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From the Old World to the New World: A Molecular Chronicle of the Phylogeny and Biogeography of Hystricognath Rodents

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Hystricognath rodents include Old World Phiomorpha and New World Caviomorpha. These two groups have an enigmatic biogeographical history. Using a nuclear marker, the exon 28 of the von Willebrand Factor gene (vWF), we reconstructed the phylogenetic relationships among 23 Hystricognathi species. These taxa encompass the complete familial diversity of the Hystricognathi. Our results indicate a basal trifurcation of hystricognaths leading to Hystricidae, Phiomorpha *s.s.* (Bathyergidae, Thryonomyidae, and Petromuridae), and Caviomorpha. The monophyly of caviomorphs is robustly supported, confirming a single colonization event of South America by hystricognaths. Caviomorpha are divided into four lineages: Cavioidea, Erethizontoidea, Chinchilloidea, and Octodontoidea. Furthermore, we suggest that (1) Chinchillidae and Dinomyidae are sister clades, (2) Abrocomidae is a true Octodontoidea, and (3) Capromyidae, Echimyidae, and Myocastoridae cluster together. Surprisingly, Erethizontidae does not appear to be the most diverged caviomorph lineage. The molecular results are discussed in the light of previous paleontological and morphological observations. Local molecular clocks are used to estimate divergence dates among hystricognath lineages. An Asian origin is suggested for Caviomorpha, and a colonization route through Australia and Antarctica is indicated as an alternative to the hypothesis of a transatlantic migration of Caviomorpha from Africa to South America.

Key Words: **phylogeny; nuclear gene (vWF); Rodentia; Hystricognathi; Caviomorpha; Dinomyidae; Chinchilloidea; Phiomorpha; biogeography; South America; Antarctica.**

INTRODUCTION

South America was an isolated continent during the Tertiary. This isolation from the Late Eocene to the Mio–Pliocene produced a variety of endemic mammalian groups belonging to marsupials, edentates, ungulates, primates, and rodents. The Cenozoic mammalian evolution in South America may be summarized into three-phases (Flynn and Wyss, 1998): establishment of archaic groups during the Paleocene (various marsupials, xenarthrans, notoungulates, and litopterns), arrival of two new immigrants (i.e., primates [Platyrrhini] and rodents [Caviomorpha]) at the Eocene–Oligocene transition, and establishment of modern faunas with Northern invaders crossing the Panamanian land bridge during the Great American Interchange at the Mio–Pliocene. The isolation of South America raises intriguing questions about the biogeographical origins and phylogenetic relationships of platyrrhines and caviomorphs: (i) Are they monophyletic lineages, arising from a single invasion event, or polyphyletic groups, possibly stemming from multiple invasions? (ii) Are they, as it is generally supposed, closely related to African forms (Catarrhini primates and Phiomorpha rodents)? (iii) What were their migration routes from the Old World to the New World?

In this study, we focus on Rodentia, an order representing 43% of the 4629 recognized living mammalian species (Wilson and Reeder, 1993). Among rodents, the South American caviomorphs belong to the infraorder Hystricognathi. Hystricognaths (230 species in 68 genera and 17 families) are well defined by morphological (Luckett and Hartenberger, 1993) and molecular (Catzeflis *et al.*, 1995; Nedbal *et al.*, 1996; Huchon *et al.*, 1999) characters. The hystricognath sister group is represented by a family of North African rodents, the Ctenodactylidae—or gundis—(Bryant and McKenna, 1995; Huchon *et al.*, 2000). Hystricognathi is divided into two well-defined biogeographical taxa: the Old World Phiomorpha (*sensu* Lavocat, 1973) and the New World Caviomorpha (*sensu* Wood, 1955). Caviomorphs suddenly appeared and diversified in South America in

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the Early Oligocene (31 Myr ago; Wyss *et al.*, 1993; Vucetich *et al.*, 1999). Their route of colonization is fully enigmatic as hystricognath fossils have been identified only in South America, Africa, and Eurasia. A North American origin of caviomorphs was proposed (e.g., Wood, 1985) but this hypothesis might be based on erroneous interpretation of fossil characters (e.g., Meng, 1990; Martin, 1994). The prevailing hypothesis suggests an African origin of caviomorphs and a transatlantic migration (e.g., Lavocat, 1969). Moreover, the number of migration events is debated, and some phylogenetic studies based on morphology suggest that caviomorphs are paraphyletic and have arisen from two independent colonizations (Bugge, 1985; Woods and Hermanson, 1985; Bryant and McKenna, 1995; McKenna and Bell, 1997) or even more (Landry, 1999). Most studies rather suggest a more parsimonious scenario, with a single origin of caviomorphs (Wood and Patterson, 1959; Nedbal *et al.*, 1994).

Old World hystricognaths (Phiomorpha *sensu lato*) include four extant families (Wilson and Reeder, 1993; McKenna and Bell, 1997): Thryonomyidae (cane rats) and Petromuridae (dassie rats), which are considered to cluster together into the superfamily Thryonomyoidea (Lavocat, 1973; Nedbal *et al.*, 1994); Bathyergidae (African mole rats); and Hystricidae (Old World porcupines). The fossil record indicates that Thryonomyidae arose in the Late Eocene–Early Oligocene of Africa. Petromuridae is a recent family appearing in the Pleistocene (Winkler, 1994; McKenna and Bell, 1997). The origin of Bathyergidae is debated, with either an African or an Asian Miocene origin (Winkler, 1994). Hystricidae is the most enigmatic. It presents many ancestral characters but their predecessors are unknown, and the earliest fossils are recorded only since the Early Miocene of Europe (McKenna and Bell, 1997). However, paleontologists hypothesized that hystricids have an Asian or Indian origin (e.g., Hussain *et al.*, 1978; Wood, 1985; Winkler, 1994). Caviomorpha are more diversified, with 13 extant New World families (Wilson and Reeder, 1993) grouped into four superfamilies: Erethizontoidea (New World porcupines), Cavioidea (guinea pigs), Octodontoidea (spiny rats), and Chinchilloidea (chinchillas).

Because the morphological evolution of rodents is characterized by high levels of homoplasy (Wood and Patterson, 1959; Jaeger, 1988), an alternative way to understand the origin and diversification of South American hystricognaths is to explore their phylogeny with molecular characters. Studies based on the mitochondrial 12S rRNA gene failed to give robust phylogenetic results on the relationships among the main caviomorph and phiomorph lineages (Nedbal *et al.*, 1994; Catzeflis *et al.*, 1995). Studies based on exon 28 of the nuclear von Willebrand factor (vWF) gene appeared promising but included only a few hystricognath species (Huchon *et al.*, 1999, 2000). To accurately estimate the number of South America colonizations by rodents, it is important to sample all the currently available hystricognath biodiversity, without exception. To reach this goal, we increased the vWF data bank to obtain a complete set of orthologues sequences. It is the first time, to our knowledge, that at least one representative of each of the 13 caviomorph and of each of the 4 phiomorph families has been sampled in a study at the nucleotide level.

This study addresses three questions about rodent evolution. (1) How many waves of hystricognath rodents colonized South America? A single colonization event implies that caviomorphs are monophyletic. (2) What is the closest sister clade of caviomorphs? Its identification could help to understand the caviomorph routes of migration. (3) What is the timing of these colonization events as deduced from molecular data? If some nucleotide substitutions or amino acid replacements behave clock-like in the vWF molecule, a temporal framework will be drawn to better understand the evolution of hystricognaths.

MATERIAL AND METHODS

Species Sampling

The dataset includes at least one representative for each hystricognath family (Table 1). Because there is a general agreement about the number of rodent families and their content (e.g., Hartenberger, 1985), it seems reasonable to consider any species sampled a fair representative of its family. Two to four species were considered for the Echimyidae, the Bathyergidae, and the key Old World and New World porcupine families. Ctenodactylidae (gundis) were chosen as a close outgroup because it has been shown that they are the sister group of hystricognath rodents on the basis of paleontological (Bryant and McKenna, 1995), morphological (Landry, 1999), and molecular (Huchon *et al.*, 2000) characters. Because Robinson *et al.* (1998) suggested that for distance analysis the most reliable outgroups are those closely related to the ingroup and slowly evolving, *Aplodontia* (mountain beaver; Aplodontidae) and *Spalax* (blind mole rat; Spalacidae) were selected as more distant outgroups because of their slow-evolving vWF exon 28 (Huchon *et al.*, 2000).

DNA Sequencing of vWF Exon 28

Tissue samples were derived mostly from the Collection of Mammalian Tissues of Montpellier (France) (Catzeflis, 1991). Taxonomy, origin, and references of the tissues are indicated in Table 1. DNA extractions and vWF exon 28 amplifications with V1/W1 primers ($V1_{direct}$ = 5′-TGTCAACCTCACCTGTGAAGCCTG-3′ and $W1_{reverse}$ = 5′-TGCAGGACCAGGTCAGGAGCCTCTC-3′) were conducted according to Huchon *et al.* (1999). For *Atherurus macrourus*, V1 was replaced by

TABLE 1

Taxonomic Frame for the Hystricognath Rodents Studied, following McKenna and Bell (1997) for Caviomorpha Superfamily Content and Wilson and Reeder (1993) for Family Names and Numbers of Genera/Species (between Parentheses)

Genera/species	Latin name	Common name	Accession	Origin (donator)/references
Phiomorpha (10/26)				
Hystricidae (3/11)	*Atherurus macrourus*	Brush-tailed porcupine	AJ251131[a]	Vietnam (J. L. Patton)/T-1751
	Trichys fasciculata	Long-tailed porcupine	AJ224675	Huchon *et al.* (1999)
Petromuridae (1/1)	*Petromus typicus*	Dassie rat	AJ251144[a]	Zoological Society of Philadelphia, USA (R. Hoyt)
Thryonomyidae (1/2)	*Thryonomys swinderianus*	Cane rat	AJ224674	Huchon *et al.* (1999)
Bathyergidae (5/12)	*Cryptomys hottentotus*	Common mole-rat	AJ251132[a]	Pietermaritzburg, South Africa (E. Nevo)/T-266
	Heliophobius argenteocinereus	Silvery mole-rat	AJ251133[a]	Kenya (C. G. Faulkes)/T-1846
	Heterocephalus glaber	Naked mole-rat	AJ251134[a]	Kenya (C. G. Faulkes)/T-1848
	Bathyergus suillus	Dune mole-rat	AJ238384	Huchon *et al.* (2000)
Caviomorpha (59/208)				
Erethizontidae (4/12)	*Erethizon dorsatum*	North American porcupine	AJ251135[a]	(J. A. W. Kirsh)/T-1789
	Coendou melanurus	Prehensile tailed porcupine	AJ224664	Huchon *et al.* (1999)
Cavioidea				
Agoutidae (1/2)	*Agouti paca*	Paca	AJ251136[a]	Petit Saut, French Guyana (J.-C. Vié)/T-1555
Dasyproctidae (2/13)	*Dasyprocta leporina*	Agouti	U31607	Porter *et al.* (1996)
Dinomyidae (1/1)	*Dinomys branickii*	Pacarana	AJ251145[a]	Cleveland Metroparks Zoo, USA (T. L. Bettinger)
Caviidae (5/14)	*Cavia porcellus*	Guinea pig	AJ224663	Huchon *et al.* (1999)
Hydrochaeridae (1/1)	*Hydrochaeris hydrochaeris*	Capybara	AJ251137[a]	Petit Saut, French Guyana (J.-C. Vié)/T-1618
Octodontoidea				
Octodontidae (6/9)	*Octodon lunatus*	Degu	AJ238386	Huchon *et al.* (2000)
Ctenomyidae (1/38)	*Ctenomys maulinus*	Tuco-tuco	AJ251138[a]	Talca, Chili (L. Contreras)/T-1005
Echimyidae (21/80)	*Proechimys oris*	Spiny rat (or casiragua)	AJ251139[a]	Breeding colony, Brazil (F. Petter)/T-0311
	Echimys chrysurus	White-faced tree rat	AJ251141[a]	Petit Saut, French Guyana (F. Catzeflis)/A-024
Myocastoridae (1/1)	*Myocastor coypus*	Nutria	AJ251140[a]	Gard, France (B. de Sousa)/T-1811
Capromyidae (12/25)	*Capromys pilorides*	Cuban hutia	AJ251142[a]	Zoo Viena, Austria (H. Burger and A. Kuebber-Heiss)/T-1845
Chinchilloidea				
Chinchillidae (3/7)	*Chinchilla lanigera*	Chinchilla	AJ238385	Huchon *et al.* (2000)
Abrocomidae (1/3)	*Abrocoma bennettii*	Chinchilla rat	AJ251143[a]	Parque National Fray Jorge, Chili (L. Contreras)/T-1004
Outgroups				
Ctenodactylidae	*Ctenodactylus vali*	Gundi	AJ238387	Huchon *et al.* (2000)
	Massoutiera mzabi	Gundi	AJ238388	Huchon *et al.* (2000)
Aplodontidae	*Aplodontia rufa*	Mountain beaver	AJ224662	Huchon *et al.* (1999)
Muridae	*Spalax polonicus*	Blind mole-rat	U31621	Porter *et al.* (1996)

Note. Information provided: Latin name, common name, EMBL data bank accession numbers for vWF exon 28 sequences, origin of the animal (name of the donator)/reference number in the collection of mammalian tissues of the "Institut des Sciences de l'Evolution of Montpellier" (Catzeflis, 1991).

[a] This paper.

primer $V5_{direct}$ (5′-GGAGGCCTGGHRGTGCCCCC-3′) (Huchon *et al.*, 2000). For most species, smaller overlapping PCR products were obtained after a reamplification step with V1 (or V5)/W2 and V2/W1 pairs of primers ($V2_{direct}$ = 5′-CCCTCAGAGCTGCGGCGCA-T-3′ and $W2_{reverse}$ = 5′-ACGTCCATGCGCTGGATCA-CCT-3′) (Huchon *et al.*, 1999). For *Echimys chrysurus*, *Abrocoma bennettii*, *Myocastor coypus*, and *Capromys pilorides*, one part of the vWF exon 28 was cloned in the pCR 2.1 plasmid vector with the Original TA cloning kit (Invitrogen, Carlsbad, CA) and bacterial transformation in *Escherichia coli* strain INVαF′. PCR products and recombinant plasmids were purified and directly sequenced on both strands with [α^{33}P]ddNTP and the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham, Cleveland, OH). The exon 28 vWF DNA sequences have been deposited in the EMBL/GenBank/DDBJ databases (Table 1).

Sequence Alignment

Sequences were aligned by hand with the ED editor (MUST package; Philippe, 1993) and compared with published orthologues. The alignment was unequivocal, with only one deletion of 1 codon for *Proechimys ori* and of 10 codons for *Petromus typicus*. In the subsequent analyses, all sites were kept, and gaps were coded as missing data. Among the 1263 aligned positions, 664 and 455 were, respectively, variable and parsimony informative.

Phylogenetic Analyses

Data were analyzed by neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) methods with PAUP* (Swofford, 1998), versions 4.0b2 and 4.0b4a. Complementary ML analyses, with the quartet puzzling method (ML_Q), and the Kishino and Hasegawa (1989) tests were conducted with TREE-PUZZLE 4.0.1 (Strimmer and von Haeseler, 1996). For ML analyses, different models of sequence evolution were compared with the likelihood ratio test (LRT) following the approach of Sullivan and Swofford (1997). After these comparisons, the most general model of sequence evolution available under the program considered was chosen (see Results for details): GTR under PAUP 4.0b2 and TN93 under TREE-PUZZLE 4.0.1 for nucleotides, and JTT for amino acids. Rate variation among sites was described by a gamma distribution with eight categories [Γ_8] (Yang, 1996). GTR and gamma parameters were optimized during a ML heuristic search with NNI branch swapping.

Neighbor-joining reconstructions were performed on GTR distances with gamma (Γ) rates or on constant site removal LogDet (LD) distances. Description of the substitution rate heterogeneities among sites by a gamma distribution improves phylogenetic reconstruction and dating estimation (Yang, 1996). Use of LogDet distances allows management of a data matrix for which the assumption of stationary base frequencies is violated (Lockhart *et al.*, 1994).

For standard MP analyses (i.e., equal weights for all nucleotide changes), heuristic searches were done with the TBR branch swapping option and 100 random addition of sequences. For weighted MP analyses (MP_w), each of the six nucleotide changes (e.g., A $\leftrightarrow$ G) at each of the three codon positions was weighted according to the product CI $\times$ S (e.g., for A $\leftrightarrow$ G changes at the first codon position, CI represents the consistency index excluding uninformative characters of the most parsimonious cladogram reconstructed only from first codon position A and G states, i.e., coding C and T as missing data in the matrix), and S represents the slope of the saturation plot between observed changes against inferred substitutions (Hassanin *et al.*, 1998a,b; Hassanin and Douzery, 1999). The cladograms used for calculating CI and S may be different for each of the 18 pairs of nucleotide changes. This point allows an *a priori* measurement of the homoplasy and saturation of each nucleotide change, instead of an *a posteriori* evaluation of the levels of homoplasy and saturation on a single MP tree (e.g., derived from a standard MP analysis).

The robustness of the nodes of the trees was assessed by (i) bootstrap percentages (BP; with PAUP 4.0b2) after 1000 replicates of resampling for NJ, 1000 for MP (one random addition of sequences; TBR branch swapping), and 100 for ML (NJ starting tree; TBR branch swapping); and (ii) reliability percentages (RP; with TREE-PUZZLE 4.0.1) estimated after 1000 ML quartet puzzling steps.

MOLPHY 2.3b3 (Adachi and Hasegawa, 1996) was used to write all the bifurcating topologies connecting the various major caviomorph and phiomorph clades. Likelihood scores were then compared by the one-tailed normal approximation test of the difference (δ) of two log-likelihoods (Kishino and Hasegawa, 1989).

Molecular Evolutionary Rates and Molecular Dating

The two-cluster and branch-length tests of the LINTRE package (Takezaki *et al.*, 1995) were used to identify species evolving significantly slower and faster relative to the others. Because of the substitution rate differences observed between vWF sequences, we adopted a compromise between constraining a unique substitution rate for all branches of the ML tree (i.e., setting a global clock) and having independent rates for each branch (i.e., no clock), by using the local clocks approach proposed by Yoder and Yang (2000). Local clocks were enforced with PAML, version 3.0b (Yang, 2000). Likelihood ratio tests (Felsenstein, 1988) between log-likelihoods of clock-constrained and non-clock-constrained trees allowed testing for the hypothesis of local molecular clocks in the vWF data.

Few data are available about the origin of the current hystricognath families, and a single calibration point was available for our clock-like tree: the caviomorph radiation at 31 Myr. The choice of this date is justified by (i) the occurrence of the first caviomorph fossil in the Tinguiririan (South American land mammal age, SALMA; 31–37 Myr; Wyss *et al.*, 1993), whereas all South American rodent families are identified in the Deseadan (SALMA; 24.5–29 Myr; e.g., Walton, 1997); and (ii) its compatibility with other mammalian divergence dates (Huchon *et al.*, 2000).

RESULTS

Phylogenetic Reconstructions

The vWF of *Heliophobius* was the single sequence among the 27 sequences having a base composition deviating from the frequency distribution assumed in the ML model (1% significance of a χ^2 test in TREE-PUZZLE 4.0.1). If the third codon positions were re-

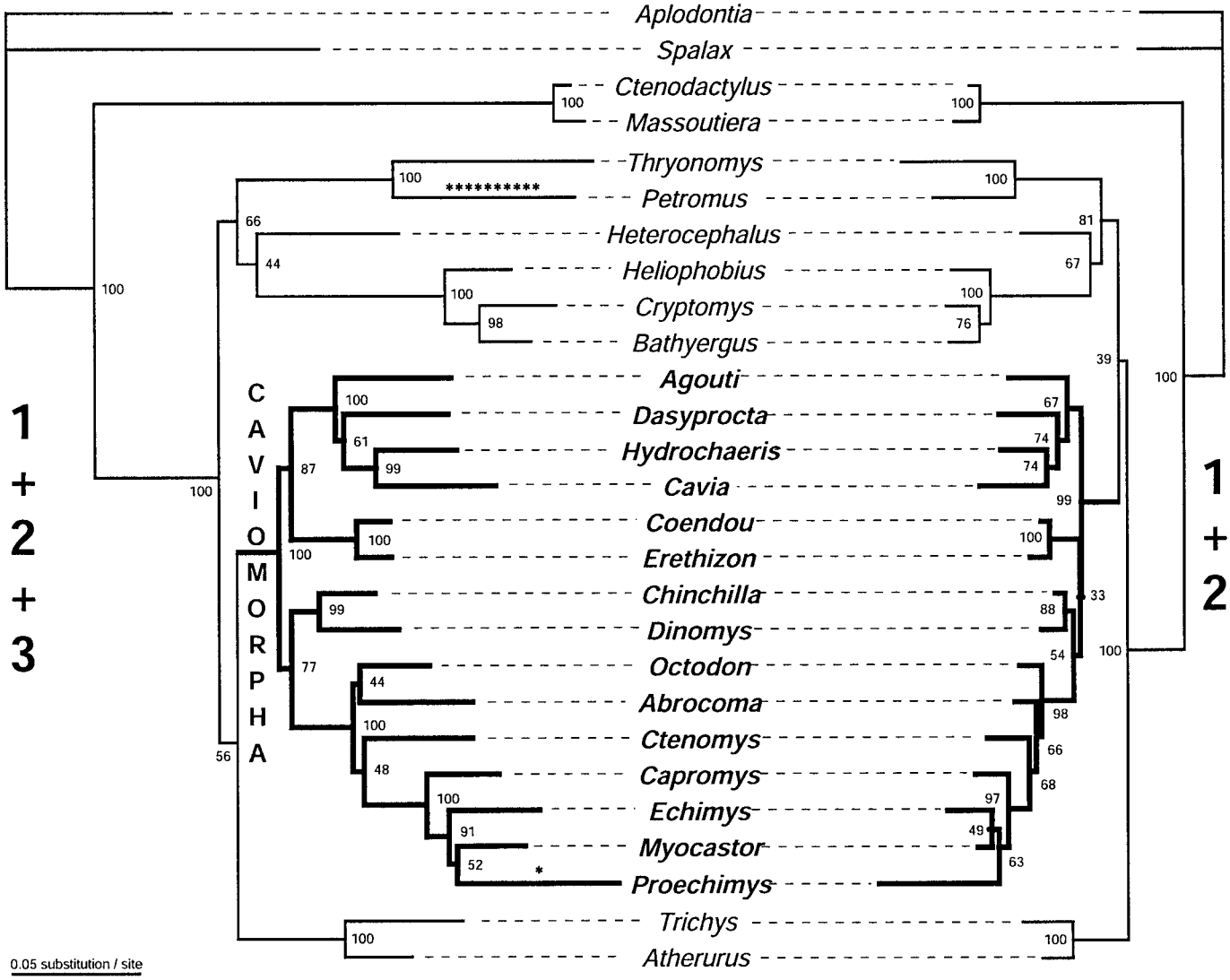

FIG. 1. Highest-likelihood phylograms of 23 hystricognath and 2 ctenodactylid vWF exon 28 sequences, rooted by *Aplodontia* and *Spalax*, reconstructed from all codon positions (left) and from first and second codon positions (right). The GTR model of sequence evolution with eight-categories gamma rates (parameter α) has been used for the two maximum-likelihood (ML) reconstructions, with the following values: (i) rate matrix = (1.27 [A ↔ C substitutions], 6.77 [AG], 0.90 [AT], 1.25 [CG], 9.28 [CT], 1.00 [GT]), and $\alpha = 0.49$ for all positions (lnL = −9599.86); (ii) rate matrix = (2.42, 8.02, 1.09, 2.76, 5.97, 1.00), and $\alpha = 0.50$ after removal of third codon positions (lnL = −4528.15). ML bootstrap percentages after 100 replicates (NJ starting trees and TBR branch swapping) are given at the nodes. Each star indicates one codon deletion. Branches leading to caviomorph species are thickened. Note that the scale is the same for both phylograms.

moved, no base composition deviation was observed. To evaluate the impact of third codon positions and the presence of *Heliophobius*, analyses were conducted with and without these characters and this taxon.

Different ML models have been compared to describe the data. First, the GTR model fitted the complete vWF better than TN93 when the highest-likelihood phylogram was considered (LRT statistics = 13.22, $P = 0.001$). Second, GTR + Γ_8 was better than GTR without rate heterogeneity (LRT = 997.20, $P < 0.001$). Third, GTR + Γ_8 + I was marginally not better than GTR + Γ_8 without invariable sites (LRT = 3.38, $P = 0.07$). Fourth, when third codon positions were excluded, the same results were observed. To run ML analyses with and without third codon positions, we chose the GTR + Γ_8 model as it resulted in a significantly better fit of the data relative to models with fewer parameters.

ML phylograms were rooted with *Aplodontia* and *Spalax* (Fig. 1). All ML reconstructions indicated that (i) Ctenodactylidae was the sister group of all hystricognaths which clustered in an unambiguously monophyletic group; (ii) Caviomorpha, here represented by at least one member of each living family, was monophyletic; (iii) Phiomorpha *s.l.* was composed of three distinct clades; Hystricidae, Thryonomyidae + Petromuridae (Thryonomyoidea), and Bathyergidae; the lat-

TABLE 2

Robustness Estimators for Representative Nodes of the Hystricognath vWF Tree, Deduced from Three Data Matrices: All Nucleotide Positions, First and Second Codon Positions, Amino Acids

Nodes	All codon positions					Codon positions one and two					Amino acids		
	NJ_Γ	NJ_{LD}	MP	MP_W	ML	NJ_Γ	NJ_{LD}	MP	MP_W	ML	NJ	MP	ML_Q
Petromus + *Thryonomys*	100	100	100	100	100	100	100	100	100	100	100	99	89
Bathyergidae	36	54	13	28	44	79	85	35	64	67	66	*	44
Phiomorpha *s.s.*	49	24	55	47	66	65	65	74	53	81	51	55	84
Caviomorpha	100	100	99	100	100	97	98	98	93	99	95	97	98
Caviomorpha + Phiomorpha *s.s.*	14	*	51	17	33	30	25	37	11	39	26	33	80
Caviomorpha + Hystricidae	80	92	36	68	56	53	67	37	27	48	67	47	*
Cavioidea	97	94	95	95	100	64	57	77	16	67	66	81	90
Chinchilla + *Dinomys*	91	98	90	99	99	82	89	86	85	88	95	79	84
Octodontoidea	100	100	100	100	100	98	98	100	97	98	95	98	94
Echimyidae + *Capromys* + *Myocastor*	100	100	100	100	100	92	88	95	95	97	87	93	91
Cavioidea + Erethizontidae	91	86	80	66	87	17	11	29	28	16	*	*	9
Chinchilla + *Dinomys* + Octodontoidea	*	*	50	62	77	30	29	44	15	54	19	54	10
Chinchilla + *Dinomys* + Erethizontoidea	*	*	*	*	*	36	44	11	8	*	67	24	73

Note. Bootstrap percentages are obtained from the majority-rule consensus trees. Minority percentages for alternative branchings are underlined. A star (*) indicates that the node is not supported by the corresponding bootstrap analysis. The different robustness estimators were obtained after bootstrap resampling with the following phylogenetic reconstructions (from left to right in the table): neighbor-joining (NJ_Γ) on gamma distances ($\alpha = 0.49$ or 0.50, with or without third codon positions), NJ on LogDet distances (NJ_{LD}), standard maximum-parsimony (MP), MP weighted (MP_W) by the CI × S product (see text for details), maximum-likelihood (ML) with heuristic searches, NJ on mean character changes (NJ), and ML with quartet puzzling (ML_Q).

ter two were moderately associated in a clade here called Phiomorpha *sensu stricto*; (iv) within Bathyergidae the relationships conformed to Faulkes *et al.* (1997) observations based on cytochrome *b* and 12S rRNA; *Bathyergus* and *Cryptomys* clustered together, with *Heliophobius* being outside, and *Heterocephalus* being in a very distant sister group position; (v) Caviomorpha contained four major clades; New World porcupines (Erethizontidae), Cavioidea, Octodontoidea, and an unexpected association between *Chinchilla* and *Dinomys*; (vi) within Cavioidea, *Agouti* and *Dasyprocta* were sister group to a *Cavia* plus *Hydrochaeris* clade; and (vii) within Octodontoidea, *Octodon*, *Abrocoma*, and *Ctenomys* were sister group to a robustly defined clade including Capromyidae, Echimyidae, and Myocastoridae: the Echimyidae *sensu lato*.

Comparison of the phylogenies reconstructed with and without third codon positions reveals a general agreement, with exceptions at two levels (Fig. 1). At the topological level, the removal of third positions induced several shifts: (i) Hystricidae moved from a sister relationship with caviomorphs to a basalmost position among hystricognaths; (ii) Erethizontidae moved from a sister relationship with Cavioidea to one with Octodontoidea + *Chinchilla* + *Dinomys*; and (iii) *Myocastor* moved from a clustering with *Proechimys* to one with *Echimys*. At the node robustness level, the removal of third codon positions (i) increased the support for Bathyergidae (BP = 67 vs 44) and Bathyergidae + Thryonomyoidea (BP = 81 vs 66) and (ii) decreased the support for Cavioidea (BP = 67 vs 100) and Octodontoidea + *Chinchilla* + *Dinomys* (BP = 54 vs 77).

All (other) phylogenetic reconstructions—based on nucleotides with and without *Heliophobius* sequences (data not shown), third codon positions, or amino acids—evidenced the same robust nodes (Table 2). The best-supported clades (BP > 85) were Thryonomyoidea, Caviomorpha, Octodontoidea, and Echimyidae + Capromyidae + Myocastoridae, and then *Chinchilla* + *Dinomys* (99 > BP > 79). Few discrepancies occurred for weakly supported interrelationships, such as those between the main hystricognath lineages and the caviomorph superfamilies (Table 2).

Tests of Alternative Hypotheses

The reference topology used for all statistical comparisons was rooted by the four sciurognath sequences (two ctenodactylids, one aplodontid, and one spalacid), and weakly supported nodes were represented by multifurcations. Three data matrices were used: nucleotides with or without third positions and amino acids.

Within hystricognaths, phylogenetic analyses identified seven major clades of family or superfamily rank: three belonged to Phiomorpha *s.l.* (Hystricidae, Bathyergidae, and Thryonomyoidea) and four to Caviomorpha (Cavioidea, Erethizontidae, Chinchillidae + Dinomyidae, Octodontoidea). The 10,395 bifurcating topologies connecting these seven clades were evaluated by ML with TREE-PUZZLE 4.0.1; the TN93 and gamma rates parameters were set to the values estimated for the best quartet puzzling tree. At the nucleotide level with all codon positions, the best alternative tree showing the paraphyly of Caviomorpha had a log-likelihood 2.09 standard error (SE) worse (probability

of the one-tailed Kishino–Hasegawa test: $P_{KH} < 0.02$) than the log-likelihood of the best tree (Fig. 1). Similarly, for codon positions one and two and at the amino acid level, the disruption of the caviomorph monophyly involved a dramatic drop in log-likelihood, which was, respectively, at least 2.29 SE and 2.24 SE worse ($P_{KH} < 0.02$) than that of the best tree. Altogether, the vWF data show that the hypothesis of a paraphyly of caviomorphs is strongly rejected.

After log-likelihood ranking of the 10,395 topologies, we independently evaluated ML parameters with PAML 3.0b for all codon positions on three selected trees: the best tree (lnL = −9605.61, Ti/Tv = 3.341, $\alpha = 0.492$), the first tree disrupting Caviomorpha monophyly (lnL = −9626.46 [$P_{KH} = 0.01$], Ti/Tv = 3.339, $\alpha = 0.489$), and the worst tree (lnL = −9668.48 [$P_{KH} < 0.0001$], Ti/Tv = 3.366, $\alpha = 0.477$). It appeared *a posteriori* that variations in parameter estimates were very slight. Identical results were obtained for codon positions one and two and amino acids. It is therefore likely that the confidence probabilities of the Kishino–Hasegawa comparisons were not affected by the use of the same ML parameters for different topologies.

The identification of the phylogenetic relationships among the three phiomorph clades and the caviomorphs was less conclusive. None of the 15 topologies connecting these four lineages appear to be significantly worse than the best tree at the 5% threshold. The two best competing topologies showed either Hystricidae or Phiomorpha *s.s.* (Thryonomyidae + Bathyergidae) as the basalmost clade among hystricognaths (Fig. 1). We note that the Kishino–Hasegawa tests rank similarly the competing topologies (except for the best trees), irrespective of the inclusion or exclusion of third codon positions. In contrast, alternative topologies are not similarly ranked when amino acids are used. For this reason no reliable conclusion can be drawn concerning the order of the first hystricognath splits.

About the caviomorph relationships, various hypotheses suggested by morphology and paleontology were also tested. (i) The grouping of *Abrocoma* and *Chinchilla* (e.g., McKenna and Bell, 1997) was significantly worse than the reference topology clustering *Chinchilla* and *Dinomys* ($P_{KH} < 0.002$), whatever the data matrix considered and whatever the phylogenetic position of the *Abrocoma* + *Chinchilla* clade among the other caviomorph lineages (i.e., Dinomyidae, Cavioidea, Erethizontoidea, and Octodontoidea). (ii) The association of Chinchillidae, Dinomyidae, Agoutidae, and Dasyproctidae in the same clade (e.g., Wood and Patterson, 1959) is also significantly rejected ($P_{KH} < 0.02$). (iii) The clustering of Dinomyidae with Erethizontidae (Grand and Eisenberg, 1982) was significantly worse than the best tree when all codon positions were considered ($0.002 < P_{KH} < 0.03$), but marginally worse with codon positions one and two ($0.04 < P_{KH} < 0.09$) and with amino acids ($0.02 < P_{KH} < 0.09$). (iv) The affinity between Dinomyidae and Cavioidea (McKenna and Bell, 1997) yielded a topology significantly worse ($P_{KH} < 0.05$) than the best one associating Dinomyidae + Chinchillidae, when all codon positions were analyzed. When codon positions one and two or amino acids were considered, all trees disrupting the *Chinchilla* + *Dinomys* clade were not significantly different from the best tree ($P_{KH} < 0.16$). (v) Among Octodontoidea, the 3 possible topologies associating *Proechimys*, *Echimys*, and *Myocastor* were not significantly different ($P_{KH} > 0.25$). This was also the case for the 15 topologies associating Octodontidae, Abrocomidae, Ctenomyidae, and Echimyidae *s.l.* ($P_{KH} > 0.06$). It was noteworthy that with the nucleotide data sets, the best topologies describing the relationships among the four main Octodontoidea taxa always clustered either *Ctenomys* or *Abrocoma* with the Echimyidae *s.l.* In other words, the vWF analyses suggested a basal emergence of the Octodontidae among the Octodontoidea.

Finally, the likelihood of the 15 possible trees clustering the four caviomorph lineages were compared. None of them was significantly better than the others, but all topologies having a $\delta \ln L < 1$ SE (whatever the characters considered) clustered Octodontoidea with Chinchillidae + Dinomyidae.

Seeking a Molecular Clock

The highest-likelihood phylograms reconstructed from codon positions one and two (Fig. 1) and amino acids (best topology identified by the Kishino–Hasegawa tests) were taken as a reference, as both displayed the same topology. The two slowest-rate species (*Aplodontia*, *Spalax*) and the fastest species (*Proechimys*; Fig. 1) were first discarded to maximize the probabilities of obtaining a clock-like tree. Then, the two-cluster and the branch-length tests indicated that several branches and taxa displayed significantly contrasted amino acid vWF substitution rates: slower for *Chinchilla* + *Dinomys* ($P_{LINTRE} < 0.01$), *Trichys* + *Atherurus* ($P_{LINTRE} = 0.02$), and *Heterocephalus* ($P_{LINTRE} = 0.03$), and higher for *Echimys* ($P_{LINTRE} < 0.01$). Finally, local clocks corresponding to the heterogeneous evolving branches were enforced with PAML 3.0b.

The hypothesis of five local clocks was accepted for amino acids, with rates of $r_1 = 0.41$ (*Trichys* + *Atherurus*), $r_2 = 0.55$ (*Heterocephalus*), $r_3 = 0.34$ (*Chinchilla* + *Dinomys*), $r_4 = 3.41$ (*Echimys*), and $r_0 = 1.00$ (default rate for the remaining species): lnL = −4090.59 with clock vs −4070.65 without clock (LRT statistics = 39.88, $df = 27$, $P = 0.05$). For nucleotides (codon positions one and two), local clocks were enforced for the same taxa, but the clock hypothesis was

accepted only after the introduction of an additional rate (r_5) for *Coendou* + *Erethizon*, i.e., the two slowest-evolving caviomorphs after *Chinchilla* + *Dinomys* (cf. Fig. 1, right). The following rates were calculated: $r_0 = 1.00$, $r_1 = 0.64$, $r_2 = 0.54$, $r_3 = 0.45$, $r_4 = 2.01$, and $r_5 = 0.56$ (lnL = −5544.07 with clock vs −5523.75 without clock; LRT statistics = 40.64, $df = 28$, $P = 0.06$).

After the local clocks were calibrated by the caviomorph radiation at 31 Myr, close divergence date estimates were computed from either amino acids or codon positions one and two. Except for erethizontoids, chinchilloids, and octodontoids, amino acids gave older date estimates, and the deeper the node the greater the difference of estimation (Fig. 2). According to the vWF clocks, the first splits in the Hystricognathi tree leading to the hystricid, phiomorph *s.s.* lineages, and caviomorphs occurred during Paleocene to Middle Eocene (63–43 Myr). Bathyergidae split from Thryonomyoidea in the Middle Eocene (48–41 Myr). From 43 Myr (younger estimate) to 31 Myr, Caviomorpha did not produce lineages that are still living. Actually, their first diversification occurred after colonization of South America, during the Late Oligocene. Octodontoid radiated at the Early/Middle Miocene transition. Closer genera—such as *Coendou* and *Erethizon* or *Myocastor* and *Echimys*—separated during Late Miocene or Plio–Pleistocene.

DISCUSSION

A Single Colonization Event of South America by Caviomorphs

The sampling of all living caviomorph and phiomorph families warranted an exhaustive consideration of Caviomorpha monophyly. The vWF phylogeny strongly supports the monophyly of Caviomorpha, and all alternatives are significantly less likely. This indicates that all living caviomorphs have a single origin, therefore reflecting a single colonization event of South America by hystricognath rodents. This molecular result contradicts the view of a reciprocal paraphyly of caviomorphs and phiomorphs, and of a double invasion of hystricognaths into South America, suggested for example by myology (Woods and Hermanson, 1985), arterial patterns (Bugge, 1985), cranial characters (Bryant and McKenna, 1995), and parasitology (Quentin, 1973; Hugot, 1982). One should note that these studies, supporting caviomorph paraphyly, are not mutually congruent. Studies on muscles, arteries, skulls, and teeth all suggest that Erethizontidae might be one of the earliest (or the earliest) hystricognath lineages (Woods and Hermanson, 1985; Bryant and McKenna, 1995). However, parasitology gives a different conclusion because Dinomyidae, Erethizontidae, Hystricidae, and the sciurognath Pedetidae share closely related endoparasites (Hugot, 1982).

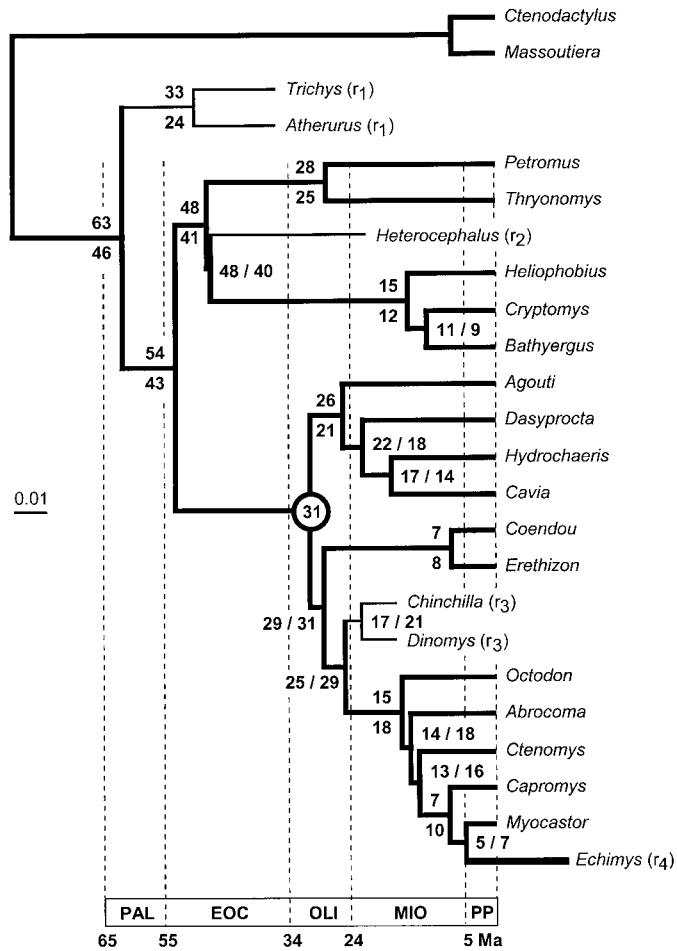

FIG. 2. Local clocks in the vWF maximum-likelihood tree showing the time frame for the radiation of the main hystricognath families. Divergence ages in million years deduced from amino acids and codon positions one and two are, respectively, given above and below branches or at the left and right of slashes. The white circle indicates the calibration point: diversification of caviomorph lineages at 31 Myr. The ML topology is deduced from analysis of amino acids and nucleotides, and branch lengths are computed after the amino acids matrix (scale: number of substitutions per site). The thickness of branches is proportional to the amino acid local clock rates: $r_1 = 0.41$, $r_2 = 0.55$, $r_3 = 0.34$, $r_4 = 3.41$ (see the text for details). The vertical dashed lines indicate the time frame for the remaining species, all evolving with the default local clock ($r_0 = 1.00$). The absolute ages (Ma) and the name of the main Tertiary divisions are given (epochs: PAL, Paleocene; EOC, Eocene; OLI, Oligocene; MIO, Miocene; PP, Plio–Pleistocene).

Subsequent Adaptative Radiations of Caviomorphs in the New World

Caviomorph invaders likely replaced South American endemic species of the Paleocene and Eocene in their ecological niches (Flynn and Wyss, 1998). Their arrival has been thought to have caused the extinction of small mammals, such as rodent-like marsupials. The success of caviomorphs in the South American environment led to their diversification into four extant groups that are identified since the Early Oligocene: erethi-

zontoids, cavioids, chinchilloids, and octodontoids (Fig. 1). Disagreements between morphopaleontological systematics (e.g., McKenna and Bell, 1997; Table 1) and molecular data (Fig. 1) involve the content of these four groups.

Chinchilloidea (Dinomyidae + Chinchillidae). The systematic position of Dinomyidae has been intensely debated. Our vWF results suggest the clustering of Dinomyidae (pacaranas) with Chinchillidae (chinchillas) to form the Chinchilloidea. This rejects the possibility of a sister group relationship between Dinomyidae and Erethizontidae and indicates that the fusion of the second and third cervical vertebrae shared by *Dinomys, Erethizon*, and *Coendou* (Ray, 1958; cited in Woods and Hermanson, 1985, p. 533) likely represents a convergence, as for their many other morphological similarities (reviewed in Grand and Eisenberg, 1982). Likewise, the fact that Dinomyidae and Erethizontidae share the same pinworm parasites (Nematoda: *Wellcomia*; Quentin, 1973; Hugot, 1982) can be explained by a horizontal transfer. For example, Patterson and Wood (1982, p. 474) indicate that the pinworm of *Erethizon* also infects the domestic cat.

Dinomyidae has also been considered to be related to Cavioidea or even included within them (McKenna and Bell, 1997). Wood and Patterson (1959) identified similarities in the dental patterns of Dinomyidae, Agoutidae, and Dasyproctidae together with Chinchillidae. Later, Patterson and Wood (1982) estimated that the three former families should in fact be the sister clade of Caviidae + Hydrochaeridae. In the present study, all alternative phylogenetic positions of Dinomyidae were significantly less likely than those of the best tree when the three codon positions were analyzed, but not when the two first codon positions alone or the amino acids were considered. Consequently, even if the vWF strongly supports the unexpected relationship of Chinchillidae with Dinomyidae, it will need to be confirmed by additional molecular analyses based on genetically independent genes.

The first Dinomyidae fossils were discovered in the "Mayoan" Middle Miocene (10–12 Myr; Vucetich *et al.*, 1999). This paleontological date is younger than our molecular estimation of a *Chinchilla–Dinomys* split between 17 and 21 Myr (Fig. 2), therefore suggesting that there is a gap in the Dinomyidae fossil record.

The second new phylogenetic result suggested by vWF sequence comparisons is the strong rejection of a relation between Chinchillidae and Abrocomidae (e.g., McKenna and Bell, 1997). We rather suggest the inclusion of Abrocomidae among Octodontoidea as previously suggested by dental characters (Martin, 1994).

Octodontoidea. Octodontoidea is the most diversified caviomorph clade, with a first radiation having produced the three extant families Octodontidae, Abrocomidae, and Ctenomyidae and a fourth lineage, which subsequently diversified into Capromyidae, Myocastoridae, and Echimyidae (Fig. 1). The existence of the latter subclade is well defined by vWF (Table 2) and supported by dental and myological studies (Woods and Hermanson, 1985). Myocastoridae has been considered either an independent family or part of the Capromyidae or an Echimyidae subfamily (e.g., Patterson and Wood, 1982; Wilson and Reeder, 1993; McKenna and Bell, 1997). In conjuction with immunological data (Sarich, 1985), the vWF results suggest the merging of Myocastoridae within Echimyidae (Fig. 1). Similarly, Capromyidae has been thought to be an Echimyidae subfamily (e.g., Patterson and Wood, 1982). The vWF trees here suggest a distinct position for *Capromys*, possibly at the family level (Fig. 1).

Based on the local clock analysis, the split between Capromyidae and Echimyidae + Myocastoridae is estimated to be 7–10 Myr old (Fig. 2). These dates are younger than the paleontological record, which gives an Early Miocene age for the first Myocastoridae (21–19 Myr) and Capromyidae (19–16.3 Myr) fossils (McKenna and Bell, 1997). This indicates that the molecular clock might not click regularly along this lineage (cf. the long *Echimys* branch) and/or that the taxonomic position of fossils may need to be reexamined (at least for the first Myocastoridae). Anyway, the close molecular relationship among these species and the fact that echimyid fossils are known since Late Oligocene (29–24 Myr) suggest that Echimyidae, Myocastoridae, and Capromyidae are part of the same taxonomic group, which should be further sampled in future phylogenetic studies.

Ctenomyidae was considered either an independent family or an Octodontidae subfamily (Pascual *et al.*, 1965; cited in Lessa and Cook, 1998). In agreement with Nedbal *et al.* (1994) and Lessa and Cook (1998), our results support the ranking of ctenomyids at the familial level (Fig. 1). Furthermore, Abrocomidae (chinchilla rats) might be more related to Octodontidae than to Ctenomyidae (analysis of all codon positions). *Abrocoma* is a chinchilla-like animal, living in the Cordillera from south Peru and Bolivia to north Argentina and Chile. Only a few morphological studies have addressed the issue of Abrocomidae origins (e.g., Martin, 1994), and we note that this family was not investigated in Luckett and Hartenberger (1985). We here include for the first time one *Abrocoma* sequence in a molecular analysis. We suggest that *Abrocoma* is one of the main lineages produced by the Octodontoidea radiation. Consequently, it appears important that future evolutionary studies involving octodontids also include Abrocomidae representatives.

The association of Abrocomidae with Octodontidae is surprising because the latter family shares various synapomorphies with Ctenomyidae, such as highly derived kidney-shaped molars. One should note that the grouping of Octodontidae with Ctenomyidae is not a

significantly less likely alternative than groupings in the best tree and that all taxa at the base of the Octodontoidea radiation are slowly evolving, most particularly the Octodontidae. We cannot exclude the possibility that the topology within Octodontoidea is the result of a long-branch attraction phenomenon, clustering Ctenomyidae with the fast-evolving Echimyidae *s.l.* Additional sampling within these families might help to resolve this issue.

Cavioidea. The molecular trees robustly cluster Caviidae, Hydrochaeridae, Dasyproctidae, and Agoutidae to form the Cavioidea clade (Fig. 1, all codon positions). This is in agreement with the morphology-based systematics, except for the Dinomyidae (see above). Among the former four families, Caviidae and Hydrochaeridae appear to be a sister group, as suggested by morphological studies (Wood and Patterson, 1959). Dasyproctidae has been regarded an Agoutidae subfamily (see comments in Wilson and Reeder, 1993; McKenna and Bell, 1997). The vWF analysis suggests the ranking of Dasyproctidae at the family level as this taxon does not cluster with Agoutidae. However, alternative hypotheses are not significantly less likely, indicating that longer nuclear sequences are required to reach higher confidence levels.

Erethizontoidea. It should be noted that South American porcupines are not the most divergent hystricognaths as suggested by morphological studies (Bryant and McKenna, 1995; Lavocat and Parent, 1985). Erethizontoids are part of the caviomorph radiation, but additional molecular studies should be conducted to evaluate whether they diverged slightly before the other caviomorph superfamilies as suggested by Bugge (1985).

Finally, the pattern of radiation of the four major caviomorph clades—Chinchilloidea, Octodontoidea, Cavioidea, and Erethizontoidea—is difficult to establish because of the lack of robust resolution and conflicting branching order between topologies reconstructed with and without third codon positions (Fig. 1). An association between Chinchilloidea and Octodontoidea is, however, suggested at the DNA level, with greater support when third codon positions are included (Fig. 1).

Timing of the Caviomorpha Radiations

The Caviomorpha subtree presents three ambiguous branching orders, representing either a lack of resolution of the vWF and/or different radiation events. The three branching points are those of the Caviomorpha superfamilies, the Octodontoidea families, and the Echimyidae genera (including *Myocastor*). The two former events might be related to two ecological events. (i) The arrival of caviomorphs in South America corresponds to a cooling period (36–25 Myr) in which indigenous species developed hypsodont teeth as an adaptative response to environmental modifications (Kay *et al.*, 1999). The first caviomorph fossils are high crowned (i.e., hypsodont; Wyss *et al.*, 1993) and many genera from the Deseadan display a high degree of hypsodonty (Vucetich *et al.*, 1999). Climatic changes may have been responsible for the success of the caviomorphs and their fast diversification. (ii) Important climatic changes contemporary to the Quechua phase of the Andean orogeny have been described during Middle Miocene (e.g., Vucetich *et al.*, 1999). Such climatic perturbations may have allowed for the occurrence of species adapted to new ecological conditions, in association with environmental barriers preventing north–south intermigration that have been described at the same time (16–11 Myr) (Walton, 1997). Our datings suggest 13–18 Myr for the origin of modern octodontoids (Fig. 2), in agreement with Vucetich *et al.* (1999), who correlated the diversification of Octodontoidea with the Middle Miocene climatic changes.

What Does vWF Tell Us about Phiomorpha Phylogenetics and Evolution?

The vWF data confirm that the two African families Petromuridae and Thryonomyidae are closely related and justify their grouping into the Thryonomyoidea superfamily, as previously indicated by fossil and mitochondrial data (Lavocat, 1973; Nedbal *et al.*, 1994). The sister group of Dassie rats and cane rats is shown to be the Bathyergidae, as previously suggested by Lavocat (1973) and Nedbal *et al.* (1994). One should note that the association of Bathyergidae and Thryonomyoidea suggests an African but not an Asian origin of Bathyergidae (Winkler, 1994).

Highest-likelihood phylograms suggest that Phiomorpha *s.l.* contains two major clades (Hystricidae, Bathyergidae + Thryonomyoidea) whose relationships with caviomorphs are sensitive to the molecular characters considered (Fig. 1). Consequently, it would be better to restrict the use of the term "Phiomorpha" to the Thryonomyoidea + Bathyergidae clade, excluding the Hystricidae. The latter family is a puzzling taxon with an enigmatic origin. Hystricidae fossils are known only since the Miocene, when they appear simultaneously in Asia, Europe, and Africa (McKenna and Bell, 1997). vWF sequences indicated that hystricids are part of the basal hystricognath radiation, suggesting that the origin of the group is much older than the Miocene and is possibly of Eocene age.

All the molecular dates for the Phiomorpha *s.s.* splits (Fig. 2) appear to be older than the relevant dates in the fossil record. The vWF sequences propose (1) an Early to Middle Eocene age for the Thryonomyoidea/Bathyergidae divergence vs a Late Eocene–Early Oligocene paleontological record for the first hystricognath, (2) an Eocene age for the first Bathyergidae split (but this might reflect the deep clustering of *Heterocephalus* with the remaining Bathyergidae) with first

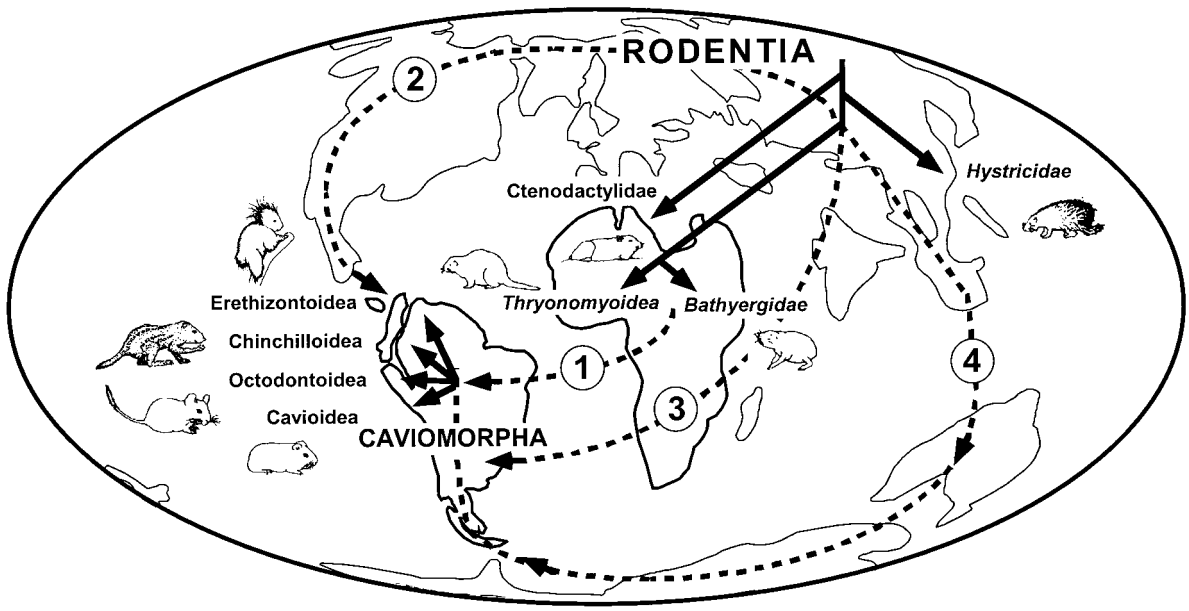

FIG. 3. Four hypotheses for the migration routes of the hystricognath rodents during the Eocene (55–34 Myr). The vWF phylogenetic tree for amino acids and codon positions one and two was superimposed on a Middle Eocene tectonic plate distribution map (after Smith *et al.*, 1994) according to the localization of the fossils of each family at the end of the Eocene. Phiomorpha *s.l.* rodents are italicized. The solid arrow lines indicate the currently recognized routes of migration. The dashed lines suggest four hypothetical routes of migration of the caviomorphs. Route 1: Caviomorpha originated from Africa and directly crossed the Atlantic ocean to reach South America (Lavocat, 1969). Route 2: Caviomorpha originated from Asia (e.g., Hussain *et al.*, 1978) and migrated through North America (cf. Wood, 1985). Route 3: Caviomorpha originated from Asia (cf. Hussain *et al.*, 1978) and migrated to South America through Africa (cf. Lavocat, 1969). Route 4: Caviomorpha originated from Asia and migrated to South America through Australia and Antarctica, after two ocean crossings (cf. Houle, 1999). One should note that the possibility of migration from Africa to Antarctica and then to South America has never been proposed. These migration routes are drawn to explain the current distribution of living phiomorphs and caviomorphs in Africa and South America (bold outlines). Note that some living hystricids and erethizontids are also distributed, respectively, in Africa and North America.

Bathyergidae fossils known only since the Early Miocene, and (3) a Petromuridae–Thryonomyidae split during the Oligocene vs a Pleistocene age for the first fossil Petromuridae. Gaps in the fossil record can be invoked to explain these discrepancies. However, the fast-evolving Bathyergidae and Thryonomyoidea sequences (cf. Huchon *et al.*, 2000) might have increased the depth of the split between the two groups and subsequently affected the divergence date estimations.

The Potential Colonization Routes of Caviomorphs to South America

Based on paleontological data, an Asian origin for hystricognaths is suggested (Flynn *et al.*, 1986; Bryant and McKenna, 1995). In sharp contrast, however, the migration patterns to explain the current distribution of most phiomorphs in Africa and caviomorphs in South America remain moot. The current consensus (Lavocat, 1969; Martin, 1994) proposed that caviomorphs originated from an African phiomorph stock and directly migrated to South America by rafting over the Atlantic ocean (Fig. 3, route 1). Despite the fact that Africa and South America were separated by the Atlantic oceanic barrier (i.e., at least 1000 km) when colonization took place, the probability of a successful colonization event might have been increased by the existence of marine currents, paleowinds, "stepping stone" islands, and rafts carried by tropical rivers, combined with dramatic climatic and oceanographic changes at the Eocene/Oligocene transition (Wyss *et al.*, 1993; Flynn and Wyss, 1998; Houle, 1999).

Our molecular data indicate an almost contemporary origin for hystricognaths (46–63 Myr) and caviomorphs (43–54 Myr). Given the probable Asian origin of hystricognaths and the short time span between the origin of hystricids and that of caviomorphs (cf. Fig. 2), our data suggest an Asian rather than an African origin for the Caviomorpha. This hypothesis has already been suggested by paleontology. Hussain *et al.* (1978) proposed an Asian origin for hystricognaths, with subsequent colonization(s) in South America through North America for caviomorphs (Fig. 3, Route 2) and migration in Africa for phiomorphs. The drawback of this scenario is the lack of obvious hystricognath fossils in North America (Meng, 1990; Martin, 1994). Given this, we cannot exclude the possibility that Caviomorpha might also have originated in Asia (cf. Hussain *et al.*, 1978) but migrated through Africa (Fig. 3, Route 3) to South America (cf. Lavocat, 1969). This would involve the paraphyly of African hystricognath fossil taxa. Interesting to note, another alternative, namely a

southern migration route for eutherian mammals from Asia to South America, through Australia and Antarctica, has never been extensively investigated (Fig. 3, Route 4). Until the Late Eocene (41–34 Myr), ecological conditions in both Australia and Antarctica were favorable for placental fauna, and Antarctica was connected to South America (Houle, 1999; Kay *et al.*, 1999). To date, no rodent fossils have been found in Australia or Antarctica, but the mammalian fossil record is scarce for the Early and Middle Eocene for these continents. All Eocene Australian fossils come from the Murgon faunal zone, which has a minimum age estimate of 54.6 Myr (Godthelp *et al.*, 1992), and all Tertiary mammalian fossils found in Antarctica date to the Late Eocene deposits at Seymour Island (Kay *et al.*, 1999). Our data indicate a minimum time lag of 12 Myr (43 to 31 Myr; Fig. 2) between the divergence of caviomorphs relative to phiomorphs and their subsequent South American diversification. We therefore suggest that Caviomorpha might have originated in Asia and followed an Australian–Antarctic migration route to reach South America (Fig. 3, Route 4) during the 12 Myr in the Middle and Late Eocene (Fig. 2).

CONCLUSIONS

Nedbal *et al.* (1994) concluded from their mitochondrial 12S rRNA analysis that the rapid radiation of the caviomorphs, the number of taxa analyzed, and the long branches in the ingroup might explain the lack of resolution for some caviomorph relationships. In their caviomorph subtrees including 10 families, a single node (Octodontoidea) was supported by more than 60% of bootstrap. Our improvements in the reconstruction of Caviomorpha relationships indicate that, despite all these constraints, the use of a nuclear DNA marker, here the vWF, provides molecular signal pertaining to the phylogeny of this group.

Our main results involve (1) the division of Hystricognathi into three clades, Hystricidae, Phiomorpha *s.s.*, and Caviomorpha; (2) the validation at the molecular level of a four-clade division within Caviomorpha; (3) the grouping of Dinomyidae with Chinchillidae; (4) the recognition of the monophyly of Echimyidae + Capromyidae + Myocastoridae; and (5) the suggestion that Caviomorpha might not have an African but rather has an Asian origin. These results will need to be validated by additional independent molecular data and by paleontological and morphological observations. The study of the phylogeny of Primates, with special focus on Platyrrhini and Catarrhini, appears complementary to better understand the biogeographical relationships between South America and the other land masses during the Tertiary.

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