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On the possibility of identifying pikas, *Ochotona* (Lagomorpha: Ochotonidae), on the basis of the morphology of the occlusal surface of permanent teeth

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ABSTRACT. Variation in the morphology of the occlusal surface of the third lower premolar tooth was examined in 69 skulls from adult specimens of four pika species. Variation was examined using standard geometric morphometric techniques as well as analysis of distances between landmarks (with and without accounting for the size factor). The results were checked on a sample of 46 specimens, specifically by comparing inter-species variance in dental and cranial features. Interspecies variation was low in the case of shape-only analysis of features of the third lower premolar, independent of the method used. Adding the size factor to the analysis notably increased the interspecies variation in teeth. Analysis of cranial data displayed more prominent interspecies variation compared to dental data, both with and without information on skull size. Sexual variation was minimal in all analyses. Thus, we suggest using information on tooth size for the purpose of species identification on the basis of the morphology of the occlusal surface of permanent teeth in pikas. In this case, morphological study of the occlusal surface of teeth could contribute to *Ochotona* species identification.

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KEY WORDS: *Ochotona*, Lagomorpha, teeth, skull, geometric morphometrics.

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О возможности определения видовой принадлежности пищух *Ochotona* (Lagomorpha: Ochotonidae) на основании признаков морфологии жевательной поверхности зубов

А.А. Лисовский, А.А. Кадетова

РЕЗЮМЕ. На основании выборки из 69 черепов взрослых пищух четырех видов была изучена изменчивость морфологии жевательной поверхности третьего нижнего предкоренного зуба. Изменчивость была изучена как при помощи стандартных методов геометрической морфометрии, так и с применением анализа дистанций между метками; с учетом и без учета размера зубов. Результат был проверен на выборке из 46 экземпляров, путем сравнения межвидовой дисперсии зубных и краниальных признаков. Независимо от метода, межвидовая изменчивость была низкой в случае анализа признаков формы третьего нижнего предкоренного, без учета размера зуба. Добавление в анализ размерного фактора заметно повысило межвидовую дисперсию. Анализ краниальных данных во всех вариантах показал большие значения межвидовой дисперсии, чем анализ дентальных данных. Половая изменчивость во всех случаях была минимальной. Мы рекомендуем обязательно использовать размерные показатели зубов для целей определения вида пищух по признакам морфологии жевательной поверхности. При таком подходе изучение морфологии жевательной поверхности зубов может оказаться полезным для определения видовой принадлежности экземпляров *Ochotona*.

КЛЮЧЕВЫЕ СЛОВА: *Ochotona*, Lagomorpha, зубы, череп, геометрическая морфометрия.

Introduction

Pikas (*Ochotona* Link, 1795) are hamster-size lagomorphs with relatively homogenous morphology. They are common in recent mountainous communities of Asia and North America within the Holarctic region. In some cases, morphological identification of pikas is not an easy task (Lissovsky, 2014), even when the whole animal is available. Geographic distribution of the genus

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was even greater in the Pleistocene era (Erbajeva, 1988; Angelone, 2008), but species identification requires extended knowledge of morphology of separated bony remains. For pika fossils, tooth morphology plays a major role in species identification (Erbajeva, 1988; Čermák *et al*., 2006; Fostowicz-Frelik, 2008; Čermák & Rekovets, 2010). In theory, tooth morphology could also represent an additional feature in recent pika identification, but existing descriptions about variations in this feature are not yet sufficient.

Our previous paper (Volkova & Lissovsky, 2018) contained unusual results of very low interspecies variation together with very high intrasample variation in the shape of the occlusal surface of permanent teeth from four pika species. Such results ostensibly contradict hands-on experience in identifying pika remains. Therefore, we attempted to analyse previous results using a slightly different set of methods. Initially, we hypothesised that the main culprit of high intrasample variation is a Pinocchio effect (Chapman, 1990; Cramon-Taubadel *et al*., 2007) that shifts the results of Procrustes analysis and increases intrasample variance. Thus, we compared the results of common geometric morphometric methods with analysis of distances between landmarks that are independent of the alignment method. These results were compared to cranial variation.

Material and methods

We studied the third lower premolar (p_3) in 69 skulls of adult pikas (the first set) from a craniological collection of the Zoological Museum of Moscow State University (ZMMU, Moscow, Russia) and Zoological Institute of Russian Academy of Science (ZIN, Saint Petersburg, Russia; see Appendix). Forty-six intact pika skulls (the second set) were included in the comparison of dental and cranial variations. The modest sample size was limited due to natural reasons, namely the presence of intact skulls with teeth from four different pika species in museum collections. The sample comprised four species: *Ochotona hyperborea* (Pallas, 1811), *O. mantchurica* Thomas, 1909, *O. macrotis* (Günther, 1875) and *O. rutila* (Severtsov, 1873). One of these species had two remote geographic groups (sample sizes for the first and second sets are shown in parentheses):

O. hyperborea: Russian Federation, Amur Region, Zeyskiy District, Zeyskiy Nature Reserve (set 1: 13; set 2: 6); Russian Federation, Sakha Republic, lower Indigirka River, Shamanovo (set 1: 11; set 2: 8).
O. mantchurica: Shilka and Argun Rivers interfluve and Greater Khinggan (set 1: 21; set 2: 16).
O. macrotis: Pamir (set 1: 15; set 2: 12).
O. rutila: North Tian Shan Mountains (set 1: 9; set 2: 4).

The teeth were prepared for analysis as described by Volkova & Lissovsky (2018). The objects in this study were represented by two-dimensional projections of permanent teeth rows on the right side of the lower jaw from the occlusal (chewing) surface. Photos of the tooth surfaces were taken using an Olympus SZX10 binocular microscope and a DeltaPix Invenio II camera (magnification ×12.5) in ZMMU and using a Canon EOS 60D digital camera combined with a binocular LOMO Micromed MSP-1 via an optical adapter AOT-1C in ZIN. Tooth photos from both museums were transformed to the same scale. The lower jaws were fixed in place using plasticine as follows: the dental row was manually positioned so that the buccal side of the right dentition was perpendicular to the focal plane of the lens. Since no positioning error was found in the previous study (Volkova & Lissovsky, 2018), we did not account for this factor. There was also no issue with the lack of focus depth with one tooth.

We studied the configuration of 20 landmarks for the p_3 (Fig. 1). The landmarks were located on the images of the teeth using TPSdig v.1.40 (Rohlf, 2004). To obtain the variables of shape that are independent of the position of the tooth in the picture (Procrustean coordinates, PrC), the landmark configurations were aligned using the principal axes option of MorphoJ software (Klingenberg, 2011). The PrC were not normalised. Forty variables (X and Y PrCs for each landmark) that characterised the shape of the occlusal surface and centroid size as a measure of tooth size were used in the analyses. An additional dataset was obtained that calculated distances between landmarks' PrCs. Such distances set was independent of tooth size because they were calculated on the basis of PrCs. They were also free of aligning error (Pinocchio effect) because they contained no landmark coordinates. We calculated 33 distances in total, 20 that describe the perimeter of the occlusal surface and 13 that cross the teeth in several directions: 1–13, 1–20, 1–4, 1–12, 4–8, 4–20, 6–18, 8–11, 8–16, 9–11, 9–14, 11–14, 16–20, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 9–10, 10–11, 11–12, 12–13, 13–14, 14–15, 15–16, 16–17, 17–18, 18–19, 19–20, 20–1 (numbers are landmark IDs from Fig. 1).

Skull size and shape were studied on the basis of 14 standard measurements taken using calipers (0.01 mm accuracy) according to Lissovsky (2014). These measurements were: condylobasal length (KBD), length of palatine foramen (DNOTV), upper diastemal length (DIAST), alveolar length of maxillary toothrow (DVKR), rostral length (DLITS; from the anterior edge of premaxillary bones to the posterior edge of maxillary toothrow alveoli), length of auditory bulla (DBAR), maximal length of orbit (DG), maximal width of orbit (WG), interorbital constriction (MG), zygomatic breadth (SKW), postorbital constriction (ZG), maximal width between lateral edges of auditory bullae (WSLB), general skull height (H) and general height of mandible (HNCH) (Lissovsky, 2014; Fig. 1). These measurements, without any transformation, comprised one "shape and size" dataset. The skull size factor was calculated as a square root from the sum of quadrates of the measurements divided by the number of measurements. The "size-free" dataset comprised the measurements divided by the size factor.

The main analysis method was a multivariate analysis of variance (MANOVA) variant, namely the maximum likelihood algorithm of the variance component analysis (after Lissovsky & Pavlinov, 2008). This analysis allows estimation of the percentage variance associated with

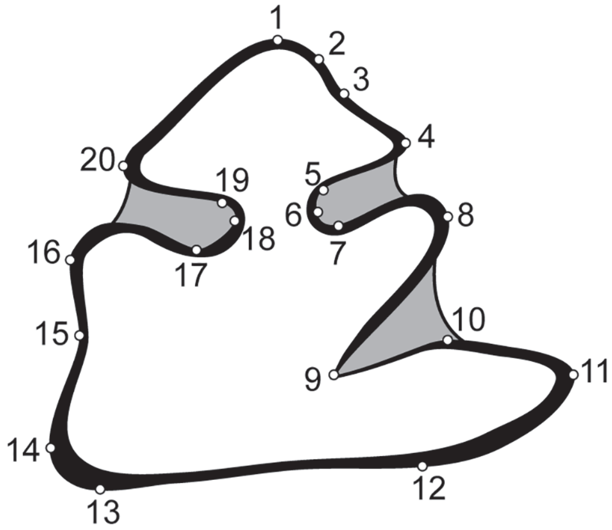

Fig. 1. Location of landmarks on the third lower premolar tooth used in the analysis.

various factors. Two factors (SEX and SPECIES) were considered to be non-fixed. A previous study (Volkova & Lissovsky, 2018) showed a lack of sexual variation in pika teeth, but we used the gender factor to compare with other factor contributions. There were five variants of dependent variables:

1. the set of tooth PrCs;
2. the set of tooth inter-landmarks distances;
3. the set of tooth PrCs together with centroid size;
4. the set of skull measurements;
5. the "size-free" set of skull measurements.

The contribution (S_j) of each factor (j) to the total diversity of all variables (i) was calculated using the formula:

$$S_j = \frac{\sum_{i=1}^{K} s_{ij}}{\sum_{j=1}^{K} \sum_{i=1}^{C} s_j} \quad ,$$

where s_{ij} is the proportion of variance for one dependent variable (i) for each factor or the within-group variation, C is the number of dependent variables and K is the number of factors plus 1 (for within-group variation). The ranges of the variance components were estimated using a subsampling approach. We generated 50 replicas by randomly removing one element from each sex/species group. The fixed rate of removal was due to the small sample size. Assuming that sexual variation could be sample-specific (Thorpe, 1976), we used a nested design (SEX nested in SPECIES).

We used STATISTICA ver.13.0 for all calculations, mainly the Variance Components module. Original algorithms were written by A.A. Lissovsky using Statistica Visual Basic language.

Results

Analysis of the shape of the occlusal surface of the third lower premolar of four pika species using landmark PrCs confirmed the predominance of within-group variance. Interspecies variance accounted for approximately 40% of general variation (Table 1). Analysis of distances between landmarks provided the same results: variation ranges of the resulting values intersected entirely in these two kinds of analysis. Adding tooth size into the analysis radically altered the results: interspecies variance increased to approximately 77%.

Comparison of morphological variation in tooth occlusal surface and skull measurements of the same specimens demonstrated that interspecies variance in skull analysis was notably larger (Tables 2, 3). Variation ranges of skull-based interspecies variance did not intersect with the same ranges of tooth-based analysis (Table 2). Although considering the skull in

Table 1. Variance proportion (%) of interspecies (SPECIES), sexual (SEX) and within-group (Error) variation in the third lower premolar of adult specimens of four pika species (SEX is nested in SPECIES; variation range is indicated in parentheses). Notes: GM — analysis of landmark Procrustes coordinates; LD — analysis of distances between landmarks after Procrustes analysis; centroid size was used as the tooth size measure.

Analysis	Form of variability		
	SPECIES	SEX	Error
Tooth shape (GM)	40.7 (37.9–43.3)	1.0 (0.5–1.7)	58.3 (56.0–61.6)
Tooth shape (LD)	41.0 (37.6–44.2)	0.8 (0.4–1.4)	58.2 (55.0–61.8)
Tooth shape (GM) and size	76.8 (74.0–79.9)	0.0 (0.0–0.0)	23.2 (20.1–26.0)

Table 2. Variance proportion (%) of interspecies (SPECIES), sexual (SEX) and within-group (Error) variation in the third lower premolar and skull of adult specimens of four pika species (SEX is nested in SPECIES; variation range is indicated in parentheses) calculated on the same specimens. Tooth shape was analysed on the basis of the landmark Procrustes coordinates; centroid size was examined as tooth size measurements. Skull shape was studied on the basis of standard skull measurements, and the skull size was reduced by dividing it by the centroid size.

Analysis	Form of variability		
	SPECIES	SEX	Error
Tooth shape	42.0 (38.3–45.4)	1.1 (0.5–2.3)	56.9 (53.2–60.2)
Tooth shape and size	77.9 (74.0–83.3)	0.0 (0.0–0.4)	22.1 (16.7–26.0)
Skull shape	86.3 (85.4–88.0)	0.2 (0.1–0.5)	13.5 (11.5–14.3)
Skull shape and size	88.9 (87.4–91.0)	0.1 (0.0–0.7)	11.0 (8.4–12.6)

Table 3. Variance proportion (%) of interspecies variation in the third lower premolar characteristics (below diagonal) and skull characteristics (above diagonal) of adult specimens of four pika species, calculated on the same specimens. Tooth shape was analysed on the basis of landmark Procrustes coordinates; centroid size was used as the tooth size measure. Skull shape was studied on the basis of standard skull measurements.

	O. hyperborea	*O. mantchurica*	*O. macrotis*	*O. rutila*
O. hyperborea		71.9	87.3	91.6
O. manchurica	69.5		70.4	81.9
O. macrotis	68.8	62.3		73.6
O. rutila	82.8	39.8	75.7	

the analysis increased interspecies variance, the difference between “pure shape”-based and “shape and size”-based results was not as large as in the case of dental analysis. Variation ranges of this parameter even slightly intersected. Sexual variation was minimal in all analyses.

Discussion

Our analysis confirmed our previous findings (Volkova & Lissovsky, 2018) on very low interspecies variance in the shape of the pika occlusal surface of the third lower premolar. This study utilised a slightly different set of species compared to Volkova & Lissovsky (2018): two species (*O. hyperborea* and *O. macrotis*) were represented in the previous paper, but this study analysed a different population set, and two species (*O. mantchurica* and *O. rutila*) were not previously studied. Thus, these results were obtained from two different data sets. Changing the approach of analysis of shape did not alter the result. Therefore, our initial hypothesis that large within-group variation was caused by the Pinocchio effect was rejected.

Adding the size factor to variance analysis increased the percentage of interspecies variance approximately two-fold. Thus, we can assume that the shape of the enamel loops alone represents a poor parameter for pika species identification. Combining size and shape parameters strengthened the decision on species classification two-fold. Nevertheless, it was not obvious which value of the proportion of interspecies variance was sufficient to allow stable species identification. In order to evaluate this factor, we compared the results on dental variation with the same results on skull variation. Parameters of metric skull variation allowed successful identification of the majority of pika species (Lissovsky, 2014). Thus, comparison with skull variation could be a good feature to predict the identification importance of dental characteristics.

Interspecies variance was larger in all cases of cranial analyses (Table 2). Nevertheless, the values obtained on the basis of the combined dataset of the shape of the occlusal surface and size of the teeth were similar to the results of cranial analyses, albeit without intersection of the variation range. Thus, we hypothesise that although dental analysis tends to provide less informative results for pika species identification, combining size and shape parameters generates results comparable to cranial analysis. In some cases (Table 3; *O. rutila*–*O. macrotis*), dental variation can display comparable or even better results than cranial analysis. Future detailed quantitative pairwise comparison of pika species in the size and shape of the occlusal surface of teeth could contribute to pika species identification.

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Appendix. List of specimens, analyzed in the study. Specimens with tooth and skull analysed are marked with asterisk. Museum IDs and sex are listed

Ochotona hyperborea

Russian Federation, Amur Region, Zeyskiy District, Zeyskiy Nature Reserve: ZMMU S-150667*, female; ZMMU S-150658*, male; ZMMU S-150626*, male; ZMMU S-150649*, female; ZMMU S-150637*, female; ZMMU S-150661*, female; ZMMU S-150630, female; ZMMU S-150631, female; ZMMU S-150634, male; ZMMU S-150645, female; ZMMU S-150647, female; ZMMU S-150651, male; ZMMU S-150663, female.

Russian Federation, Sakha Republic, lower Indigirka River, Shamanovo: ZMMU S-65245*, male; ZMMU S-65240*, male; ZMMU S-65231*, male; ZMMU S-65230*, female; ZMMU S-65229*; ZMMU S-65228*, female; ZMMU S-65242*, female; ZMMU S-65238*, female; ZMMU S-65233, male; ZMMU S-65237, female; ZMMU S-65239.

O. mantchurica

Shilka and Argun Rivers interfluve (Russian Federation, Zabaykalskiy Territory) and Greater Khinggan (China, Inner Mongolia): ZMMU S-82585*, male; ZMMU S-178617*, male; ZMMU S-178616*, female; ZMMU S-178618*, male; ZMMU S-82584*, male; ZMMU S-82594*, male; ZMMU S-134660*, male; ZMMU S-82830*, female; ZMMU S-178615*, female; ZMMU S-14806*, female; ZMMU S-82583*, female; ZMMU S-14805*, female; ZMMU S-175365*, male; ZMMU S-17277*, female; ZMMU S-134658*, male; ZMMU S-30110*, male; ZMMU S-15648, male; ZMMU S-82831, female; ZMMU S-178619, male; ZMMU S-182047, female; ZMMU S-178621, female.

O. macrotis

Tajikistan, Pamir: ZIN 40520*, male; ZIN 40519*, male; ZIN 86586*, female; ZIN 40517*, female; ZIN 86583*, female; ZIN 40518*, female; ZMMU S-144172*, male; ZIN 86584*, female; ZIN 20299*, female; ZMMU S-144154*, female; ZIN 86585*, female; ZIN 86587*, male; ZMMU S-144173, female; ZIN 22563; ZIN 23150.

O. rutila

North Tian Shan Mountains (Kazakhstan, Kirgizia): ZMMU S-139611*, male; ZMMU S-163699*, male; ZMMU S-14927*, female; ZMMU S-64872*, male; ZMMU S-14926, male; ZMMU S-14929, male; ZMMU S-14930; ZMMU S-60601, female; ZMMU S-82829, female.