

Detecting biological distinctiveness using geometric morphometrics: an example case from the Vancouver Island marmot

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The Vancouver Island marmot (*M. vancouverensis*) consists of a small insular population with an uncommon dark-brown pelage colour. This population provides an excellent example of how morphology and molecules can tell very different stories about the evolution of a group. Mitochondrial DNA indicates that the degree of divergence of *M. vancouverensis* is minimal and actually comparable or even smaller than among populations of continental species of marmots. Accurate geometric morphometric analyses on crania and mandibles from modern and subfossil samples, in contrast, convincingly demonstrate that the Vancouver Island marmot is one of the most distinctive populations among all living marmots. Thus, *M. vancouverensis* is not simply a dark variant of its sister species *M. caligata* but represents a highly morphologically derived population whose evolutionary significance is overlooked by molecular analyses. In this study, we show how geometric morphometrics can complement studies of genetic divergence by measuring the phenotypic dis-

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tinctiveness of a population. This was done by performing cross-validated discriminant analyses on mandibular shape in the Vancouver Island marmot and its closest Nearctic relatives. Thus, we found that using mandibles we can achieve in *M. vancouverensis* a classification accuracy as high as for 'good species' (i.e., species which are also significantly divergent in molecular analyses) and much higher than in populations within those same species. The approach used in this study is simple, effective and, at least in this specific case, fairly robust to sampling error in small samples. Thus, such morphological analysis seems promising as a tool complementary to molecular studies to aid detection of biological distinctiveness in natural populations. We suggest that its potential should be explored and its usefulness examined also in Palearctic marmots and other mammals. If successful, the 'geometric morphometric approach to the study of biological diversity' would add a phenotypic dimension to the characterization of the evolutionary significance of populations. In turn, this would allow a more inclusive understanding of the complexity of adaptive variation with potential implications for conservation biology.

KEY WORDS: biological diversity, conservation, discriminant analysis, DNA barcoding, mandible, *Marmota vancouverensis*, shape.

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INTRODUCTION

Different species of mammals (BRADLEY & BAKER 2001) and other vertebrates (AVISE & WALKER 1999) typically show at least 2% divergence at the mitochondrial cytochrome b. The Vancouver Island marmot (*M. vancouverensis*) has been considered a 'good species' based on its distinctive external morphology (HALL 1981, BARASH 1989). However, the analysis of marmot phylogenetic relationships indicated that the divergence of its mitochondrial cytochrome b DNA from *M. caligata*, its closest living relative, is only 1.2% (STEPAN et al. 1999). Thus, STEPPAN et al. (1999) concluded that the Vancouver Island marmot "is an insular allospecies of the superspecies *caligata*" based on phenotypic differences, but the amount of genetic divergence for this population is smaller than the 2% threshold mentioned above, even smaller than the minimum of 6% divergence observed in other marmot species and clearly within the range of within species variation for the genus *Marmota* (range 0-3.5%, mean = 1.4%). It could be argued that the Vancouver Island marmot may simply represent an insular population of its continental sister species

(*M. caligata*) in which genes for a dark pelage colour, occasionally observed in other populations (NAGORSEN 1987, ARMITAGE 2009), have become fixed in the gene pool and in which the alarm call has been modified from the continental call (BLUMSTEIN 1999),

Indeed, HOFFMANN et al. (1979) found strong similarities between the Vancouver Island marmot and *M. caligata* not only in their karyotype but also in the skull morphology which they described using linear measurements. It was, thus, a surprise when *M. vancouverensis* was shown to have the most distinctive mandibular and molar morphology among all living marmots (CARDINI 2003, POLLY 2003). The morphological distinctiveness of *M. vancouverensis* was reconfirmed by later studies on crania (CARDINI & O'HIGGINS 2004; CARDINI et al. 2005, 2007).

Differing from the original linear measurement study by HOFFMANN et al. (1979), the more recent analyses used geometric morphometrics, a suite of methods developed over the last two decades that have been considered to be a 'revolution in morphometrics' in terms of accuracy, statistical power and visual effectiveness (ROHLF & MARCUS 1993, ADAMS et al. 2004). Geometric morphometric methods employ Cartesian coordinates of anatomical landmarks (instead of interlandmark distances or angles as in traditional morphometrics, MARCUS 1990) to efficiently separate and accurately quantify size and shape components of form differences. Thus, by using appropriate statistical methods, the geometry of spatial relationships of anatomical landmarks is preserved and variation among individuals can be visualized using a variety of diagrams (ADAMS et al. 2004).

Although molecular and morphological evolution are often argued to be only loosely coupled (AVISE 2004: 48-49), striking contradiction between the morphological distinctiveness of *M. vancouverensis* and its minimal mtDNA divergence and putatively young age (12,000-13,000 years; NAGORSEN 2005) requires a convincing explanation and raises questions about risks of underestimating biological diversity by focussing on a single point of comparison like mitochondrial DNA for taxonomic differences.

Researchers have suggested that the unusually distinctive shape of *M. vancouverensis* results from the action of strong selective pressures in a small insular population during a time of pronounced environmental change (CARDINI et al. 2007). This explanation fits well with the pattern of rapid morphological evolution observed in insular mammals (MILLIEN 2006). However, the evolutionary acceleration only occurred in the shape of the phenotype, whereas size of the skull and mandible of *M. vancouverensis* are within the range of variation observed in continental populations of *M. caligata* and does not follow the prediction of size reduction according to the 'island rule' for large insular mammals (LOMOLINO 2005). The lack of differentiation in size is likely to be related to the advantages of a large size to survive long hibernation periods (ARMITAGE 1999, ARMITAGE & BLUMSTEIN 2002). The fact that size often dominates the pattern of form variation in traditional morphometrics may explain why HOFFMANN et al. (1979) failed to detect aspects of the morphology of the skulls of *M. vancouverensis* which are not found in other marmots.

A simpler explanation of the distinctive shape of *M. vancouverensis* skulls and teeth is available, however, that has little to do with evolutionary

processes on islands: unusual morphologies can sometimes be an artefact of sampling error. For instance, CARDINI & ELTON (2007) showed that in small samples of skulls one third or more of the shape distance between means of phylogenetically and ecologically well separated monkey species can be accounted for by sampling error. Indeed, all studies which first recognized *M. vancouverensis* skull and teeth as highly distinctive were performed on a small sample ($N < 10$) of specimens which were mostly collected from the same locality at the beginning of the 20th century. Thus, the putative morphological distinctiveness of skulls and teeth of the Vancouver Island marmot might have actually been due to poor sampling of closely related individuals, whose unusual shape simply represented a case of local transient variation of little demographic and evolutionary relevance.

To be able to discriminate patterns that truly reflect significant evolutionary processes from patterns that stem from chance sampling, it is crucial to improve sampling in terms not only of size but also of representation of the whole population geographic range. With this aim, NAGORSEN & CARDINI (2009) analysed a sample of *M. vancouverensis* using the same mandibular landmark configuration as in CARDINI (2003). However, they included a larger number of specimens from different localities and also examined variation over time by including Holocene subfossils. With this study, they confirmed that *M. vancouverensis* is strongly differentiated in mandibular shape from continental marmots and differences are not simply due to sampling error. Also, they found that temporal variation in mandibular form was minimal over the past 2500 years and suggested that morphological divergence may have occurred soon after Vancouver Island was isolated from the mainland at the end of the last glaciation. Thus, both geographical isolation and rapid environmental change may have contributed to accelerating the rate of morphological evolution on the island.

The case of the Vancouver Island marmot is an exemplar of the potential of powerful quantitative methods of shape analysis for identifying unique aspects of biological variation (CARDINI & O'HIGGINS 2004). Indeed, had it not been for its uncommon (ARMITAGE 2009) dark pelage, the Vancouver Island marmot may not have been designated an endangered species in 1978 and might have followed the fate of other small insular populations of uncertain taxonomic status like *M. caligata sheldoni*, which was last reported on Montague Island, Alaska, at the beginning of the previous century (LANCE 2002). Interestingly, however, the contribution of geometric morphometrics to the identification of biological diversity within closely related taxa has never been carefully examined. In contrast, molecular data have become the primary and often sole source of evidence for this purpose, despite warnings from numerous scientists against the limitations and risks of a unilateral approach (e.g., WILL & RUBINOFF 2004, DESALLE et al. 2005, RUBINOFF 2006, ELIAS et al. 2007, TREWICK 2008). How biodiversity is assessed has relevance for taxonomy and more crucially for conservation biology. What the units are that make up the diversity of life is essential information in establishing conservation priorities. Limiting an assessment to one or only a few genetic markers is unlikely to provide an accurate description of biodiversity. CRANDALL et al. (2000) stressed the importance of conserving heritable variation in phenotypes because it

is expression of adaptive characters “shaped by gene flow, genetic drift and natural selection”. Thus, by focussing on functional rather than just historical diversity, information is obtained about the evolutionary processes that led to different adaptations and the preservation of these processes allows to maintain the potential for evolution.

With the present communication we are not providing another point of view on the pros and cons of the use of molecular data in taxonomy and conservation biology nor are we suggesting an approach for setting conservation priorities. These topics are beyond the scope of our study and well covered in a variety of publications. Instead, we aim to use the study of Vancouver Island marmot morphology as an example of how to explore the potential of geometric morphometrics for the characterization of morphological distinctiveness, which we define as the occurrence within a group of closely related populations of measurable differences in morphology comparable in magnitude to those observed between ‘good species’ (i.e., populations whose eco-ethological, morphological and molecular divergence leaves no doubt of their specific status as reproductively isolated communities and evolutionary independent lineages). Thus, we use cross-validated discriminant analyses on shape data as a tool to detect morphological distinctiveness by comparing classification accuracy across populations of *Marmota* (*Petromarmota*). The population whose distinctiveness needs to be assessed (*M. vancouverensis*, in our example) will be considered significantly morphologically distinctive if discriminant functions predict specimen affiliation with an accuracy comparable to and larger than that observed for ‘good species’ and their subspecies. Cross-validated discriminant analyses using geometric morphometric data on shape are simple and fast, and their predictions have been shown to be accurate when applied to groups of species difficult to discriminate by eye and often overlapping for morphological disparity (e.g., CARDINI & ELTON 2007, 2009). This approach could easily complement analyses of evolutionary divergence using molecular data, and thus provide a second line of evidence for a fairly rapid but highly informative assessment of population adaptive variation using morphological characters.

METHODS

The samples comprise 239 mandible specimens of adult modern marmots. Samples include museum specimens of all representatives of the subgenus *Petromarmota* (i.e., *M. caligata*, *M. flaviventris*, *M. olympus* and *M. vancouverensis*). Samples were available for four subspecies of *M. caligata* and three of *M. flaviventris*. Images were captured using a flatbed scanner at a resolution of 300 dpi. Mandibles were laid horizontally on the labial side. Nine anatomical landmarks used by CARDINI (2003), CARDINI & TONGIORGI (2003) and CARDINI & O’HIGGINS (2005) were digitized on the hemimandible labial side. A two dimensional approach to the study of three-dimensional structures implies a loss of information which is minimal for a nearly flat object, like a marmot hemimandible. Further details on samples, the landmark configuration and measurement error can be found in NAGORSEN & CARDINI (2009).

Analyses were performed using geometric morphometrics (ROHLF & MARCUS 1993, ADAMS et al. 2004). Specimens were described using Cartesian coordinates of ana-

tomical landmarks. Configurations were optimally superimposed in TPSSmall (ROHLF 2003) using generalized Procrustes analysis (ROHLF & SLICE 1990) to obtain a matrix of shape coordinates. Discriminant analysis (DA) was performed in SPSS 15 (2006) and used to estimate percentages of individuals correctly classified to their group (hit ratios) based on shape coordinates. DA was cross-validated using a jackknife 'leave-one-out' approach. This allows group affiliation for a specimen to be predicted using discriminant functions (DFs) built on specimens other than the one which is predicted. Thus, a cross-validated DA avoids the risk of over-fitting the data, which would lead to circular reasoning and potentially spuriously high hit ratios.

DA was also performed on random subsamples of five specimens for each group to build DFs. These were used to classify the remaining specimens. This approach is equivalent to a hold-out sample cross-validation (HAIR et al. 1998) for the purpose of assessing the effect of very small sample sizes. As with the jackknife cross-validation (above), over-fitting is avoided by predicting group affiliation using DFs based on samples which do not include the specimens which are being classified. The hold-out cross-validation using the protocol described above was repeated four times on four sets of almost non-overlapping random subsamples. This series of cross-validated DAs allowed us to explore the robusticity of predictions where sampling error is very large as may happen when poorly studied populations are included in the analysis.

Only cross-validated results from this (hold-out samples) and the previous (jackknife approach) analysis are presented and discussed. Minimum, maximum and average hit ratios were computed for both subspecies and species of *Petromarmota*, and compared to those of *M. vancouverensis*. To exemplify the outcome of analyses when population differences are expected to be small, hit-ratios were also computed for two subspecies samples (*M. c. okanagana* and *M. f. flaviventris*) within the two main species and compared to those of all other populations.

RESULTS

Percentages of individuals classified using shape according to groups (subspecies and species) are shown in Table 1a (jackknife cross-validation) and Table 1b (hold-out samples cross-validation). For the hold-out samples, results were averaged across the four repetitions.

Results are summarized in Fig. 1a-b, which presents average, minimum and maximum hit ratios within subspecies, within species (*M. vancouverensis* excluded) and in *M. vancouverensis*. Within subspecies, hit ratios ranged between less than 20% and almost 60%. Within species (excluding *M. vancouverensis*), hit ratios scored about 80% (Fig. 1a-b). Species hit ratios from hold-out samples cross-validated DAs, however, ranged from about 60 to 90% (Fig. 1b). Ranges of hit-ratios for species showed no overlap with subspecies, although in hold-out samples DAs the largest subspecies hit-ratio (50%) was close to the lowest species hit-ratio (56%).

Marmota vancouverensis had hit-ratios slightly larger than other species (Fig. 1a-b). In the hold-out sample DAs (Fig. 1b), the range of hit-ratios for *M. vancouverensis* overlapped with that of *M. caligata*, *M. flaviventris* and *M. olympus*, but did not overlap with the range of hit-ratios in subspecies of *M. caligata* and *M. flaviventris*. In contrast, hit ratios for *M. c. okanagana* and *M. f. flaviventris* (Fig. 1c-d) were well below the lower extreme for species hit-ratios and clearly within the range of subspecies hit-ratios.

Table 1.

Percentages of individuals classified according to groups computed using (a) jack-knife and (b) hold-out sample cross-validated DAs of mandible shape (percentages of correctly classified individuals are emphasized in bold; species are emphasized using different shades of grey; sample size, N, is also shown).

Taxon	N	1,1	1,2	1,3	1,4	2,1	2,2	2,3	3	4
a) <i>M. caligata caligata</i>	1.1 40	57.5	10	7.5	15	5	2.5	2.5	0	0
<i>M. c. cascadenensis</i>	1.2 30	30	30	13.3	13.3	3.3	0	3.3	3.3	3.3
<i>M. c. okanagana</i>	1.3 8	25	12.5	12.5	25	0	0	25	0	0
<i>M. c. oxytona</i>	1.4 21	14.3	23.8	19	33.3	0	4.8	0	4.8	0
<i>M. flaviventris avara</i>	2.1 36	0	5.6	8.3	0	44.4	19.4	22.2	0	0
<i>M. f. flaviventris</i>	2.2 15	0	6.7	0	0	13.3	40	33.3	6.7	0
<i>M. f. nosophora</i>	2.3 52	0	1.9	7.7	1.9	32.7	30.8	23.1	1.9	0
<i>M. olympus</i>	3 14	0	7.1	7.1	0	0	0	0	78.6	7.1
<i>M. vancouverensis</i>	4 23	0	0	0	0	0	0	0	4.3	95.7
b) <i>M. caligata caligata</i>	1.1 40	26.4	23.6	14.3	17.1	4.3	6.4	5	1.4	1.4
<i>M. c. cascadenensis</i>	1.2 30	12	37	23	12	2	1	5	6	2
<i>M. c. okanagana</i>	1.3 8	8.3	16.7	33.3	25	0	8.3	0	8.3	0
<i>M. c. oxytona</i>	1.4 21	15.6	28.1	18.8	31.3	1.6	4.7	0	0	0
<i>M. flaviventris avara</i>	2.1 36	6.5	4	7.3	0.8	35.5	17.7	21	3.2	4
<i>M. f. flaviventris</i>	2.2 15	5	5	0	0	27.5	25	30	2.5	5
<i>M. f. nosophora</i>	2.3 52	4.8	8.5	8.5	3.2	26.1	22.3	20.2	4.3	2.1
<i>M. olympus</i>	3 14	0	11.1	8.3	0	0	0	0	63.9	16.7
<i>M. vancouverensis</i>	4 23	2.8	0	0	0	0	0	1.4	11.1	84.7

DISCUSSION

Our study shows that population distinctiveness in quantitative morphological characters can be fruitfully investigated using geometric morphometrics. The endemic and presumably recently isolated population of *M. vancouverensis* produced very high hit ratios (> 80%) even when few specimens were available to build discriminant functions. Hit ratios were comparable to those of 'good species' with a long history of genetic isolation (since early-mid Pleistocene at least, according to STEPPAN et al. 1999) and clearly larger than those of subspecies. This implies that Vancouver Island marmot (STEPPAN et al. 1999) displays levels of morphological variation as large as or even larger than those observed among other species of the same subgenus despite its low divergence in mtDNA. This finding is not entirely surprising if one considers that divergence in mitochondrial DNA tends to overlook new and rapidly evolved species (MORITZ & CICERO 2004; see also below). However, it is interesting to observe that results of standard discriminant analyses of shape data from a simple structure (the hemimandible) and very small samples (hold-out analyses) were fully congruent with previous studies using more sophis-

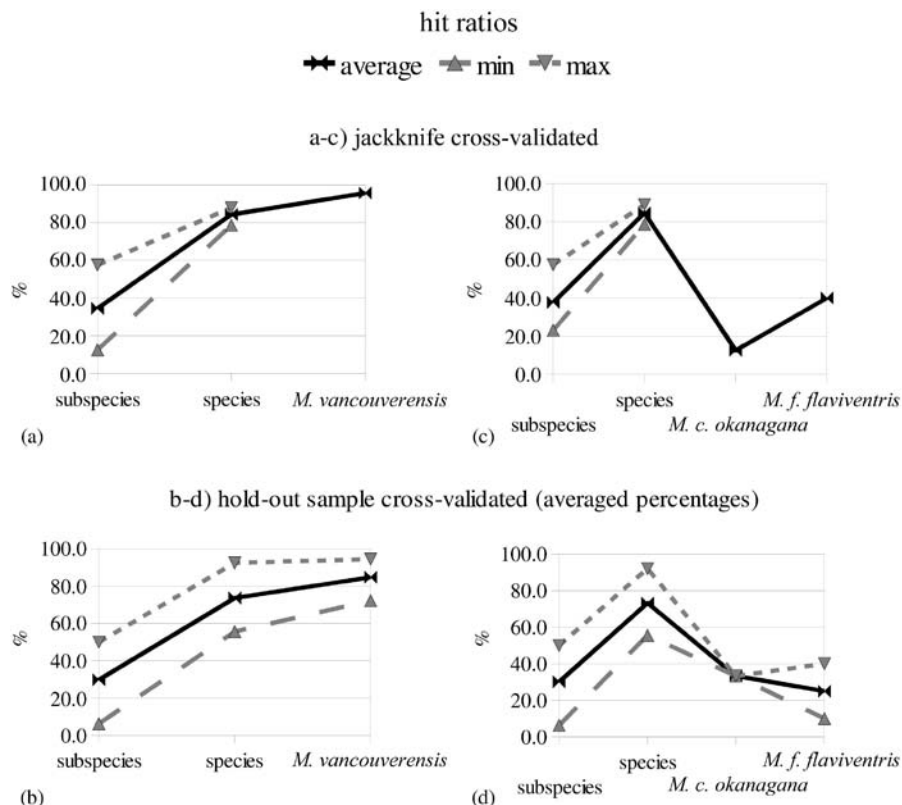


Fig. 1. — Percentages of correctly identified individuals (hit ratios).

ticated techniques on two- or three-dimensional cranial landmarks (CARDINI & O'HIGGINS 2004; CARDINI et al. 2005, 2007). Traditional morphometrics on form (HOFFMANN et al. 1979), that are heavily influenced by size in contrast, failed to reveal anything unusual in the morphology of *M. vancouverensis* mandibles and crania compared to continental populations of *M. caligata*. Thus, geometric morphometrics may represent a powerful tool to explore biological variation in closely related taxa and detect population distinctiveness even when sampling error is large.

In principle, the approach used in our study is straightforward. It only requires a minimal understanding of Procrustes-based methods for the analysis of anatomical landmark coordinates and a basic knowledge of standard multivariate statistics. The landmark configuration has to be designed carefully, but advice on this can be found in the literature, which is rich in examples of landmark configurations from a variety of mammals (and other organisms), and this step has to be taken just once in any analysis. A protocol similar to the one we used here on shape could be extended to the analysis of size, and other methods,

like disparity analysis applied to size and shape data (CARDINI et al. 2007, FRANKLIN et al. 2009, NOWAK et al. 2009), could also be effective in extracting information on morphological distinctiveness. All these approaches, however, implicitly rely on a few assumptions. The first assumption is that the samples used must adequately represent variation within the study groups; the second is that a criterion for delimiting the a priori groups that will serve as units of analysis is needed; and the third is that knowledge about related species, whose taxonomic status has already been confidently assessed, must be available.

For samples that adequately represent variation in the study groups, museum collections offer an excellent source of data and, at least for simple and relatively flat structures like hemimandibles in most rodents and other mammals, digital images can easily be obtained using standardized protocols, which minimize measurement error and reduce differences among operators. This would potentially allow low cost data collection from major institutions, which already have very large collections (e.g., the Smithsonian National Museum of Natural History in Washington, DC), and via group specialists and field biologists, who may have access to samples of populations poorly represented in major museums. Indeed, structures like mandibles and crania are not only the most commonly available mammal material in museums, but may also often be obtained from field collections of animals who died of natural causes. For instance, about half of the sample of the rare and endangered Zanzibar red colobus (*Procolobus kirkii*) analysed by NOWAK et al. (2009) consisted of individuals who were incidentally found in the field during a behavioural study. Similarly, several specimens of *M. marmota* analysed by CARDINI (2003) were collected by rangers of the Gran Paradiso National Park, who found them during field patrols and made them available to scientists. Also, most of the sample of *M. vancouverensis* from the Royal British Columbia Museum, which hosts the largest collection of this species and provided the largest number of specimens of this rare endemic population for our study, were from carcasses found in the field by biologists or naturalists. Thus, networks of group specialists may directly (e.g., by looking for specimens and taking pictures) or indirectly (e.g., by providing local support or getting information on samples) contribute to data collection. Co-operations can be fruitfully set up so that field biologists, local communities and institutions help in finding additional data yielding material from rare populations. Besides the benefits of boosting national and international cooperation and the possibility of increasing awareness on biodiversity conservation by getting local people involved in the research project, this strategy could improve sampling without the need for expensive field trips to collect specimens with often unnecessary and hardly justifiable sacrifice of animals.

Decisions for delimiting a priori groups may be based on a variety of criteria among which geographic distribution is likely to be the main one (DESALLE et al. 2005). Populations with allopatric or parapatric ranges can be selected as a priori groups to test geographic hypotheses. Of course, this does not exclude the possibility of cryptic variation within geographical units. The occurrence of unusual phenotypes and higher-than-average variance in size or shape may provide clues about the presence of cryptic diversity and the need to refine a priori groups. Other sources of information (external morphology,

behaviour, molecular data, etc.) can be used to aid the establishment of a priori groups, and a multidisciplinary approach is indeed likely to provide the most accurate results (DESALLE et al. 2005). For instance, STRUSHAKER (1981) and COLYN (1991) used vocalizations and biogeographic data together with information on pelage colour to tell populations of red colobus apart. These were later tested by COLYN (1991) and CARDINI & ELTON (2009) for differences in skull form, and examined by TING (2008) for molecular divergence.

Knowledge about related species whose taxonomic status is uncontroversial will vary from taxon to taxon, but for our approach to work a set of species (ideally closely related to the study populations) must be available for which species status is well established. This will provide the background against which to compare the magnitude of morphological distinctiveness estimated by hit ratios from cross-validated discriminant analyses. Thus, highly distinctive populations will have cross-validated hit ratios comparable to and larger than those found respectively between and within 'good species'. That these ranges show minimal overlap is supported by our study and is a reasonable expectation as, even in the case of convergence or retention of plesiomorphic characters, it is highly unlikely that lineages with a long history of reproductive isolation (e.g., *M. caligata* and *M. flaviventris*) have evolved nearly identical morphologies (POLLY 2004). Nevertheless, this observation cannot be generalized until it has been verified in other groups (e.g., different orders of mammals). Testing this hypothesis might be particularly valuable since the occurrence of several closely related populations provides a situation in which DNA barcoding seems to be less reliable (ELIAS et al. 2007) whereas the morphometric approach may be most effective in detecting their subtle differences. For instance, an accurate morphometric analysis may reveal the outcome of an evolutionary process like rapid speciation, which may be 'cryptic' for most molecular markers (MORITZ & CICERO 2004, MACE & PURVIS 2008) but is likely to leave a signature in quantitative characters like the sizes and shapes of bones (NAGORSEN & CARDINI 2009).

Palaearctic marmots have a wider distribution and more species than Nearctic marmots. That they may exhibit cryptic diversity has recently been shown by karyotypic studies (BRANDLER et al. 2008), who suggested that *M. baibacina kastschenkoi* may actually represent a separate species. Palaearctic marmots also include several poorly studied populations, whose distributions are either parapatric or allopatric. Sometimes, for instance in *M. camtschatica* and *M. marmota*, populations may be found within the same species separated by hundreds of kilometers with no record of intervening populations in historical times. This geographic complexity and the lack of a clear understanding of many aspects of the systematics of the genus *Marmota* makes this group an ideal candidate to explore the usefulness of geometric morphometrics in the analysis of evolutionary distinctiveness. The group is promising not only in exemplifying the potential for discovering cryptic evolutionary variation, but also because of the availability of an international network of marmot specialists (<http://www.cons-dev.org/marm/MARM/EMARM/framarm/framarm.html>) which can provide invaluable support. The network has been active for about two decades and has successfully managed to promote international scientific cooperation, and communication between scientists and the general public, to

achieve a better understanding of the biology and conservation of marmots. The international marmot network thus offers an excellent starting point to bring together scientists from different countries to provide local support to find samples of rare populations, help with data collection and the identification of study groups, perform analyses, interpret results etc. This would greatly facilitate a more extensive pilot study on the potential of geometric morphometrics for discovering distinctive aspects of biological diversity which may be missed by the exclusive adoption of a molecular approach.

CONCLUSION

Even proponents of DNA barcoding acknowledge that genetic diversity is only one aspect of evolution and “the task of identifying and describing new species is ultimately achieved through comprehensive taxonomic work” (HAJIBABAEI et al. 2007). This is important for the accuracy and meaning of taxonomy and even more for the effectiveness of conservation biology. If evolutionary processes are to be preserved, their multifactorial nature must be taken into account and this means that aspects other than the divergence of short molecular sequences need to be considered and their potential to inform about population uniqueness evaluated.

That a pronounced phenotypic differentiation may occur without corresponding divergence in mtDNA, as in the example of *M. vancouverensis*, may not be so uncommon after all. Besides the already mentioned observation of frequent acceleration in morphological evolution of insular mammals (MILLIEN 2006), a textbook example of minimal genetic divergence in a highly diverse clade is that of the Lake Victoria cichlids. In this group, MEYER et al. (1990) found an average divergence in mitochondrial DNA sequences of less than 1% (RUBINOFF 2006) despite their extraordinary ecomorphological radiation. This small degree of molecular divergence was consistent with findings from previous electrophoretic studies of nuclear gene proteins. However, there was almost no sharing of DNA types across species and that strongly suggested that representatives of this morphologically and ecologically disparate group were not simply morphotypes within a single biological species. Significant evolutionary divergence which was not detected using a strict barcoding approach has been shown also in invertebrates. TREWICK (2008) showed how well defined grasshopper morphospecies with narrow geographic ranges and clear ecological differences may have various degrees of haplotype sharing with the most widespread species in the group. He suggested that these morphotypes may arise through stochastic fixation of morphological variation in small populations and raised the question of whether morphospecies of this kind should be more or less significant than haplogroups based on a barcoding approach. Following a similar line of reasoning, CRANDALL et al. (2000) and AGAPOW et al. (2004) observed that species, especially among mammals and birds, often consist of populations whose borders can be fuzzy and whose gene pools may be characterized by high levels of gene flow. For populations of this kind, genetic data alone are unlikely to produce satisfactory answers to

questions of taxonomic diversity, and ecology, behaviour and morphology are needed to help in identifying unique aspects of their biology. Molecular divergence is becoming the primary criterion for species recognition and there are potentially several benefits from careful use of molecular tools for this purpose. However, it is generally acknowledged that whether populations truly deserve special attention should be assessed by integrating different sources of data from a variety of disciplinary sectors (MORITZ & CICERO 2004), and this means that other quantitative tools should be explored for identifying components of biological diversity. MACE & PURVIS (2008) pointed out in a review on the role of evolutionary biology in conservation that, to incorporate information on evolutionary processes by maximizing phylogenetic distinctiveness, branch lengths from molecular phylogenies can be used by conservation biologists as a proxy for disparity. Disparity, however, could be estimated using other metrics, and there are several options available for this use of morphology (FOOTE 1997). If the aim is to preserve as much disparity (genetic, ecological, behavioural, morphological, etc.) as possible to buffer against the loss of evolutionary potential in a heavily anthropized and rapidly changing world, direct or indirect clues on how easily a population may be replaced by another one in terms of its ecological role and evolutionary history must be obtained using as much information as possible. Populations at range margins are often believed to have a particular adaptive potential, but their genetic diversity may easily go undetected because the molecular markers being used may evolve too slowly or simply miss the regions of the genome which responded adaptively to the local environmental conditions or were shaped by founder effects and drift (MACE & PURVIS 2008). This is an instance where approaches like the analysis of morphological variation with statistically powerful methods like geometric morphometrics may offer a new perspective and provide at least an initial glimpse of population distinctiveness. If this tool is shown to be easily applicable, in a good number of cases it will provide an independent and potentially highly informative source of data on evolutionary and ecological processes to complement genetic analyses, thus offering a broader view of the biology of a group and increasing the accuracy of data available to conservation biologists in setting their targets.

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