

European non-volant mammal diversity: conservation priorities inferred from phylogeographic studies

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Received 30 December 2008; Accepted 15 June 2009

A b s t r a c t. The increasing use of phylogeographic studies, based on mitochondrial DNA, in European mammals not only provides the genetic structure of the populations and a reconstruction of the evolutionary history of each taxon but is also useful in identifying cryptic species and areas that may represent conservation priorities. We reviewed the published data (about 60 articles) reporting phylogeographic studies, based on sequences of mtDNA genes, in order to identify those populations representing putative species, not yet formally described among European mammal species of all orders, with the exception of Chiroptera. A DNA taxonomic approach and the value of subspecies are also discussed in relation to conservation activities.

Key words: conservation priorities, taxonomy, mtDNA

Introduction

The European Union has recently set up the ambitious goal to stop biodiversity loss by 2010. To do this, a network of protected areas (Natura 2000 Network) has been developed using a list of specially protected species included in the Habitat and Birds Directives. Generally, biodiversity status and trends are better measured at the species level. However, for the European mammals, the current debate over species concepts and its consequences for biological conservation (Rojas 1992, Hey et al. 2003, Isaac et al. 2004, Agapow et al. 2004) has received scarce attention by the scientific community. Mammal diversity in Europe has still been described using the Biological Species Concept (BSC), while there is increasing evidence that other species concepts, including e.g. the Evolutionary Species Concept (ESC), the Phylogenetic Species Concept (PSC) and the Genetic Species Concept (GSC), may better describe the current biodiversity (Cotterill 2003, Baker & Bradely 2006). Furthermore, following national legislations, considerable attention has been spent outside Europe to identify units that are important for conservation efforts below the species level (Evolutionary Significant Units – ESUs, and Management Units – MUs; Moritz 1994) and to discuss the conservation significance of subspecies (O’Brien & Mayr 1991). For various reasons, European mammalogy (but not herpetology; cf. Veith 1996) has never been properly discussed on these issues. This does not imply lack of improvements in the taxonomic knowledge of European mammals in the last decades, rather a lack of discussion about theoretical properties of species (and subspecies), having consequences for conservation strategies. However, it is well established that taxonomy

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and preferred species criteria can have deep consequences on the selection of conservation priorities (Meijaard & Nijman 2003). In fact, the use of BSC species lists is prone to overlook important regions of endemism when utilised to prioritize areas to be protected (Peterson & Navarro Sigüenza 1999). It is interesting to note that while several European mammals have been intensively studied from a phylogeographic perspective (reviewed in Bilton et al. 1998, Taberlet et al. 1998, Hewitt 1999, Randi 2007), with many divergent phylogroups identified, very rarely taxonomic conclusions were formalised. In the U.S., Genetic Species Concept (GSC) has been conceptualised and consistently used (Bradley & Baker 2001, Baker & Bradley 2006), leading to the hypothesised presence of a large number of undescribed cryptic species among mammals. In this regard, it should be however noticed that not all the examples proposed by Baker & Bradley (2006) were really ‘cryptic’ (i.e. *Loxodonta cyclotis*), since they were already described on morphological evidence. The aim of the present work is to review the available literature on phylogeographic research to help identify new species of European mammals.

Material and Methods

We obtained an up to date list of all the European non-volant mammals species following the taxonomy proposed in Wilson & Reeder (2005). One single species, *Mus cypriacus*, was formally described after the publication of this book (Cucchi et al. 2006). For each species, we selected papers dealing with data on phylogeography based on mtDNA, cytochrome *b* or control region (that some author report as D-loop). We retrieved 59 papers. For each analysed species we extracted the following information: 1, completeness of the distribution of the sampled populations (i.e. if the sample covers the range of the species in Europe, with particular attention to putative refugial areas); 2, presence and number of allopatric or parapatric phylogroups found within Europe; 3, localisation of the phylogroups; 4, genetic divergence (Kimura 2-Parameter model) among phylogroups, calculated only for cytochrome *b*; we used the “net between group mean”, that considers the within-group polymorphism.

We used only cytochrome *b* data for genetic divergence because the use of control region does not allow the comparison of genetic divergence due to the non homogeneous mutation rates along the sequences (Saccone et al. 1991) and the different sequence length used in different papers.

The use of a single measure, such as a genetic divergence of cytochrome *b*, as a taxonomic tool has been criticized (Ferguson 2002). However, this procedure may be predictive and constitute the starting point for future studies (Vogler & Monaghan 2007). In evaluating genetic divergence as a taxonomic tool, we followed the comparative approach of Bradley & Baker (2001) and Baker & Bradley (2006), involving comparison of the genetic divergence of the cytochrome *b* of the species under study with an appropriate set of other well recognized, and genetically studied species that share the same life histories and biological characteristics.

To individuate the putative unrecognized species among the identified phylogroups, we compared the genetic divergence among phylogroups, with the distribution of pairwise genetic divergence between sister species, congeneric with the species under study. In a few cases we considered sister species belonging to related genera or included in the same family of the species under study. We preferred using pairwise genetic divergence between sister

species rather than the overall pairwise genetic divergences, because it better represents the true genetic difference attained since the dichotomous speciation event. These two values can be very different one from the other; for example, values of genetic divergences between pairs of sister species in mammals varies between 3 and 8%, while, considering all the congeneric species these values range between 8 and 10% (see Johns & Avise 1998).

We tentatively identified a phylogroup as a putative new species if its genetic divergence with respect to the sister group is similar or greater than the mean pairwise divergence among sister species. We would also like to stress that the identification of a putative new species should be taken only in a probabilistic view. Such a procedure simply indicates those phylogroups that could represent new species confirmed after additional data on nuclear genes (according with the Genetic Species Concept *sensu* Barker & Bradley 2006).

We identified pairs of sister species after consulting recently published phylogenetic analyses of the following genera: *Crocidura* (Motokawa et al. 2000, Oehdachi et al. 2004, 2006, Duhé et al. 2006, 2007), *Sorex* (Fumagalli et al. 1996, Shaffer & Stewart 2007), *Mus* (Suzuki et al. 2004, Chevret et al. 2005), *Apodemus* (Michaux et al. 2002, Suzuki et al. 2003, Liu et al. 2004), *Microtus* (Jahirova et al. 2004, Martíková et al. 2007), *Lepus* (Yamada et al. 2002, Wu et al. 2005) and for the family Cervidae (Pitra et al. 2004).

Table 1. Genetic divergence (Kimura 2-Parameter model) between pairs of sister species in different mammalian taxa (mean \pm standard error).

	Pairs of sister species	Genetic divergence
<i>Erinaceus</i>	1	4.6%
<i>Crocidura</i>	3	6.5 \pm 2.6%
<i>Sorex</i>	7	6.0 \pm 3.8%
<i>Lepus</i>	3	4.3 \pm 1.7%
<i>Microtus</i>	11	5.7 \pm 2.1%
<i>Apodemus</i>	4	8.9 \pm 2.2%
<i>Mus</i>	6	8.0 \pm 5.6%
Cervidae	6	5.4 \pm 3.8%
Mammals	73	6.3% (0.3-19.5)*

*mean (range), data from Baker & Bradley (2006)

Results

The values of divergences (Kimura 2-Parameter model) among pairs of congeneric sister species are rather homogeneous (Table 1). Moreover, these values are only slightly higher, on average, than the 5% value arbitrarily chosen by Barker & Bradley (2006) for species status. The highest inter-specific values have been found in *Apodemus* (8.9%) and the lowest values in *Lepus* (4.3%).

Lagomorpha can be considered well studied while 41% of European species of 'Insectivora' (Soricomorpha, Erinaceomorpha) have still to be studied. However, among all the species studied, 46% (34% of rodents, 46% of shrews, and 50 % of Artiodactyla) requires more data because the range of the species has not been adequately sampled, especially because it does not cover all the hypothetical refugial areas. The 48% of sampled species shows phylogroups within the European boundary obtaining a total of 87 allo-parapatric phylogroups (Table 2).

Table 2. Summary of the phylogeographic studies in non-volant European mammal species including the number of mitochondrial phylogroups and the number of putative unrecognized species (see text for explanation).

	Rodentia	Insectivora	Lagomorpha	Carnivora	Artiodactyla
Total no. species in Europe	62	31	7	22	12
no. studied species	32	13	6	15	6
no. species which more data are needed	11	6	2	7	2
no. species with phylogroups	15	6	3	2	3
no. of phylogroups	47	21	6	5	8
no. of putative new species	4	3	1	0	0

For Artiodactyla, Carnivora and Lagomorpha, the papers are referred mainly to D-loop investigations. This is because there is a generally lower intraspecific genetic differentiation among large and vagile mammals in respect to small mammals. Considering only cytochrome *b*, according to our comparative approach, we identified eight phylogroups that we consider new putative species representing 10% of the total studied species and 18% of the species for which the distribution of the sampled populations is satisfactory. These phylogroups are indicated in Table 3, together with the genetic divergence from sister clades and their geographic localisation.

For rodents the most divergent phylogroups have been found within the following species: *Dinaromys bogdanovi* (Balkans – Kryštufek et al. 2007), *Microtus savii* (Sicily – Castiglia et al. 2008), *Microtus agrestis* (Southern Europe – Jarrola & Searle 2004), *Arvicola amphibius* (Italian Peninsula – Taberlet et al. 1998). For shrews divergent phylogroups have been found for *Crocidura suaveolens* group (Cyprus and South-western Europe – Dubey et al. 2007) and for *Neomys anomalus* (Iberian Peninsula – Castiglia et al. 2007). For Lagomorpha, *Oryctolagus cuniculus* shows a divergent phylogroup (Iberian Peninsula – Branco et al. 2000).

For *M. agrestis* there are also data available concerning nuclear genes (X and Y chromosome markers) that allow firmly the existence of a new species of mammal to be invoked (Hellborg et al. 2005). On the contrary, for some phylogroups, i.e. *Microtus savii*, *Arvicola amphibius*, *Neomys anomalus* divergent cytochrome *b* haplotypes were found only in a single population.

Table 3. The European non-volant mammal species showing highly divergent phylogroups.

Species	Genetic divergence from the sister group	Range
<i>Crocidura suaveolens</i>	8.3%	South-western Europe
<i>C. suaveolens</i>	7.2%	Cyprus
<i>Neomys anomalus</i>	8.3%	Iberian peninsula
<i>Oryctolagus cuniculus</i>	7.6%	Iberian peninsula
<i>Arvicola amphibius</i>	5.0%	Italian peninsula
<i>Dinaromys bogdanovi</i>	5.8%	Balkans
<i>Microtus savii</i>	7.2%	Sicily
<i>M. agrestis</i>	5.2%	Southern Europe

Discussion

The values of the genetic divergence we obtained to identify a putative non-volant mammal species in Europe are very similar to those obtained by Baker & Bradely (2006) at a more wide scale, with a mean value of 6.3% for sister species and 12.8% for overall intra-generic comparison.

In the analysed literature (see Table 2) we observed that for a high number of species more data are necessary to have a good and representative sampling data set. We would also recommend to pay more attention to evaluating samples from possible refugia; because the risk of a wrong or incomplete scenario.

Another important aspect is the identification of priority conservation areas or areas of endemisms. Adopting the Biological Species Concept, such areas could be underestimated. For example, if we consider the species richness of European mammals map, based on the species' ranges of European atlas (Mitchell-Jones et al. 1999) and we add the putative species that we identified (see Table 3), the scenario will change to revealing a higher rate of richness and endemism in the Mediterranean Peninsulas (see also Bilton et al. 1998). More in detail, a pioneering attempt in prioritising important conservation areas in Europe is a work based on the vertebrate and vascular plant atlas projects (Wylliams et al. 2000), that revealed that both diversity and endemism are high in and around the mountains of Central Europe (Pyrenees, Alps, Balkans). On the other hand, diversity and endemism become lower in the north and the south. However, while the species richness reduction may be realistic (but southern regions, such as southern Italy, may be undersampled, i.e. Aloise & Gagnin 2003, Scaravelli et al. 2004, Aloise et al. 2005, Ribas et al. 2005), the highest endemism rates are found in and around the mountains of Central Europe, thus openly contradicting the 'southern Pleistocene refugia' paradigm proposed after a number of phylogeographic studies, mainly obtained through mtDNA (Hewitt 1996, 1999, Kotlik et al. 2006). Thus, it may be hypothesised that failing to recognise the taxonomic specific individuality of many southern populations represents the main cause of this result. It is worth noting that while subspecies, especially of large mammals, may be included in protective legislation such as the Berne Convention (thus protected whatever their effective taxonomic status is), they are of no relevance in present priority setting exercises that use only *species* distribution.

Although the subspecies concept has been hotly criticised (i.e. Cronin 2006), it is often considered useful to illustrate intraspecific variability and subspecies are often the target of conservation plans (Haging et al. 2006). One of the main problems is that current subspecies may well indicate a true species (especially when consistently recognised following well conducted classic taxonomic revisions under the BSC in the last 50 years) or, alternatively, variability of morphological characters of no evolutionary meaning. The latter situation is often the result of lack of competent revisions, so that a plethora of names often based on a single studied specimen described more than a century ago remain potentially valid and 'available'. However, it has been recently recognised that a main problem was borne out from lack of consistency in the use of the subspecies category in conservation lists among regions and mammal taxa (Gippoliti & Amori 2007). In particular, subspecies are often used to describe the diversity of populations of large mammals but, in a few countries like the U.S. (e.g. Vignieri et al. 2006) it is rarely adopted for groups, for example, like rodents and 'insectivores' in international documents such as the IUCN Red List. With respect to phylogeographic research on European mammals, it is often observed

a similar trend, and a parsimonious approach prevails in taxon description (Brünnner et al. 2002, Heuborg et al. 2005). A clear example is represented by the recent notable discovery of the existence on Cyprus of an endemic form of the *Crocidura suaveolens* group (Duby et al. 2007) that is maintained only at subspecific level while the other Mediterranean Pleistocene survivors are considered as full species (*Crocidura sicula*, *C. zimmermanni*, *Mus cypriacus*). On the contrary, the identification of genetically isolated subclades in large mammals has often been considered as an adequate proof of subspecific validity (e.g. Lorenzini & Lovari 2006).

Why phylogenies of European mammals are hardly translated into taxonomy? Palearctic species often have huge distributions across several countries. Therefore, each country developed a ‘national’ mammal taxonomy not always properly known internationally, due also to linguistic diversity across the Continent. This fact, coupled with a lumping attitude that goes back to the classic reference of Ellerman & Morrison Scott (1951), led to synonymy of most of the latin names proposed since Linnaeus in agreement with the BSC (Corbett 1997). However, the subdivision of Europe into many separate countries made (and still makes) wide-scale taxonomic revisions a huge and impractical endeavour, while some areas are still undersampled. Therefore, it should be not surprising that at the end of the 1970s, some clearly distinct lineages like *Lynx pardina* and *Rupicapra pyrenaica* were still unrecognised (Corbett 1978). In the last two decades, molecular studies investigated the genetic structuring of populations of mammals. These phylogenetic studies have been incorrectly assumed sometimes as being a substitute for taxonomic revisions (Wheeler 2004). While they furnish invaluable information and may represent a first step in the identification of cryptic species, only a classic descriptive taxonomic revision may be able to provide physical discriminating characters and the geographic limits of named taxa. Scientific names facilitate communication and thus render possible conservation assessments of taxa and their inclusion in protective legislation and priority setting exercises, both based on internationally recognised checklists such as Wilson & Reeder (2005). Usually, European mammalogy suffers from the abandonment of classical taxonomy in favour of modern research themes, and this factor leads to a tendency to search for stability in taxonomy, a fact that has been discussed and criticised by Dvořák (1998) for amphibians and reptiles.

In conclusion, the exclusion of genetically well-identified taxa, with nonrecognised taxonomic status from European directives can cause a loss of cryptic species. However, our attempt should be considered as a starting-point for a wider scientific discussion of an important issue in conservation activities. Moreover a greater involvement of European mammalogists in the debate on species concepts should be welcome, as well as a stronger collaboration between phylogenetists and taxonomists. Biodiversity conservation in Europe, as elsewhere, depends on sound taxonomy, otherwise the aim of EU to stop biodiversity loss by 2010 could be an impossible goal.

Acknowledgements

We would like to thank J. B. Searle and J. Zima for the useful suggestions to an early draft of the manuscript.

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