labonne *et al.*, 2014; Boivin *et al.*, 2022). The just-mentioned studies consider the particular teeth as wholes without individuating their crown elements, which provides a simplified large-scaled picture of dental integration. However, our GM-based study of crown elements of equine dentition revealed their non-trivial covariation structure, which involves combining homologous crown elements of different teeth into dispersed modules (Pavlinov & Spasskaya, 2021). These results indicate that a similar analysis of the elements of prismatic dentition in rodents may be promising in uncovering detailed covariation patterns relevant to the analysis of the levels of integration in the prismatic mammalian dentition.

This article reports the results of our study of variation and covariation of the crown elements of upper and lower molars in the arvicoline genus *Ondatra*, taken as a typical example of the prismatic dentition. The peculiarity of our methodology is in elementaristic analysis of dental crowns including recognition, shape description, and subsequent analyses of their basic elements known as “salient triangles”. This study is conducted as a continuation of our previous research of the equine dentition, so it follows the same protocol of describing dental crowns and processing the respective data combining GM, cluster, and partly correlation analyses. This is a kind of pilot study, so the main objective of this report is to consider a possibility of such combinatorial approach to analyze a detailed covariation structure of the tooththrows for uncovering their integration patterns and modularity. The basic working hypothesis to be tested is an existence of certain levels of integration both within and among upper and lower molars in the arvicoline prismatic dentition. Along with this, we intend to consider the usefulness of a distance-based approach to study molar shape variation.

Materials and methods

The studied sample included 20 specimens of *Ondatra* collected in Odessa Obl. (Ukraine) and kept in the Zoological Museum at Lomonosov Moscow State University (Appendix 1). Their left-side upper and lower dentitions were digitized by the camera Sony DSC-HX400, with tooth orientation being standardized with respect to a plane surface.

The sample includes specimens with minimally worn molars to exclude a possible effect of age-dependent variation. The homology and nomenclature of molar crown elements were adopted following the standard scheme for generalized cricetid dentition (Vorontsov, 1982) for the sake of compatibility of our findings with the future studies on dentition of other grinding rodents. The terms for upper crown elements are ended with ‘n,’ and those for lower ones with ‘d.’ The enamel layers of dental crowns were outlined manually on their images in CorelDRAW program using the Shape tool, with vector lines of a minimum thickness being drawn along the midlines of the enamel layers. If the layer was interrupted at the tip of a salient angle, the contour line was drawn along the latter’s outer rim. The contour lines were divided into fragments corresponding to the particular salient triangles, with their boundaries being set at the maximal curvatures of re-entrant angles (Fig. 1). These fragments were considered individual shapes, 31 of them in total, to be described by the GM tools and compared numerically. The contour lines thus fragmented were then converted into high-resolution halftone raster images.

Each shape was described by an array of semilandmarks set automatically equidistantly along the contour line between two points fixed at the boundaries of the respective crown element, with their number depending on the contour line length (Fig. 1). The semilandmarks were set and their 2D coordinates were acquired by tpsDig2 program (Rohlf, 2017), they were converted into standard landmarks by tpsUtil program (Rohlf, 2019a). Each run in tpsDig2 was repeated twice and consensus configuration for each element was calculated in tpsRelw program (Rohlf, 2019b) to be used in all subsequent analyses.

The landmarked shapes were analyzed by PAST program (Hammer *et al.*, 2001). Their initial x-y-coordinates were transformed into shape variables using generalized least-square Procrustes superimposition. For each shape, pairwise Procrustes distances were calculated between specimens, the resulting distance matrices were vectorized, with each vector (column) of pairwise distances representing a particular crown element. Euclidean distances were calculated between vectors, and cluster analyses were run separately for each of the particular teeth, tooththrows, and for entire dentition, uniformly applying Ward algorithm and with bootstrap estimations of cluster support (1000 replicates). This algorithm was selected for its revealing a more “definite” similarity relations on the resulting phenograms as compared to more popular UPGMA and NJ algorithms. The respective phenograms, with the crown elements distributed on them, served as graphic representations of covariation patterns. In addition, statistical significance (at the level of $p < 0.01$) of similarity of distance matrices was estimated by Mantel test (9999 permutations), and pairwise Pearson correlation coefficients were used in comparison of shapes. Individual variation of the shape of a crown element was characterized by a parameter V calculated

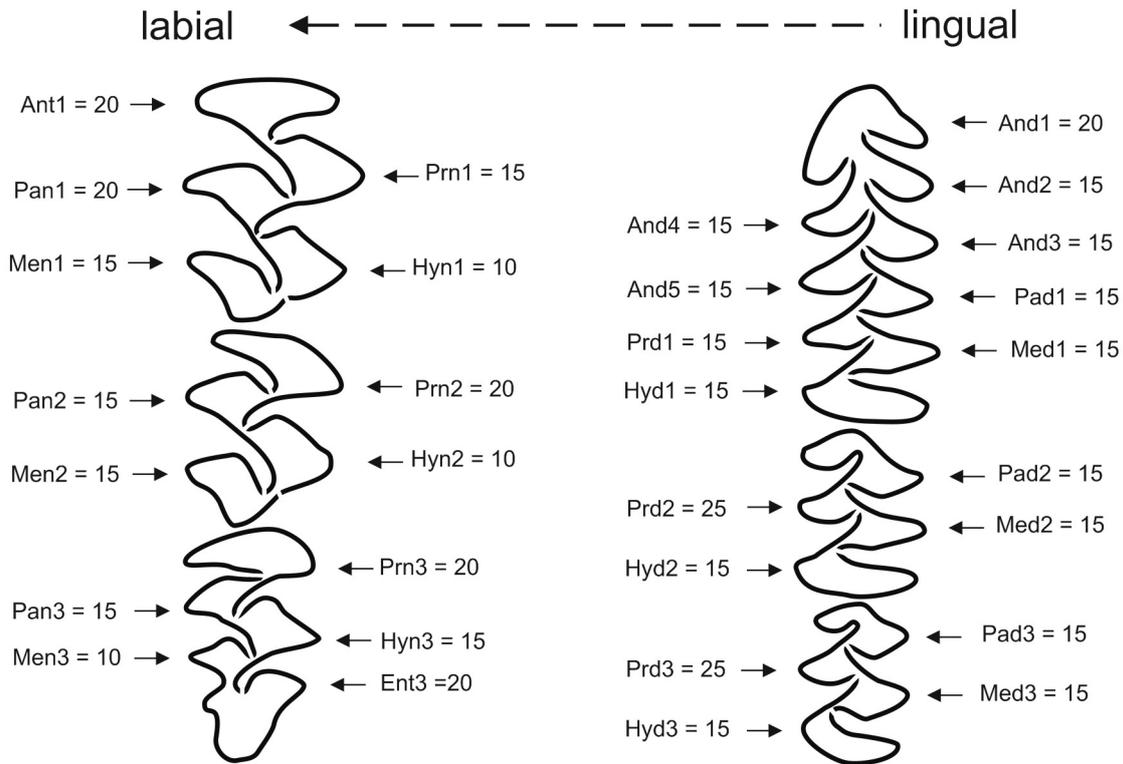


Fig. 1. Contour lines of molar crowns in *Ondatra*. Gaps in them mark boundaries between analyzed crown elements. Figures indicate numbers of semilandmarks set for the respective elements. Element designations: Ant/And — anterocon/anteroconid, Ententocon, Hyn/Hyd — hypocone/hypoconid (with posterior cingulum), Men/Med — metacon/metaconid, Pan/Pad — paracon/paraconid, Prn/Prd — protocon/protoconid; figures in the designations indicate tooth numbers M1, M2, M3 (except for enumeration of anteroconid elements).

as the average value of the pairwise Procrustes distances between respective specimens for the respective element.

Results

Individual variation of the crown elements (Fig. 2, parameter V) is the least for Pan1, Pan2, Pad1, and Med2 (0.07–0.09), and it is the highest for anteroconid angles (0.12–0.16) and especially Men3 and Ent3 (0.19–0.21).

The principal results of cluster analyses of correlations between crown elements are as follows. The bootstrap supports reach up to 98–100% for some pairs of elements, they exceed 50% for nearly all particular groupings thereof within each tooth, for about two third of such groupings within each toothrow, and for about one third of them in between-rows comparison. Regarding within-tooth comparisons (Fig. 3), the opposite (inner/outer) elements are most frequently paired. Regarding between-teeth comparisons within the same rows (Fig. 4), most of these pairs are retained, while grouping between homologous elements of M1 and M2 are additionally revealed in the upper (Pan1/Pan2, Prn1/Prn2) and partly lower (And5/Pad1–2) rows. In a total comparison (Fig. 5), upper and lower proto- and

paracones/paraconids on M1–2 were clustered together (though not with high bootstrap support), and several within-tooth (Men3/Ent3, And1/And4, Hyd1/Prd1, Hyd2/Med2, Prd2/Pad2) and between-teeth (Ant1/Prn3, And1–4/Hyn3) groupings were recognized.

Correlation coefficients between matrices of Procrustes distances are in general very low, varying from zero to 0.48 (the table is not provided here because of its large size and irrelevance of its details). Pairwise comparisons of the upper and lower crown elements provide the following results: average correlations between individual elements within each tooth vary from 0.09–0.11 (upper and lower M1, lower M3) to 0.13–0.14 (upper M2 and M3, lower M2); correlations between elements of upper and lower toothrows do not exceed 0.32 (significant for Pan1/Hyd2, Hyn2/Prd2, and Men3/Hyd3). Correlations between crown elements within each toothrow are similarly weak for the upper row (up to 0.32, significant for Ant1/Ant3, Men1/Hyn3), while they are stronger for the lower row (up to 0.48, significant for And1/And4, Prd1/Med1, Hyd2/Med2, Prd2/Pad3, Med2/Prd3, Hyd2/Prd3).

High correlation coefficients correspond to high bootstrap supports of clusters in some cases (And1/And4, Hyd1/Med1, Hyd2/Med2), while there is no

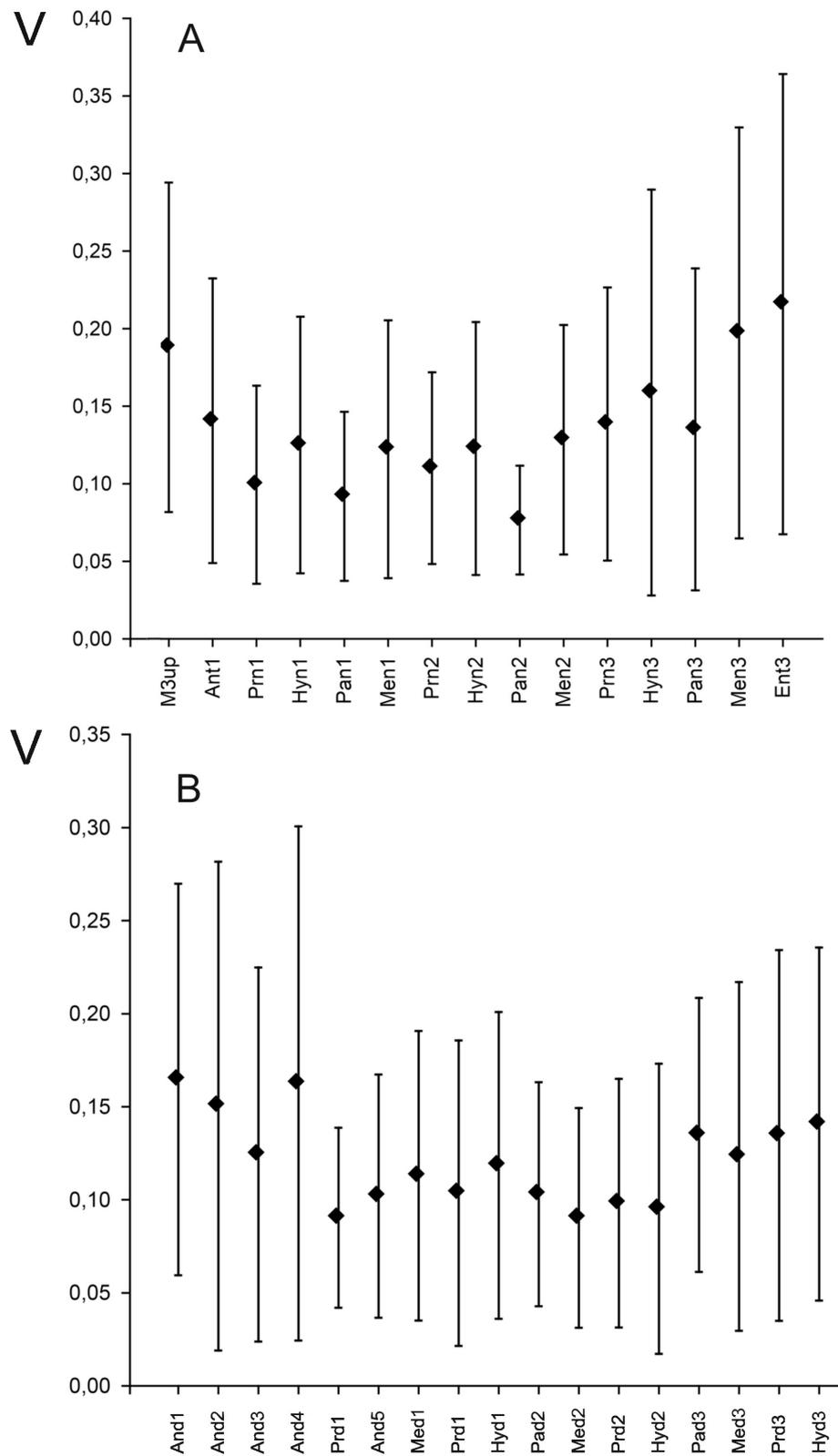


Fig. 2. Distance-based estimates (limits and averages) of variation of the crown elements of the cheek teeth of *Ondatra*. Element designations as in Fig. 1. V — individual variation of the elements (see text for explanation).

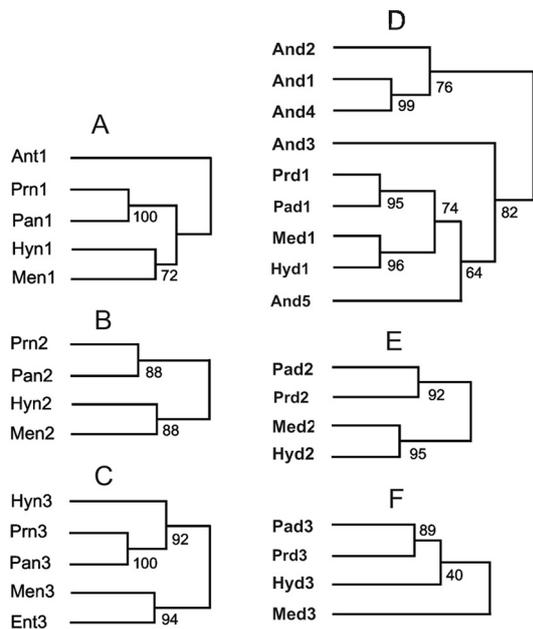


Fig. 3. Phenograms illustrating within-tooth covariations of the crown elements in *Ondatra*: A–C — upper M1–3, D–F — lower M1–3. Figures along branches are percentage of bootstrap support of respective clusters. Element designations as in Fig. 1.

such correspondence in others (Ant1/Prn3, Prn1/Pan1, Prd1/And5, Prd2/Pad2). With this, several pairs of significantly correlated elements are not distinguished by cluster analysis (Ant1/Ent3, Men1/Hyn3, Men3/Ent3, Hyn2/Prd2).

Discussion

The results of distance-based analysis of shape variation of the crown elements in *Ondatra* indicate that the most variable are those of lower M1 and upper M3 taking terminal position in them. They are similar in being underwent evolutionary complication due to the appearance of additional elements lacking in primitive bunodont dentition of lower cricetids (Gromov & Polyakov, 1977). Their high variability, as compared to other crown elements, means their developmental instability as a prerequisite of their high evolvability. Contrary to this, the elements of the second molars are the least variable: this is caused by the middle position of these teeth in the tooththrows, which limits greatly their possibility to change and predetermines their low evolvability.

By within-tooth analyses, clearly recognized are the modules that combine crown elements corresponding to the opposing pairs of cusps characteristic of generalized bunodont dentition (Vorontsov, 1982): these are protocone/paracone and hypocone/metacone in the upper M1–M2 and protoconid/paraconid and hypoconid/metaconid in the lower M1. A similar covariation pattern is observed in the lower M2, in which opposing

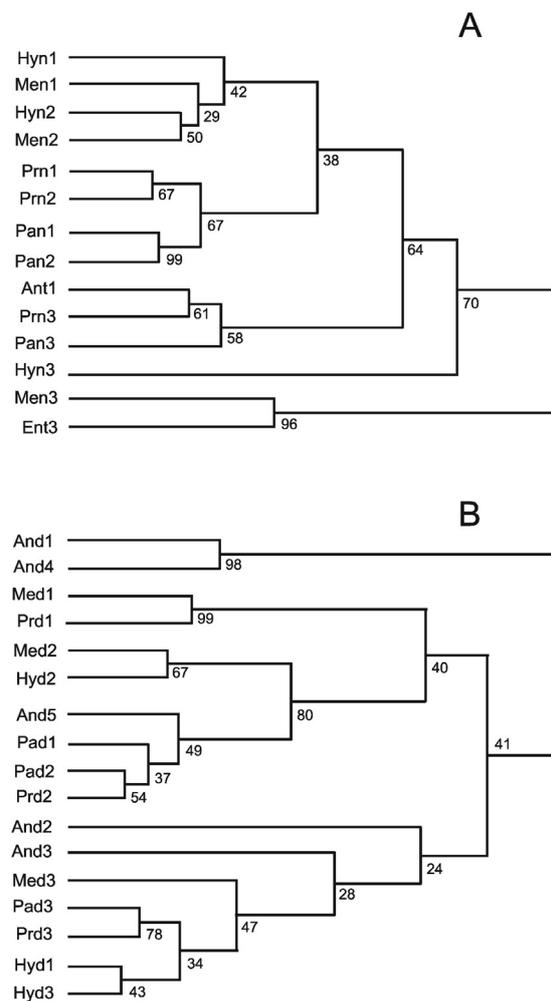


Fig. 4. Phenograms illustrating covariation of the crown elements within upper (A) and lower (B) tooththrows in *Ondatra*. Element designations as in Fig. 1, figures along branches as in Fig. 3.

protoconid/paraconid and hypoconid/metaconid constitute distinct modules; the first pair is distinguished also in the lower M3. It is these cusps that are fused first when bunodont molars turn into lophodont and prismatic ones. Thus, this level of integration of the elements of advanced prismatic dentition in arvicolines seems to reflect preservation of some basic integration features inherent in the ancestral bunodont dentition.

The complicated crown structure of the upper M3 and lower M1 each has its peculiar details. In the former, metacone is united with entocone, which develops evolutionarily as its “offshoot”. In the latter, of interest is that the posterior labial element of anteroconid (And5) is included in the same module with some basic cusps of M1–2, while its other elements (except for And3) constitute a separate module. Such covariation patterns may reflect evolutionary sequence of complication of the posterior portion of upper M3 and anterior portion of lower M1.

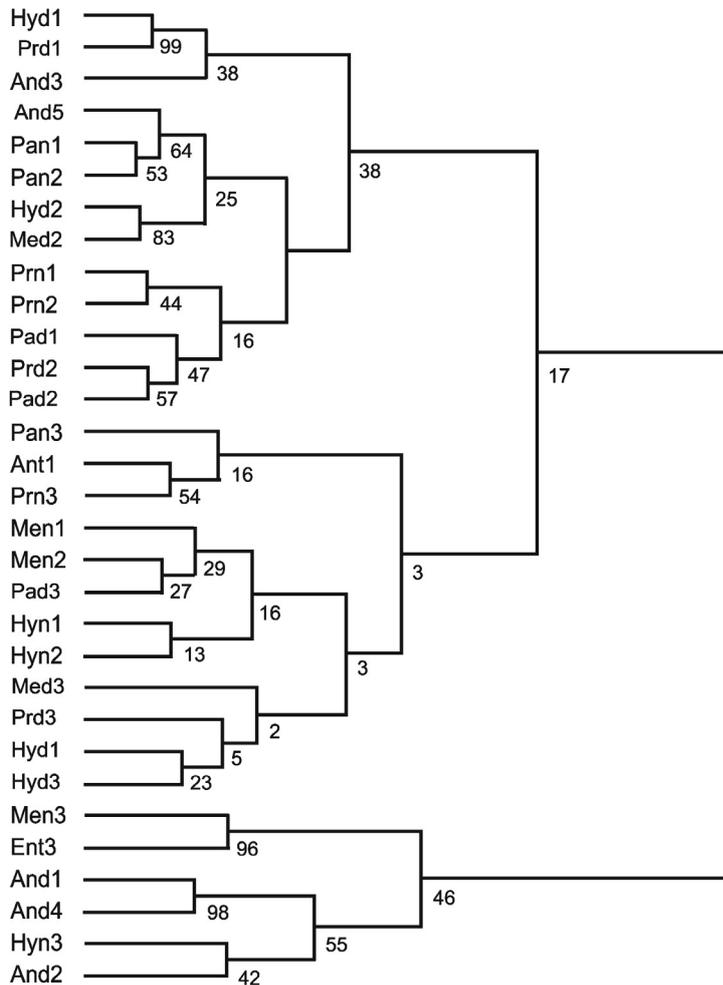


Fig. 5. Phenogram illustrating covariation of the crown elements of upper and lower tooththrows in *Ondatra*. Element designations as in Fig. 1, figures along branches as in Fig. 3.

Between-teeth analyses within each of tooththrows reveal somewhat different integration pattern. In this case, certain homologous crown elements of the adjacent teeth are first grouped to become integrated subsequently into those combined modules that were revealed by within-tooth analyses. This is most evident in the upper M1–2, in which the respective modules include (a) protocones with paracones and (b) metacones with hypocones. A partly similar pattern is observed in the lower M1–2, in which protoconids are combined in the same module with paraconid and metaconid. Along with this, several modules recognized by within-tooth analyses are also recognized by between-teeth comparisons, examples are proto- and metaconids of lower M2–3. Thus, covariation pattern revealed by between-teeth analyses allows supposing that, at least in the upper tooththrow, integration effect uniting the homologous crown elements located in different teeth into dispersed modules, is stronger than that uniting opposing elements in the same teeth. This effect largely agrees with the one shown previously for *Equus*, in which homologous (anterior and posterior) fossettes of different teeth are combined in such dispersed modules (Pavlinov & Spasskaya, 2021).

Between-rows analysis of upper and lower dentitions is most interesting in revealing a specific covariation of certain elements of anterior part of the lower M1 and posterior part of the upper M3. These two parts are known to undergo coherent complication in the arvicoline evolution (Gromov & Polyakov, 1977), and their covariation in the adult dentition of *Ondatra* may reflect their certain developmental conjugacy. However, it is questionable if it is reasonable to consider this covariation pattern a consequence of these elements (And1,2,4/Men3,Hyn3,Ent3) being parts of the same dispersed developmental module. Besides, another appealing grouping is revealed that includes crown elements of the opposing upper and lower first and second molars (Prn1–2/Pad1–2). This may reflect their strong functional interaction that is controlled developmentally.

Thus, the combinatorial approach, as applied to an elementaristic analysis of prismatic dentition in the arvicoline genus *Ondatra*, reveals a detailed covariation pattern in it, which appears to be biologically meaningful and in part non-trivial. Considering these results together with those previously obtained for dentition of the ungulate genus *Equus*, one may conclude that this methodology may provide an important tool for the exploration of integration pattern and modularity of highly specialized dentition of herbivorous mammals.

As GM, together with other numerical methods supplementing it, is new for the research of morphological integration, and it faces specific conceptual and methodological problems requiring both theoretical consideration and accumulation of extensive comparative data (Hallgrímsson *et al.*, 2009; Klingenberg, 2009, 2014; Mitteroecker & Gunz, 2009; Goswami & Polly, 2010; Lawing & Polly, 2010; Klingenberg & Marugán-Lobón, 2013; Cardini, 2019; Cardini & Marco, 2022). This evidently holds true for the combinatorial approach applied in our studies: its effective incorporation into this research program requests clarification of certain important points, before all reliable identification of the levels of integration (modularity) of crown elements, taking in consideration partial inconsistency of covariation patterns revealed by cluster and correlation analyses. The distance-based estimates of shape variation need certain standardization to be comparable

for different data sets. Of importance would be the elaboration of a unified protocol of elementaristic GM-description of various types of mammalian dentition (bunodont, selenodont, lophodont, prismatic, etc.) to make them compatible and comparable in this respect. Comparative data based on such description of prismatic dentition in rodents belonging to distant clades, such as muroids and cavioids, would be of crucial importance for testifying the validity of this methodology.

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Appendix 1. The total list of examined specimens of *Ondatra zibetica* (Linnaeus, 1766) used in the current paper. All specimens ($n = 20$) kept in the collection of Zoological Museum at Lomonosov Moscow State University (Moscow, Russia).

Locality: Odessa Obl. (Ukraine).

Specimens: ZMMU S-82414, S-82418, S-82424, S-82425, S-82426, S-82428, S-82433, S-82438, S-82441, S-82442, S-82443, S-82444, S-82445, S-82446, S-82447, S-82448, S-82450, S-82452, S-82458, S-82459.