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GENERAL BIOLOGY

Morphological Differentiation of *Clethrionomys rufocanus* Sundevall, 1846 and *Clethrionomys rex* Imaizumi, 1971 (Arvicolinae, Rodentia) in the Zone of Sympatry in the Far East

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The subgenus *Craseomys* of the genus *Clethrionomys* is represented by two species, the widespread species of the Palearctic ecozone *Clethrionomys rufocanus* Sundevall, 1846 and the species *Clethrionomys rex* Imaizumi, 1971 inhabiting only Sakhalin Island, several Japanese and the South Kuril Islands [2, 8, 11]. Their taxonomical status has been proved relatively recent by molecular genetic methods [5]. Nevertheless, the issue about their level of morphological differentiation is still open. The most obvious differences of these species are displayed in morphotypical characteristics of the third upper molar (M3), with other morphological features [1, 5–8], including the first lower molar (m1) [1, 4], being similar, which is successfully used in diagnosis of many modern and fossil species of voles. Comparison of the dental features of *Cl. rufocanus* and *Cl. rex* is connected with that the essential age-related changes of the occlusal surface pattern of molars with crown abrasion are typical of them, as well as of all forms of rhizodont voles. The detection of age variability with the use of the method based on 11 ontogenetic stages of molars [3] allowed us to estimate the levels of morphological differentiation of the species studied with respect to both M3 and m1 and find the evolutionary trends of their dental system.

Morphological characteristics of M3 and m1 of *Cl. rufocanus* and *Cl. rex* captured on Shikotan Island (the South Kuril Islands) have been studied. Samples are presented by individuals of different age (Table 1).

The M3 morphotypes of *Cl. rufocanus* and *Cl. rex* were distinguished with the use a modified method of Rörig and Börner [10], with adding of two intermediate morphotypes "sim-typ" (from "simplex" to "typica") and "duplicata 1" (from "typica" to "duplicata 2"). The m1 morphotypes were determined with the use of Niethammer's method [9]. Measurements of the morphometric features of the m1 occlusal surface were done on digital images [3] according to the scheme presented in Figs. 1a, 1b. Statistical calculations of the results were performed using the software package Statistica v.6.0.

Interspecific differences have been found both in the process of crown formation and roots of molars. In *Cl. rex*, the dentine tracts (enamel interruptions) reach the m1 occlusal surface before the completion of crown formation, whereas in the majority of *Cl. rufocanus*, the interruption of dentine tracts is coincident with the beginning of root formation (the 5th–6th stages). Among 62 individuals of *Cl. rufocanus*, only in two individuals do the dentine tracts reach the occlusal surface before the beginning of root formation. In comparison with *Cl. rufocanus*, the dentine tract on the anterior cap of *Cl. rex* is wider and more shifted to the buccal side.

Differences in root formation between the species studied are shown in the height of the tooth neck (the

Table 1. Age structure of the samples of *Cl. rufocanus* and *Cl. rex* (based on the ontogenetic stages of molars [3])

Species	Total number of individuals	Ontogenetic stage				
		3	4	5	9	10
Cl. rufocanus	62	1	35	4	17	5
Cl. rex	36	5	22	–	6	3

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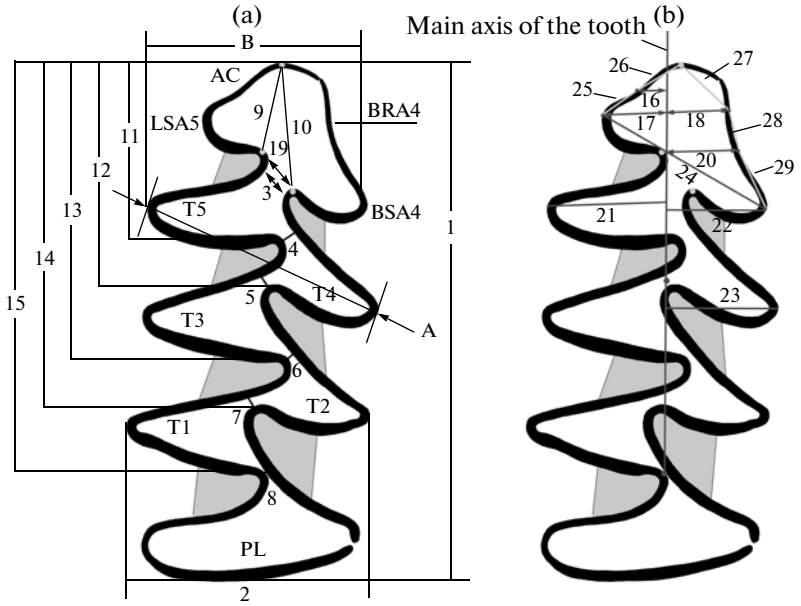

Fig. 1. The scheme of (a) measurements of the occlusal surface m1 and (b) additional measurements of the anteroconid in *Cl. rufocanus* as an example and indication of m1 elements. AC, anterior cap; PL, posterior lobe; T1–T5, dentine fields (triangle loops); BRA4, the 4th buccal reentrant angle; BSA4, the 4th buccal salient angle, LSA5, the 5th lingual salient angle.

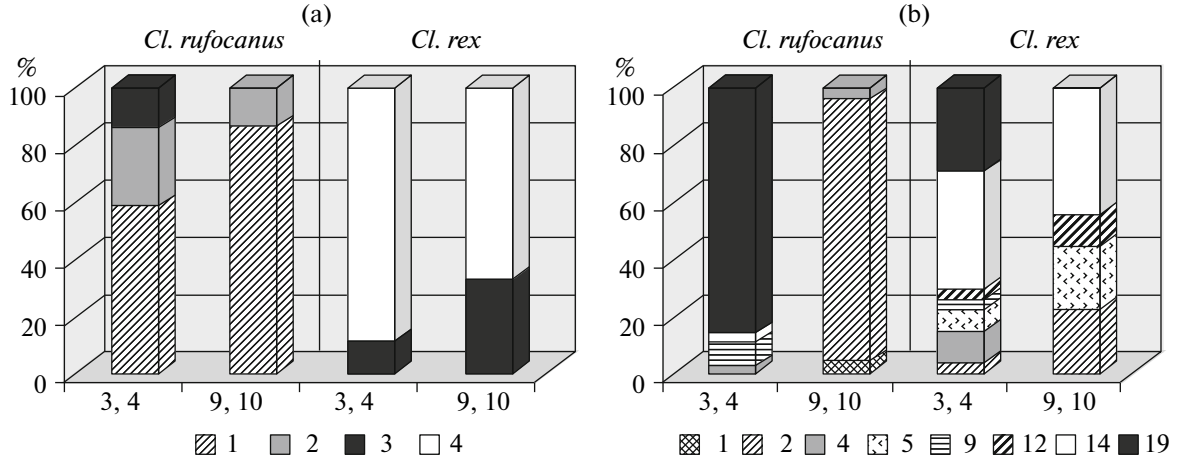

Fig. 2. Frequency distributions of the (a) M3 and (b) m1 morphotypes at different ontogenetic stages in *Cl. rufocanus* and *Cl. rex* from Shikotan Island. Indication M3 morphotypes on a: (1) simplex; (2) sim-typ; (3) duplicata 1; (4) duplicata 2.

distance from the place of root furcation to the lower border of enamel). Abe [4] showed that the formation of a high neck and small roots is typical of molar teeth of some forms of *Cl. rex* from the Japanese Islands. We have revealed significant interspecific differences in the neck height of m1 ($F(1; 11) = 12.13$, $p = 0.01$): while in *Cl. rufocanus*, the tooth neck is not high or almost not expressed ($M \pm SD$ (the 9th stage) $= 0.72 \pm 0.20$ mm; $M \pm SD$ (the 10th stage) $= 0.70 \pm 0.11$ mm), in *Cl. rex*, its height at late stages may exceed the height of roots ($M \pm SD$ (the 9th stage) $= 1.53 \pm 0.59$ mm; $M \pm SD$ (the 10th stage) $= 1.03 \pm 0.33$ mm). Thus, in comparison with *Cl. rufocanus*, the acceleration of development of dentine tracts and retardation of root formation in *Cl. rex* indicate a higher stage of hypsodonty of molars of this species.

The third stage was combined with the fourth stage; and the ninth stage, with the tenth stage in comparison with frequency distributions of the M3 and m1 morphotypes both for *Cl. rufocanus* and *Cl. rex*. Analysis of the M3 morphotypes frequencies confirmed published data about a more complicated configuration of this tooth in *Cl. rex,* compared to *Cl. rufocanus* [1, 4, 5, 8]. It is typical both of young animals (the 3rd and 4th

Table 2. Standardized coefficients of the discriminant function and means of canonical variables for *Cl. rufocanus* and *Cl. rex* from Shikotan Island

Features of m1 and stage no.	DF 1	DF 2
6	0.12	0.12
1	–2.54	–1.39
12	–0.34	–0.66
15	2.06	2.44
17	1.12	0.29
5	–0.37	–0.37
23	–1.29	0.85
B	1.62	0.88
29	1.29	–0.80
11	0.55	–0.07
A	–0.59	–1.04
24	–1.30	0.35
9	1.15	0.19
27	–0.61	–0.62
19	0.23	0.51
18	–0.15	–0.17
14	–1.05	0.05
22	0.66	–0.87
Constant	33.55	–7.52
	Means of canonical variables	
4th stage, *Cl. rex*	0.56	–4.38
9th stage, *Cl. rex*	–10.44	–0.30
4th stage, *Cl. rufocanus*	5.38	1.70
9th stage, *Cl. rufocanus*	–7.78	2.11

stages: $\chi^2 = 55.36$, $df = 3$, $p < 0.000$) and old animals (the 9th and 10th stages: $\chi^2 = 31.03$, $df = 3$, $p < 0.000$). In the sample of *Cl. rufocanus*, a simpler morphotype ("simplex") with three buccal and lingual salient angles predominates, which is absent in *Cl. rex* (Fig. 2a). Complicated morphotypes ("duplicata 1 and 2") with three or four lingual and four buccal salient angles are typical of *Cl. rex*. In *Cl. rufocanus*, the morphotype "duplicata 1" can be found at early stages of molar development (Fig. 2a).

Comparison of the m1 morphotypic characteristics of *Cl. rex* and *Cl. rufocanus* with determination of the ontogenetic stage has revealed a previously unknown significant interspecific differences of the morphotype frequencies for both young ($\chi^2 = 33.37$, $df = 6$, $p < 0.000$) and old animals ($\chi^2 = 20.16$, $df = 5$, $p < 0.001$). In the majority of young *Cl. rufocanus*, all dentine fields are merged on m1 (motphotype 19); in old animals, the m1 morphotype typical of this species with the isolation of all dentine fields except the neck of anteroconid (T5–AC) predominates (morphotype 2) (Fig. 2b). The m1 morphotype with the isolation of dentine fields at the levels T3–T2 and T1–PL (morphotype 14) is predominant in *Cl. rex* (Fig. 2b). However, m1 with isolated dentine fields may occur in young *Cl. rex* (morphotypes 2 and 19) as well as in *Cl. rufocanus*. The common morphotypes m1 in very young and old animals of these species may complicate their identification based on morphotypic characteristics.

These species also differ from each other in the shape of the m1 anterior cap. In m1 (in definitive stages) of *Cl. rufocanus* the lingval angle LSA5 and buccal angles BRA4 and BSA4 are well pronounced that underlines its "mushroom form." For m1 of *Cl. rex*, a simple configuration of the anterior cap is typical, it is morphotypically somewhat similar to m1 of the nominative subspecies *Clethrionomys*, when emergent angles on the anterior cap are almost indiscernible, and it slants to the buccal posterior direction.

The stepwise discriminant analysis has been performed for estimating the differentiation of *Cl. rufocanus* and *Cl. rex* from the zone of sympatry on morphometric features of m1 at the 4th and 9th stages, which were the best represented in the samples. The first discriminant function (DF1) is connected with age variation in the proportions the occlusal surface of m1 (characters 1, 14, 15, 23, B) and the shape of the anterior cap (characters 9, 17, 24, 29) (Table 2). Interspecific differences, observing in direction of DF2, are connected with characteristics of the anteroconid (characters 12, 22, 23, A, B), with the shape of the anterior cap (characters 27, 29) and proportions of m1 (characters 1, 15) (Table 2). The correct identification of *Cl. rufocanus* and *Cl. rex* based on these characters with molars at the 4th stage is possible with 100% accuracy. Differences between old individuals with occlusal surface abrasion (the 9th stage) are smaller (differentiation is 96%) (Fig. 3). Additional testing of the discrimination by the "sliding examination" method has shown that the average accuracy of species identification that are the best represented in the samples studied with respect to 18 characters is lower (98%) because of a decreased rate of correct identification of *Cl. rex*, but in general, it remains high (Table 2).

Thus, the presence of a high level of morphological differentiation of *Cl. rufocanus* and *Cl. rex* estimated by morphotypic and morphometric characters of M3 and m1 have been shown in the zone of sympatry on Shikotan Island. A high level of discrimination allows us to recommend the morphometric characters of m1 for species identification. Comparison of interspecific differences with their dynamics in ontogenesis confirms the assumption that these species follow different scenarios of the evolution of the dental system: more hypsodont molars of *Cl. rex* retain the common morphotypic characteristics of m1 of the nominative subspecies of the genus *Clethrionomys* to a greater extent, which is accompanied by a considerable complication of M3 because of formation of additional elements; the *Cl. rufocanus* evolutionary trends are the

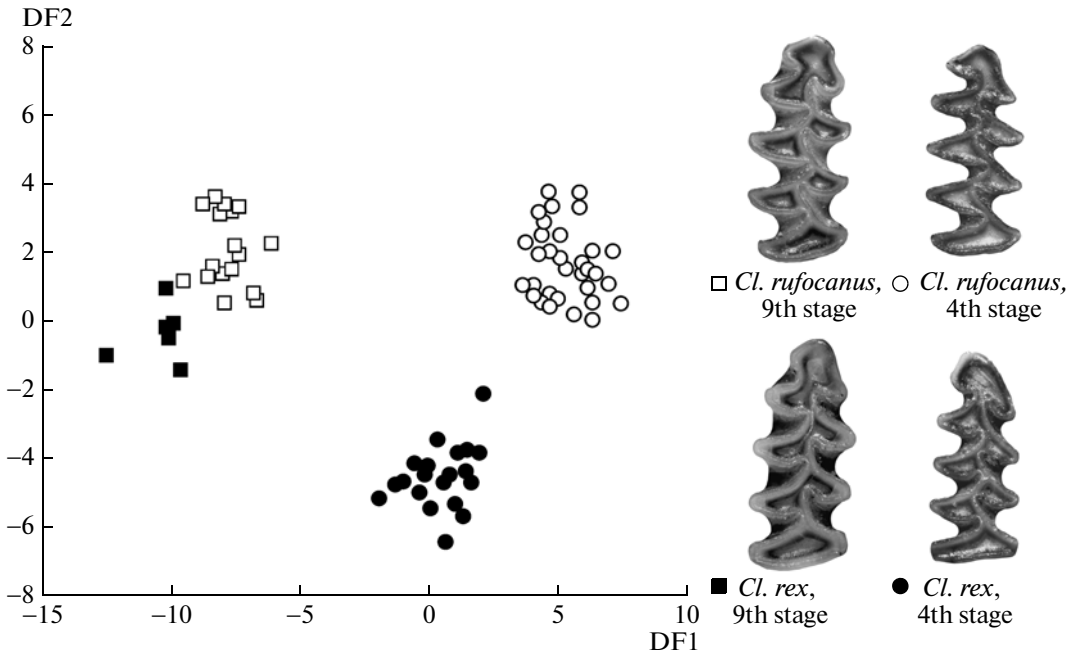

Fig. 3. Distribution of samples of the m1 (first lower molar) of *Cl. rufocanus* and *Cl. rex* from Shikotan Island at the 4th and 9th stages in the space of the first (DF1) and second (DF2) discriminant functions.

isolation of the dentine fields of the molar, preservation of the simple configuration of M3, and complication of the anteroconid elements of m1.

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