

Evolution of isoesterase tissue expression patterns in *Cavia* (Caviidae, Rodentia)

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ABSTRACT

We conducted cladistic analysis of isoesterase (EC. 3.1.1.1) gene expression in six tissues for four species of *Cavia*, to construct a phylogenetic hypothesis for these species and used it to evaluate the dynamics of differentiation in isoenzyme tissue expression patterns (TEP) and their value for phylogenetic reconstruction. The characters considered were the intensity and the presence or absence (P/A) of expression of each isoenzyme locus in each tissue. Twelve of the 19 putative loci presented interspecific variation in at least one tissue. The phylogenetic relationship obtained from 19 intensity characters and eight P/A characters was: ((*C. aperea*, *C. porcellus*), (*C. fulgida*, *C. magna*)). The cladograms' retention indices were 0.8 for the 19 unordered intensity characters and 1.0 for the eight P/A characters. The results of the present and other works suggest that patterns of gene expression may be used for phylogenetic inference to a wide range of taxonomic levels, from closely related species to the family or higher levels, depending upon the rates of evolution and the age of each group. It was also found that *C. magna* presented a significantly higher rate of evolution ($P < 0.01$) for the esterase gene expression than the sister species, *C. fulgida*. This suggests a non-uniform rate of TEP regulatory evolution and that one should not assume equal rates of evolution among lineages to infer phylogenies from this kind of data.

INTRODUCTION

Mindell and Sites (1987), studying isoenzyme tissue expression patterns (TEP) variation in 13 avian genera from two orders and Thorpe and Dickinson (1988) studying TEP in 24 Hawaiian picture-winged *Drosophila* concluded that these patterns have poor phylogenetic utility at these taxonomic levels, due to widespread homoplasies. Both studies leave open the possibility that these regulatory characters may be useful for phylogenetic studies at lower taxonomic

levels and less divergent groups. On the other hand, Whitt (1987) found multilocus isoenzyme characters useful for higher level phylogenetic inference. Kettler *et al.* (1986) found that a phylogeny based on a kind of "regulatory distance", calculated from the expression of 27 enzymes in six tissues of four species of the fish family Umbridae, was similar to that based on morphological and structural gene differences. Some investigators have suggested the existence of a kind of "regulatory clock" for the rate of evolution of gene expression in some organisms (Ferris and Whitt, 1979; Whitt, 1987). Little if any, of this kind of study has been done in mammals.

Cavia Pallas is a small South American rodent genus of the family Caviidae (see Graur, 1993, for a hypothesis that Caviomorpha originated independently from the other rodents). No recent comprehensive taxonomic revision exists for the genus and thus there

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is no consensus on the number of valid species. The Brazilian forms were revised by Ximenez (1980) who recognized three species, *C. aperea* (with two subspecies), *C. fulgida* (= *C. rufescens*), and *C. magna*. Some evidence points to the existence of a different species (*C. tschudii* = *C. cutleri*) in the central Andean region, while others suggest that these animals are conspecific with *C. aperea* (Gilmore, 1950; Hückinghaus, 1962; Weir, 1974). Central Andes is considered to be the most likely area for the beginning of the domestication process of the guinea-pig *C. porcellus* from specimens of wild *C. aperea* (or from *C. tschudii*, Gilmore, 1950; Weir, 1974). The status of the guinea pig is also controversial. While most investigators gave it a specific status as *C. porcellus* (Weir, 1974) others suggest it should be considered a subspecies of *C. aperea* (Hückinghaus, 1962).

MATERIAL AND METHODS

Adult individuals of both sexes of *Cavia aperea*, *C. fulgida*, and *C. magna* (Ximenez, 1980) were collected in the field in the following localities (n = sample size) in south and southeast Brazil: *C. aperea*: Tramandaí, Rio Grande do Sul (RS) (n = 1), Dois Irmãos, RS (n = 13); *C. magna*: Tramandaí, RS (n = 12), Criciúma, Santa Catarina (n = 2); *C. fulgida*: Roça Nova, Paraná (PR) (n = 4), Morretes, PR (n = 5); and Poços de Caldas, Minas Gerais (n = 1). The 12 individuals of *C. porcellus* were of the guinea pig laboratory strain "Dunkin-Hartley".

Blood and tissue extraction and preparation were performed as described in Schneider *et al.* (1983) and the electrophoretic determinations according to Monjeló and Cordeiro (1979), with minor modifications. The patterns of the esterase system (EC. 3.1.1.1) were determined by electrophoresis in horizontal polyacrilamide gel and staining according to Beckman and Johnson (1964), including α and β -naphthyl acetate, for each of six tissues: liver, kidney, mid intestines, pancreas, heart, and plasma. The intensity of expression of each isoenzyme was divided into four states, absent (0), weak (1), medium (2), and strong (3), by visual inspection.

For the phylogenetic analysis of the TEP data each locus/tissue combination is a character (Buth, 1984) and the character states are the four intensities of expression cited above. The multistate characters (those with more than two intensity states) were considered as unordered (that is, the change of any state of expression to any other has the same value) or ordered according to the intensity values in two

independent evaluations. An analysis was also done transforming the intensity characters in a presence/absence (P/A) data set.

The phylogenetic inferences were performed with the PAUP program (version 2.4.1, available from D.L. Swofford, Lab. of Molecular Systematics, Smithsonian Institution, Washington, D.C., USA), using the Wagner (unrestricted) parsimony criterion. For all analyses the **bandb** and **mulpars** options of tree construction were used to find all the most parsimonious trees and the **blrange** command was employed to assess the maximum and minimum changes in each branch of the trees. The retention index (RI) was calculated with the Hennig86 program (version 1.5, available from J.S. Farris, Dep. of Ecology and Evolution, S.U.N.Y., Stony Brook, NY, USA).

The two-tailed Wilcoxon matched-pairs signed-ranks test (Siegel, 1956) was used following Templeton (1983) and the binomial test (Siegel, 1956) was used following Mindell *et al.* (1989) to evaluate if the number of isoenzyme tissue expression changes differed significantly along the branches of the trees leading to sister species.

RESULTS AND DISCUSSION

Nineteen loci were determined for the cavian esterase system (Table I). Holmes and Masters (1967) proposed the existence of at least 12 esterase loci for *C. porcellus* while Monjeló and Cordeiro (1979), studying two populations of *C. aperea*, found evidence for 13 loci for this species. The higher number of loci postulated here may be explained by the fact we used four species and six tissues. However, the same 19 loci were postulated by Gimmler (1977), who studied several ontogenetic stages of *C. porcellus* and *C. aperea*.

There are clear differences among tissues in the number of isoenzyme loci expressed and in the number showing interspecific variation (Table I). Similarly, differences exist in the number of tissues in which each locus is expressed, some loci (e.g., locus 2) being expressed in just one tissue (not expressed at all in *C. porcellus*) while others (e.g., locus 16 in *C. magna* and *C. fulgida*) were expressed in five of the six tissues screened. Differences in the number of loci expressed among tissues and in the proportion of those showing polymorphism were also observed in others studies (Kettler *et al.*, 1986; Mindell and Sites, 1987; Dickinson, 1991).

Table I also shows the existence of interspecific variation in the isoenzyme locu/tissue combinations among the four species. Nineteen of the 114

Table I - Tissue expression of the 19 presumptive esterase loci for the four *Cavia* species.

Loci	Naphthyl acetate		Liver				Kidney				Intestine				Heart				Plasma				Pancreas			
	α	β	a	p	m	f	a	p	m	f	a	p	m	f	a	p	m	f	a	p	m	f	a	p	m	f
1	+		1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	+		0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
3	+		1	1	2	1	1	1	2	1	3	3	3	3	3	3	1	3	0	0	0	0	0	0	0	0
4		+	2	2	3	3	3	3	3	3	2	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0
5	+		0	0	0	0	0	0	0	0	0	0	0	2	2	0	2	3	3	0	3	0	0	0	0	
6	+	+	2	2	2	2	0	0	0	0	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0
7	+		0	0	0	0	0	0	0	0	2	2	2	2	0	0	0	0	0	0	0	0	3	3	3	3
8		+	3	3	2	3	3	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	+		0	0	0	0	0	0	0	0	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0
10	+		3	3	3	3	0	0	0	0	3	3	2	3	0	0	0	0	0	0	0	0	1	1	1	1
11	+	+	3	3	3	3	0	0	0	0	3	3	3	3	0	0	0	0	2	2	0	2	1	1	1	1
12		+	0	0	0	0	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	+		3	3	3	3	0	0	0	0	2	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0
14	+		0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
15		+	2	2	2	2	1	2	2	1	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
16	+	+	3	3	3	3	1	3	2	1	0	0	2	2	1	1	1	1	1	2	2	2	2	0	0	0
17	+		1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	2	2	0	2	0	0	0	0
18	+		1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	2	2	0	2	0	0	0	0
19	+		0	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0

0 = no expression, 1-3 = increasing expression intensity, a = *C. aperea*, p = *C. porcellus*, m = *C. magna*, f = *C. fulgida*. Boldface values indicate interspecific variability.

combinations of locus/tissue show variation in intensity while eight vary in the presence/absence comparison (Table II). Corresponding figures for other data are, respectively: 80% and 52% for the Hawaiian *Drosophila* (Dickinson, 1980), 90% and 46% for the Umbridae (Kettler *et al.*, 1986), and 86% (P/A) for two avian orders (Mindell and Sites, 1987).

Phylogenetic analysis

The interspecific variability observed in the isoesterase TEP can be used for phylogenetic analysis (e.g., Mindell and Sites, 1987; Thorpe and Dickinson,

1988). The cladogram of Figure 1, constructed using the unordered intensity data set (Table II) and rooted according to the evidence discussed below, presented a RI = 0.8 and a consistency index (CI) of 0.95, due to a homoplasious step in character 14. The phylogeny obtained with the intensity values as ordered characters (not shown) gave the same tree with one more homoplasious step (character 16). Using the characters transformed to P/A values (the eight boldface characters of Table II) resulted in a topologically identical tree (not shown) with CI and RI = 1.0 (two informative characters). The branch leading to *C. magna* has five of the eight steps of the tree and those

Table II - Data matrix of the 19 TEP characters showing interspecific variation in Table I.

Species	Characters																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>C. aperea</i>	1	1	1	3	2	2	2	3	3	3	3	2	2	1	0	1	0	2	2
<i>C. porcellus</i>	0	1	1	3	2	2	2	3	3	3	3	2	1	2	0	3	0	2	2
<i>C. magna</i>	1	2	2	1	3	3	0	0	2	2	2	0	3	2	2	2	2	0	0
<i>C. fulgida</i>	1	1	1	3	3	2	2	3	3	2	3	2	2	1	2	1	2	2	2

Symbols as in Table I. Boldface values are characters varying in a presence/absence mode.

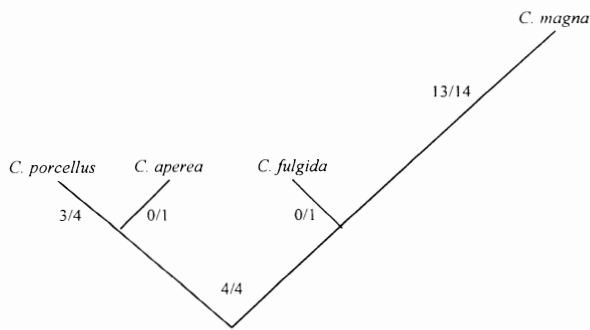


Figure 1 - Phylogenetic relationship of the four species of *Cavia* based on 19 variable unordered isoenzymes TEP (Table II) showing the minimum and the maximum number of changes in each branch. Length = 22.

leading to *C. porcellus* and *C. fulgida* have no observed character change. The branches of the tree of Figure 1 have relative lengths similar to these values, *C. magna* with more than 50% of the changes in isoenzyme intensity observed in the genus.

Rooting the phylogenetic tree

Notwithstanding the lack of TEP information available for any species close enough to be considered as an outgroup for *Cavia*, we use some biological and biogeographic information about these species to postulate a tentative root for the *Cavia* tree (Figure 1). We think that the root should not be placed at the *C. magna* or *C. porcellus* branches because both are very probably the two most recent species of the genus (see below). Similarly, the root at the *C. aperea* branch would imply the unlikely hypothesis that the domesticated *C. porcellus* is older than *C. fulgida* (distributed all over southeastern Brazil). A tree with the root in the *C. fulgida* branch implies that *C. aperea* would be as old as *C. magna*. Although this last hypothesis is plausible, it remains unlikely that *C. aperea*, which occurs over most of South America with at least two subspecies, would not be older than the postulated very recently diverged *C. magna*. Therefore, the most appropriate place for the root of the phylogenetic tree is the central branch leading to the two groups of species as depicted in Figure 1.

The relationship depicted by the phylogenetic tree, *C. porcellus* as a sister taxon of *C. aperea*, agrees with the hypothesis that the population (or populations) from which *C. porcellus* was domesticated belonged to *C. aperea* (Hückinghaus, 1962; Weir, 1974) and excludes *C. fulgida* and *C. magna* as the immediate ancestral species. However, the wild *Cavia* from Peru, one of the most accepted areas of origin of *C. porcellus* (Gilmore, 1950; Weir, 1974), are sometimes considered

a different species from *C. aperea* (Weir, 1974). Therefore, there is a possibility that another species is the most recent ancestor of *C. porcellus*, although these Peruvian animals are thought to be very closely related to *C. aperea* (Weir, 1974).

Gene silencing

We found five locus/tissue losses of expression in the branch leading to *C. magna*. In fact, all character changes in this branch in the cladogram based on the P/A matrix involved silencing of loci expression: four out of the five loci expressed in plasma and the loss of expression of locus 5 in heart. There was also a loss of the intestine specific loci 2 in *C. porcellus*.

This high degree of gene silencing observed in *C. magna* (and the one in *C. porcellus*) may be related to selection or genetic drift. For example, the high level of gene silencing in the tetraploid goldfish was explained by genetic drift through population "bottlenecks" coupled with artificial selection (Woods and Buth, 1984).

Origin of *C. porcellus* and *C. magna*

C. magna is endemic to the swamp habitats located immediately behind the beach dunes on the coast of southern Brazil and Uruguay (Ximenez, 1980). This region is supposed to be of very recent geological origin and arose after several Pleistocene marine transgressions, the last of which occurred during the great Holocene transgression about 5,500 years ago (Villwock et al., 1986). These data and the phylogenetic tree suggest that *C. magna* diverged from *C. fulgida* during one of these marine regressions, probably during the last one.

Rates of regulatory evolution

As noted above, the phylogenetic trees show large differences in the number of changes that occurred in the *C. magna* branch in relation to its sister species *C. fulgida*, and, to a lesser extent, in *C. porcellus* in relation to *C. aperea*. We tested if these differences in the number of TEP character changes were significant, i.e., if there is (or was) a differential rate of evolution of TEP between these sister species. A test with the pair *C. porcellus* and *C. aperea* was not done due to the low number of changes in these branches. Only the unambiguous character changes in the cladograms were used. The results of the tests are independent of the position of the tree root unless it is placed in the *C. magna* or *C. fulgida* branches.

For the *C. magna* and *C. fulgida* sister species, the binomial tests showed a significant difference in the number of changes between the species for both the P/A ($P < 0.05$) and the unordered intensity data cladograms ($P < 0.01$). With the Wilcoxon test, only when we use the loci as the paired observations in the unordered intensity data set cladogram was there a large enough number of changes. We treated all loci as independent observations, with the exception of loci 17 and 18, which have the same pattern of expression in all tissues. The Wilcoxon test showed that the number of changes in TEP that occurred in the branch leading to *C. magna* was significantly greater than that occurred in *C. fulgida* ($P < 0.01$).

The results of these statistical tests showed clearly that there is rate variation in the overall esterase gene expression among lineages. Consequently, the existence of a "regulatory evolutionary clock" (Ferris and Whitt, 1979; Whitt, 1987) is not empirically corroborated and therefore one should not use methods that assume equal rates of evolution among lineages (e.g., the UPGMA clustering method) to infer phylogenies with this kind of data. These tests also showed the importance of using phylogenetic methods that use character and character states along with overall distance values (e.g., Kettler *et al.*, 1986).

Adaptive pressure to the very different ecological habitat where *C. magna* lives may have accelerated its rate of evolution over that of *C. fulgida*. Besides being the most divergent in isoesterases TEP, *C. magna* is also the biggest of the four species and has enlarged digital membranes and swimming capabilities (Ximenez, 1980). A founder effect may have also contributed to the rate acceleration of *C. magna* isoesterase gene regulation. For example, Dickinson (1991) attributed the higher TEP variability of the *Drosophila* Hawaiian group in relation to the *D. virilis* group to its explosive speciation through possible predominantly founder events or to stronger selective forces.

Phylogenetic utility of regulatory data

Thorpe and Dickinson's (1988) phylogenies within *Drosophila* subgroups present CIs of about 0.9 and although not completely congruent with the standard chromosomal inversion phylogeny, they are sometimes consistent with other data, like the mitochondrial DNA phylogeny of the planitibia subgroup (DeSalle *et al.*, 1987). Mindell and Site's (1987) avian cladogram combining information from several isoenzymes reflected very accurately the traditional classification. Transforming to P/A characters the TEP

intensity data of the Umbridae (from Table II of Kettler *et al.*, 1986) we inferred a cladogram (CI = 0.95, RI = 0.84) with the same topology of the intensity data and that is consistent with other genetic and morphological information (Kettler *et al.*, 1986).

We think that these results together with the one presented here indicate that neither the too pessimistic conclusions (e.g., Mindell and Sites, 1987; Thorpe and Dickinson, 1988) nor some very optimistic expectations (e.g., Whitt, 1987) on the phylogenetic utility of TEP regulatory data were accurate. It is clear that TEP characters would suffer from saturation (loss of the phylogenetic information due to multiple changes) at some higher taxonomic level, but where this will happen would depend on the age of each group, that may be different for the same categories of different organisms, and on the rates of evolution.

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RESUMO

Foram realizadas análises filogenéticas utilizando a expressão de isoesterases em seis tecidos em quatro espécies de *Cavia*. Foi construída uma hipótese de relacionamento filogenético entre essas espécies e avaliada a dinâmica de diferenciação dos padrões de expressão isoenzimática tissular (TEP) e seu valor na reconstrução filogenética. Para as análises filogenéticas foram utilizadas a intensidade e a presença/ausência (P/A) de expressão de cada locus esterásico em cada tecido determinada através de eletroforese em gel de poliacrilamida. Doze dos 19 loci encontrados apresentaram variação interespecífica em pelo menos um tecido, totalizando 19 caracteres de intensidade e oito caracteres de P/A. As espécies do gênero apresentaram o seguinte relacionamento filogenético: ((*C. aperea*, *C. porcellus*), (*C. fulgida*, *C. Magna*)). O índice de retenção dos cladogramas derivados dos 19 caracteres de intensidade e dos oito caracteres de P/A foram de 0,8 e de 1,0, respectivamente. Os resultados do presente trabalho e de outros sugerem que padrões de expressão gênica podem ser utilizados para inferência filogenética em vários níveis taxonômicos, de espécies próximas até famílias ou ainda a categorias superiores, dependendo das taxas de evolução e da idade dos grupos. *C. magna* apresentou uma taxa evolutiva para a expressão tissular das

esterases significativamente maior ($P < 0,01$) que a sua espécie irmã *C. fulgida*. Tais fatos sugerem que as modificações de TEP não evoluem de maneira constante e que portanto para inferência de filogenias com esse tipo de informação não se deve utilizar métodos que assumam igualdade nas taxas evolutivas entre linhagens.

REFERENCES

- Beckman, L.** and **Johnson, F.M.** (1964). Esterase variations in *Drosophila melanogaster*. *Hereditas* 51: 212-220.
- Buth, D.G.** (1984). The application of electrophoretic data in systematic studies. *Annu. Rev. Ecol. Syst.* 15: 501-522.
- DeSalle, R., Friedman, T., Prager, E.M.** and **Wilson, A.C.** (1987). Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *J. Mol. Evol.* 26: 157-164.
- Dickinson, W.J.** (1980). Evolution of patterns of gene expression in Hawaiian picture winged *Drosophila*. *J. Mol. Evol.* 16: 73-94.
- Dickinson, W.J.** (1991). The evolution of regulatory genes and patterns in *Drosophila*. *Evol. Biol.* 25: 127-173.
- Ferris, S.D.** and **Whitt, G.S.** (1979). Evolution of the differential regulation of duplicate genes after polyploidization. *J. Mol. Evol.* 12: 267-317.
- Gilmore, R.M.** (1950). Fauna and ethnography of South America. In: *Handbook of South American Indians*, Volume 6 (Steward, J.H., ed.). Smithsonian Institution, Washington, D.C., pp. 345-360.
- Gimmler, M.C.** (1977). Diferenciação qualitativa e quantitativa de isoesterases na ontogênese de *Cavia aperea pamparum* e *C. porcellus*. Master's Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.
- Graur, D.** (1993). Molecular phylogeny and the higher classification of Eutherian mammals. *Trends Ecol. Evol.* 8: 141-147.
- Holmes, R.S.** and **Masters, J.C.** (1967). The developmental multiplicity and isoenzyme status of cavian esterases. *Biochem. Biophys. Acta* 132: 379-399.
- Hückinghaus, F.** (1962). Vergleichende Untersuchungen über die formenmannigfaltigkeit der unterfamilie Caviinae Murray 1886. (Ergebnisse de Südamerika-expedition Herre/Rohrs 1956-57). *Z. wiss. Zool.* 166: 1-98.
- Kettler, M.K., Ghent, A.W.** and **Whitt, G.S.** (1986). A comparison of phylogenies based on structural and tissue-expressional differences of enzymes in a family of Teleost fishes (Salmoniformes: Umbridae). *Mol. Biol. Evol.* 3: 485-498.
- Mindell, D.P.** and **Sites Jr., J.W.** (1987). Tissue expression patterns of avian isoenzymes: a preliminary study of phylogenetic applications. *Syst. Zool.* 36: 137-152.
- Mindell, D.P., Sites Jr., J.W.** and **Graur, D.** (1989). Speciation evolution: a phylogenetic test with allozymes in *Sceloporus* (Reptilia). *Cladistics* 5: 49-61.
- Monjeló, L.A.S.** and **Cordeiro, A.R.** (1979). Tissue distribution and population variability of esterases in *Cavia aperea*. *Rev. Brasil. Genet.* 2: 211-222.
- Schneider, M.P.C., Cordeiro, A.R.** and **da Silva, A.C.** (1983). Variability and tissue distribution of lactate dehydrogenase in four species of the genus *Cavia*. *Rev. Brasil. Genet.* 6: 71-80.
- Siegel, S.** (1956). *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York, pp. 312.
- Templeton, A.R.** (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221-244.
- Thorpe, P.A.** and **Dickinson, W.J.** (1988). The use of regulatory patterns in constructing phylogenies. *Syst. Zool.* 37: 97-105.
- Villwock, J.A., Tomazelli, L.J., Loss, E.L., Dehnhardt, E.A., Horn, N.O., Bachi, F.A.** and **Dehnhardt, B.A.** (1986). Geology of the Rio Grande do Sul Coastal Province. In: *Quaternary of South America and Antarctic Peninsula*, Volume 6 (Rabassa, J., ed.). A.A. Balkema, Rotterdam, pp. 79-97.
- Weir, B.J.** (1974). Notes on the origin of the domestic guinea-pig. *Symp. Zool. Soc. Lond.* 1974: 437-446.
- Whitt, G.S.** (1987). Species differences in isoenzyme tissue patterns: their utility for systematic and evolutionary analyses. In: *Isozymes: Current Topics in Biological and Medical Research, Volume 15: Genetics, Development, and Evolution* (Markert, C.L., ed.). Liss, New York, pp. 1-26.
- Woods, T.D.** and **Buth, D.G.** (1984). High level of gene silencing in the tetraploid goldfish. *Biochem. Syst. Ecol.* 12: 415-421.
- Ximenez, A.** (1980). Notas sobre el género *Cavia* Pallas con la descripción de *Cavia magna* sp.n. (Mammalia-Caviidae). *Rev. Nordest. Biol.* 3(especial): 145-179.

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