

Evolution of marmots (Rodentia, Sciuridae): combining information on labial and lingual sides of the mandible

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The morphology of the lingual side of the mandible was analysed in the genus *Marmota*. Adults from 12 of 14 living species were compared using geometric morphometric techniques. The information on the lingual side was then combined with that of the labial side from a previous analysis. The combined dataset is the most complete description of a marmot mandible ever used in an interspecific comparison in this genus and it represents the second morphological study ever realized that includes a large sample with all marmot species. The study confirmed the uniqueness of *M. vancouverensis* for mandible morphology, with atypical traits likely to have evolved in a relatively young but small population of marmots isolated on the Vancouver Island since the end of the Pleistocene (10 000–100 000 ybp), stressing the importance of a strong effort to save this species from extinction. *Marmota olympus* also has a distinctive mandibular trait undetected in a previous analysis using information on the lingual side only. The Olympic marmot has a longer evolutionary history but it is presently found only in the Olympic Peninsula and shares with *M. vancouverensis* a similar history of geographic isolation and population bottlenecks. Genetic bottlenecks might have profoundly affected marmot morphological evolution and may have increased the rate of shape change in marmot radiations. Both the analysis of the lingual side alone and the combined dataset (lingual and labial information) failed to clearly discriminate the two main marmot subgenera but the combined description of the mandible supports the similarity of the two main species (*M. caligata* and *M. flaviventris*) of the subgenus *Petromarmota*, and the proximity of the North American *M. monax* to Palaearctic marmots. Surprisingly, the analysis of the lingual side indicated that *M. camtschatica* and *M. marmota* have unusual traits for the subgenus *Marmota*. At least for the latter species, this finding is congruent with peculiarities in fur and parasitic relationships, and may be related to an ancient phylogenetic origin for *M. marmota*.

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Introduction

The genus *Marmota*, a member of the sciurid family (Hoffmann *et al.* 1993), includes the largest mammals capable of true hibernation (Armitage 2000). Most of its 14 species are social rodents, living in colonies that occupy mountains or high latitude meadows in the Holarctic.

Marmots have been the focus of great interest among sociobiologists studying the evolution of mammal societies. The variable degree of sociality found in different marmot species was correlated to environmental harshness (Barash 1974) providing interesting clues about selective forces influencing mammal social evolution (Armitage 2000). Sociobiological studies need to be placed in an evolutionary context, but only recently have extensive studies on marmot molecular (Kruckenhauser *et al.* 1999, Steppan *et al.* 1999) and morphological (Cardini 2003, Polly 2003, Cardini and Slice 2004) evolution been undertaken. Morphological analyses of marmot skeletal characters are also aimed at testing the hypothesis of a presumed propensity of the sciurid skeleton to homoplasy due to size or ecological similarities (Hafner 1984, Roth 1996, Velhagen and Roth 1997).

Steppan *et al.* (1999) compared mitochondrial cytochrome b sequences among all living marmots, and found that two main radiations can be hypothesized. The subgenus *Marmota* includes all Palaearctic species plus two North American ones, *M. broweri* and *M. monax*. The subgenus *Petromarmota* consists of four Nearctic species (*M. caligata*, *M. flaviventris*, *M. olympus*, and *M. vancouverensis*) inhabiting the Rocky Mountains (To avoid confusion between *Marmota* as a genus and *Marmota* as a subgenus, the latter will be indicated as *Marmota*_{SG}).

Cardini (2003) reported on a geometric morphometric analysis of the labial side of the hemimandible of all marmot species (ingroup) and a few other sciurids (outgroup). The mandible labial morphology supported *Marmota*_{SG} and the strict relationship of the two main species (based on distribution and population size) of *Petromarmota* (ie *M. caligata* and *M. flaviventris*). Morphological convergence due to size similarity was not evident in the marmot and outgroup mandibles. *Marmota vancouverensis* had a distinctive mandible morphology despite its close genetic similarity to *M. caligata* (Steppan *et al.* 1999). The uniqueness of *M. vancouverensis* morphology was not detected by Hoffmann *et al.* (1979) in a morphometric comparison of Amphiberingian marmots using cranial and mandibular characters. Cardini (2003) interpreted the atypical features of *M. vancouverensis* mandible shape as a by-product of its geographic isolation on Vancouver Island during the last 10 000–100 000 years. Genetic drift during severe population bottlenecks acted as an accelerator of morphological evolution making this young species the most divergent marmot for mandible shape (Cardini 2003), melanistic fur (Barash 1989) and unusual components in its alarm calls (Blumstein 1999). In contrast, *M. olympus*, which also is believed to have evolved in a Pleistocene glacial refugium, although earlier than *M. vancouverensis*, does not display unusual characters and its mandible combines traits from both marmot subgenera. This led *M. olympus* to cluster with *Marmota*_{SG} species in the mandibular phenogram and ordinations (Cardini 2003).

The posterior region of the labial side of the marmot mandible was investigated by Cardini and Slice (2004) with an analysis of the outlines (mathematical curves that stands for the two-dimensional image of a physical boundary). Two adult specimens were chosen as representative of each marmot species and compared

with other marmots and with outgroup species. In contrast to Cardini's findings using landmarks spanning the entire labial side of the mandible, the use of the posterior region results in a poor separation of marmot subgenera and even of species belonging to different sciurid genera.

An analysis of the outlines was performed also by Polly (2003) for comparing the shape of the lower third molar (M_3) in subspecies of five North American marmots, including fossil specimens. Polly (2003) employed phylogenetic methods for analysing the continuous variation of the outlines. *Marmota monax* was clearly separated from all other lineages, but subspecies of *M. caligata* were mixed with those of *M. flaviventris*. *Marmota broweri* was similar to *M. flaviventris engelhardti*, and *M. vancouverensis* was sister group to *M. caligata nivaria*. However, a very long branch leading to *M. vancouverensis* in Polly's phylogram indicated "that its molar shape is more divergent than other samples".

These studies on marmot evolutionary history reflect a broader interest on marmot biology in the recent years. Indeed, marmots are of great importance not only for sociobiologists and evolutionary biologists, but also for conservationists (*M. vancouverensis* is one of the most endangered mammal species in the world), anatomists (the first detailed book on marmot anatomy is about to be published – Bezuidenhout and Evans 2003), and physiologists, who have found marmots to be excellent models for the study of the hepatitis B virus (Tennant *et al.* 2002) and hibernation mechanisms (Arnold 1990, papers by Armitage *et al.*, Florant *et al.*, Ruf and Arnold, Woods and Salsbury cited in Heldmaier and Klingenspor 2000; Armitage *et al.* 2003) with possible applications to human medicine.

Despite the growing interest in marmot biology, few comparative studies have been performed that include all marmot species. Cardini's (2003) comparison of marmot mandibles is the only morphological study including a large sample of all species. However, the two-dimensional geometric morphometric analysis of marmot mandibles by Cardini did not investigate the morphology of the lingual side. Although several anatomical landmarks are in common between the two mandible sides, others are available on only one side. For instance, the anterior tip of the incisor alveolus is often broken on the labial side, while this area on the lingual side is generally unbroken due to the robust symphysis. This region is highly informative for its functional role in the incisor bite.

In order to verify Cardini's (2003) findings, a geometric morphometric analysis of the lingual side of the mandible was performed. A configuration of 10 landmarks (ie a set of topographically corresponding anatomical points) was used to describe the geometric shape and size of adult mandibles in 12 marmot species (all living species except *M. broweri* and *M. sibirica*), and five outgroup species.

The present study is aimed at answering the following questions: (1) Does the lingual side of the adult marmot mandible produce phenetic groups congruent with marmot phylogeny? (2) Is there any morphological pattern in the evolution of the lingual side of the marmot mandible that can be correlated to size similarity and, thus, support the hypothesis of a sciurid propensity to homoplasy of skeletal

characters? (3) Are the phenetic interspecific relationships suggested by the lingual side of the mandible congruent with those shown by the labial side? (4) Does the combined information on the labial and lingual sides offer further insights into the understanding of marmot morphological evolution?

Material and methods

Techniques for data collection were described in detail in Cardini and Tongiorgi (2003), to which the reader is referred for more information. Lingual side (LING) of left hemimandibles of 144 adult marmot specimens belonging to all marmot species except *M. broweri* and *M. sibirica* were photographed (Appendix; Table 1). Also, seven specimens belonging to four other sciurid genera were included in the analysis as 'outgroups'. Data ($n = 419$) for the labial side (LAB) of the left hemimandible were available from a previous study (Cardini 2003). Adults were discriminated following Cardini (2003) that employed information on premolar wear, mandible size and shape, to sort out homogeneous groups of putative adults.

Mandibles were photographed under standardized conditions and at a distance that minimizes photographic distortions (Cardini and Tongiorgi 2003). Images were digitized at high resolution directly from the negative. Sciurid hemimandibles are almost flat (thickness 1 cm), and thus suitable for a two-dimensional morphometric study (Velhagen and Roth 1997).

Landmarks were digitized (TPSDig 1.31, Rohlf 2002a) on the lingual and labial side of the mandible (Fig. 1, Table 2) and their precision tested as described in Cardini and Tongiorgi (2003). As most hemimandibles in museum collections are united at the symphysis, sample size for LING is smaller than for LAB. In two species, *M. broweri* and *M. sibirica*, no specimen with separated

Table 1. LING sample size and hemimandible size ('?' indicates specimens of unknown sex). * Total includes also specimens for which information on sex is missing.

Species	Abbreviations	Number			Centroid size (mm)
		Total*	Females	Males	
<i>Marmota baibacina</i> Kastschenko, 1899	<i>bai</i>	12	2	5	85.4
<i>M. bobak</i> (Müller, 1776)	<i>bob</i>	8	2	4	82.7
<i>M. caligata</i> (Eschscholtz, 1829)	<i>cal</i>	16	8	7	84.5
<i>M. camtschatica</i> (Pallas, 1811)	<i>cam</i>	5	?	2	81.8
<i>M. caudata</i> (Geoffroy, 1844)	<i>cau</i>	19	10	9	85.2
<i>M. flaviventris</i> (Audubon and Bachman, 1841)	<i>fla</i>	11	6	4	71.9
<i>M. himalayana</i> (Hodgson, 1841)	<i>him</i>	14	5	7	89.4
<i>M. marmota</i> (Linnaeus, 1758)	<i>mar</i>	27	6	5	83.8
<i>M. menzbieri</i> (Kashkarov, 1925)	<i>men</i>	2	?	?	71.7
<i>M. monax</i> (Linnaeus, 1758)	<i>mon</i>	14	8	2	76.3
<i>M. olympus</i> (Merriam, 1898)	<i>oly</i>	8	4	4	89.6
<i>M. vancouverensis</i> Swarth, 1911	<i>van</i>	8	4	4	85.6
<i>Spermophilus undulatus</i> (Pallas, 1778)	<i>speu</i>	1	?	?	40.0
<i>Cynomys ludovicianus</i> (Ord, 1815)	<i>cynl</i>	1	?	?	55.0
<i>Tamias (Eutamias) amoenus</i> Allen, 1890	<i>euta</i>	2	1	1	23.9
<i>T. (E.) sibiricus</i> (Laxmann, 1769)	<i>euts</i>	1		1	30.0
<i>Sciurus vulgaris</i> Linnaeus, 1758	<i>sciv</i>	2	2		43.7

hemimandible was available, and they were thus excluded in the LING comparison. Geometric morphometric techniques allow the comparison of the geometric shape of organisms or their organs (Bookstein 2000). Geometric morphometrics analyses were performed with programs of the TPS Series (Rohlf 2002a), Morphueus *et al.* (Slice 1999) and Morphologika (O'Higgins and Jones 1999). Statistical analyses were performed with NTSYSpc (Rohlf 2002b) and STATISTICA (1997).

Landmark based methods use Cartesian coordinates of topographically corresponding points (Marcus *et al.* 2000) to describe the form of anatomical structures. Differences due to rotation and translation (position) or size are removed by superimposing specimens (generalized Procrustes analysis or GPA, Rohlf and Slice 1990) and standardizing their size (unit centroid size, ie squared root of the sum of squared distances between each landmark and the centroid of the landmark configuration). Landmark configurations are then projected in a Euclidean space that approximates the curved shape space to aid statistical analyses. This approach is satisfactory when variations are small, as in the present data (results not shown). Shape variables are computed as linear combinations of the original Cartesian coordinates, and used for displaying shape differences with deformation grids (thin plate spline or TPS, Bookstein 1991) or for comparing specimen shape with multivariate statistical

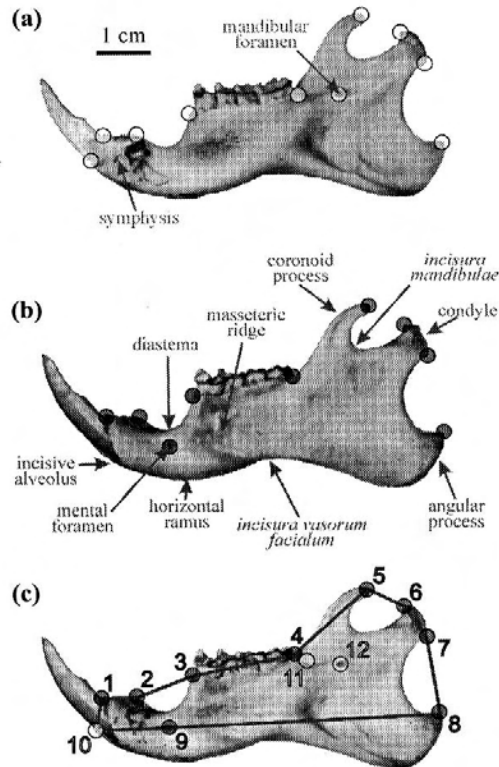


Fig. 1. Marmot mandible anatomical regions and landmarks for: (a) LING, transparent white, picture reflected; (b) LAB, transparent grey; (c) BS, LING and LAB are made transparent and superimposed so that both sides are visible; the wireframe used for 'stylised' drawings (Figs 5–6) is shown.

Table 2. Hemimandible landmarks on lingual (LING), labial (LAB) or on both sides (BS). Landmark identification numbers are referred to Fig. 1c.

No.	LING	LAB	BS	Definition
1			*	Upper extreme anterior part of the incisor alveolus
2			*	Anterior top of the mandibular symphysis
3			*	Anterior extremity of the maxillary tooththrow (premolar alveolus)
4		*		Intersection of the dental ridge with the dorsal portion of the masseteric ridge (base of the coronoid process)
5			*	Tip of the coronoid process (region of insertion of the temporal muscle)
6–7			**	Anterior and posterior tip of the condyle
8			*	Posterior extremity of the angular process (region of insertion of the superficial masseter)
9		*		Mental foramen (for the trigeminal nerve)
10	*			Anterior lower extremity of the mandibular symphysis
11	*			Posterior end of the tooththrow
12	*			Mandibular foramen

techniques. Transformations of a reference shape (the average of that sample) into a target shape (one of the specimens) are often magnified in figures in order to make shape changes more evident.

Analysis of variance (ANOVA) sex $\times$ species for centroid size was used as a test for size differences between sexes and among species. *Marmota camtschatica* and *M. menzbieri* were excluded from this analysis as information on sex was missing. Since sample size was not adequate for statistical tests of shape sexual dimorphism, mean shapes for males and females of each species were computed for both LING and the combined information on both sides (BS), and they were compared with an UPGMA (unweighted pair-group method using arithmetic average) cluster analysis of the matrix of Procrustes distances (pairwise distances computed in the shape space, Rohlf 2002a). Procrustes distance is the distance measure that defines the shape space and it has been employed in several geometric morphometric analyses (for instance, see Marcus *et al.* 2000, and Milne and O'Higgins 2002).

The phenetic relationships among BS and LING mean shapes (pooled sexes) were investigated with cluster analyses and principal component analyses (PCA). A canonical variate analysis (CVA) was performed for testing species differences on the matrix of LING shape variables. *Marmota menzbieri* was not included in the CVA because of its small sample size ($n = 2$). Also, CVA has been widely used in morphometrics (for instance, see Rohlf *et al.* 1996, Duarte *et al.* 2000, and Corti and Rohlf 2001).

Seven landmarks are common to both LING and LAB configurations. In order to combine the information on both LING and LAB sides, mean shapes were computed separately in each dataset, then superimposed using a common baseline (landmark 1 – landmark 8). Then, in each species LING coordinates for landmark 10, 11 and 12 (not visible on LAB) were appended to the matrix of LAB coordinates (LAB coordinates for the seven common landmarks were used in the BS configuration as they are more accurate due to larger LAB samples). The resulting BS configuration contains combined information on both mandible sides for 12 marmot species (Fig. 1c). *Marmota broweri* and *M. sibirica* have missing landmarks on the LING side. For these two species, the average coordinates for landmarks 10–12 in the genus *Marmota* were used as estimate of LING landmarks (Slice 1999).

Ordination techniques and cluster analyses are both useful for summarising multivariate data (see Rohlf 1998 for an overview of statistical techniques applicable to shape variables in geometric morphometrics). The former generally provide a more accurate reconstruction of phenetic relationships and the latter allow a better discrimination of species clusters. Most of the discussion will focus on groups that are found with both techniques.

	<i>baidF</i>	<i>baiM</i>	<i>bobF</i>	<i>bobM</i>	<i>calF</i>	<i>camM</i>	<i>cam</i>	<i>cauF</i>	<i>cauM</i>	<i>flaF</i>	<i>flaM</i>	<i>himF</i>	<i>himM</i>	<i>marF</i>	<i>monF</i>	<i>olbF</i>	<i>olbM</i>	<i>varF</i>			
<i>baidF</i>	0.0319	0.0000																			
<i>baiM</i>	0.0291	0.0265	0.0000																		
<i>bobF</i>	0.0214	0.0235	0.0126	0.0000																	
<i>bobM</i>	0.0389	0.0287	0.0234	0.0264	0.0000																
<i>calF</i>	0.0360	0.0352	0.0264	0.0283	0.0161	0.0000															
<i>camM</i>	0.0348	0.0408	0.0288	0.0260	0.0364	0.0395	0.0000														
<i>camF</i>	0.0410	0.0479	0.0447	0.0359	0.0489	0.0522	0.0310	0.0000													
<i>cauF</i>	0.0406	0.0308	0.0249	0.0229	0.0315	0.0367	0.0269	0.0389	0.0000												
<i>cauM</i>	0.0391	0.0371	0.0300	0.0252	0.0346	0.0378	0.0238	0.0310	0.0146	0.0000											
<i>flaF</i>	0.0300	0.0228	0.0246	0.0221	0.0284	0.0286	0.0419	0.0501	0.0354	0.0375	0.0000										
<i>flaM</i>	0.0286	0.0284	0.0311	0.0234	0.0303	0.0314	0.0371	0.0385	0.0342	0.0327	0.0231	0.0000									
<i>himF</i>	0.0352	0.0331	0.0313	0.0252	0.0302	0.0289	0.0344	0.0405	0.0275	0.0293	0.0308	0.0253	0.0000								
<i>himM</i>	0.0385	0.0254	0.0311	0.0261	0.0349	0.0360	0.0408	0.0438	0.0262	0.0317	0.0285	0.0284	0.0202	0.0000							
<i>marF</i>	0.0411	0.0370	0.0312	0.0267	0.0313	0.0362	0.0287	0.0288	0.0286	0.0284	0.0393	0.0317	0.0287	0.0311	0.0000						
<i>marM</i>	0.0397	0.0448	0.0347	0.0300	0.0358	0.0382	0.0276	0.0286	0.0351	0.0322	0.0445	0.0346	0.0330	0.0404	0.0147	0.0000					
<i>monF</i>	0.0322	0.0205	0.0206	0.0214	0.0253	0.0309	0.0344	0.0496	0.0321	0.0378	0.0205	0.0311	0.0346	0.0317	0.0351	0.0402	0.0000				
<i>monM</i>	0.0269	0.0360	0.0283	0.0249	0.0366	0.0361	0.0296	0.0373	0.0387	0.0378	0.0350	0.0349	0.0366	0.0364	0.0307	0.0324	0.0296	0.0000			
<i>olbF</i>	0.0274	0.0347	0.0275	0.0241	0.0289	0.0311	0.0308	0.0428	0.0392	0.0393	0.0339	0.0329	0.0377	0.0437	0.0311	0.0278	0.0266	0.0299	0.0000		
<i>olbM</i>	0.0330	0.0488	0.0403	0.0358	0.0448	0.0427	0.0406	0.0432	0.0507	0.0477	0.0462	0.0466	0.0474	0.0530	0.0403	0.0339	0.0426	0.0359	0.0245	0.0000	
<i>varF</i>	0.0439	0.0536	0.0412	0.0384	0.0552	0.0548	0.0338	0.0488	0.0438	0.0458	0.0509	0.0480	0.0490	0.0512	0.0433	0.0394	0.0437	0.0407	0.0414	0.0483	0.0000
<i>varM</i>	0.0402	0.0547	0.0409	0.0375	0.0497	0.0471	0.0317	0.0455	0.0439	0.0433	0.0511	0.0458	0.0428	0.0512	0.0413	0.0326	0.0454	0.0420	0.0363	0.0376	0.0238

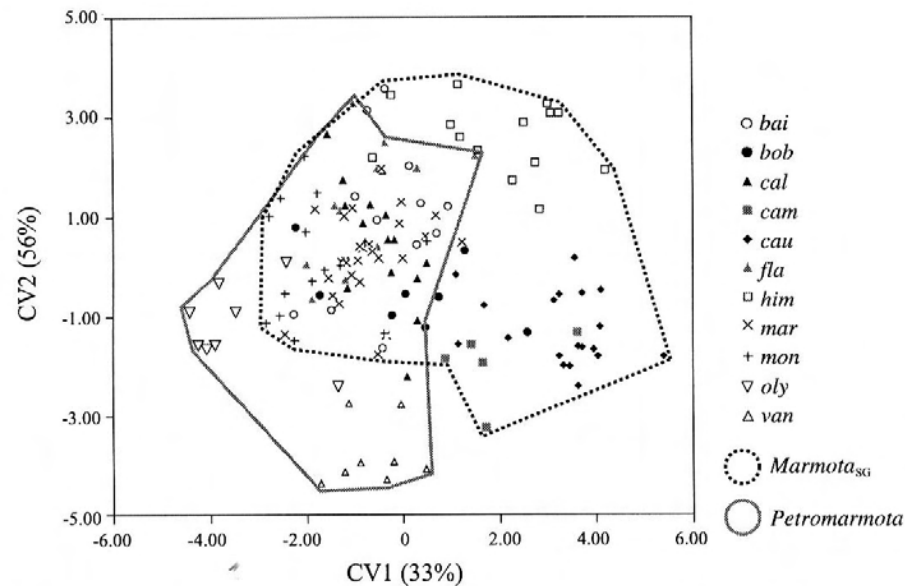


Fig. 3. Ordination of the LING landmark configurations of the marmot specimens along the first two CVA axes (percentages of variance are shown in parentheses). See Table 1 for explanation of species name abbreviations.

very moderate for the remaining species, and representatives of the two marmot subgenera do not form distinct clusters.

Indeed, species belonging to *Marmota*_{SG} and *Petromarmota* are mixed in the phenogram of the mean shapes (Fig. 4a). Principal component analysis (Fig. 4b) of the mean shapes provides a slightly better separation of marmot subgenera, with *M. vancouverensis* and *M. olympus* isolated from all other species, *M. caligata* fairly separated from the *Marmota*_{SG} cluster while *M. flaviventris* lies halfway between *M. caligata* and *Marmota*_{SG}.

The phenogram and the PCA plot both indicate the unique LING shape of *M. vancouverensis*. *Marmota monax* and *M. baibacina* have similar LING shape, and also *M. camtschatica* and *M. marmota* are fairly similar. In the PCA plot also *M. caudata* and *M. menzbieri* are relatively close to each other. Shape clusters do not reflect size differences among species (Fig. 4a; eg the large mandible of *M. baibacina* is similar to the small one of *M. monax*).

Combined analysis of LING and LAB information

Information on both sides of the mandible is combined using average shapes for LING and LAB, and estimating missing landmarks for *M. broweri* and *M. sibirica*. The resulting 12 landmark configuration is analysed with PCA and cluster

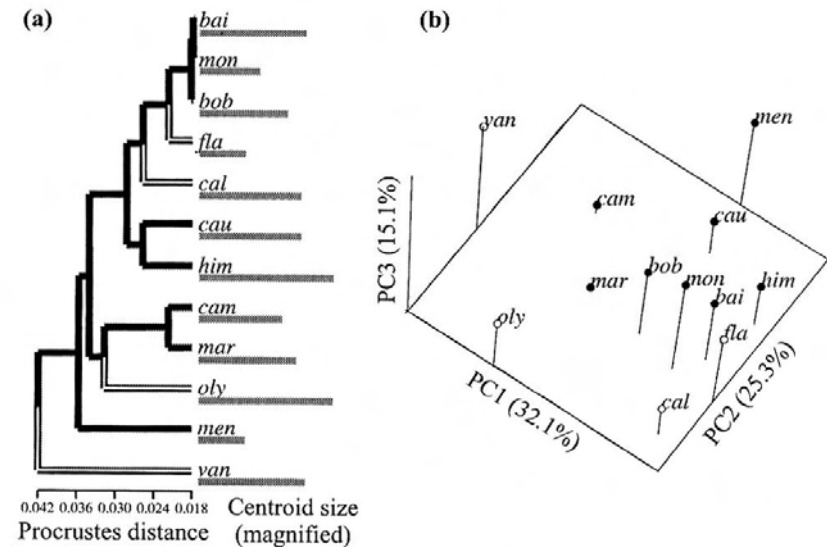


Fig. 4. Similarity relationships for LING mean shapes of marmot mandibles. (a) UPGMA cluster analysis of the matrix of Procrustes distances. Branches for *Petromarmota* species are emphasized with white lines in this and other phenograms; grey lines by species abbreviated names are proportional to LING mandible average centroid size (magnified) for each species. (b) Ordination of the species mandibles in the space of the first three principal components of the shape variables (explained variance is given in parentheses). See Table 1 for explanation of species name abbreviations.

analysis. Since both UPGMA and WPGMA (weighted pair-group method using arithmetic average) phenograms have similar cophenetic correlation to the original matrix of Procrustes distances (respectively, $r = 0.76$ and $r = 0.75$), a strict consensus tree between UPGMA and WPGMA phenograms is presented (Fig. 5). In WPGMA the size of clusters is used as a weight during computations. This method should be used when cluster sizes are suspected to be uneven as it may happen for marmots where one subgenus has more than twice as many species as the other one. Also, mean BS shapes of marmots are projected onto the first three PCA axes (Fig. 6) that explain 62.7% of the variance.

PCA ordination and phenogram are fairly concordant about how marmot species should be clustered. *Marmota vancouverensis* with its recurved coronoid process (landmark 5), and flat top of the mandibular symphysis (landmark 2), is the most atypical marmot species (Fig. 5). The plot of the first three PCA axes (Fig. 6) suggests that all species of *Marmota*_{SG} group together, except *M. camtschatica* and *M. marmota*, which are relatively close to each other and in between the main *Marmota*_{SG} cluster and *M. vancouverensis* and *M. olympus*. *Marmota caligata* is next to *M. flaviventris*, which, in turn, is relatively close to the main *Marmota*_{SG} cluster. The phenogram (Fig. 5) supports a cluster including most *Marmota*_{SG}

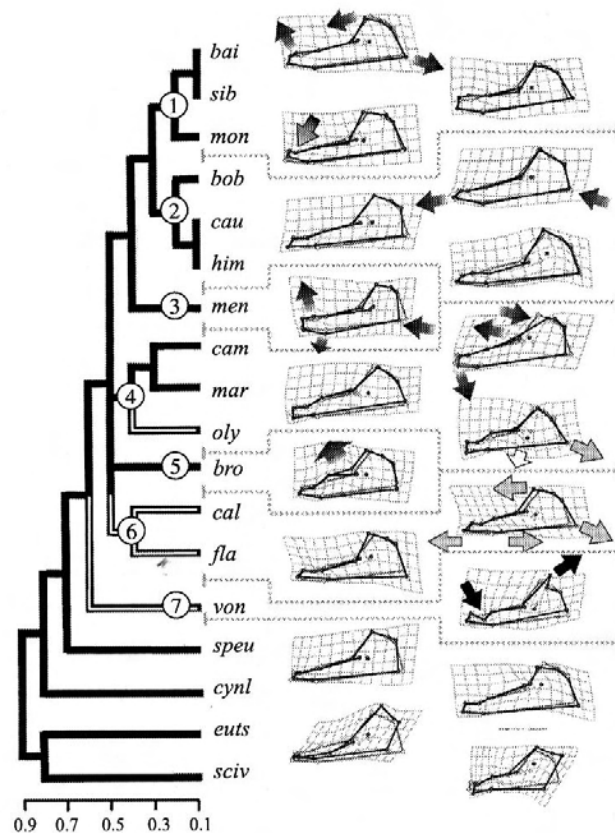


Fig. 5. Phenogram illustrating similarity relationships among mean BS shapes of marmot mandibles. TPS deformation grids (magnified 4× for marmots, and 2× for outgroup species) showing shape features of mean marmot mandibles (in grey – average *Marmota* mandible, in black – transformed into the mean shape of each marmot species; for the outgroup, the average of *Marmota* plus outgroup species is used as starting shape). Main marmot clusters are indicated with numbers, and arrows help detection of their characteristic mandibular traits (shaded grey for *Marmota*_{SG}, light grey for *M. caligata*, *M. flaviventris* and angular process of *M. olympus*; white for *M. olympus* autoapomorphic position of the mandibular foramen; black for *M. vancouverensis*). See Table 1 for explanation of species name abbreviations.

species. *Marmota camtschatica* and *M. marmota* are separated from it, and group together with *M. olympus*. *Marmota broweri* too does not cluster together with the majority of *Marmota*_{SG} species and it has the lowest score on PC3. *Marmota caligata* and *M. flaviventris* are relatively similar and separated from other marmots.

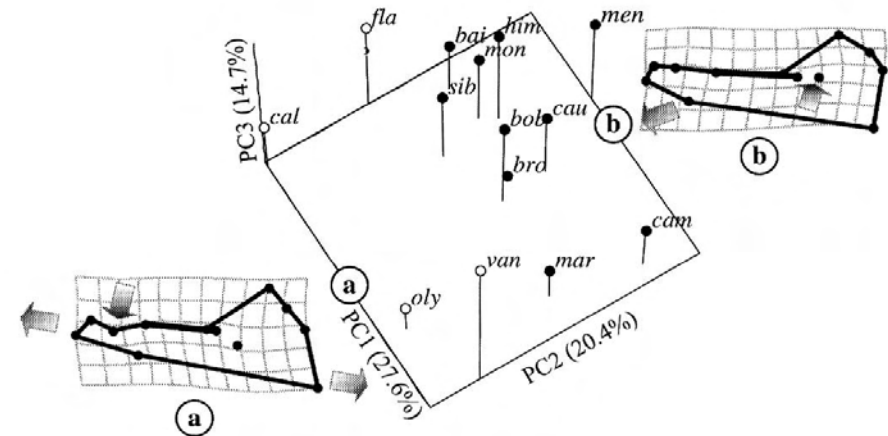


Fig. 6. Ordination of the mean BS shapes of marmot mandibles in the space of the first three principal components of the shape variables (percentage of variance in parentheses). TPS deformation grids (magnified 3×) for extreme specimens (a, b) along PC2, which provides partial separation of the marmot subgenera, are shown; shaded arrows emphasize main differences between the two subgenera. See Table 1 for explanation of species name abbreviations.

Some discrimination of the marmot subgenera is achieved on PC2, and magnified (3×) deformation grids for extreme points (highest and lowest PC2 scores) along this axis (Fig. 6) suggest that, generally, *Petromarmota* (a) has an elongated angular process (landmark 8), while the area between the mental and mandibular foramina (landmarks 9, 12) is contracted. Opposite traits are found in *Marmota*_{SG} (b), which has also a more pronounced top of the mandibular symphysis (landmark 2). Also, the ventral half of the incisor alveolus (between landmark 9 and 11) is elongated in *Marmota*_{SG}, and the upper tip of the incisor alveolus (landmark 1) is posteriorly displaced. Distinctive features of the mandibular shape for the main clusters are emphasized with arrows in Figure 6.

The genus *Marmota* and the tribe Marmotini [ie marmots, prairie dogs (*Cynomys*) and ground squirrels (*Spermophilus*)] are clearly separated by the mandible shape (Fig. 5).

Discussion

Sexual dimorphism

Adult sexual dimorphism in LING mandibles is moderate compared to inter-specific differences. Even if a powerful test for sexual dimorphism could not be performed in the LING sample due to its relatively small (compared to the number of shape variables) sample size, this result is congruent with Cardini's (2003) findings on LAB.

An apparent discrepancy between LING and LAB (Cardini 2003) analysis relates to size. Cardini, using much larger samples, showed that males are always significantly larger than females for LAB size. Differences in LING size between sexes are close to significance ($p = 0.089$), and, indeed, despite sampling error and low statistical power in small samples, in seven species out of 10 males average larger than females. Results of ANOVA for LING size may have also been influenced by the violation of the assumption of homoscedasticity.

The topology of the BS phenogram for mean marmot mandibles with separate sexes (Fig. 2) is different from that with pooled sexes (Fig. 5). This difference is not surprising and can be partly related to the inclusion of specimens of unknown sex in the computation of mean shapes regardless of sex. Due to the smaller sample size, BS phenogram with separate sexes should be less accurate in reconstructing similarity relationships than BS phenogram with pooled sexes. Nonetheless, the former does a better job at discriminating marmot subgenera. An exception is the puzzling position of *M. baibacina* males, apparently close to *Petromarmota* species. Also, the Procrustes distance between male and female mean shapes of *M. baibacina* is the larger than those between sexes of every other species. *Marmota baibacina* may be a problematic species since its mandibular shape is influenced by plague epidemics. Pole and Bibikov (1991 quoted in Armitage 2000) showed that certain shape changes in *Marmota baibacina* mandible can be correlated to plague epidemics, and suggested that mandible phenotype may be related to genetic differences that confer resistance to plague but that are not equally advantageous in healthy populations. Studies of *M. baibacina* are further complicated by uncertainties in its taxonomic status. A subspecies, *M. baibacina kastschenkoi*, has been recently suggested to represent a different species with 36 chromosomes instead of 38 typical of *Marmota*_{SG} (Brandler 2003).

LING landmark analysis

LING is less effective than LAB in discriminating the two marmot subgenera. Given the number of landmarks in common in the two analyses, and the fairly large LING sample sizes of *M. caligata* ($n = 16$) and *M. flaviventris* ($n = 11$), the two main species of *Petromarmota*, it is quite surprising that the strong similarity between their mandibles is not supported by the LING analysis. On the other hand, *M. vancouverensis* is confirmed as the most divergent marmot for mandible morphology, which is also congruent with its other atypical traits and related to severe genetic bottlenecks during its recent evolutionary history (Cardini 2003).

The LING phenogram fails to detect *Marmota olympus* as being strikingly dissimilar to all other species, and second only to *M. vancouverensis* for mandible peculiarities. *M. olympus* is clearly isolated in the plot of the first three PCA axes, which gives a more reliable representation of LING phenetic relationships (higher correlation to matrix of Procrustes distances). A pronounced expansion of the area between the mandibular foramen (landmark 5) and the coronoid and condylar processes, visualized in the TPS plot of BS mean shapes as a ventral displacement

of the mandibular foramen (Fig. 5, white arrow), is likely to represent a unique feature of *M. olympus*.

Marmota monax is confirmed to be fairly similar to *M. baibacina* as in Cardini's LAB study. Since the former has small mandibles and the latter very large ones, size homoplasies can be excluded as a possible reason of this puzzling morphological resemblance in species clearly separated in both geographic distribution and phylogenetic relationship (Steppan *et al.* 1999). Retention of plesiomorphic traits or convergence due to factors other than size can be involved in the development of similar mandibular shapes in *M. monax* and *M. baibacina*.

As in Cardini (2003), no shape clusters clearly reflecting size similarities have been found that can support the hypothesis of presumed propensity of the sciurid skeleton to convergence induced by size similarities (Hafner 1984, Roth 1996, Velhagen and Roth 1997). However, the evolution of homoplastic characters may have been promoted by the colonization of similar ecological niches. This hypothesis is not tested in the present study as a detailed comparative study of marmot ecology (especially concerning diet) is lacking.

Combined analysis of LING and LAB information

The analysis of the BS dataset provides the most complete information available for the interspecific comparison of marmot mandibles. Results, however, need to be interpreted with caution. Indeed, adding the 'LING-specific' landmarks (10–12) to the LAB mean shapes may have introduced a source of error especially in those species for which very small LING samples were available (eg *M. menzbieri*). LING-specific landmarks of *M. broweri* and *M. sibirica* had to be estimated using average values, and this also can flaw results. Finally, in this section of the Discussion, as well as in the previous one and in the corresponding sections of the Results, the gene tree proposed by Steppan *et al.* (1999) provides the phylogenetic context in which the mandible phenetic relationships need to be interpreted. However, Steppan *et al.* (1999) phylogeny is based on just one gene and even well supported clades need to be confirmed by future studies using other characters. Bearing in mind these caveats, some observations can be made.

First, *M. vancouverensis* emerges as a very distinctive species for mandibular traits, strengthening Cardini's (2003) findings. Indeed, despite the close genetic similarity of *M. vancouverensis* and *M. caligata*, the former species is unique among marmots for morphological and behavioural characters, and it is likely to represent an interesting model of accelerated (and possibly not yet complete) speciation due to genetic drift. Severe bottlenecks during the short evolutionary history of *M. vancouverensis* made it the most atypical living marmot population. In this view, a strong conservation (Bryant 2002) effort to save this species is invaluable to preserve its unique combination of genes.

Integrating the LAB data with LING information was basic in discovering distinctive traits in *M. olympus* too. The markedly atypical (compared to other marmots) position of the mandibular foramen is an autoapomorphic character for

this species, that, in other mandibular regions, mixes traits found in *Marmota*_{SG} species (posteriorly bent coronoid process found also in *M. camtschatica* and *M. marmota*) with other traits (elongated angular process) characteristic of the typical *Petromarmota* species (ie *M. caligata* and *M. flaviventris sensu* Cardini 2003). The evolutionary history of *M. olympus* is longer than that of *M. vancouverensis*, but both species may have evolved in isolated populations during glacial or interglacial times (Steppan *et al.* 1999), and genetic drift may have contributed to model at least some features (mandibular foramen) of the Olympic marmot mandible.

The BS mandible analysis does a poor job, compared to the LAB analysis, in giving support to *Marmota*_{SG}. Actually, all Palearctic marmots plus *M. monax* exhibit small differences in mandibular shape. The two exceptions are represented by *M. camtschatica* and *M. marmota*, clearly separated from other marmots in the BS analysis. *Marmota marmota* may indeed be unusual among Eurasiatic marmots. Its relationship to the other species of *Marmota*_{SG} is unresolved in Steppan *et al.* (1999) phylogeny, but they see it as an ancient offshoot of the Palearctic lineage. *Marmota marmota* was considered quite peculiar by Rumiantsev and Bibikov (1994) too, who noted its uniqueness among Palearctic species for fur morphology and parasitic relationship with fleas.

Of the two Nearctic species belonging to *Marmota*_{SG}, similarities of *M. monax* to Palearctic species are corroborated by the inclusion of LING information. In contrast, nothing can be added to Cardini's (2003) observations about *M. broweri*, since LING landmarks were not visible in the available specimens and average values for the whole genus were used to replace these missing landmarks.

Conclusions

Four questions inspired this study, and they can be now concisely answered:

(1) The LING marmot mandible leads to similarity groups largely incongruent with marmot phylogeny. Phylogenetic signal in LING seems thus quite weak.

(2) No shape groups reflecting size similarities could be found in the analysis of the labial side and combined dataset. The hypothesis of sciurid propensity to skeletal homoplasy in species with similar size is not supported by the analysis of the marmot mandible.

(3) LING and LAB phenetic relationships are only partially congruent. The stronger phylogenetic signal found in the LAB analysis and the incongruencies between the two datasets in spite of having several landmarks in common, suggest that very large samples are needed for powerful morphological comparisons in the genus *Marmota*.

(4) The BS dataset provides some stimulating insights into marmot morphological evolution. Two findings are particularly interesting as they concern species where sample sizes for LING and LAB were similar, and, thus, they might be less affected by errors due to the smaller samples generally available for LING. *Marmota olympus* turns out to have a very unusual position of the mandibular foramen,

and this may be related to its evolution as peripheral isolate. *Marmota marmota* is seen as one of the most atypical species in *Marmota*_{SG}, which could be due to its long evolutionary history and supports previous findings about its unusual parasitic relationships and fur morphology (Rumiantsev and Bibikov 1994).

The analysis of LING and BS dataset provide useful insights for the study of marmot morphological evolution. *M. vancouverensis* is confirmed as the most atypical marmot in mandibular shape, and its morphological uniqueness makes it valuable both for conservationists and evolutionary biologists. *Marmota olympus* too emerged as fairly unusual. These two species are likely to offer interesting clues for understanding the evolution in peripheral isolates during the Pleistocene glaciations.

Finally, the LING analysis warns caution in interspecific comparisons involving species like the Palearctic marmots, that are believed to have radiated from North American ancestors that migrated to Asia at the end of the Pliocene. Interspecific differences among these species may be really small, although significant, and large samples are required for robust reconstructions of their morphological relationships. Accelerated rate of morphological evolution after genetic bottlenecks, as exemplified by *M. vancouverensis*, may have also contributed to create phenetic patterns incongruent with phyletic relationships.

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Appendix. Museum catalog numbers.

Abbreviations: USNM – National Museum of Natural History (Washington, USA); BMNH – British Museum of Natural History (London, UK); ZIN – Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia); MSNM Ma – Museo di Storia Naturale di Milano; PNGP – National Park Gran Paradiso (Torino, Italy); SC – collection of Dino Scaravelli; DBA UMR – Department of Animal Biology, University of Modena and Reggio Emilia; MVZ – Museum of Vertebrate Zoology (Berkeley, USA).

Marmota baibacina: 14.5.10.74, 12.4.1.42, 14.5.10.73, 8.3.2.74, 12.4.1.40 BMNH; 31871, 46147, 50165, 63930, 63931, 63932, 84030 ZIN. *M. bobak*: 19.7.7.3325, 594a BMNH; 15459, 44204, 44236, 50287, 50289, 50296 ZIN. *M. caligata*: 135161, 135163, 88005, 88006, 42638, 90132, 221012, 101300, 202790, 90134, 137319, 226148, 96206, 146449, 146449, 174503 USNM. *M. camtschatika*: 58.11.18.3, 1937.6.12.10 and 15084, 69.4.20.1 BMNH; 57880, 78912 ZIN. *M. caudata*: 97.10.3.54, 10.12.2.25, 8.7.6.27, 69.493, 1937.6.12.7, 8.10.3.16, 92.1.1.7 BMNH; 62114, 62115, 62118, 173383, 35500, 173384, 353196, 353197, 353199, 353200 USNM; 27912, 40553 ZIN. *M. flaviventris*: 99759, 234960, 168884, 157828, 158033, 168799, 128754, 93689, 42112, 133505, 202937 USNM. *M. himalayana*: 11.2.1.95, 11.2.1.94, 96.11.4.2, 92.1.1.4, 8.7.6.30, 23.9.1.38, 23.9.1.40, 8.2.29.1, 8.7.6.20, 5.12.5.3 BMNH; 84104, 144039, 198638, 573031 USNM. *M. marmota*: 1.1.195, 2.8.4.31, 2.8.4.30, 2.8.4.32 BMNH; 3 specimens not in catalog DBA UMR, 165, 12, 173, 175(I), 178, 168, 159(I), 167, 21B, 17, 171, 39, 159(II), 161, 149, 158, 15, 36B, 32, 38B SC. *M. menzbieri*: 31704, 65852 ZIN. *M. monax*: 19.7.7.2552, 36.11.6.31, 36.11.6.34 BMNH; 4550 MSNM Ma; 243949, 36.11.6.33, 242724, 96574, 283359, 77142, 77143, 25184, 35361, 227256 USNM. *M. olympus*: 66950, 67612, 241657, 241658, 241947, 241948, 241659, 242102 USNM. *M. vancouverensis*: 12091, 12092, 12090, 12099, 12100, 12098, 12093, 12095 MVZ. *Spermophilus undulatus evermanni* 12.4.1.21 BMNH. *Cynomys ludovicianus* 19.7.7.2841 BMNH. *Tamias (Eutamias) amoenus* 208398, 231929, 231931 USNM. *T. (N.) sibiricus*: 200617 USNM. *Sciurus vulgaris* 47.11.15, 25.7.1.9 BMNH.

