



The evolution of behavioural systems: a study of grooming in rodents

J. Malange^{a,b,*}, C.C. Alberts^c, E. Spinelli Oliveira^{a,d} and H.F. Japyassú^{a,b}

^a Programa de Pós-Graduação em Neurociências e Comportamento, NeC,
Instituto de Psicologia, Universidade de São Paulo, São Paulo, Brazil

^b Núcleo de Etologia e Evolução, Instituto de Biologia,
Universidade Federal da Bahia, Bahia, Brazil

^c Universidade Estadual Paulista, São Paulo, Brazil

^d Departamento de Biologia, Faculdade de Filosofia, Universidade de São Paulo,
Ciências e Letras de Ribeirão Preto, São Paulo, Brazil

*Corresponding author's e-mail address: jumalange@usp.br

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Abstract

In the present paper we focus on the study of complex behavioural systems, within an explicit phylogenetic framework. We reconstruct the phylogeny of rodents using grooming sequences from 12 terminals. Using a method derived from graph theory, we decompose complex behavioural systems into strings of behavioural units (behavioural routines) which are then used as behavioural characters to compose the phylogenetic matrix in addition to three mitochondrial markers as molecular characters (the cytochrome *b* gene (*cytb*), the 16S ribosomal RNA gene and the 12S ribosomal RNA gene). Our results point to a highly structured behavioural morphospace: only a few characters have been selected for, within the total space of possibilities. The optimization of hundreds of non-homoplastic routines onto three distinct phylogenies (behavioural, combined data and the molecular supertree of Fabre et al., 2012) reveals the same evolutionary trend from simple to complex: while simple behavioural routines (zero- or first-order sequences) are synapomorphies at basal levels of the phylogeny, progressively more complex behaviours evolve later, appearing closer to the tips of the phylogeny. Also, the optimization shows that the organisation of units into modules of coordinated action patterns first evolved around large body parts, namely the head and the trunk, modules that were later fused into one single organising module among rodents. We support the use of complex behavioural systems as a promising tool in the study of evolutionary scenarios and discuss the role of routines length and microstructure to provide phylogenetic information and elucidate evolutionary processes.

Keywords

Grooming, evolution, behavioural systems, rodents.

1. Introduction

Complex behavioural systems are not as easy to study in a comparative perspective as are simple, reflex-like units of behaviour. When we add complexity to a system, it usually shows more context sensitivity, a property that is not much appreciated in phylogenetic studies. This is probably at the root of the diminishing numbers of papers dealing with phylogenetic information in behavioural journals (Price et al., 2011).

Extreme context sensitivity is probably the reason behind the long standing controversy regarding the use of behavioural data to estimate phylogenies, a controversy revolving mainly around the alleged inherent plasticity of behaviour and the inapplicability of the homology criteria to this evanescent database (Lorenz, 1941; Atz, 1970; Baroni Urbani, 1989). There have been many successful empirical studies deploying phylogenetical relationships from behaviour (McLennan, 1993; Paterson et al., 1995; Alberts, 1996; Slikas, 1998; Stuart & Hunter, 1998; Bucheli et al., 2002; Noll, 2002; Desutter-Grandcolas & Robillard, 2003; Robillard et al., 2006; Cap et al., 2008), but these studies are mostly concerned with simple behavioural patterns, simple units of species typical behaviour.

The success of the use of simple, reflex-like behavioural units within an explicitly phylogenetic context has encouraged the use of other, more complex aspects of the performance, such as the sequential organisation of these units (Robillard et al., 2006; Legendre et al., 2008), but this is still a quite unusual approach. Even more unusual is the simultaneous use of simple units, short and long sequences of behaviour in a phylogenetic context; such an approach could possibly give a picture of the evolution of complexity within behavioural systems. It would allow one to answer questions regarding possible evolutionary trends from simple to complex systems, or vice versa. Also, it would help to settle the question of the suitability of behaviour as a phylogenetic character to its highest level: if even complex behavioural systems (as for example long sequences of behaviour) prove to be phylogenetically informative, then the relationship between behavioural plasticity and evolutionary processes (West-Eberhard, 2003, pp. 337–352) must be readdressed. In the present paper we deal with these questions tracing the evolution of varying levels of behavioural complexity in the phylogeny of rodents.

Rodents have a quite long history of phylogenetic reconstructions. Some molecular studies have considered them as a polyphyletic group (Graur et al., 1991; Li et al., 1992; D'Erchia et al., 1996; Reyes et al., 2000; Adkins,

2001, 2003; Arnason et al., 2002; Lin et al., 2002), while other studies have favoured their monophyly (Martignetti & Brosius, 1993; Quintana, 1998; da Silva Neto, 2000; Reyes et al., 2000; Adkins, 2001, 2003; Huchon & Douzery, 2001; Arnason et al., 2002; Lin et al., 2002; Rowe & Honeycutt, 2002; Reyes et al., 2004; Narita et al., 2006; Poux et al., 2006). Nevertheless, recent papers have converged on the monophyly hypothesis (Blanga-Kanfi et al., 2009; Fabre et al., 2012). In this paper, we present grooming as a new, potentially rich database for rodent phylogeny, and explore this database in under sampled rodent taxa.

Grooming is a well-defined and described behaviour (Eisenberg, 1963; Roth & Rosenblatt, 1966, 1967) that has been studied with regard to its function (Cromwell & Berridge, 1996; Shanias & Terkel, 1996; Ferkin et al., 2001; Greer & Capecchi, 2002; Wolff et al., 2002; Kalueff & Tuohimaa, 2004; Berridge et al., 2005; Hawlena et al., 2007), hierarchical (Fentress, 1972, 1973, 1987; Dawkins & Dawkins, 1976) and sequential organisation (Berridge, 1990; Colonnese et al., 1996; Bursten et al., 2000), and also with regard to its evolutionary pattern (Farish, 1972; Sachs, 1988; Berridge, 1990). Grooming occurs among vertebrates and invertebrates, and seems to be an old and evolutionarily conservative behavioural pattern (Berridge, 1990; Colonnese et al., 1996). Despite this evolutionary conservatism, grooming units and their sequential organisation show enough variability as to be potentially informative for phylogenetic studies (Dawkins & Dawkins, 1976; Berridge, 1990; Bursten et al., 2000).

When dealing with behavioural sequences, it is desirable to take into consideration the probabilistic nature of this kind of data, a condition that has been recently treated by the direct optimization of small behavioural sequences (Japyassú & Machado, 2010). The use of longer behavioural sequences would be welcome, since it would result in an exponentially larger numbers of phylogenetic characters (Japyassú & Viera, 2002), but the extension of this direct optimization method to longer behavioural sequences is computationally intensive, and has not been developed up to now. In this paper we follow a different analytical procedure to circumvent the computational difficulties of the direct optimization approach. We use a method derived from graph theory to extract behavioural sequences that will be subsequently used as characters in a phylogenetic analysis (Alberts, 1996; Japyassú et al., 2006). We focus on sequences of an easily observable behaviour (self-grooming) in order to understand the evolution of this complex

behavioural system, mapping the behavioural characters in three distinct tree topologies.

2. Methods

2.1. Behavioural data

Grooming is a well-known behaviour, and its main units, as described for *Rattus norvegicus* (Silverman, 1978), are ‘wash’, ‘groom’, ‘scratch’ and ‘lick penis’ (urinary papilla). We complemented this behavioural catalogue with other units obtained from a review of the literature and from preliminary observations of the animals under study, so that the new catalogue comprises a total of 27 behavioural units (Appendix A). We did not consider variations in the form of each behavioural unit because we focus not on the evolution of the units themselves, but rather on the evolution of the routines, that is, of the probabilistic sequences of the units.

A total of 42 specimens comprising 12 terminals were videotaped. The terminals analyzed were *Calomys callosus* Rengger, 1830 (2♀, 2♂), *Mesocricetus auratus* Waterhouse, 1839 (2♀, 1♂) and *Meriones unguiculatus* Milne-Edwards, 1867 (1♀, 2♂), of the superfamily Muroidea; *Cavia aperea* Erxleben, 1777 (3♀, 2♂), *C. intermedia* Cherem, Olimpio e Ximenez, 1999 (3♀, 4♂) and *C. porcellus* Linnaeus, 1758 (1♀, 2♂), of the family Caviidae; *Trinomys yonenagae* Rocha, 1995 (1♀, 1♂), *Thrichomys pachyurus* Wagner, 1845 (1♀, 1♂) and *T. laurentius* Thomas, 1904 (2♀, 2♂), of the family Echimyidae. Specimens of the species *T. laurentius* were further subdivided in two distinct terminals, because they exhibit different behavioural and morphometrical features (Reis et al., 2002; Basile, 2003; Mendes et al., 2004), and also because they come from isolated populations of different biomes and distant geographic areas, one from São Raimundo Nonato (Piauí; 1♀ and 1♂; 09°00'55"S, 42°41'58"W) and another from Caetité (Bahia; 1♀ and 1♂; 14°04'10"S, 42°28'30"W). *Felis silvestres f. catus* Linnaeus, 1758 (4♀, 3♂) (order Carnivora) and *Oryctolagus cuniculus* Linnaeus, 1758 (1♀, 2♂) (order Lagomorpha) constituted outgroups in the analysis.

C. callosus, *M. auratus*, *M. unguiculatus*, *C. aperea*, *C. porcellus*, *T. yonenagae*, *T. pachyurus*, *T. laurentius* and *O. cuniculus* were kept under standard laboratory conditions with controlled climate parameters (21–24°C, 45–55% humidity and 12/12 h light/dark cycle). All animals were provided with food and water ad libitum. The rodents were placed in acrylic cages (41 × 34 ×

20 cm). These cages were kept at the laboratory where the animals belonged (Adolfo Lutz Institute at São Paulo; Psychology Institute and FFCLRP at the University of São Paulo; Oswaldo Cruz Institute at Rio de Janeiro). *C. intermedia* were filmed at their natural habitat (Moleques do Sul Island, Santa Catarina State, Brazil, 27°51'S, 48°26'W). *F. catus* were also filmed at their natural, domestic habitat (in São Paulo city, Brazil).

Grooming was videotaped in focal animal method (Lehner, 1996). We sampled a total of approximately 75 h, resulting in nearly 2000 events for each terminal. All terminals reached a plateau when plotted on accumulation curves, revealing a sufficient and comparable sampling effort (Appendix B). The sessions were transcribed with the aid of the program EthoLog (Otoni, 2000).

2.2. Obtaining behavioural routines

First-order transition matrices were analysed using the software EthoSeq, available online at <http://www2.assis.unesp.br/cats/ethoseq.htm>. The details of the software algorithm are described elsewhere (Japyassú et al., 2006), but below we provide a brief description of the main steps in the graph analysis. This graph analysis results in probabilistic behavioural sequences (routines). In order to obtain these routines, EthoSeq builds a first-order transition matrix from the raw sequence data, for each species. Next, it searches, in each matrix, for the most probable directed hierarchical graph, given the dyadic association between the behavioural units in the transition matrix. Behavioural units are the vertices (V), and transition probabilities the arrows (A), in each of the possible directed hierarchical graphs $G(V, A)$ present in the transition matrix. Each behavioural unit appears only once in a $G(V, A)$, which connects all units in one single hierarchy. EthoSeq simply chooses the most probable hierarchical graph for a given matrix and a given initiating behaviour (the root, r), and it does so by maximizing the transition probabilities between behavioural units, with the function:

$$P(r, \dots, l) = P(r) \prod_{(i,j) \in (r, \dots, l)} C(i, j),$$

where $P(r)$ is the unconditional probability of the behavioural unit r , at the root of the hierarchical graph; $C(i, j)$ is the conditional probability of the behavioural unit i given the unit j ; $P(r, \dots, l)$ is the probability of the linear branch from the behavioural units r through l (from the root to the tip of the hierarchy). The routines then are the set of these linear branches within the most probable graphs found for each species.

2.3. Phylogenetic analyses

The behavioural phylogenetic matrix with informative routines (available as supplementary material in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>) was analysed using the software TNT (v. 1.1, Goloboff, Farris Nixon, 2008), using heuristic search (RAS + TBR) parameters, 10 replications, to search the most parsimonious tree. Bootstrap (Felsenstein, 1985) and jackknife (Farris et al., 1996) were used as measures of clade support (100 replications).

In order to investigate the association between the size of the routine and its position (from the root to the tips of the tree) as a synapomorphy on the phylogeny, we performed a Spearman's correlation test (Spearman, 1904).

For the molecular analysis we used three mitochondrial markers, the cytochrome b gene (*cytb*) (approximately 1140 bp), the 16S ribosomal RNA gene (approximately 1560 bp) and the 12S ribosomal RNA gene (approximately 1020 bp). The molecular data were obtained from GenBank (Table 1). While for *cytb* most species presented the complete sequence, for 16S and 12S only partial sequences were obtained, or no data at all.

To align molecular sequences and to reconstruct the phylogeny using mitochondrial data via dynamic optimization we employed the POY software, version 3.0.11 (Wheeler, 2003). The molecular data undergone a complete search and for the jackknife analysis the command jackboot was used with the 100 replicates parameter.

Table 1.

GenBank accession numbers for the molecular data according to each taxon and to each mitochondrial gene used on this study.

Species	<i>cytb</i>	16S	12S
<i>Felis catus</i>	AB194812.1	DQ334823.1	AY012149.1
<i>Oryctolagus cuniculus</i>	AY292717.1	DQ334838.1	AY292691.1
<i>Thrichomys apereoides laurentius</i>	AY083332.1	n/a	n/a
<i>Thrichomys pachyurus</i>	AY083329.1	n/a	n/a
<i>Trinomys yonenagae</i>	AF194295.1	AF422899.1	AF422865.1
<i>Meriones unguiculatus</i>	AB381901.1	EU078984.1	AJ851249.1
<i>Calomys callosus</i>	DQ447282.1	n/a	n/a
<i>Mesocricetus auratus</i>	AJ973379.1	DQ334843.1	X84390.1
<i>Cavia aperea</i>	GU136743.1	n/a	AF433908.1
<i>Cavia porcellus</i>	DQ017040.1	DQ334847.1	AF433909.1

n/a, not available.

We also used POY to reconstruct a phylogeny based on the concomitant use of both molecular and behavioural data employing the program's Combined Analysis.

3. Results

3.1. Phylogeny of rodents

From the 6083 grooming routines, 2832 were phylogenetically informative. The analysis of the character matrix with these informative routines (see supplementary material in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>) resulted in one single most parsimonious tree (Figure 1).

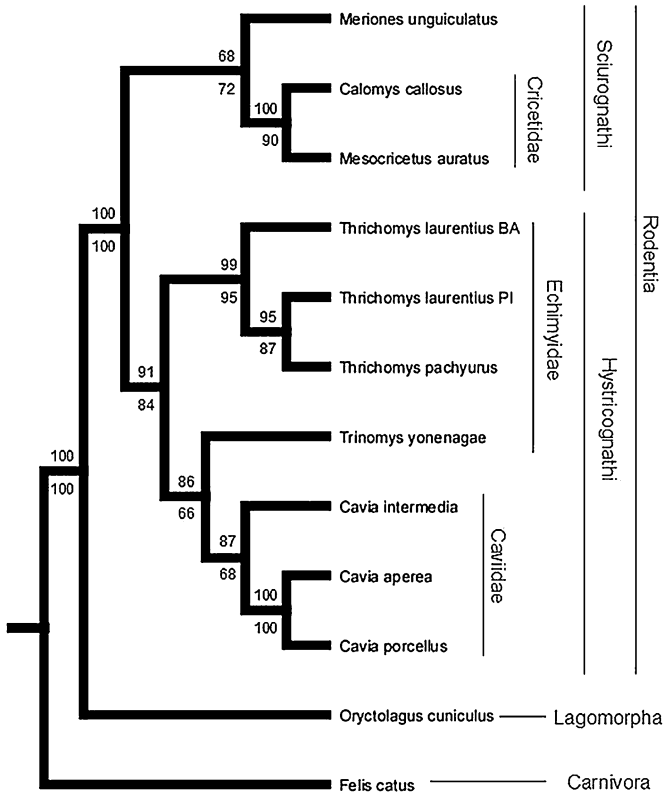


Figure 1. Single most parsimonious tree resulting from the analysis of 2832 informative grooming sequences (6970 steps; CI = 40; RI = 31). Jackknife and bootstrap values are shown above and below the branches, respectively.

High jackknife (above branches) and bootstrap (below branches) values indicate strong confidence in all the internal nodes of the cladogram. We have recovered traditional groupings, such as the rodent suborders Sciurognathi and Hystricognathi, and also the monophyly of the families included in the analysis. The single discrepancy with traditional groupings is the position of *Trinomys*. The two isolated and geographically distant populations of *Thrichomys laurentius* are not sister groups.

The monophyly of rodents is not recovered in the tree for molecular data (Figure 2), since the position of the rabbit *Oryctolagus* as sister of Caviidae breaks up the group. The position of *Trinomys* is also different from that in the behavioural data tree. On the other hand, all other terminals are positioned in a very similar way, if not identical, as in the behavioural tree.

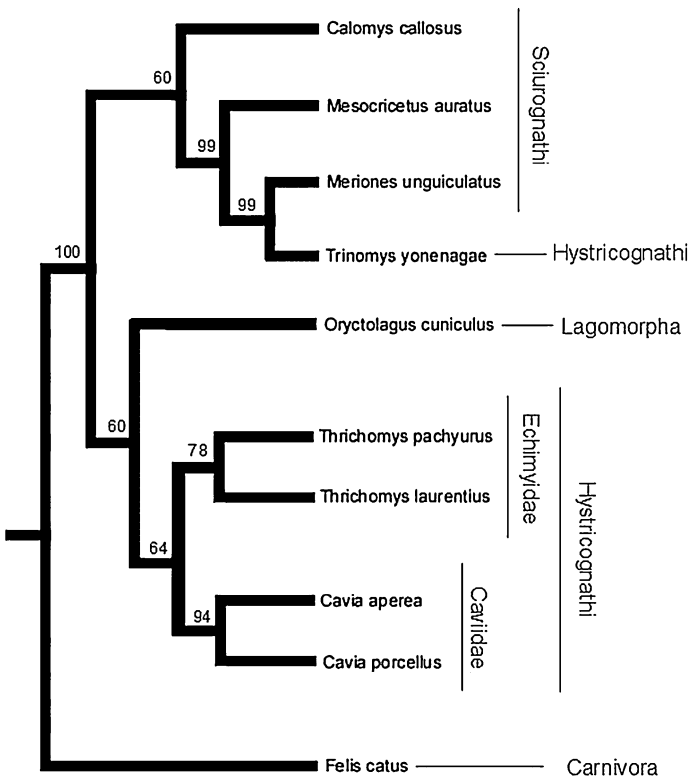


Figure 2. Most parsimonious tree resulting from mtDNA sequences alignment and tree construction via dynamic optimisation of the 12S, 16S and *cytb* genes. Jackknife values are shown at the nodes.

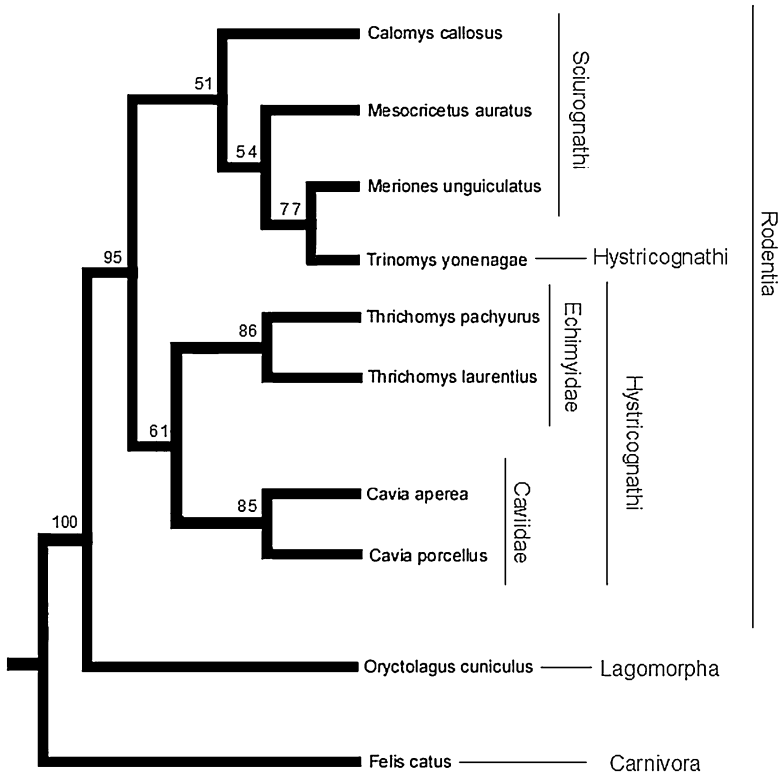


Figure 3. Most parsimonious tree combining molecular and behavioural data. Jackknife values are shown at the nodes.

The combined analysis of both molecular and behaviour data recovered again the monophyly of rodents (Figure 3). Excluding the differences due to the reduced number of taxa, the reconstruction is very similar to the behaviour tree. The only one dissimilitude is the position of *Trinomys*, which remained within the Sciurognathi as in the molecular tree.

3.2. The evolution of grooming

In order to have a grasp on the evolution of grooming routines in rodents, we optimised onto the behavioural phylogeny the 359 routines that do not present homoplasy. The optimization is very similar when performed on the combined data tree (behavioural plus molecular), but we have more taxa in the behavioural tree, due to the lack of molecular information for some of the species. Thus, the behavioural tree provides more information regarding

the evolution of grooming sequences, including the interesting non-sister relationship between the two populations of *Thrichomys laurentius*.

Overall, this analysis shows a striking difference between the grooming behaviour of rodents and that of the outgroup taxa, and this result holds irrespective of which of the outgroup taxa we choose to root the cladogram. Both *F. catus* and *O. cuniculus* (possible outgroup species) usually perform facial and body grooming in separate behavioural bouts, while rodents mostly perform complete grooming sequences, involving both the head and the rest of the body. For example, in one single bout cats and rabbits groom only body parts alternating between licking and biting the flank, and licking and biting the belly. Rodents do not present this routine, and instead they make regular use of complete sequences such as rub ear/rub eye/rub vibrissae/lick forelimb/lick back/lick flank/bite flank/lick belly; they perform these routines quickly, especially in the species of the suborder Sciurognathi. Cats and rabbits frequently show long pauses between grooming sequences, while rodents mostly perform uninterrupted sequences. Thus, it seems that rodent evolution resulted in the unification of two previously distinct behavioural domains: the head and the body grooming modules. Notwithstanding this evolutionary unification of two modules into a single structure, at a larger scale there is still a similarity between the plesiomorphic and the apomorphic organisation, in the sense that both groups perform grooming sequences in a head-tail direction.

In the rodent clade, we remark that histricognathids frequently freeze in the middle of their grooming sequences. For example, histricognathids frequently display the routine scratch head/bite hind foot/lick tail/freeze/wash anogenital area.

Hystricognathid rodents are also characterised by grooming sequences intermingled with non-grooming behaviour, as for example, in the routine rub vibrissae/lick forelimb/exploration/walking/exploration/bite belly. Also, hystricognathids have lost many routines that sciurognathids share with the outgroups. These are usually small routines such as, for example, the routine bite/lick/bite (the belly).

Within sciurognathids, cricetids differ markedly from the other rodents (they present 56 synapomorphies). One major difference is the intense use of the hind legs to scratch other body parts, composing routines such as scratch head/bite hind legs/scratch belly/bite hind legs/scratch flank. Also, besides using the usual head-tail overall direction of grooming activities, cricetids additionally make use of a dorsoventral flow of behaviours.

3.3. Evolution of complexity in grooming behaviour

Our main question relates to the evolution of complexity in this behavioural system, which can be assessed by a correlation between the size of the routine and the level in the phylogeny where this routine appears as a synapomorphy. In order to evaluate this correlation we selected from the available routines the ones without homoplasy (CI = 100, $N = 359$ for the behavioural phylogeny, $N = 263$ for the combined data phylogeny, and $N = 388$ for the topology of Fabre et al., 2012), and inspected the level of the three phylogenies, from the root to the tip of the tree, where they stuck as synapomorphies (to see synapomorphies for the major clades, check Table 2). In all analyses, the distribution of the behavioural characters follow a common, very similar pattern: short routines are more informative at the basal nodes of the phylogeny, medium-sized routines inform the intermediate nodes, and large routines are synapomorphies mainly at the more distal nodes (near the tips) of the phylogeny (Figure 4). There is a significant positive correlation between the complexity (size) of the routine and its position on the phylogeny ($N = 359$; $p < 0.01$; $r = 0.244$).

There are routines of all kinds, from sequences of up to nine behavioural units connected in a string, to simple dyads (a sequence of only two units) or even monads (zero order sequences, or isolated units). The number of possible sequences increases exponentially according to the function $R = k(k - 1)^{(n-1)}$, where k is the number of behavioural units present in the whole repertoire, and n is the size of the sequence (number of behavioural units in the sequence). There is a strong disconnection between the number of possible sequences and the number of actual routines uncovered by our data mining analyses (Figure 5): from a total of 5.9×10^{12} possible grooming sequences of up to 9 units (up to 8th-order sequences), EthoSeq has uncovered only 6083 grooming routines actually exhibited by rodents.

This result points to a highly structured behavioural morphospace. There are actually very few long routines in the repertoire of rodents, what amounts to saying that there are very few long behavioural sequences that are biologically structured. One could wonder if the low number of long routines could be due to insufficient sample size. If the reason for the presence or absence of long routines was low sample size, the resulting sequences mined by the graph algorithm method would be a collection of randomly assorted routines, with no phylogenetic signal. Instead, our results show that the long routines

Table 2.

Synapomorphic characters with consistency index equal to 100% for the major groups in the study: Order Rodentia, Suborder Sciurognathi and Suborder Hystricognathi.

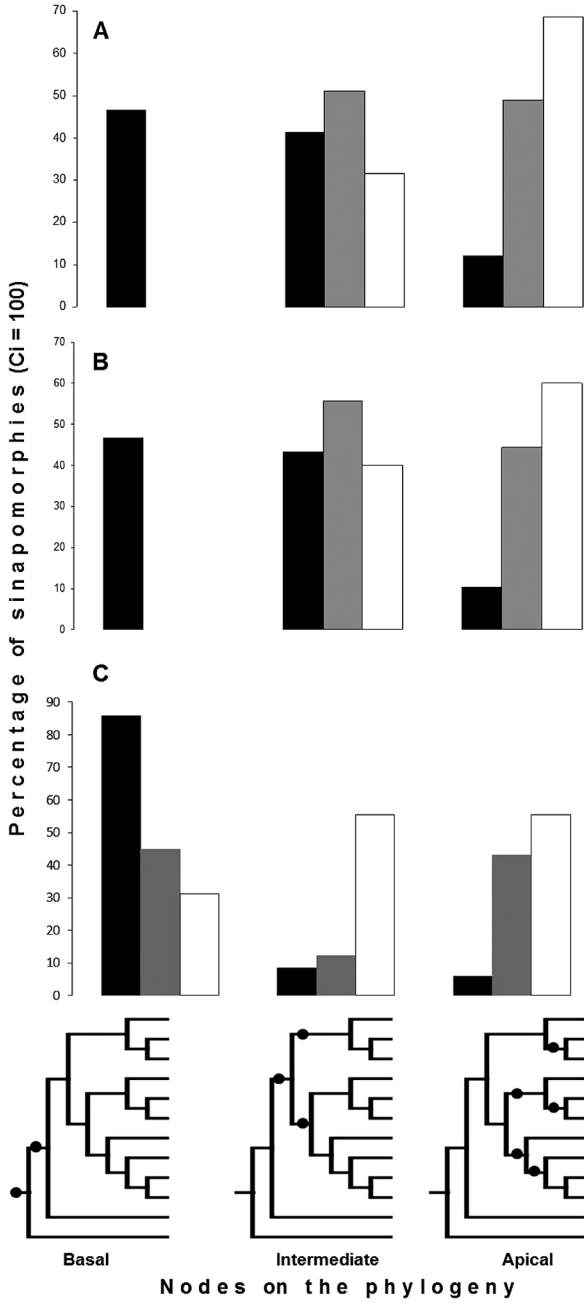
(Sub)Order		State
Rodentia		
bit_hindf, bit_forelimb	stch_head, bit_hindf, stch_head	+
bit_forelimb, exploration	lick_forelimb, lick_belly, lick_flanc	+
stch_flanc, exploration	lick_forelimb, bit_forelimb, exploration	+
drinking, walking, exploration	exploration, bit_hindf, shake	+
walking, lick_flanc	stch_flanc, exploration, walking, exploration	+
drinking, wash_anoge	bit_forelimb, exploration, walking, exploration	+
stch_belly, lick_hindf	exploration, walking, exploration, bit_forelimb	+
lick_belly, lick_forelimb, bit_forelimb		+
pause, lick_belly	drinking, lick_forelimb	-
pause, lick_flanc	lick_flanc, exploration	-
pause, lick_back	walking, eating, shake	-
lick_hindf, pause	pause, lick_forelimb, walking	-
lick_flanc, stopped	lick_hindf, walking, exploration	-
lick_back, pause	bit_forelimb, lick_forelimb, shake	-
lick_flanc, pause	shake, eating, shake	-
lick_back, shake	shake, eating, walking	-
lick_flanc, wash_anoge	bit_back, lick_back, lick_hindf	-
stch_belly, pause	lick_back, lick_flanc, bit_flanc	-
bit_tail, exploration	bit_hindf, lick_hindf, walking	-
bit_forelimb, lick_hindf	shake, walking, eating	-
pause, stch_belly	lick_hindf, bit_hindf, exploration, walking	-
mord_flanc, stch_flanc	eating, walking, exploration, shake	-
bit_tail, lick_tail	shake, walking, eating, walking	-
stopped, lick_forelimb	walking, exploration, walking, bit_flanc	-
bit_hindf, exploration	shake, eating, walking, exploration	-
lick_hindf, lick_belly		-
Sciurognathi		
stch_flanc, bit_hindf, bit_forelimb		+
walking, stch_flanc, exploration		+
walking, stch_flanc, exploration, walking		+
lick_hindf, lick_forelimb, rub_eye, rub_vibss		+
rub_eye, rub_vibss, lick_forelimb, bit_forelimb, lick_forelimb		+
rub_eye, rub_vibss, lick_forelimb, exploration, walking		+
rub_vibss, lick_forelimb, bit_forelimb, exploration, walking		+
lick_forelimb, rub_eye, rub_vibss, lick_forelimb, wash_anoge		+
rub_eye, rub_vibss, lick_forelimb, exploration, walking, exploration		+
rub_vibss, lick_forelimb, rub_eye, rub_vibss, lick_forelimb, lick_belly		+
rub_eye, rub_vibss, lick_forelimb, pause, lick_forelimb, rub_eye		+

Table 2.
(Continued.)

(Sub)Order	State
walking, exploration, lick_forelimb, pause, lick_forelimb, rub_vibss	+
eating, shake	-
shake, stch_head	-
stopped, shake	-
shake, stopped	-
bit_forelimb, lick_forelimb, exploration	-
exploration, eating, shake	-
rub_eye, rub_vibss, lick_forelimb, shake	-
Hystricognathi	
stch_head, stopped	+
bit_forelimb, exploration, stopped	+
stopped, bit_forelimb, stopped	+
stopped, stch_head, stopped	+
stch_head, bit_hindf, stopped	+
bit_forelimb, wash_anoge, pause	+
wash_anoge, exploration, shake	+
bit_hindf, exploration, stopped	+
lick_forelimb, exploration, stch_flanc	+
exploration, bit_forelimb, shake	+
pause, bit_forelimb, pause	+
bit_forelimb, stopped, rub_vibss	+
pause, bit_forelimb, lick_forelimb	+
bit_hindf, rub_vibss, lick_forelimb, bit_forelimb	+
exploration, rub_vibss, lick_forelimb, bit_forelimb	+
stch_head, bit_hindf, shake, walking	+
wash_anoge, exploration, shake, exploration	+
bit_hindf, stch_head, bit_hindf, stopped	+
stopped, exploration, lick_forelimb, exploration	+
bit_belly, lick_belly	-
lick_belly, bit_belly	-
lick_flanc, lick_hindf, lick_forelimb	-

The behavioural characters were optimised onto the topology of Fabre et al. (2012). The states of the characters mean presence (+) or absence (-).

found are the very synapomorphies that shape our behavioural reconstruction (Figure 1), which is almost identical to the topology that can be seen in phylogenies reconstructed using completely different characters (e.g., Fabre et al., 2012). Our few long routines do structure the phylogeny and, thus, cannot be the result of random processes.



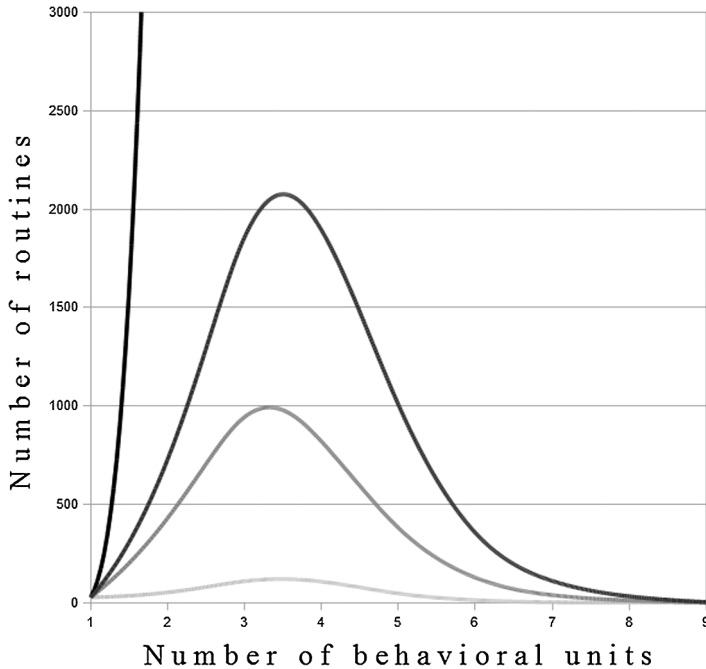


Figure 5. Behavioural morphospace: number of grooming routines as a function of the complexity of the routine (number of behavioural units in the routine). The black line shows the number of possible routines, or the total morphospace. The grey lines show the number of actual routines performed by the animals. Dark grey represents the total number of routines actually performed (including informative and non-informative ones); medium dark grey represents the phylogenetically informative routines; and light grey shows the number of non-homoplastic routines.

4. Discussion

4.1. Phylogeny of rodents

The behavioural and combined data phylogeny show rodents as a monophyletic group (Figures 1 and 3). The monophyly of rodents is also supported

Figure 4. Evolution of the complexity on the behavioural system. Simple, short routines (black) are more informative at basal levels of the phylogeny; large, or complex routines (white) are more informative at the upper levels of the phylogeny; routines of intermediate size (grey) show an intermediate pattern. The circles drawn at the branches of the hypothetical phylogeny illustrate the level where the characters stuck as synapomorphies. This pattern was observed in the behavioural (A), the combined data (B) phylogenies, and (C) in the topology of a recent rodent phylogeny (Fabre et al., 2012).

by morphological (Carleton & Musser, 2005) and many other molecular phylogenies (Nedbal et al., 1996; DeBry & Sagel, 2001; Reyes et al., 2004; Blanga-Kanfi et al., 2009), and is also recovered in the recent comprehensive meta-analysis of Fabre et al. (2012).

Within rodents, our behavioural data support the monophyly of Sciurognathi, a result that is mostly uncontroversial, since many authors agree on a sister group relationship for the families Cricetidae and Muridae (McKenna & Bell, 1997; De Brye et al., 2001; Carleton & Musser, 2005), both included in the present study. Also, many authors agree that the families Echimyidae and Caviidae are embedded within Histricognathi (Carleton & Musser, 2005), a result that was recovered only on our behavioural tree (Figure 1). The only topological difference between behavioural and recent reconstructions (Fabre et al., 2012) is the position of *T. yonenagae*, a poorly known, and recently described rodent species. So it seems that our behavioural characters (routines), recovered by the graph algorithm (Japyassú et al., 2006), do indeed provide accurate phylogenetic information.

The two populations of *T. laurentius* are possibly two different species. There is clearly need for more data in order to confirm this result, but we are confident of it because we have observed further morphological and behavioural differences between them. For example, specimens from the Piauí population are smaller, more silent, and less aggressive towards humans, than are the specimens from Bahia. Also, these populations differ in food and water intake, urine concentration (Mendes et al., 2004) and anatomy (Reis et al., 2002; Basile, 2003).

4.2. The evolution of grooming

Units of grooming behaviour are uninformative at the level of the order Rodentia, implying that they are possibly older than the whole group. Many studies show that grooming units are present in many different rodent species (Steiner, 1973; Borchelt et al., 1976; Lacher Jr., 1981; Randall, 1981; Geyer & Kornet, 1982; Chou et al., 1985; Messias, 1995; Shanas & Terkel, 1996; Bursten et al., 2000; Manaf & Spinelli Oliveira, 2000; Sabatini & Costa, 2001; Stopka & Gracíasová, 2001), and it seems that these units have been used for more than 50 million years, before the separation between the altricial and precocial rodents (Colonnese et al., 1996). Grooming units can be shared even among tetrapods (Sachs, 1988), a possibility that extends their origin to an even deeper evolutionary point.

Present in such a wide range of taxa, grooming probably exerts important adaptive functions. Within mammals it is related to obvious functions such as ectoparasite or dirt removal (Geyer & Kornet, 1982; Hart, 1990; Mooring & Hart, 1997; Hawlena et al., 2007) and bacterial control by saliva (Kohari et al., 2009), but also to presumably derived functions such as thermoregulation (Shanas & Terkel, 1996), and even communication (Ferkin et al., 1996, 2001; Wolff et al., 2002) or ritualised territory defence (Bursten et al., 2000).

Among these functions, we suggest that the reason behind the evolutionary merging of head and trunk grooming modules in rodents is an increase in the efficiency of ectoparasite removal. It is well known that ectoparasites have a stronger effect on smaller mammals, a condition known as the 'body size principle' (Hart et al., 1992; Mooring et al., 2000). The reason behind this principle is that smaller mammals are more affected by ectoparasites due to their higher surface/volume relationship, a condition that should select for more effective ectoparasite removal. Enhanced parasite infestations, due to habitat selection or social vigilance stress, for example, select for higher grooming rates in ungulates (Mooring et al., 2004).

Enhanced parasite load, due to the reduced rodent size, could also select for more efficient grooming patterns. The all-body bouts of grooming behaviour performed by rodents (starting on the head and ending in the trunk) could indeed be more effective by reducing the chances of crossed infestations between the head and the trunk. The idea of cross-infestation relates to the possibility that, right after a head cleaning bout, trunk parasites could, for example, re-infect the head. This would be especially common in smaller mammals, because ectoparasites like ticks could more rapidly find their way to uninfected, recently cleaned areas. In this situation, there would be selective pressure for whole body (head plus trunk), complete cleaning bouts. Thus, our point is that, besides the evolutionary enhancement of the frequency of grooming in smaller mammals (Hart et al., 1992; Mooring et al., 2004), there would also be selective pressure for a more efficient pattern of grooming in these smaller animals, and the head-trunk, fused grooming pattern, would be more efficient in preventing possible crossed-infestations between distinct body parts.

In line with this reasoning, we see that within rodents, the smaller ones, like the sciurognathids, perform all-body grooming sequences in a presumably more efficient way: they perform very quick all-body grooming sequences, and they make use of an additional overall direction of grooming

activities (they make use of a dorsoventral flow of behaviours in addition to the usual head-tail flow); these results are also accounted for by the body size hypothesis, and accordingly we suggest that body size is an important factor also in the evolution of the pattern of grooming in rodents.

4.3. Evolution of complexity in grooming behaviour

Our results point to an increase of behavioural complexity through time. As a general trend, rodent behaviour evolves from short to long grooming routines, adding each time more connectivity to the emerging behavioural system. This is a very robust result, since the pattern remains when we optimise the routines in three distinct tree topologies (Figure 4), including a recent comprehensive molecular meta-analysis (Fabre et al., 2012).

This result has implications for the delimitation of behavioural characters: short sequences should be preferred if one is looking for deep phylogenetic structure, that is, if one is interested in the phylogeny of very ancient taxa. The other way around, long behavioural routines should be preferred if one is willing to uncover historical relationships between recent taxa. This is analogous to what happens with molecular data, where some sequences are used to reveal the topology of ancient taxa, while others reveal relationships between populations of a single species (Michot et al., 1990; Nee et al., 1998). This result points to the possibility of using hyper-variable behavioural sequences for inferring rapid evolutionary events among very recent taxa.

The exponentially growing function of the number of possible behavioural routines contrasts markedly with the bell shaped function of the number of actual behavioural routines (Figure 5). The exponentially growing function refers to the total space of possibilities, that is, the complete behavioural morphospace. The bell shaped function represents the routines that are biologically structured, the ones that have been shaped by evolutionary processes that acted upon rodent grooming behaviour. The strong difference between these curves means that evolutionary processes have selected for a very small portion of the complete behavioural morphospace. Selection or behavioural constraints are particularly harsh for long behavioural routines, since the number of actual routines declines pronouncedly for sequences with more than four behavioural units, implying that there is a restriction for the increase of complexity in this group. So it seems that, besides the general trend for the progressive increase in behavioural complexity, there are upper biological limits to complexity.

4.4. Upper bounds to complexity

Although there are numerous papers detecting significant evolutionary trends in the number of behavioural units (Ord & Blumstein, 2002; McComb & Semple, 2005; Japyassú & Caires, 2008; delBarco-Trillo et al., 2012; Freeberg et al., 2012; Gustison et al., 2012; Ord & Garcia-Porta, 2012; Pollard & Blumstein, 2012), only a few of them look for the evolution of syntax, trying to detect patterns in the organisation of the behavioural units. This is usually the case for communication sequences repertoire size, for example, in birds (Ord & Garcia-Porta, 2012), where some groups have even been shown to have open ended song repertoires (Hailman et al., 1985; Freeberg & Lucas, 2012). Traditionally, song and repertoire size in songbirds have been shown to be sexually selected markers of male quality, based on the notions that learning more song elements is cognitively difficult (see review in Catchpole & Slater, 2008), a task that gets additionally expensive when consistency in the repetition of songs is relevant (Sakata & Vehrencamp, 2012). Thus, the evidence we have so far about syntax is that the evolution of open ended repertoires is rare, and that the use of long song streams is energetically costly, not only due to motor performance per se, but more generally because it requires more neural tissue to be maintained even in quiet moments (Brenowitz, 2004; Van der Linden et al., 2009). Longer routines, that are usually connected to larger behavioural repertoires (Hailman et al., 1985), thus, have additional costs that could help explain the clear absence of very long grooming routines (Figure 5).

Besides the physiological costs, another possible selective pressure against very long routines could be related to a trade-off with vigilance. There is evidence that grooming frequencies are smaller among territory holding male cervids, than among females or bachelor males (Hart et al., 1992). This is because territory holding males allocate more time to vigilance (either against possible contesting males, or in the search for reproductive females) and, thus, reduce grooming frequency and efficiency (Mooring et al., 2004). This suggests an indirect cost for enhancing grooming rates: the negative payoffs of the associated decreased vigilance rates. Decreased vigilance is an important selective factor that affects time allocation not only for grooming, but for a range of other time consuming activities and, thus, can potentially have a more general impact on fitness (Dukas & Clark, 1995). Thus, the trade-off between vigilance and grooming could select not only

for reduced grooming rates, but also against very long grooming bouts, thus posing a higher cost for these longer routines.

4.5. Concluding remarks

The organisation of behavioural units into modules of coordinated patterns first emerged around large body parts, such as the head and the trunk, modules that were later fused. The evolution of higher complexity within this system is possibly connected to the selective pressures promoting size reduction within rodents, with indirect effects on the increase of selective pressures related to parasite load. The increase in the complexity of grooming seems subjected to upper limits, which may be due to physiological and/or vigilance constraints.

The modular organisation, and the evolution of the connectivity within behavioural systems, show that we can move from the use of reflex-like units of behaviour as taxonomic indicators, a very successful tradition reminiscent from classical ethology (McLennan, 1993; Paterson et al., 1995; Alberts, 1996; Slikas, 1998; Stuart & Hunter, 1998; Bucheli et al., 2002; Noll, 2002; Quadros, 2002, 2008; Desutter-Grandcolas & Robillard, 2003; Robillard et al., 2006; Cap et al., 2008), to an evolutionary analysis of the complex behavioural systems, such as foraging or mating systems, a tradition more akin to behavioural ecology studies. This approximation between the research agendas of classical ethology and behavioural ecology will require an expansion of the focus in this last discipline: from the modelling of alternative and evolutionarily stable behavioural strategies in a few, to the comparative study of large groups of species, within an explicitly phylogenetic framework.

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Appendix A.

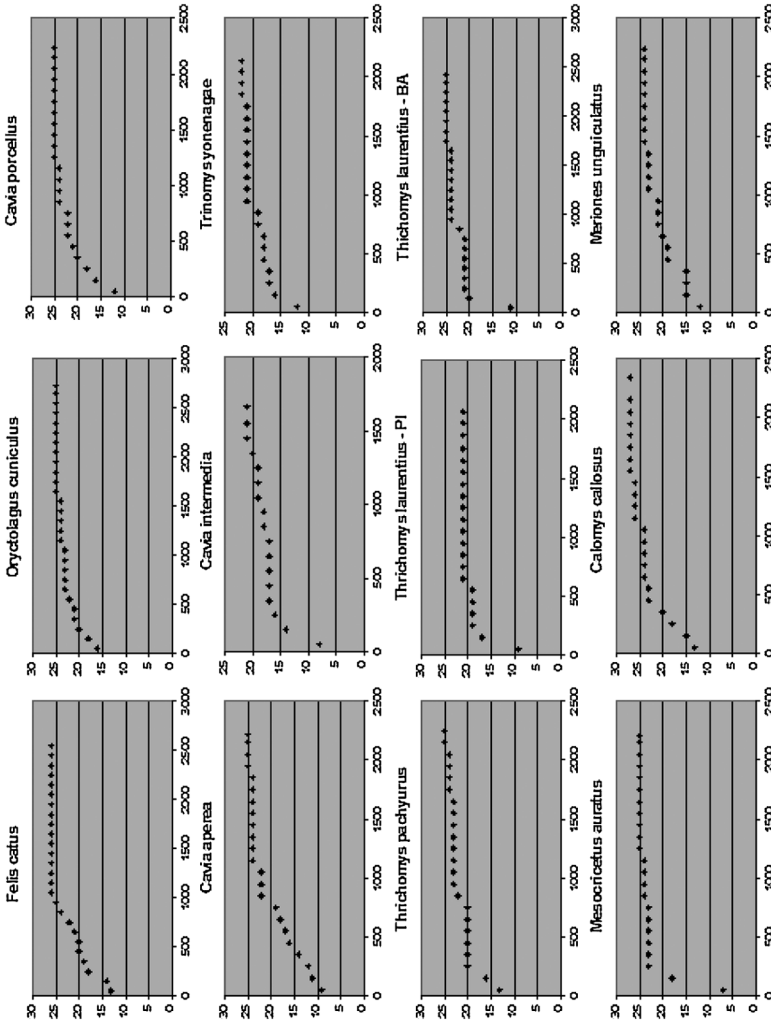
The ethogram with 27 behavioural categories was accomplished through the observation of grooming behaviour in the 12 taxa studied, and also from descriptions available in the literature. Silverman (1978) defined the major units of grooming behaviour described for *Rattus norvegicus* (Wistar), supported by the descriptions of Grant, Mackintosh and Draper (*apud* Silverman, 1978). According to them, washing is the alternation among licking forelimbs and rubbing head with them; combing is lick and comb (bite) flank hair, hindfoot, belly and tail with teeth; scratching is defined as the action of combing flank and back hairs with hindfeet; lick urogenital area is followed by covering the female and, often, by ejaculation.

Lick forelimb (lick_forelimb)	On its hind feet, the animal raises its forelimb(s) to the mouth (together or one limb at a time) and lick them, or the animal turns down its head and conduces its mouth and tongue to the forelimb(s). Lick is the same to “wash” according to Silverman’s (1978) terminology.
Lick hind foot (lick_hindf)	The animal raises its hind foot (feet) to the mouth or it can turn down to the foot (feet) region and lick them, always one foot at a time.
Lick belly (lick_belly)	The animal displays a dorsoventral curvature and lick belly region at the same time that it moves its head.
Lick back (lick_back)	The animal turns over its body and head in order to reach its body back and lick this region.
Lick flank (lick_flank)	The animal turns over the body to the flank region and licks it.
Lick tail (lick_tail)	The animal holds its tail by forelimbs or it can turn down to tail and lick it. This movement is often performed in a distal-proximal direction.
Biting forelimb (bit_forelimb)	On its hind feet, the animal raises its forelimbs to the mouth (together or one limb at a time) and bite them, or the animal turns down its head and conduces its mouth and tongue to forelimb(s). This behavioural category is almost interpolated to “lick forelimb” and correspond to the “comb” according to Silverman’s (1978) terminology.
Biting hind foot (bit_hindf)	Raising hind foot (feet) to the mouth or turn down to foot (feet), the animal bites foot, always one at a time.
Bitting belly (bit_belly)	On its hind feet, the animal displays a dorsoventral curvature in order to bite its ventral region and comb ventral hair in the direction the hair grows.
Biting back (bit_back)	On its hind feet, the animal turns over the body and head in order to reach its back. The animal bites its dorsal region and combs the hair in the direction the hair grows.
Biting flank (bit_flank)	The animal turns over its body and head in order to reach flank region and bites and combs it in the direction the hair grows.
Biting tail (bit_tail)	The animal holds its tail with the forelimbs or it can turn down to the tail and bite it. This movement is often performed in a distal to proximal direction.
Scratch head (stch_head)	The animal scratches its head, including the ears, the snout and the top of the head, always by one foot at a time. The animal can displays a head curvature to hind feet.

Appendix A.

(Continued.)

Scratch belly (stch_belly)	The animal scratches its belly always by one foot at a time.
Scratch back (stch_back)	The animal scratches its back always by one foot at a time.
Scratch flank (stch_flank)	The animal scratches its flank always by one foot at a time.
Wash anogenital region (wash_anoge)	The animal displays a dorsoventral curvature and turns over its neck to the genital region and licks it, following a down to up direction. Coprophagy (faeces intake) can be observed in this context.
Shake (shake)	The animal shakes the head or the wide body one to another side (right and left) in a rapid way.
Rubbing eye (rub_eye)	On its hind feet, the animal bends the forelimb(s), touches the eye area and rubs the eyes with ventral side of its limb(s). The movements are performed in a distal proximal direction by one or both forelimbs at a time.
Rubbing ear (rub_ear)	On its hind feet, the animal bends the forelimb(s), touches the ear area and rubs the ears with ventral side of its limbs while its head exhibit a dorsoventral motion. This behavioural category is performed in a distal proximal direction.
Rubbing vibrissae (rub_vibss)	On its hind feet, the animal bends the forelimb(s), touch face area and rubs vibrissae and nose with ventral side of its limbs in circular movements.
Drinking (drinking)	The animal puts the tongue out and licks the drinking place. Movements on water surface can help to observe this behaviour.
Eating (eating)	The animal reaches the feedbox or takes a food by its forelimbs on the cage and eats them. It is possible to observe chewing performance (chewing behaviour). Coprophagy (faeces intake) wasn't included in this behavioural category.
Environmental exploration (exploration)	The animal can be foraging in the cage or just explore the place when it is making steady movements with the nose and vibrissae, walking or is stopped on its hind feet in the ground. The animal can display rearing, and sometimes scratches the ground too.
Walking (walking)	The animal gets around the cage without exploring or foraging or displaying rearing.
Stopping and freezing (stopped)	There's no observable movement by the animal who can be sleeping or displaying freezing behaviour. This behavioural category was considered when the animal stays fixed for more than 30 s (random). Freezing can occur in the middle of a complete grooming sequence, while sleeping can only end such a sequence.
Pause (pause)	Pause was considered when the animal stays in inactivity by a period shorter than 30 s. This behavioural category was included to admit the counting of the same behavioural categories more than once in a sequence.



Appendix B. Accumulation curves indicating that all terminals reached a plateau, indicating a sufficient and comparable sampling effort. Each graphic below represents the cumulative number of behavioral units types (for a description of each behavioral unit see Appendix A), as a function of the total number of behavioral units (events) observed and registered, for each terminal (approximately 2000 events in 76 h).