

# The Input of DNA Sequences to Animal Systematics: Rodents as Study Cases

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## 1. Introduction

### 1.1 General context

The advent of molecular techniques, and especially the possibility to get DNA sequences that can then be compared between individuals of any taxon using more and more powerful algorithms of analysis, has represented a kind of revolution in systematics (Lecointre et al., 2006; Giribet et al., 2007). As in other groups, mammalian systematics was for long only based on morphological and anatomical characters. A brief history of Mammal taxonomy has been provided by Wilson & Reeder (2005: xxiii), starting by early works of Trouessart (1898-99, 1904-5) and ending by the compilation by Mc Kenna & Bell (1997). Since the latter, a huge quantity of data including a significant proportion of molecular ones has accumulated, that have greatly improved our view of the relationships between main mammalian groups (Springer et al., 2004), and increased the number of individual species in each of them (Wilson & Reeder, 2005). Rodents represent the most diverse order of Mammals, comprising around 40% of both generic and specific mammalian diversity (Wilson & Reeder, 2005). In one of the first significant contribution to the study of their classification, Tullberg (1899) subdivided them into two suborders, the Sciurognathi and Hystriognathi (see Hautier et al., 2011), based on morpho-anatomical characteristics of their skull. Several decades of research have led to significant advances in our understanding of rodent evolutionary systematics that were synthesized in Luckett & Hartenberger (1985) major contribution. Again, arrangement of the diversity at the various taxonomic levels, as well as species characterization and delimitation in this group, have greatly improved with the growing use of nuclear and mitochondrial gene sequence data since the 80's, and more importantly the 90's (Catzefflis et al., 1992; Carleton & Musser, 2005).

This review aims at showing how DNA sequences have re-boosted rodent systematics, bringing new data in support or in contradiction to traditional ones, but finally leading to a much better supported classification of this order, via a more accurate characterization and delimitation of its constituent subgroups, down to the species level. It is not intended to be a

plea for what has been critically quoted as “the molecularisation of taxonomy” by Lee (2004), but rather to acknowledge the progress in systematics (using rodents as model cases) that DNA sequence data have brought in an integrative context. Indeed, it is clearly within this philosophy of “delimit[ing] the units of life’s diversity from multiple and complementary perspectives” (*sensu* Dayrat, 2005: 407) that spectacular advances have been made in recent years in the characterization of taxa and the assessment of their phylogenetic relationships. Saying that implicitly implies that phylogeny should serve as basis for taxonomy, a principle which is underlying most of the current works and findings in these disciplines and which will not be discussed further here.

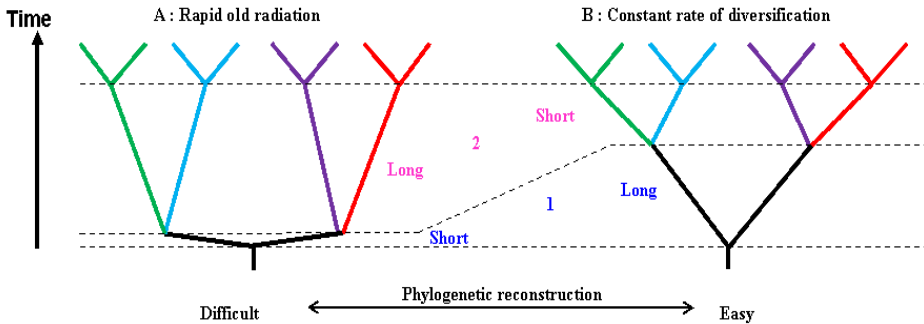
After some methodological considerations, this chapter will follow a top-down organization in taxonomic ranking, from higher taxa to species, and will be based on numerous examples taken in various rodent groups, and studied with a variety of DNA sequence data.

## 1.2 Advantages, drawbacks and progress of molecular data

Evolutionary relationships between organisms are generally expressed in phylogenetic trees which then may serve as basis for classification and systematics. A phylogenetic tree is termed fully resolved when only two descending branches are issuing from each node. Such a node is a dichotomy and it represents the speciation event that occurred between two taxa from their hypothetical most recent common ancestor. If there are more than two descending branches, the node is a polytomy or a multifurcation. In this case, the interpretation is more difficult because it can actually mean that an ancestral taxon has split into several descendant taxa but it can also signify that the data used do not resolve the dichotomous branching orders between taxa, and the node is thus unresolved. The concepts of soft and hard polytomies have been introduced (Maddison, 1989; Poe & Chubb, 2004; Walsh et al., 1999) to distinguish between phylogenetic irresolutions due to inadequate data and/or methods, and rapid radiations. The term radiation is used to describe a phase of “divergent evolution of numerous related lineages within a relatively short time” (Futuyma, 1998 : 117; Figure 1A). In the case of soft polytomies, increasing the number of characters analyzed should bring more information and thus solve dichotomous nodes previously masked. If the increase of the number of characters used does not help to decipher phylogenetic relationships, then hard dichotomies should be suspected. However, the question will remain as to know if enough data have been added to resolve the multifurcation. Different molecular studies have shown that an increase in the number of characters combined to the analysis of new genes (in general nuclear exons or introns) have effectively helped to disentangle polytomies that were reputed hard or at least very difficult to solve. Among them are the relationships among the different orders of mammals (Madsen et al., 2001; Murphy et al., 2001), the different families of birds of the super-order Neoaves (Ericson et al., 2006), or the different families of rodents (see paragraphs 2.2.2 and 2.3.1). In these different examples, molecular studies led to some inferences about lineage relationships that were never previously hypothesized from morphological characters.

Advantages of molecular data over traditional morphological characters have rapidly been identified: they represent objective characters whose number can be increased nearly to infinity, and they allow comparisons between phylogenetically distant organisms even when there is no comparable morphological character between the corresponding taxa. However, more time has been needed to realize that homoplasy (convergence and reversal

of characters) was a major issue that could blur the phylogenetic signal and thus lead to erroneous groupings (see paragraph 2.1.2). This is particularly problematic in the case of rapid radiations when phylogenetic signal has to be recorded during short time intervals (internodes) whereas this signal might be eroded during the long time span of individual lineage evolution (Whitfield & Lockhard, 2007; Figure 1). The art of phylogeny consists in establishing robust relationships, especially in such circumstances, while avoiding biases due to homoplasy.



1: Divergence time between basal nodes; 2: Divergence time between terminal nodes

Fig. 1. Some phylogenetic reconstructions are harder to solve than others (modified from Whitfield & Lockhard, 2007).

Various tools are nevertheless available to avoid the misleading effects of homoplasy. The first one is *the choice of the gene*. The molecular era started with the sequencing of mitochondrial genes (cytochrome b, 12S rRNA, control region), mainly because they were easily amplified (several copies by cell as compared to only one copy of nuclear DNA). Several years passed before it was realized that the mitochondrial genome is not adapted to address questions related to ancient divergence events, such as for example the diversification of mammalian orders (Honeycutt & Adkins, 1993) or the radiation of Rodentia (see paragraph 2.1.2). The reason is that the rate of evolution of mitochondrial genes is generally much higher than the nuclear ones. The consequence is that homoplasy accumulates more rapidly (especially at third position of coding genes), thus hiding the phylogenetic signal. The advent of nuclear gene sequencing allowed to resolve many polytomies because of a lesser homoplastic signal. This does not mean that mitochondrial genes have to be banished but rather that it is necessary to evaluate what gene(s) will be the most adapted to the phylogenetic questions asked. In short, rapid-evolving gene will fit for recent phylogenetic separation and slow-evolving genes for ancient radiation.

*The choice of the reconstruction method* is also of prime importance to avoid artificial groupings. The first molecular trees were produced using the same reconstruction methods as for morphological characters, namely cladistic analysis. However, as soon as 1978, Felsenstein drew the phylogeneticist's attention about artifacts of reconstruction, among which the famous "long-branch attraction", that is the grouping of unrelated taxa on the basis of convergent mutations due to high rates of evolution. The advent of

probabilistic methods (Maximum likelihood and Bayesian inference) based on explicit models of sequence evolution greatly improved tree reconstructions (see Felsenstein, 2004). These models include different parameters, such as base frequency, type of substitution or substitution-rate heterogeneity. More parameters will likely be included in the future as their influence on sequence evolution is discovered.

Consequently, *the choice of the model of sequence evolution* is also a decisive stage to cope with homoplasy, as the main objective is to correct effects of hidden multiple substitutions leading to underestimation of the real distance between taxa. Estimation of the model of sequence evolution and associated parameters the best adapted to the dataset is now the first step when starting a phylogenetic analysis. These models not only concern the whole studied genes but also partitions of them according to their functional structure (or any other kind of partition). For example, if the analysis is based on several coding genes, it can be more fruitful to partition the genes according to the three coding positions than considering the whole genes separately. The main inconvenience is that most softwares do not implement these partitioned models yet, but this is a promising way to handle sequence homoplasy. More recently, mixture models have been developed that are based on site-specific patterns of substitution (see Philippe et al., 2005) allowing a better description of the substitution process, thus a reduction of systematic errors in tree reconstructions.

As the number of possible trees is nearly infinite (more than 30 millions of rooted trees for 10 taxa), *the test of node robustness* quickly appeared as a necessity. The most widespread method is the bootstrap (Felsenstein, 1985) which rebuilds phylogenetic trees from artificial matrices created after character resampling (drawing of  $n$  sites among  $N$ ). The number of times (in percentage) each majority grouping has been observed among the reconstructed trees is then indicated at nodes. Another method consists in comparing the best tree to one or several alternative hypotheses. This method arose from the development of probabilistic analyses that attribute a likelihood value (conditional probability) to each tree. Then, it is possible to test if the difference in loglikelihood between two trees is statistically significant or not, and thus to answer the question: is the alternative tree significantly worse than the null hypothesis (in general the optimal phylogenetic tree)?

The advent of phylogenomics and Next Generation Sequencing (NGS) combined with sophisticated probabilistic methods of tree-building using complex models of sequence evolution, implicitly led to the belief that the era of biased and artefactual reconstructions was over. However, increasing the number of nucleotides does not necessarily resolve phylogenetic incongruence but, on the contrary, may lead to incorrect although well-supported trees (Delsuc et al., 2005; Jeffroy et al., 2006). So, it is still important to test the quality of the dataset by estimating its homoplasy content. Some studies recommend the removal of factors of inconsistency, such as fast-evolving positions, or positions with compositional biases (see Philippe et al., 2005). Given the increasing number of available nucleotides, removing some parts of the sequences appears feasible because theoretically enough informative positions should remain to ensure consistency of the phylogenetic signal. It is thus likely that in the future, combining an increasing number of molecular characters through NGS with powerful phylogenetic analyses will conduct to reduce again the number of polytomies, if any are left!

## 2. Higher levels of Rodentia systematics

### 2.1 Systematic position of the order Rodentia among mammals and the question of rodent monophyly

#### 2.1.1 The consensual pre-molecular era

From a morphological point of view, the main character defining Rodentia is the presence of a unique large evergrowing chisel-like incisor by half-jaw. This is however not the only distinctive character as no less than six other characters were listed in Hartenberger (1985: 10). Moreover, all these features are clearly derived eutherian characters (synapomorphies), thus making monophyly of Rodentia strongly supported. By contrast to other mammalian orders (for example Artiodactyla or Carnivora) that were delimited with more difficulty (see Simpson, 1945), Rodentia appeared morphologically well characterized. This concerns not only living rodents but more surprisingly also fossils. This was clearly stated by Simpson (1945: 198): "...the order is exceptionally clear cut. There is not, even among fossils, any question as to whether a given animal is or is not a rodent, however doubtful its position in the order may be." So, before the nineties, no scientist contested the monophyly of the group and the main questions regarding Rodentia concerned higher (identification of its sister taxon) or lower (relationships between families; see paragraph 2.2) taxonomic levels.

The question of the systematic position of Rodentia among the other mammalian orders, although more discussed than rodent monophyly, did not raise much contradictory debates. In fact only two hypotheses have been advanced as to rodent origin and close relationships. The first allies rodents with primates (McKenna, 1961), and the second is the classical rodent-lagomorph relationship, at the basis of the concept Glires since the earliest classifications of mammals (for example Gregory, 1910). This latter hypothesis quickly became the prevailing one as more data and synapomorphic characters accumulated (Hartenberger, 1985; Luckett, 1977; Novacek, 1985).

#### 2.1.2 Contribution of molecular data: Regression and progression of the debate

From a molecular point of view, the question of the systematic position of Rodentia among mammals is indisputably linked to the question of the order monophyly. As a matter of fact, in 1991, Graur et al. published a paper entitled "Is the guinea-pig a rodent?" that sounds as thunder in the peaceful life of rodentologists! This study was based on 15 protein sequences representing 1 998 aligned amino acids for four lineages: Primates, Artiodactyla, Rodentia (the guinea pig and a myomorph: rat or mouse or hamster) and one outgroup (marsupial or bird or toad). Maximum-parsimony analyses supported a tree in which rodents were not monophyletic because the guinea pig branched outside the clade ((Artiodactyla, Primates), Myomorpha). Later, this paper found an echo in "The guinea-pig is not a rodent" published by D'Erchia et al. (1996). This study was based on complete mitochondrial genome sequences of 16 taxa, representing five Primates, two Carnivora, two Cetartiodactyla, one Perissodactyla, one Insectivora, one Lagomorpha and three Rodentia (guinea pig, mouse and rat), with one Marsupialia as outgroup. Here again Rodentia did not appear monophyletic but contrary to the previous study, the clade *Mus-Rattus* appeared as the sister taxon to all other eutherian orders (except Insectivora). In this study the question of Glires was unsettled because the position of the rabbit was not robustly supported.

This challenge of rodent monophyly can retrospectively be considered as a textbook case, cumulating a number of molecular pitfalls that have since been well identified. First, the sampling question has immediately been underlined by some authors (Luckett & Hartenberger, 1993): the huge diversity of rodents (more than 2000 species) cannot be reduced to three taxa without consequences on phylogenetic inferences. This point was later confirmed by Lecointre et al. (1993) showing that reconstructed trees are highly sensitive to taxon sampling and that obtaining a reliable phylogeny necessitates to choose several taxa per presumed monophyletic lineage, as well as in the outgroups. Moreover, several authors (Cao et al., 1998; Philippe & Douzery, 1994) came to the conclusion that phylogenies based on four taxa (quartet analysis) can be highly misleading. The second problem concerned the methods used for phylogenetic inferences. Both studies refuting rodent monophyly were based on maximum parsimony analysis, a method already known to be subject to tree-reconstruction artefacts, such as “long branch attraction” (Felsenstein, 1978). Sullivan & Swofford (1997) reanalyzed the same datasets with more sophisticated probabilistic methods and showed the importance of using an adequate model of sequence evolution. According to their results, phylogenetic reconstructions can converge to a wrong tree, in particular if the model is oversimplified as in the studies of Graur et al. (1991) and D’Erchia et al. (1996). Among others, heterogeneity of substitutions between sites (modeled by a Gamma distribution) appeared as a particularly important factor to take into consideration for recovering a correct phylogeny.

After these two studies, more complete mitochondrial genomes were sequenced in rodents as well as in diverse mammalian orders (see references in Reyes et al., 2004). However, increasing the number of complete mitochondrial genomes or analyzing the data with probabilistic methods and adapted models did not change the first result: mitochondrial DNA proved to be unable to recover the monophyly of Rodentia (see Arnason et al., 2002). In all these studies, myomorphs (the rat and mouse lineage) appear as outside the rest of the rodents. Later the studies of Reyes et al. (2004) and Kjer et al. (2007) finally recovered a monophyletic Rodentia clade, probably because enough myomorph taxa were included to break the long branch leading to the Muridae (mouse and rat) family and also because effective methods such as Bayesian analysis and adapted models of sequence evolution were used. In the mean time, however, more and more studies based on nuclear genes invariably concluded that rodents are monophyletic (Adkins et al., 2003; Amrine-Madsen et al., 2003; DeBry & Sagel, 2001; Huchon et al., 2002). This result was usually achieved using extensive rodent sampling but also much less nucleotides than when whole mitochondrial genomes were considered. These contrasted results clearly showed that nuclear genes are much less affected by homoplasy than mitochondrial sequences, and thus appeared more appropriate to recover the phylogenetic signal for deep-level relationships (Springer et al., 2001). In fact, the debate was definitively closed in 2001 when two different studies (Murphy et al., 2001; Madsen et al., 2001) based on approximately 10 000 base pairs resolved most of the mammalian phylogenetic tree. Both papers came to the conclusion that Rodentia is a monophyletic group, to which Lagomorpha is the sister taxon, a result that finally reconciled morphologists and paleontologists with molecularists after 10 years of keen debate!

## 2.2 Rodent families and their phylogenetic relationships

### 2.2.1 The pre-molecular era: 100 years of work in diverse disciplines

Thirty-four living rodent families are currently recognized when including the Diatomyidae, a family recently reactivated (Huchon et al., 2007) to include *Laonastes aegnimamus* discovered in Laos in 1996 and described by Jenkins et al. (2005). Surprisingly, the number of rodent families has stayed relatively stable since the pioneer work of Brandt in 1855, i.e. approximately between 30 and 35 (Anderson & Jones, 1984; Hartenberger, 1985; Simpson, 1945; Wilson & Reeder, 1993, 2005). By contrast, interfamilial relationships were much more debated, which led to various proposals as to suprafamilial groupings. The earliest classifications of Brandt (1855) and Tullberg (1899) recognized two (Sciurognathi and Hystricognathi) and three (Hystricomorpha, Myomorpha and Sciuromorpha) major groups, respectively, but subsequent works identified more divisions (with the rank suborder, infraorder, or superfamily), the number of which ranged “anywhere from five to 16” (Carleton & Musser, 2005).

As early as in the Early Eocene, 11 families of rodent are already recognized (Hartenberger, 1998). Rodents most likely originated in Central Asia (Hartenberger, 1996) and within a few millions of years they diversified and dispersed on all continents to the exception of Antarctica and South America (Hartenberger, 1998). This radiation, that took place about 55 to 65 millions of years ago according to paleontological data or even earlier according to molecular data (between 70 and 80 Mya; Huchon et al., 2007; Montgelard et al., 2008), occurred so quickly that it was qualified as “explosive” (Hartenberger, 1996). Rapidly after their emergence, rodents also appeared ecologically diversified, and they currently occupy a tremendous variety of habitats in nearly all the ecosystems. This rapid geographical and ecological diversification is probably one of the reasons why numerous characters are homoplastic, precluding their use in phylogenetic studies. Before reaching this conclusion, a huge diversity of morphological and anatomical characters have been studied in the hope to discover that some of them escaped homoplasy: dental (Butler, 1985; Flynn et al., 1985; Marivaux et al., 2004) or cranial (Novacek, 1985) characters, cephalic arterial patterns (Bugge, 1985), middle-ear features (Lavocat & Parent, 1985), enamel (Martin, 1997) or placental characteristics (Luckett, 1985), paleontological data (Jaeger, 1988; Vianey-Liaud, 1985)... After 100 years of research, the consensual relationships were nevertheless very few. Well-supported evolutionary relationships concerned the close affinity between Geomyidae and Heteromyidae (Falbusch, 1985), Aplodontidae and Sciuridae (Lavocat & Parent, 1985; Vianey-Liaud, 1985; Wahlert, 1985), Dipodidae and Muroidea (a superfamily including six families; see paragraph 3) as well as the split of the Hystricognathi suborder in old world Phiomorpha (four families) and new-world Caviomorpha (13 families). Some putative affinities were also put forward, but less convincingly, such a sister group relationship between Gliridae and Sciuridae + Aplodontidae (Bugge, 1985; Lavocat & Parent, 1985), Ctenodactylidae and Hystricognathi (Jaeger, 1988; Luckett, 1985) or Anomaluridae and Pedetidae (Bugge, 1985; Lavocat & Parent, 1985). Other proposed relationships were more speculative (see Luckett & Hartenberger, 1985) and a number of families stayed as *incertae sedis* because of inconclusive results. Finally, no strong hypothesis has ever been put forward concerning suprasubordinal relationships or the position of the root of the rodent tree based on traditional morpho-anatomical characters.

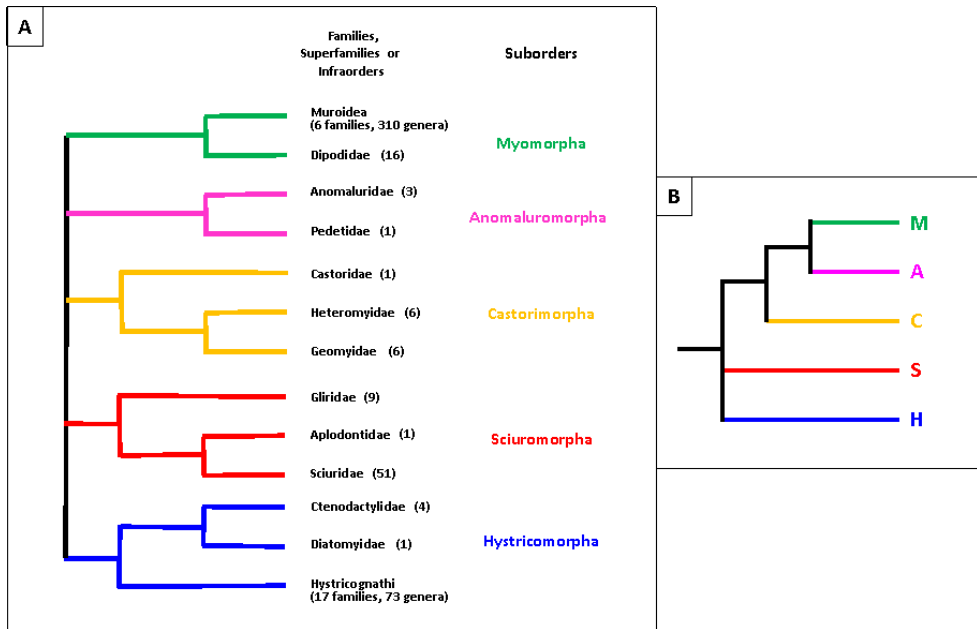


Fig. 2. A: The 34 rodent families and their relationships in five suborders (Carleton & Musser, 2005); B: Likely phylogenetic arrangement of the five suborders in three major lineages (Blanga-Kanfi et al., 2009 ; Montgelard et al., 2008).

### 2.2.2 Contribution of molecular data: Reaching a consensus in five clades

In this context of uncertainty about suprafamilial relationships, the contribution of molecular data was essential, not only for selecting between the diverse prevailing hypotheses but also because new relationships emerged that had never been advanced by previous morphological or paleontological analyses. The first studies were based on mitochondrial genes (cytochrome b and 12S rRNA; Nedbal et al., 1996; Montgelard et al., 2002) but several nuclear genes have then been sequenced intensively: the von Willebrand Factor (vWF; Huchon et al., 1999), the interphotoreceptor retinoid-binding protein (IRBP; Huchon et al., 2002; DeBry, 2003), the alpha 2B adrenergic receptor (A2AB; Huchon et al., 2002), the growth hormone receptor (GHR; Adkins et al., 2003), a breast and ovarian cancer susceptibility gene (BRCA1; Adkins et al., 2003), or the apolipoprotein B (APOB; Amrine-Madsen et al., 2003), among others. Contrary to what happened at the beginning of the molecular era (see above), all these molecular studies included a substantial number of rodent taxa and were mostly congruent (at least for the groupings strongly supported). In particular, all well-supported associations previously mentioned (see paragraph 2.2.1 and figure 2) were confirmed, allowing to invalidate some alternative morpho-paleontological propositions such as for example Ctenodactylidae as the first emerging group among rodents (Hartenberger, 1985) or a group including Muridae, Dipodidae, Heteromyidae and Geomyidae (*Myomorpha sensu* Carleton, 1984). Even if the phylogenetic signal appears less altered in nuclear than in mitochondrial genes (see Springer et al., 2001), these relationships were often evidenced by the combination of several genes.



Finally, most recent molecular studies on rodent phylogeny (Blanga-Kanfi et al., 2009; Huchon et al., 2007; Montgelard et al., 2008) agree on the identification of five major clades among Rodentia (Figure 2A): Myomorpha (Muroidea and Dipodidae), Anomaluromorpha (Anomaluridae and Pedetidae), Castorimorpha (Geomyidae, Heteromyidae, Castoridae), Sciuromorpha (Aplodontidae, Sciuridae and Gliridae) and Hystricomorpha (Hystricognathi, Ctenodactylidae and Diatomyidae). These groupings, which are given suborder ranks in Carleton & Musser (2005) are very dissimilar in terms of taxonomic diversity: from only two families and four genera for Anomaluromorpha to seven families and 326 genera for Myomorpha which represents nearly 70% of rodent diversity.

## 2.3 Relationships between the five major clades of Rodentia

### 2.3.1 Influence of factors of inconsistency on rodent suprafamilial relationships

As stated in the introduction, molecular phylogeny can be subjected to different biases and lead to erroneous trees, such as described about the monophyly of rodents (see paragraph 2.1.2). A number of factors have been identified as major sources of inconsistency (Philippe et al., 2005): variation in nucleotide composition, across-site heterogeneity, shifts in the evolutionary rate among lineages, or heterotachy (rate variation across a site through time). Two studies (Blanga-Kanfi et al., 2009; Montgelard et al., 2008) included representatives of all rodent families and analyzed the influence of different factors of inconsistency on the relationships between the five major clades of rodents. The paper of Blanga-Kanfi et al. (2009) is based on six nuclear protein-coding genes (6 255 positions) whereas the paper of Montgelard et al. (2008) used two mitochondrial genes (2 126 sites), two nuclear exons (2 571 sites) and four nuclear introns (2 897 sites). With the aim to increase the ratio between phylogenetic and nonphylogenetic signal, both studies analyzed different methods for thwarting factors of inconsistencies, among which only the effect of removing fast-evolving positions will be reported here. In the two studies, the five major rodent clades are highly supported as well as a super-clade including Myomorpha + Anomaluromorpha + Castorimorpha. This grouping has never been suggested from morpho-anatomical or paleontological data and is referred as "the Mouse-related clade" (Huchon et al., 2002) pending a true binomial denomination. Inside this clade, a sister group relationship between Anomaluromorpha and Myomorpha is privileged over the other two possibilities. This clade is highly supported by the intron data in the study of Montgelard et al. (2008) whereas other datasets (including the six nuclear genes of Blanga-Kanfi et al., 2009) only moderately supported this relationship, whatever the analyses considered. From there, rodent phylogeny at the highest taxonomic level can thus be reduced to three major lineages: Sciuromorpha, Hystricomorpha and the Mouse-related clade (Figure 2B).

The next step was to try resolve the order of divergence between these three lineages, that is to identify the root of Rodentia. The study by Blanga-Kanfi et al. (2009) does not support any of the three possibilities (one of the three lineages at the base of rodents) when all the nucleotide dataset is considered and whatever the analyses performed (different types of models). Conversely, when fast-evolving positions are removed from their complete dataset (1114 nucleotides deleted on 7594 deleted), Montgelard et al. (2008) obtained a strong support for the clade Hystricomorpha + Sciuromorpha, to which the Mouse-related clade thus showed a basal position. By contrast, removal of the fastest-evolving sites (258 positions on 6255) moderately improved the support for a basal position of Sciuromorpha in

the paper of Blanga-Kanfi et al. (2009) but this position is reinforced when the protein sequences are analyzed with sophisticated evolutionary models.

We can finally report the study of Churakov et al. (2010) who addressed the question of the root of the rodent tree on the basis of the insertion of SINEs (short interspersed repetitive elements). These are mobile elements whose insertion is considered as a unique and irreversible event at the genome scale. Thus they are regarded as virtually free of homoplasy, making SINEs efficient tools for reconstructing phylogenetic relationships (Serdobova & Kramerov, 1998). Concerning Rodentia, Churakov et al. (2010) found eight retroposon insertions and two indels as characterizing the clade Hystricomorpha+Mouse-related, thus making Sciuromorpha the first offshoot among rodents, in accordance with Blanga-Kanfi et al.'s (2009) results. However, the hypothesis of a basal position of the Mouse-related clade is not totally refuted as they also identified two SINES and one indel shared by Hystricomorpha and Sciuromorpha. The authors put forward two hypotheses to explain these data: an incomplete lineage sorting or introgressive hybridization occurring in the early stages of these two main rodent lineages. Another hypothesis, not invoked by Churakov et al. (2010) is that SINES would be homoplastic as already described in the literature (Nishihara et al., 2009).

### **2.3.2 Future prospects for molecular, morphological and paleontological data**

Concerning molecular data, we could be tented to conclude that relationships between the three main rodent lineages would represent a real hard polytomy (that is they diverged simultaneously) because as much as 6000 characters cannot definitely solved the relationships between them. However, it could also be that the nucleotide dataset considered is not large enough. Indeed, 16 kb of DNA sequences have been necessary to solve the phylogenetic relationships between the different families of Madagascar's lemurs (Horwath et al., 2008) and 24 000 nucleotides succeeded to fix ratite relationships (Harshman et al., 2008)!

As to anatomical, morphological or paleontological data, their future input to phylogenetical and systematic studies likely lies in a reanalysis of characters in the light of molecular phylogenies with the major challenge to understand morphological evolution. Moreover, it should not be forgotten that very important technical progresses have been performed (such as geometric morphometric methods) allowing the quantification of shape modifications (see for example Hautier et al., 2011). In the current frame of knowledge of higher-level rodent relationships, studies can now be conducted with the scope to understand if characters common to different lineages do represent real homologies (common ascendance) or not. In this context, fossils could constitute temporal landmarks in order to trace morphological modifications. Finally, the advancement of new disciplines such as evolutionary developmental biology ("evo-devo" in short) will probably link molecular and morphological disciplines, allowing to understand the respective roles of ascendance (phylogeny) and ecology (selection) during evolution of morphological characters (see for example Renvoise et al., 2009).

## **3. Intermediate taxonomic levels (families – subfamilies – tribes) of the Muroidea superfamily**

Within each of the five rodent suborders, a wealth of molecular studies based on DNA sequences has been devoted to determine the systematic arrangement around the familial

level. As an example, we will examine the systematic relationships among the superfamily Muroidea, which includes six families (Calomyscidae, Platacanthomyidae, Spalacidae, Nesomyidae, Cricetidae and Muridae), and represents by far the most speciose group of rodents (Figure 3). Muroidea has been the focus of a number of molecular studies based on various mitochondrial and nuclear genes (Jansa et al., 2009; Jansa & Weksler 2004; Michaux & Catzeflis, 2000; Michaux et al., 2001; Steppan et al., 2004). Figure 3 schematically illustrates the most probable relationships between all the families and subfamilies currently recognized in this superfamily. This arrangement served as a basis for the systematic classification retained by Musser & Carleton (2005), and resumed in Figure 3 (see \* in the legend for departures to this arrangement). We will here review, for each family, their content and organization on the basis of the different molecular analyses that have been performed. It can also be mentioned that these studies had for consequence the reanalysis of dental character evolution in the light of molecular phylogenies. For example, the study by Lazzari et al. (2008) highlighted the weight of functional constraints and revealed numerous dental homoplasies among the different morphological grades observed in the course of muroid evolution.

### 3.1 Calomyscidae and Platacanthomyidae

As compared to other muroid families (see Figure 3), Calomyscidae (1 genus, 8 species) and Platacanthomyidae (2 genera, 2 species) are small families and only a few studies integrated them in large-scale analyses to precise their phylogenetic position within the Muroidea. In the case of the Asian family Platacanthomyidae, a basal position has been evidenced for *Typhlomys* among muroid rodents, a result that led Jansa et al. (2009) to propose a Eurasian origin for the Muroidea. Calomyscidae appeared as an isolated lineage in all molecular studies (Jansa et al., 2009; Jansa & Weksler 2004; Michaux et al., 2001; Steppan et al., 2004). Molecular data clearly grouped Calomyscidae with Dendromuridae, Muridae and Cricetidae, but no study has yet resolved the branching pattern within this group

### 3.2 Spalacidae

In all phylogenetic reconstructions based on molecular data, fossorial Spalacidae appeared as an early differentiated lineage in the Muroidea, of Eurasian origin (Jansa et al., 2009; Jansa & Weksler, 2004; Robinson et al., 1997; Steppan et al., 2004). The content and internal relationships of Spalacidae have long been debated (see Gogolevskaya et al., 2010 for details), until Jansa & Weksler (2004) and Norris et al. (2004) recognized Myospalacinae, Rhizomyinae, and Spalacinae as distinct, but closely related subfamilies of Spalacidae within the Muroidea based on nuclear as well as mitochondrial sequence comparisons. This result was later confirmed by Gogolevskaya et al. (2010) who showed that representatives of these subfamilies shared the same variants of small genetic sequences, namely the B1 small interspersed elements (SINES) and the 4.5S<sub>1</sub> small nuclear RNA. This study confirmed the role of SINES in rodent phylogeny, as suggested by Serdobova & Kramerov (1998), already using Spalacidae. The distinctiveness of Tachyoryctinae (African mole rats), especially with respect to Rhizomyinae (Asian bamboo rats), is currently mainly supported by morpho-anatomical arguments (Musser & Carleton, 2005). On the other hand, molecular data gathered to date rather showed the close relatedness of *Rhizomys* and *Tachyoryctes*, that both Jansa & Weksler (2004) and Steppan et al. (2004) considered as belonging to the Rhizomyinae, an hypothesis that would mean the disappearance of the Tachyoryctinae subfamily as a taxonomic rank. Finally, Norris et al. (2004) and Jansa et al. (2009) evidenced

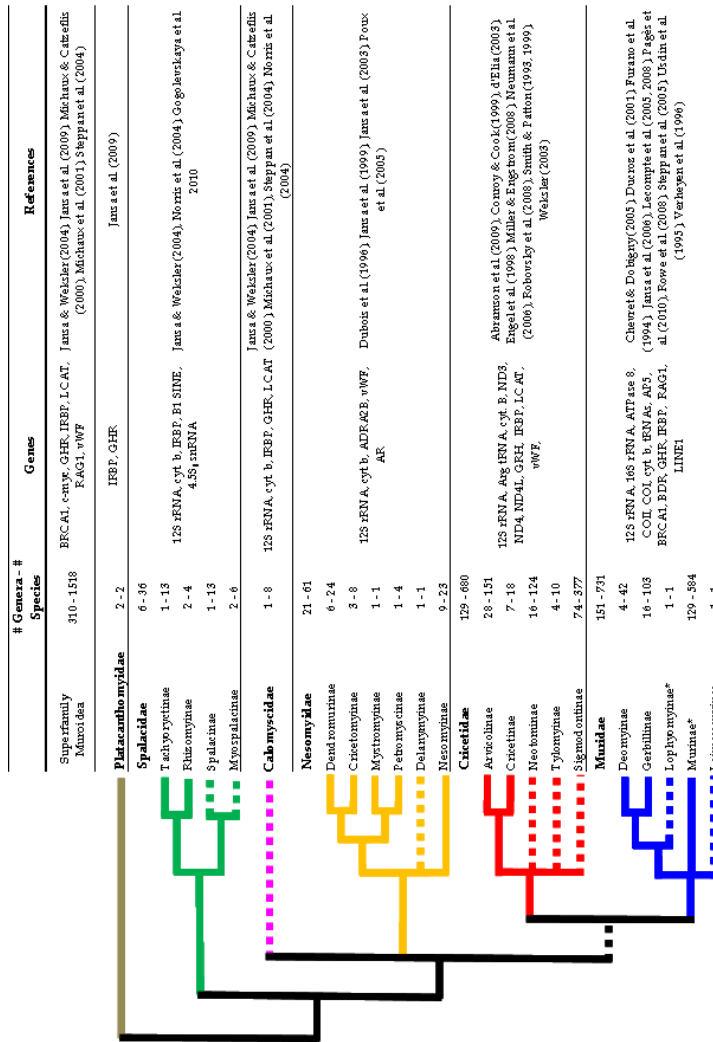


Fig. 3. Strongly supported (in bold) and hypothetic/conflictual (dotted lines) phylogenetic relationships between families / subfamilies of Muroid rodents as established following various studies using DNA sequences. \* Otomyini included in Murinae, and Lophomyinae included in Muridae, as in Jansa & Weksler (2004) / Jansa et al. (2009). 4.5S1 snRNA = 4.5S1 small nuclear RNA; ADRA2B = alpha 2B adrenergic receptor; AP5 = Acid Phosphatase type V; AR = androgen receptor; Arg tRNA = Arginine tRNA; B1 SINE = B1 short interspersed element; BDR = Borrelia direct repeat; BRCAL = Breast Cancer Gene 1; c-myc = proto-oncogene; COI and COII: Cytochrome Oxidase subunits I and II, cyt b = cytochrome b; GRH = Growth Hormone Receptor; IRBP = Interphotoreceptor Retinoid Binding Protein; LCAT = Lecithin Cholesterol Acyl Transferase; LINE1 = Long Interspersed repeated DNA; ND3, ND4, ND4L = subunits of nicotinamide dinucleotide dehydrogenase; RAG1 = Recombination Activating Gene 1; vWF = von Willebrand Factor

a sister group relationships between Myospalacinae and Spalacinae, an affinity that is however not strongly supported.

### 3.3 Nesomyidae

The current contours of the family Nesomyidae owe much to molecular data. Six subfamilies (Cricetomyinae, Delanyomyinae, Dendromurinae, Mystromyinae, Nesomyinae, and Petromyscinae) are currently recognized, a result that echoes some of the hypotheses brought forward by Lavocat (1973, 1978) based on palaeontological evidence. Some Malagasy endemic Nesomyinae were first found related to *Cricetomys*, an African representative of the Cricetomyinae (Dubois et al., 1996) based on 12S rRNA mitochondrial gene. The results obtained by Jansa et al. (1999) were more ambiguous as to the relationships between Nesomyinae and other muroid subfamilies, probably because they were based on cytochrome *b*, a too rapidly evolving gene for addressing adequately issues related to such ancient events. Indeed, subsequent studies by Jansa & Weksler (2004), Michaux & Catzeflis (2000), Michaux et al. (2001) and Steppan et al. (2004), all based on nuclear genes, clearly showed that the Nesomyidae as defined here above represent a well supported natural group. Within it, the Malagasy endemic Nesomyinae would be the sister group to a clade including two pairs of taxonomically equivalent groups, namely the Cricetomyinae and Dendromurinae on the one hand, and the Mystromyinae and Petromyscinae on the other hand (see Jansa & Weksler, 2004; Jansa et al., 2009). The Delanyomyinae, with only one secretive species, has never been involved in phylogenetic analyses based on molecular data, and its inclusion within the Nesomyidae mainly rests on its previous association with either Dendromurinae or Petromyscinae based on morpho-anatomical characters (Musser & Carleton, 2005).

Studies focusing on Nesomyidae subfamilies mainly concerned Nesomyinae and Dendromurinae. In the former, Jansa et al. (2009) and Jansa & Carleton (2003) privileged the hypotheses of a unique event of colonization of Madagascar by this endemic group, an hypothesis congruent with the monophyly of Nesomyinae regularly evidenced using nuclear gene sequences (Steppan et al, 2004; Poux et al, 2005). Dendromurinae was the topic of various molecular studies which, together with morphological re-analyses, finally led to precise its generic content (see Musser & Carleton, 2005). In particular, Verheyen et al. (1996) using mitochondrial cytochrome *b* sequences, then Michaux & Catzeflis (2000) using the nuclear gene *LCAT*, definitely proved that *Deomys* should not be considered as a member of the Dendromurinae, confirming earlier results obtained by Denys et al. (1995) based on morphological and DNA-DNA hybridization data. As currently understood, the Dendromurinae subfamily now comprises the species-rich genera *Dendromus* and *Steatomys* as well as the monospecific genera *Dendroprionomys*, *Malacothrix*, *Megadendromus*, and *Prionomys*.

### 3.4 Cricetidae

The family Cricetidae has long been considered as a subgroup of an extended Muridae family, and is still a matter of controversy as far as its limits are concerned (Musser & Carleton, 2005). From a molecular perspective, earlier studies were also quite hesitant in their conclusions, as various lineages of current Cricetidae and Muridae appeared to have emerged nearly simultaneously, giving polytomies that were interpreted as resulting from adaptive radiations (see Conroy & Cook, 1999; Michaux & Catzeflis, 2000). Jansa & Weksler

(2004) and Steppan et al. (2004), based on nuclear gene sequences and a relatively comprehensive taxonomic sampling, evidenced a monophyletic group corresponding to Cricetidae in their “modern” meaning, which includes the subfamilies Arvicolinae, Cricetinae, Neotominae, Sigmodontinae, and Tylomyinae. If each subfamily appeared well supported by molecular data, affinities between them are uncertain: A relatively poorly supported clade joining the Holarctic Arvicolinae and the Palearctic Cricetinae was found by Steppan et al. (2004) based on a combination of nuclear gene sequences. Alternatively, Jansa et al. (2009) suggested a sister relationship between Sigmodontinae and Neotominae, and a basal position of Cricetinae among all other Cricetidae but here again with relatively low support.

The content and organization of Arvicolinae has since then been the subject of many molecular studies, including those by Abramson et al. (2009) and Robovsky et al. (2008), who tried to arrange the numerous tribes constituting this species-rich subfamily. Interestingly, the distribution of satellite DNA also proved to be informative at the infra-subfamilial level, bringing support to the tribe Arvicolini (Acosta et al., 2010). The Cricetinae have been studied via mitochondrial and nuclear sequences by Neumann et al. (2006) who evidenced a well-supported phylogenetic structure in three main lineages that would have diverged during the late Miocene (7-12 Myr ago). Sigmodontinae, the New World Cricetidae, were also shown to comprise three monophyletic clades by Jansa & Weksler (2004) and Steppan et al. (2004), a finding that was already apparent in an earlier study by Engel et al. (1998) based on various mitochondrial genes. Among them, Sigmodontinae “*sensu stricto*” represent the South American offshoot of the family, whose colonization between 5 and 9 Myr ago of the subcontinent from North America is a remarkable example of the filling of an empty niche following a fortuitous invasion (Engel et al., 1998). The internal organization of this highly diverse subfamily (> 300 species) has been studied using mitochondrial and nuclear gene sequences by various authors, including Smith & Patton (1993, 1999), d’Elia et al. (2003), and Weksler (2003). The Neotominae mostly represent the North American branch of the Cricetidae, among which the so-called Peromyscine rodents is by far the most speciose group. Through a combination of mitochondrial and nuclear sequences, Miller & Engstrom (2008) ended up to a well-resolved phylogeny confirming the Reithrodontomyini as the major tribe of this subfamily, with Baiomyini, Ochrotomyini and Neotomini as successive sister taxa. Previously, Bradley et al. (2004) based on cytochrome *b* gene data, quite convincingly showed that the Tylomyinae (then treated as the tribe Tylomyini) appeared as basal to the other Neotominae considered.

### 3.5 Muridae

A monophyletic group equivalent to the Cricetidae (see above) emerged in molecular phylogenetic analyses conducted at the Muroid level (Jansa et al., 2009; Jansa & Weksler, 2004; Michaux et al., 2001), that was proposed as the Muridae family by Steppan et al. (2004). The combination of molecular results and other sets of characteristics (Musser & Carleton, 2005) led to organize this speciose assemblage into five main subfamilies (Leimacomyinae, Deomyinae, Gerbillinae, Lophomyinae, Murinae). Leimacomyinae only comprise the monospecific genus *Leimacomys*, known by two specimens caught in 1890. Its inclusion in the Muridae still awaits confirmation, especially from a molecular perspective. Deomyinae (corresponding to the former Acomyinae, see Musser & Carleton, 2005 for details) emerged following various molecular analyses (including DNA-DNA hybridization

experiments; Chevret et al., 1993) which confirmed that i) *Acomys* should not be considered as a Murinae (see Dubois et al., 1999 for instance), and ii) *Deomys* was closely related to *Acomys*, but also to *Lophuromys* and *Uranomys* (Michaux et al., 2001; Steppan et al., 2004, 2005; Verheyen et al., 1996). These four genera comprise a well-supported clade which should be named Deomyinae, with the Gerbillinae as sister group. The latter subfamily represents a rather homogeneous group from a morpho-anatomical point of view, whose content was not adequately surveyed using molecular markers before Chevret & Dobigny (2005). Based on mitochondrial gene sequences, these authors specified the generic content of the group, identified three main clades that may correspond to distinct tribes, and suggested an African origin for the Gerbillinae, with subsequent migration events towards Asia. The monospecific Lophomyiinae, considered as belonging to the Cricetidae based on skull and dental characteristics (Musser & Carleton, 2005), is here placed within the Muridae, following Jansa & Weksler (2004) who found it to be the sister group to the Deomyinae + Gerbillinae clade, although this relationship would need to be strengthened.

The Murinae represents a huge assemblage of species (Figure 3). For this reason, it has seldom been considered exhaustively in molecular works. However numerous studies focused either on taxonomical or geographical subgroups of murine rodents, progressively leading to significant advances in the systematics of this subfamily. Nuclear and mitochondrial sequence data were predominantly used, but interspersed repeated DNA (LINE-1 or Lx family) also proved to be useful as heritable characters in defining the murine lineage or some of its sub-parts (Furano et al., 1994; Usdin et al., 1995). Steppan et al. (2005), based on separate and combined analyses of mitochondrial and nuclear genes sequenced in most major murine groups, showed the basal split of a clade of Philippine old endemics (corresponding to Phloeomyini *sensu* Lecompte et al., 2008), the remaining taxa being organized within at least 7 geographically structured lineages. Among them, a South-east Asian "*Rattus*" clade (Rattini tribe *sensu* Lecompte et al., 2008) has recently been analyzed by Pagès et al. (2010) in a thorough phylogeny-based taxonomic revision. Another of these lineages, known as the Sahul (Australia + New Guinea) old endemic rodents, appeared as the sister group to another lineage of Philippine old endemics (Jansa et al., 2006; Rowe et al., 2008). Both clades probably derived from a single colonization event of New Guinea from the West, during the Late Miocene - Early Pliocene period. Lecompte et al. (2008) proposed the tribal name Hydromyini for this entire assemblage including the Australo-Papuan and Philippine murine radiations. Ducroz et al. (2001), using complete cytochrome b, and partial 12S and 16S ribosomal RNA mitochondrial sequences, evidenced a major clade of mostly African genera to which they proposed the tribal name Arvicanthini. Another important lineage of African rodents was defined as "the *Praomys* group" by Lecompte et al. (2005), before being formally named Praomyini (Lecompte et al., 2008). A number of other, either Eurasian (Apodemini, Millardini, Murini) or African (Otomyini, Malacomyini), tribes were also advanced by Lecompte et al. (2008). Overall, these authors proposed that the Murinae diversity be organized in at least 10 tribes, and, given the pattern of relationships observed between them, that multiple exchanges occurred between Eurasia and Africa in this subfamily. The first colonization event of Africa would have taken place around 11 Myr ago, followed by a major period of diversification between 7-9 Myr ago.

As apparent following this rapid survey, significant progress has been made within the last 15-20 years regarding the content of, and pattern of relationships between, the main murid rodent lineages. This result has been achieved through the use of adequate genetic markers

coupled to large taxonomic sampling representative of the diversity of the groups under scrutiny. Beside their purely taxonomic outputs, these studies have also yielded a wealth of knowledge on the biogeographical history of muroid rodents throughout the World. From a temporal point of view, the basal radiation of Muroidea would have taken place around 25 Myr ago, then major lineages (families and subfamilies) progressively differentiated during the following 10-12 Myr (Steppan et al., 2004). The generic diversification occurred at various periods, but was clearly enhanced (adaptive radiations) when new spaces were colonized, such as South America and Africa. This mainly Miocene story has taken place over nearly all the continents, with migration/colonization events that proved to be crucial in the evolution of the group towards its current diversity.

#### **4. The species level in rodent taxonomy**

Until recently, systematic and taxonomic proposals based on sequence data at the species level often arose as a by-product in studies focusing either on phylogenetic relationships or evolutionary processes of a given supraspecific group. However, molecular data now tend to be integrated in multidisciplinary studies explicitly devoted to species characterization / description. This trend especially develops in groups where cryptic and sibling species are numerous, and where the use of genetic markers rapidly proved to be of paramount importance. This was accompanied by a conceptual evolution in the field of species concepts that now take into account molecular and other genetic characters in species delimitation. From a practical point of view, the use of sequence data has also prompted a debate on how genetic distances and genetic characters should be considered in species delimitation and description. These reflections have logically resulted in the recent proposals centred on the idea of DNA taxonomy, among which DNA barcoding has gained most of the attention.

##### **4.1 Contribution of sequence data to cryptic and sibling species identification**

As defined by Knowlton (1986), sibling species represent a particular class of cryptic species, i.e. those that are phylogenetically closely related (often sister species). These morphologically very similar species have for long been identified as a major obstacle to the application of the morphological species concept (see Mayr, 1948). The increasing recognition of cryptic taxa, undoubtedly linked to the generalization of genetic tools (including DNA sequencing), poses various questions concerning their distribution across taxa and biomes, their conditions of emergence, and their impact on biodiversity estimates (Bickford et al., 2007).

Patterson (2000) reviewed recent discoveries in Neotropical mammals, and highlighted the fact that among the “newly recognized species”, the re-evaluation of already collected and studied materials outnumbered real *de novo* descriptions. According to Patterson (2001: 195), this trend to resurrect synonyms is jointly attributable to “continued morphological study, higher resolution genetic analyses and a shift toward a phylogenetic species concept (and away from polytypic species)”. Rodents represent sixty percent of these new species, i.e. a much greater proportion than their actual share in the Mammalia class (ca. 40% of genera or species). Given the current activity of description / recognition of rodent species in Asia, one may hypothesize that the same trends would be found on this continent. In Africa, rodents and primates are the two mammalian groups in which the largest numbers of new



species have been described in the decade 1989-2008 (ca 33% each; Hoffmann et al. 2009). In both groups, numerous of these novelties have been characterized thanks to genetic methods, including cytogenetics (Taylor, 2000; Granjon & Dobigny, 2003) and sequence-based analyses (see hereafter).

In African rodents, beside re-evaluation of some groups using traditional methods, molecular phylogenetic studies have opened the way to many species resurrections, particularly in speciose groups such as the "*Arvicanthis* division" (Ducroz et al., 2001), the *Praomys* group (Lecompte et al., 2002, 2005) or the genus *Lophuromys* (Verheyen et al., 2007). Phylogeographical studies are also the occasion for taxonomic revisions, including the finding of cryptic and sibling species, as acknowledged by Avise (2000: 204). The recent work by Bryja et al. (2010) on the *Praomys daltoni* complex both confirmed the synonymy of *P. daltoni* and *P. derooi* and strongly suggested the existence of a new, sibling species for this West African murine rodent. It has to be underlined that the "lumping" of taxa previously thought to represent distinct species could also result from phylogeographical surveys, as in Nicolas et al. (2009) where the wide-ranging deomyine *Acomys airensis* was shown to represent a junior synonym of the localized (but previously described) *A. chudeaui*.

#### 4.2 Genetic clusters and species concepts

Species concepts have multiplied along the history of evolutionary sciences, from the unique typological and mainly morpho-anatomically based species concept to a variety of concepts reflecting both the discipline of predilection and the school of thoughts of their promoters (see the useful reviews by Harrison [1998] and De Queiroz [2007, 2011]). Among them, those referenced as the "genealogical" and the "diagnosable" species concepts are both considered as phylogenetic by De Queiroz (2007). They are also strongly linked to the development of molecular studies because exclusive coalescence of alleles and diagnosability (via qualitative, fixed difference), are considered as the main properties of species. One of the main practical problems produced by this proliferation of species concepts is the variability in the number of species inferred by any of them. As exemplified by De Queiroz (2011), the criterion of fixed character state differences (the basis of phylogenetic species concepts) commonly leads to the recognition of more species taxa than the criterion of intrinsic reproductive barriers (the basis of the biological species concept). A shift toward a phylogenetic species concept was one of the reasons invoked by Patterson (2001) to explain the current trend in Neotropical species number increase (see above). The "genetic species concept" of Dobzhansky (1950), revisited by Bradley & Baker (2001) and Baker & Bradley (2006) even goes further, considering as genetic species all genetically defined phylogroups, especially when based on DNA sequence analyses. This concept, which explicitly focuses on genetic isolation rather than on reproductive isolation, would mainly concern morphologically non-differentiated species. Strictly applied, it might lead to an increase of >2,000 species in mammals only (Baker & Bradley, 2006).

Hopefully, after a period of more or less anarchic burgeoning of such "specialized" species concepts, the current trend is now to try reconciling them into a unified species concept where species are considered as "evolving metapopulation lineage" that can be diagnosed via one or (better) several properties. These properties could be drawn from any discipline, provided it will furnish convincing lines of evidence of species delimitation (De Queiroz, 2007). In this frame, molecular data, when correctly interpreted, can of course represent

strong arguments in favour (or not) of the recognition of an independent lineage as a true species. Interestingly, this new approach emerges at the same time as integrative systematics or taxonomy (Dayrat, 2005; Lecompte et al., 2003) that recommend the integrated use of data from various fields in order to adequately and critically assess hypotheses on species delimitation and characterization. However, when diagnostic characters have to be identified, which is one of the main tasks of taxonomists, sequence data may present some inherent difficulties. This inconvenience should be overcome, or at least critically taken into account when the corresponding results have to be interpreted in a systematic context (see hereafter).

### **4.3 Genetic distances and characters: Their practical use in systematics and taxonomy**

The use of genetic data to delimitate species has for long been a matter of debate. In the 70's and 80's when protein electrophoresis was the main tool used to compare genetic diversity within and between species, genetic distances were generally computed, and interpreted as measuring the level of differentiation of populations / species. Correlations between such distances and the taxonomic ranks of the groups under study were examined. Important overlaps were then observed between genetic distance ranges and taxonomic levels (from subspecies to well-differentiated species via sibling species), that preclude definitive taxonomic conclusions based on such correlations (Zimmerman et al., 1978; Avise & Aquadro, 1982; Graf, 1982). Similarly, when sequence data have started accumulating, various authors have compiled sequence divergence values (especially K2P [Kimura two parameter] distance from cytochrome b gene data) *versus* taxonomic ranks, showing the same pattern of overlap (see Bradley & Baker, 2001 for Mammals; Johns & Avise, 1998 for Vertebrates). As acknowledged by various authors, and recently summarized by Coleman (2009: 197): "Nucleotide change does occur, genera differ more than do their component pairs of species, but the nucleotide change is continuous, with no gap, no point of reference correlatable with some facet of speciation." Under the biological species concept, where the achievement of reproductive isolation is the major criterion to define species, the use of such distance data appears widely equivocal (Ferguson, 2002): recently diverged species (showing reproductive isolation) can exhibit a smaller genetic divergence than conspecific populations genetically differentiated (e.g. because of geographic distance), but still reproductively compatible. Conversely, a small genetic distance between two individual sets of any given taxon, even if they show statistically well-supported reciprocal monophyly, may cast doubt on the distinct specific status of these groups in absence of other convincing diagnostic criteria (see Ferguson, 2002 for some examples). These criteria should better be structural (morpho-anatomical, karyological...), whereas size or shape variation may represent inadequate characters, being often subject to local selection linked with geographical, environmental or biotic factors. Thus, while recommending the general procedure followed by Gündüz et al. (2007) who considered molecular subdivision as an indicator of reproductive isolation for recognizing species boundaries, then used geometric morphometrics and external morphology to assess the amount of phenotypic partitioning among the species identified, we nevertheless insist on the importance of putting forward unambiguous, selection-free, diagnostic characters to characterize new species.

The identification of molecular apomorphies (or “fixed genetic characteristics”, Ferguson, 2002) could represent an alternative to the sole use of genetic distance to characterize a new species. This was achieved, among others, in the recent rodent species descriptions by Pardiñas et al. (2005), Goodman et al. (2009), and Jayat et al. (2010). However, as underlined by the latter authors themselves, these character states should be taken with caution when sequences of some related species are not included, and/or when only a few haplotypes of the new species are considered. Another perspective, proposed by Coleman (2009) in a “biological species concept” frame, would be to use genes either involved in the reproductive barrier (sexual behaviour, gamete approach / fusion...), or for which sequence variation would accompany the separation of clades into sexually isolated subclades. The Internal Transcribed Spacer 2 region (ITS2) of the nuclear ribosomal cistrons, one of the most studied nuclear species-level molecular marker (Hajibabaei et al., 2007), seems a promising candidate in this respect. This gene displays both sequence and secondary structure variations that are highly correlated with taxonomic classification. Thus, it might represent a powerful tool because not only sequences can be compared but also some aspects of the secondary structure formed by the initial RNA transcript (Coleman, 2009).

#### 4.4 DNA barcoding and DNA taxonomy

The concomitant loss of taxonomic expertise, the need for large-scaled biodiversity evaluation, and the development of both molecular techniques and computing power have prompted the emergence of DNA taxonomy, where nucleotide sequences are, as a first approximation, taken as diagnostic characters of the species under study (Blaxter, 2003, 2004). Within this frame, the objective of DNA barcoding is to provide a simple diagnostic tool, based on the correspondence between DNA sequences (generally of the cytochrome oxidase I mitochondrial gene) and species as defined via traditional systematics (Hebert et al., 2003). The progress of DNA barcoding, and its contribution to the description of species, especially of cryptic and sibling species have been acknowledged by Frézal & Leblois (2008). This study however, identified several crucial pitfalls, such as those linked to the representativity of species diversity on the one hand, and those associated with the peculiarities of mitochondrial DNA (maternal inheritance, risk of nuclear copies, variations in rate of evolution across taxa) on the other hand. The necessity for adequate sampling (of taxa within a given group and of individuals within a species), and for sequencing of nuclear markers, appear as useful recommendations to overcome these potential biases (Frézal & Leblois 2008). Enlarging sample sizes may also help to distinguish between intraspecific variations and species-level dichotomies. Pons et al. (2006) addressed this point in their “general mixed Yule coalescent” (GMYC) model, which combines models of stochastic lineage growth with coalescence theory to develop a new likelihood method that determines the point of transition between species-level (interspecific long branches) and population-level (short burgeoning branches) evolutionary processes. Pagès et al. (2010) recently adopted this approach, which does not require defining entities *a priori*, for the murine rodent tribe Rattini, a group in which species identification is difficult to assess through morphological determination. Pagès et al. (2010) thus recovered 24 putative species, to which they could *a posteriori* attribute an unambiguous species name to 18 of them. The remaining six groups either corresponded to small samples for which insufficient data was available to choose a valid name, or to potentially new species. Hence, although being in essence derived from purely molecular procedures, this phylogeny-based method for

delimiting species also includes an integrative taxonomy approach when it comes to taxa characterization and naming.

#### 4.5 Sequence data and recent species description

To check for the importance of molecular data in recent descriptions of new rodent species, two distinct datasets were analyzed. First, the list of muroid rodent species described between 1990 and 2003 was drawn up from Musser & Carleton (2005). Second, all scientific papers describing new rodent species published between 2004 and 2011 were tentatively gathered. In both cases, the relative importance of DNA sequence data in the characterization of new species was evaluated. In the description of new species, morphological information still has a major place, as a detailed description of type series (and especially of the holotype) is required as well as a comparison of the new species structural characteristics relative to related species. However, information from additional fields are now regularly added, that can be of prime importance in the new species diagnosis.

One hundred and seven species of muroid rodents were described between 1990 and 2003, according to Musser & Carleton (2005). Most of them (i.e. > 94%) belong to the families Cricetidae (N = 52) and Muridae (N = 49), in agreement with their importance within the superfamily Muroidea, where they represent nearly 94% of the total number of species (see Figure 3). Musser & Carleton (2005) quoted some of these new species as debatable because either based on a very small number of specimens (sometimes only one), or in need of further and more detailed comparison with already existing species. Morphological and, most of the time, morphometrical data constitute the basis of these descriptions, although in a few cases this information appears hardly useful, or even useless, for species diagnosis. This is the case for instance for *Taterillus tranieri*, an example of sibling species whose main characteristic consists in its karyotype (Dobigny et al., 2003). It is noteworthy that chromosomal features represented the most frequently included character (in addition to morphological ones) in species descriptions, being present in at least 31 of the cases (i.e. nearly 30%). This highlights the importance of karyotypic data as species-specific diagnostic characters in rodents, a group where chromosomal variability has already been underlined in several occasions (see Granjon & Dobigny, 2003 and Taylor et al., 2000, for recent examples in African rodents). By contrast, DNA sequence data were only provided as a support to the new species description in 10 cases (i.e. 9.3%). Musser & Carleton (2005) mentioned that in at least 8 other instances, molecular data came soon after species descriptions, generally confirming the validity of the concerned species. Interestingly, protein electrophoresis data were still used in a small number of these descriptions (less than 10).

In the period 2004-2011, we identified 55 new rodent species descriptions in the literature (Table 1). Although all regions of the world are concerned, the tropical belt concentrates most of these biological novelties. Among them, 20 concerned the family Cricetidae and 26 the family Muridae. Sequence data proved to be directly involved in 30 of these descriptions (i.e. nearly 55%). Interestingly, a more regular use of molecular data is observed in the description of New World Cricetidae, as compared to Old World Muridae. This probably reflects the implication of distinct working groups, each having their own procedures for the taxonomic study of these rodent families. In some cases, molecular studies identified new

Species	Family / Subfamily	Region	Main characteristics studied (diagnostic in bold)	Sources	Role of DNA sequence
<i>Spermophilus taurensis</i>	Sciuridae / Sciurinae	Turkey	<b>geometric crano-dental morphometry - karyology - DNA sequence data (cyt b, D-loop, tRNAs, X and Y chromosome sequences)</b>	Gündüz et al. (2007)	Prominent
<i>Heteromys catopterus</i>	Heteromyidae / Heteromyinae	Venezuela	<b>external and crano-dental morphology / morphometry</b>	Anderson & Gutiérrez (2009)	None
<i>Dendromus ruppi</i>	Nesomyidae / Dendromurinae	Sudan	<b>external and crano-dental morphology / morphometry</b>	Dieterlen (2009)	None
<i>Eliurus carletoni</i>	Nesomyidae / Nesomyinae	Madagascar	<b>external and crano-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Goodman et al. (2009)	Important
<i>Eliurus danieli</i>	Nesomyidae / Nesomyinae	Madagascar	<b>external and crano-dental morphology / morphometry</b>	Carleton & Goodman (2007)	Suggestive (prior to description)
<i>Voalavo antsahabensis</i>	Nesomyidae / Nesomyinae	Madagascar	<b>external and crano-dental morphology / morphometry</b>	Goodman et al. (2005)	None
<i>Proedromys liangshanensis</i>	Cricetidae / Arvicolinae	China	<b>external (incl. penis) and crano-dental morphology / morphometry</b>	Liu et al. (2007)	None
<i>Peromyscus schmidlyi</i>	Cricetidae / Neotominae	Mexico	external and crano-dental morphology / morphometry - <b>karyology - DNA sequence data (cyt b)</b>	Bradley et al. (2004)	Important
<i>Abrawayaomys chebezi</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and crano-dental morphology / morphometry</b>	Pardiñas et al. (2009)	None
<i>Akodon philipmyersi</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and crano-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Pardiñas et al. (2005)	Important
<i>Akodon polopi</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and crano-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Jayat et al. (2010)	Important
<i>Akodon viridescens</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and crano-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Braun et al. (2010)	Prominent
<i>Calomys cerqueirai</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and crano-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Bonvicino et al. (2010)	Important
<i>Cerradomys goytaca</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and crano-dental morphology / morphometry - karyology</b>	Tavares et al. (2011)	None
<i>Cerradomys langguthi</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and crano-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Percequillo et al. (2008)	Suggestive (prior to description)
<i>Cerradomys vivoi</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and crano-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Percequillo et al. (2008)	Suggestive (prior to description)

<i>Drymoreomys albimaculatus</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b, IRBP)</b>	Percequillo et al. (2011)	Important
<i>Eligmodontia bolsonensis</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and cranio-dental morphology / morphometry* - DNA sequence data (cyt b)</b>	Mares et al. (2008)	Important
<i>Juliomys ossitenuis</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Costa et al. (2007)	Suggestive (prior to description)
<i>Neusticomys ferreirai</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - karyology</b>	Percequillo et al. (2005)	None
<i>Oecomys sydandersoni</i>	Cricetidae / Sigmodontinae	Bolivia	<b>external and cranio-dental morphology / morphometry</b>	Carleton et al. (2009)	None
<i>Oligoryzomys moojeni</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - karyology</b>	Weksler & Bonvicino (2005)	None
<i>Oligoryzomys rupestris</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - karyology</b>	Weksler & Bonvicino (2005)	None
<i>Phyllotis alisosiensis</i>	Cricetidae / Sigmodontinae	Argentina	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Ferro et al. (2010)	Important
<i>Rhipidomys ipukensis</i>	Cricetidae / Sigmodontinae	Brazil	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Rocha et al. (2011)	Important
<i>Thomasomys andersoni</i>	Cricetidae / Sigmodontinae	Bolivia	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Salazar-Bravo & Yates (2007)	Important
<i>Lophuromys chercherensis</i>	Muridae / Murinae	Ethiopia	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Lavrenchenko et al. (2007)	Important
<i>Lophuromys kilanzoi</i>	Muridae / Deomyinae	Tanzania	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Verheyen et al. (2007)	Important
<i>Lophuromys machangui</i>	Muridae / Deomyinae	Tanzania	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Verheyen et al. (2007)	Important
<i>Lophuromys makundii</i>	Muridae / Deomyinae	Tanzania	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Verheyen et al. (2007)	Important
<i>Lophuromys menageshae</i>	Muridae / Deomyinae	Ethiopia	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Lavrenchenko et al. (2007)	Important
<i>Lophuromys pseudosikapusi</i>	Muridae / Murinae	Ethiopia	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b)</b>	Lavrenchenko et al. (2007)	Important
<i>Lophuromys sabunii</i>	Muridae / Deomyinae	Tanzania	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Verheyen et al. (2007)	Important

<i>Lophuramys stanleyi</i>	Muridae / Deomyinae	Tanzania	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Verheyen et al. (2007)	Important
<i>Archboldomys kalinga</i>	Muridae / Murinae	Philippines	<b>external and cranio-dental morphology / morphometry - karyology</b>	Balete et al. (2006)	None
<i>Chrotomys sibuyanensis</i>	Muridae / Murinae	Philippines	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b)</b>	Rickart et al. (2005)	Important
<i>Coccymys kirrhos</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Musser & Lunde (2009)	None
<i>Grammomys brevirostris</i>	Muridae / Murinae	Kenya	<b>external and cranio-dental morphology / morphometry</b>	Kryštufek (2008)	None
<i>Grammomys selousi</i>	Muridae / Murinae	Tanzania	<b>external and cranio-dental morphology / morphometry - karyology</b>	Denys et al. (2011)	None
<i>Hydromys zieglerei</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Helgen (2005b)	None
<i>Hylomyscus arcimontensis</i>	Muridae / Murinae	Tanzania	<b>external and cranio-dental morphology / morphometry</b>	Carleton & Stanley (2005)	None
<i>Hylomyscus walterverheyeni</i>	Muridae / Murinae	Gabon	<b>external and cranio-dental morphology / morphometry - karyology - DNA sequence data (cyt b, 16S rRNA)</b>	Nicolas et al. (2008)	Suggestive (prior to description)
<i>Leptomys arfakensis</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Musser et al. (2008)	None
<i>Leptomys paulus</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Musser et al. (2008)	None
<i>Mayermys germani</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Helgen (2005a)	None
<i>Microhydromys argenteus</i>	Muridae / Murinae	New Guinea	<b>external and cranio-dental morphology / morphometry</b>	Helgen et al. (2010)	None
<i>Mus cypriacus</i>	Muridae / Murinae	Cyprus	<b>classical and geometric external, cranio- dental and dental morphometry - karyology - DNA sequence data (D-loop) and other molecular markers</b>	Cucchi et al. (2006)	Suggestive (prior to description)
<i>Musseromys gulantang</i>	Muridae / Murinae	Philippines	<b>external and cranio-dental morphology / morphometry - DNA sequence data (GHR &amp; IRBP)</b>	Heaney et al. (2009)	Important
<i>Rhynchomys banahao</i>	Muridae / Murinae	Philippines	<b>external and cranio-dental morphology / morphometry</b>	Balete et al. (2007)	None

<i>Rhynchomys tapulao</i>	Muridae / Murinae	Philippines	<b>external and cranio-dental morphology / morphometry</b>	Balete et al. (2007)	None
<i>Saxatilomys paulinae</i>	Muridae / Murinae	Laos	<b>external and cranio-dental morphology / morphometry</b>	Musser et al. (2005)	None
<i>Tonkinomys daovantieni</i>	Muridae / Murinae	Laos	<b>external and cranio-dental morphology / morphometry</b>	Musser et al. (2006)	None
<i>Laonastes aenigmamus</i>	Diatomyidae	Laos	<b>external and cranio-dental morphology / morphometry - DNA sequence data (cyt b &amp; 12S rRNA)</b>	Jenkins et al. (2005)	Important
<i>Phyllomys sulinus</i>	Echymyidae	Brazil	<b>external and cranio-dental morphology / morphometry - karyology</b>	Leite et al. (2008)	Suggestive (prior to description)
<i>Isothrix barbarabrownae</i>	Echymyidae	Peru	<b>external and cranio-dental morphology / morphometry</b>	Patterson & Velazco (2006)	None

Table 1. Rodent species descriptions in the period 2004-2011, and role of DNA sequence data among the datasets used.

lineages that were subsequently formally described as new species (these descriptions then included or not the sequence data). In most cases, the description process encompassed the DNA sequence data that sometimes represented the major characteristics of the newly described species. Indeed, both Gündüz et al. (2007) and Braun et al. (2010) when describing *Spermophilus taurensis* and *Akodon viridescens*, respectively, pointed out the difficulty of assigning individual specimens to these species based on the sole morphological and morphometrical characteristics, whereas molecular data unambiguously classify the same specimens in well-supported monophyletic clades. To a lesser extent, this is the case for the numerous species of *Lophuromys* described by Lavrenchenko et al. (2007) and Verheyen et al. (2007; Table 1). In the latter paper however, some of these new species would certainly need a stronger assessment of their status, as their current assignation relied on insufficiently supported bootstrap values in distance-based phylogenetic trees built on cytochrome b sequences (see for instance graph 10 in Verheyen et al., 2007).

In all studies using DNA sequence data but three (Cucchi et al., 2006, for *Mus cypriacus*, Heaney et al., 2009 for *Musseromys gulantang*, and Jenkins et al., 2005 for *Laonastes aenigmamus*), the molecular analyses were conducted using the cytochrome b gene. This proves again the importance of this gene at the species level in rodents (and more generally in mammals), and highlights its potential importance as a target gene for DNA barcoding / DNA taxonomy in this group (see above). Cytochrome b was regularly considered in association with other, mainly mitochondrial, genes. Karyological data were included in 21 of the 51 recent species descriptions, but proved to be not systematically diagnostic. Around 2/3 of these recent species descriptions were based on a combination of morphologic / morphometric and genetic (*sensu lato*) data that were jointly used to delimit and characterize



the new species. This again testifies for the importance of adopting an integrative approach in modern taxonomy (Dayrat, 2005; Lecompte et al., 2003), in which molecular data will undoubtedly have a growing place in conjunction with other fields including traditional ones.

## 5. Conclusion

It is now clear that classical morpho-anatomical data will not alone allow answer the numerous questions, and test the numerous hypotheses that remain in the field of animal systematics, even in structurally complex groups such as higher Vertebrates. Along the years, DNA sequence data have proven to represent a precious alternative source of information, as exemplified here above at different taxonomic levels in Rodentia. At the genus level, which has not been tackled in details here above, one may find additional examples acknowledging the same fact: genera defined on morphological grounds only often need to be revised using complementary tools, and particularly molecular ones. This aspect has been underlined at various occasions by Musser & Carleton (2005; see for instance their comments on the relatively recently described genera *Amphinectomys*, *Andalgalomys* and *Volemys*). At this important taxonomic level, the use of molecular data should also be encouraged, even if other sources of information may usefully be considered (see Ford [2006] for an interesting discussion centred on Australian murid rodents).

This overview of the role that molecular data have played in rodent systematics over the last decades reflects the ever growing importance of this kind of information in evolutionary biology as a whole. The improvement of laboratory procedures, the development of sophisticated data treatment softwares and of huge databases, together with the evolution of concepts associated with this field of research, have concurred to make DNA sequences a major source of information for disciplines such as population genetics, phylogeography and phylogeny, that all are related to some extent with systematics. From there, additionally to the classic work they need to do on voucher specimens, taxonomists now have also to get involved in various activities, or at least to consider the results from several disciplines when revising a group or describing a new species. These activities will undoubtedly continue to include the acquisition, treatment and interpretation of molecular data. In rodents and as shown above, a number of taxa (from species to higher-level supraspecific groups) are still in need of a more accurate delimitation, phylogenetic relationships of many others still remain to be established, and new species still await to be described all over the world!

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## 7. References

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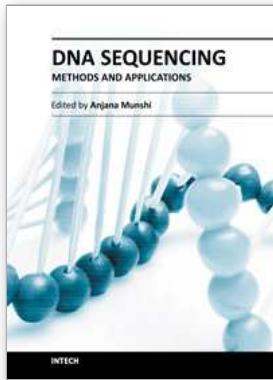
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This book illustrates methods of DNA sequencing and its application in plant, animal and medical sciences. It has two distinct sections. The one includes 2 chapters devoted to the DNA sequencing methods and the second includes 6 chapters focusing on various applications of this technology. The content of the articles presented in the book is guided by the knowledge and experience of the contributing authors. This book is intended to serve as an important resource and review to the researchers in the field of DNA sequencing.

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