

# Polymorphism of phosphogluconate dehydrogenase (PGD) in New World monkeys: taxonomic significance

Iracilda Sampaio, Maria Paula Cruz Schneider, Carmem Maria Leitão Barroso  
and Horacio Schneider

## ABSTRACT

Phosphogluconate dehydrogenase (PGD) polymorphism in 23 species of 13 genera of New World monkeys was investigated. The occurrence of 21 codominant autosome alleles may explain the total electrophoretic variation encountered. The only alleles shared by two or more genera were *PGD\*11* and *PGD\*15*, the former grouping *Aotus*, *Cacajao*, *Chiropotes*, *Saimiri*, *Cebuella*, *Callithrix* and *Saguinus*, and the latter *Alouatta*, *Lagothrix*, *Callicebus* and *Cebus*. The intergeneric groupings obtained are compared with the most recent taxonomic classifications for the New World monkeys.

## INTRODUCTION

The two most recent taxonomic arrangements for the New World monkeys are congruent in relation to the division of the 16 existent genera into two families: **Cebidae**, grouping *Aotus*, *Cebus*, *Saimiri*, *Callimico*, *Cebuella*, *Callithrix*, *Leontopithecus* and *Saguinus*, and **Atelidae**, grouping *Alouatta*, *Ateles*, *Brachyteles*, *Lagothrix*, *Pithecia*, *Chiropotes*, *Cacajao* and *Callicebus* (Rosenberger *et al.*, 1990; Schneider *et al.*, 1993a). The two classifications agree in everything except the positioning of *Aotus*, placed by Rosenberger *et al.* in the atelid clade and by Schneider *et al.* close to the cebids.

Over the last few years, our group has carried out extensive electrophoretic studies on natural populations of platyrrhini, seeking to identify markers that could help understand the relationship among genera, species and populations. In one of these studies,

3,113 animals belonging to 12 genera and 24 species were analyzed in terms of polymorphism of carbonic anhydrase II, 24 different alleles having been detected (Sampaio *et al.*, 1991a). This study revealed important intra-generic markers in *Aotus*, *Callicebus*, *Cebus* and *Saguinus*. The resolution was poor at the inter-generic relationship level: the allele *CAII\*13* groups *Alouatta*, *Ateles*, *Cacajao*, *Chiropotes*, *Pithecia* and *Saimiri*, while the remaining genera present their own markers, which are different from the above-mentioned group.

We recently presented the results of a comparative study on the variation of lactate dehydrogenase (LDH) in more than 3,000 specimens representing 15 genera of platyrrhini. The degree of polymorphism found was low both for the inter- as well as intra-generic level. Intrapopulation polymorphism was only detected in *Saimiri sciureus*, *Pithecia pithecia*, *Pithecia irrorata*, *Alouatta belzebul*, *Callithrix humeralifer*, *Callithrix kuhli* and *Callithrix penicillata*, while at the inter-specific level, the species of the group *Cebus albifrons* (*C. albifrons* and *C. nigrivittatus*) share no alleles for the locus LDHB with the group *Cebus apella* (*C. apella*) and with the other platyrrhini (Schneider *et al.*, 1994).

## MATERIAL AND METHODS

Polymorphism of erythrocytic phosphogluconate dehydrogenase (PGD) in 2,738 specimens of New World monkeys belonging to 23 species and 13 genera was studied (Table I).

PGD phenotypes were analyzed through horizontal electrophoresis in 11% potato starch (Sigma)

in three different buffer systems: (1) 40X PC (tank: 245 mM monobasic sodium phosphate/150 mM citric acid, adjusted with 10 M NaOH; Gel: 1X CP); (2) 20X PCE, pH 6.9 (tank: 110 mM monobasic sodium phosphate/75 mM trisodium citrate/2.5 mM EDTA, adjusted with 10 M NaOH; Gel: 1X CPE); (3) 10X TC, pH 5.2 (100 mM Tris, adjusted with 10 M citric acid; gel 1 TC). Beta-mercaptoethanol was added to the hemolyzates to prevent secondary bands.

**Table I** - Species of New World monkeys investigated for PGD polymorphism.

Species	Number of			
	Code	Populations	Specimens	Reference
<b>Family Atelidae</b>				
<i>Alouatta belzebul belzebul</i>	Abb	4	674	Schneider <i>et al.</i> (1991)
<i>Alouatta seniculus straminea</i>	Ass	3	107	Sampaio <i>et al.</i> (1996)
<i>Ateles paniscus</i>	Apa	1	7	Sampaio <i>et al.</i> (1993)
<i>Ateles chamek</i>	Ach	1	44	Sampaio <i>et al.</i> (1993)
<i>Lagothrix lagothricha</i>	Lla	1	8	Present paper
<i>Cacajao calvus rubicundus</i>	Ccr	1	7	Schneider <i>et al.</i> (1995)
<i>Chiropotes satanas chiropotes</i>	Csc	1	12	Schneider <i>et al.</i> (1995)
<i>Chiropotes satanas utahicki</i>	Csu	1	95	Schneider <i>et al.</i> (1995)
<i>Pithecia irrorata</i>	Pir	1	143	Schneider <i>et al.</i> (1995)
<i>Callicebus brunneus</i>	Cbr	1	166	Schneider <i>et al.</i> (1993b)
<i>Callicebus cupreus</i>	Ccu	1	33	Schneider <i>et al.</i> (1993b)
<i>Callicebus moloch</i>	Cmo	1	80	Schneider <i>et al.</i> (1993b)
<b>Family Cebidae</b>				
<i>Cebus apella apella</i>	Caa	1	148	Sampaio <i>et al.</i> (1991b)
<i>Cebus apella paraguayanus</i>	Cap	1	55	Sampaio <i>et al.</i> (1991a)
<i>Saimiri sciureus sciureus</i>	Sss	6	150	Silva <i>et al.</i> (1993)
<i>Saimiri sciureus boliviensis</i>	Ssb	2	120	Silva <i>et al.</i> (1993)
<i>Saimiri sciureus ustus</i>	Ssu	1	171	Silva <i>et al.</i> (1993)
<i>Aotus nancymai</i>	Ana	1	93	Present paper
<i>Aotus vociferans</i>	Avo	1	20	Present paper
<i>Aotus azarae</i>	Aaz	1	88	Present paper
<i>Aotus infulatus</i>	Ain	2	87	Schneider <i>et al.</i> (1989)
<i>Saguinus fuscicollis weddelli</i>	Sfw	1	136	Melo <i>et al.</i> (1992)
<i>Saguinus midas midas</i>	Smm	1	13	Melo <i>et al.</i> (1992)
<i>Saguinus midas niger</i>	Smn	1	123	Melo <i>et al.</i> (1992)
<i>Callithrix humeralifer</i>	Chu	1	14	Meireles <i>et al.</i> (1992)
<i>Callithrix emiliae</i>	Cem	1	85	Meireles <i>et al.</i> (1992)
<i>Callithrix jacchus jacchus</i>	Cjj	1	35	Meireles <i>et al.</i> (1992)
<i>Callithrix jacchus geoffroyi</i>	Cjg	1	6	Meireles <i>et al.</i> (1992)
<i>Callithrix jacchus penicillata</i>	Cjp	1	10	Meireles <i>et al.</i> (1992)
<i>Cebuella pygmaea</i>	Cpy	1	8	Present paper
<b>Total</b>		<b>42</b>	<b>2738</b>	

Allele frequencies and Hardy-Weinberg equilibrium were estimated by the program BIOSYS of Swofford and Selander (1981).

## RESULTS AND DISCUSSION

Electrophoretic variation of PGD was detected in 50% of the species analyzed: *Alouatta belzebul*, *Alouatta seniculus*, *Aotus infulatus*, *Aotus nancymai*, *Aotus vociferans*, *Callicebus brunneus*, *Callithrix humeralifer*, *Callithrix emiliae*, *Callithrix jacchus*, *Chiropotes satanas*, *Saguinus midas*, and *Saimiri sciureus*. All observed genotype distributions are in agreement with those expected assuming the two or three-allele model in

Hardy-Weinberg equilibrium. The entire variability of PGD in New World monkeys can be explained by the occurrence of 21 autosomic codominant alleles, the frequencies of which are shown in Table II. The electrophoretic patterns found for each genus are described below.

### *Alouatta*

Populations of both *A. belzebul* and *A. seniculus* were polymorphic for PGD: *A. belzebul belzebul* showed PGD 10, PGD 10-15 and PGD 15 phenotypes, and *A. seniculus*, PGD7, PGD 7-15 and PGD 15. The frequencies of the putative ancestral allele, *PGD\*15*, were markedly different in these two species (Table II). The PGD 7 of *A.*

**Table II** - Allelic frequencies of phosphogluconate dehydrogenase (PGD) in New World monkeys.

POP	Alleles of PGD																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ass							56								43						
Abb									18						82						
Lla															100						
Cbr															99			1			
Ccu															100						
Cmo															100						
Caa															100						
Cap															100						
Apa									100												
Ach									100												
Pir														100							
Aaz											100										
Ain1											88										12
Ain2											100										
Ana											99							1			
Avo						3					97										
Ccr											100										
Csc											88	12									
Csu											57	43									
Sss		5									94									3	
Ssb					1						99										
Ssu											100										
Cpy											100										
Cjj											83		17								
Cjg											100										
Cjp											100										
Chu			100																		
Cem	5		75					20													
Sfw											100										
Smm											96								4		
Smn				2							95					3					

POP = Population. See Table I for abbreviations.

*belzebul* and PGD 10 of *A. seniculus* were previously reported as having the same electrophoretic mobility in PCE buffer, pH 6.9 (Lima *et al.*, 1990), but we tested this variant in CP buffer, pH 5.9, and found that PGD 7 was more anodic than PGD 10.

### *Aotus*

PGD 11 was the most common phenotype in all species of *Aotus*, being monomorphic in *A. azarae* and in *A. infulatus* from the left bank of Tocantins river. Species-specific alleles, however, were present in *A. nancymai*, *A. vociferans* and in *A. infulatus* from the left bank of the Tocantins river (Table II). Interestingly, the two populations of *A. infulatus* from both banks of the Tocantins river showed marked differences in their allelic distributions: the population from the left bank (N = 55) was monomorphic for PGD 11, while the small population from the right bank (N = 16) presented the additional allele *PGD\*21* (Table II). Heterogeneity between these two populations for GPI, CA2 and PGM1 loci was previously reported (Schneider *et al.*, 1989), and the most probable explanation for these differences was the interruption of gene flow, due to the barrier represented by the Tocantins river.

### *Callicebus*

Extremely low variability was observed in this genus; only two heterozygotes (PGD 15-18) were detected in *C. brunneus*, while the remaining specimens from the three species (*C. moloch*, *C. brunneus* and *C. cupreus*) had a monomorphic pattern (PGD 15). A great deal of similarity between these three species of *Callicebus* has also been observed for 19 other loci (see Schneider *et al.*, 1993b).

### *Callithrix*

The variability detected in this analysis is congruent with the currently accepted taxonomic arrangement, dividing *Callithrix* into two groups of species (Mittermeier *et al.*, 1988; Meireles *et al.*, 1992). In the *C. argentata* group, *C. humeralifer* had only PGD 3, while *C. emiliae* showed PGD 1-3, PGD 3 and PGD 3-8 phenotypes, the *PGD\*3* allele being the most frequent (Table II). Conversely, the three subspecies of the *C. jacchus* group did not share any PGD pattern with those from the *C. argentata* group: *C. j. geoffroyi* and *C. j. penicillata* had a monomorphic pattern (PGD 11), and *C. j. jacchus* had PGD 11 (the most common), PGD 11-13 and PGD 13 patterns. The *PGD\*11* allele seems to be the ancestral form of PGD in *Callithrix* because it was also

detected in *Aotus*, *Saimiri*, *Cacajao*, *Chiropotes*, *Cebuella* and *Saguinus*.

### *Chiropotes*

The same phenotypes were observed in *C. satanas utahicki* and *C. satanas chiropotes* (PGD 11, PGD 11-12 and PGD 12), but the allelic frequencies were markedly different (Table II). *PGD\*11* is probably the ancestral allele in *Chiropotes* as it is found in several other genera. The differences in genotypic distribution between the two subspecies are probably due to the fact that they currently are separated by the Amazon river, *C. s. chiropotes* to the north and *C. s. utahicki* to the south.

### *Saguinus*

PGD 11 phenotype was the only pattern found in *S. fuscicollis weddelli* and it was also the most common in *S. midas*. Subspecific markers at very low frequencies were present in both *S. m. midas* (PGD 4-11 and PGD 11-16) and *S. m. niger* (PGD 11-19). As in the case of *C. satanas*, the two subspecies of *S. midas* are also separated by the Amazon river (*S. m. midas* to the north and *S. m. niger* to the south). This barrier is probably responsible for the differences in the distribution of PGD. In the case of *Saguinus*, however, it is likely that the alleles *PGD\*4* (2%), *PGD\*16* (3%) and *PGD\*19* (4%) are all derived from *PGD\*11*, having appeared independently after the separation of the two subspecies.

### *Saimiri*

*PGD\*11* allele was the only marker detected in *S. sciureus ustus*, and it was the predominant form in the other two subspecies, *S. sciureus boliviensis* (*PGD\*5* = 1% and *PGD\*11* = 99%), and *S. sciureus sciureus* (*PGD\*2* = 5%, *PGD\*11* = 94% and *PGD\*15* = 1%).

The six remaining New World monkey genera showed only monomorphic patterns of PGD: *Ateles* (PGD 9), *Cebuella* and *Cacajao* (PGD 11), *Pithecia* (PGD 14), *Lagothrix* and *Cebus* (PGD 15).

Two intergeneric clusters can be identified, based on the frequencies of *PGD\*11* and *PGD\*15* alleles, the only alleles shared by more than two genera: *PGD\*11*, with frequencies ranging from 57 to 100%, groups *Cacajao*, *Chiropotes*, *Aotus*, *Saimiri*, *Cebuella*, *Callithrix*, and *Saguinus*, while the *PGD\*15* allele (43 to 100%) places together *Callicebus*, *Cebus*, *Alouatta* and *Lagothrix* (Table II). These groupings only partially agree with the proposals of Rosenberger *et al.* (1990) and of Schneider *et al.* (1993a) regarding the division of the

platyrrhine into two families, **Cebidae** and **Atelidae**. The major points of agreement are listed below:

1) The monophyletic character of the family **Cebidae**, represented here by *Cebuella*, *Callithrix*, *Saguinus*, *Aotus* and *Saimiri*, all of which share *PGD\*11* at a high frequency. The only discrepancy is the fact that *Cebus* presents the allele *PGD\*15*, typical of the atelids.

2) The close similarity between *Alouatta* and *Lagothrix* which belong to the family **Atelidae**, presenting *PGD\*15* with frequencies varying from 43 to 100%. Interestingly, the two species of *Ateles* do not share PGD alleles with any other platyrrhini. It is likely that the allele *PGD\*9* constitutes a derived form of *PGD\*15*, having appeared during the evolution of *Ateles*. Our previous study with CAII shows that *Ateles* is closely linked to *Alouatta*, corroborating the monophyletic character of these three lineages (Sampaio *et al.*, 1991b).

3) The close relationship between *Chiropotes* and *Cacajao* (*PGD\*11*), which together with *Pithecia* and *Callicebus* constitute the pitheciines. It was this group, however, that presented the poorest resolution in terms of positioning in relation to the other platyrrhines. While *Chiropotes* and *Cacajao* could be strongly linked to the Cebids (*PGD\*11*), *Callicebus* was found to be close to the atelids (*PGD\*15*), and *Pithecia* an isolated lineage with its allele *PGD\*14*, not observed in any other genus. These data are not in agreement with our previous findings with Carbonic anhydrase II, where *Pithecia*, *Chiropotes* and *Cacajao* constitute a monophyletic group, linked closely to *Ateles* and *Alouatta* (Sampaio *et al.*, 1991b).

The results of the present study demonstrated an apparent controversy regarding the dichotomy **Cebidae** (*Cebus-Saimiri-Aotus-calitrichines*) vs. **Atelidae** (atelines plus pitheciines) proposed by Rosenberger *et al.* (1990) and corroborated by Schneider *et al.* (1993a). Nevertheless, the intergeneric groupings suggested by both CAII as well as PGD should be interpreted with caution, for the following reasons: 1) because the analysis is based on a small number of markers (only two loci); 2) because due to the limits of the electrophoretic method, similar electrophoretic mobilities may not necessarily mean that the alleles are identical, and 3) as the principal platyrrhine lineages are probably separated by at least 15 or 20 million years (Schneider *et al.*, 1993a), some of the similarities observed here may be the product of evolutionary convergence.

In any case, our results show that PGD polymorphism at the intra- and interspecific level constitutes a useful tool for the comprehension of microevolutionary questions, particularly within the

genera *Alouatta*, *Aotus*, *Saimiri*, *Chiropotes*, *Saguinus* and *Callithrix*.

## ACKNOWLEDGMENTS

This work was supported by the Federal University of Pará (UFPA), Financing Agency of Studies and Projects (FINEP), National Council for Scientific and Technological Development (CNPq), and Electric Power of Northern Brazil (ELETRONORTE). We thank Dr. José Augusto P.C. Muniz from the National Primates Center (Ananindeua, Pará) for permission to collect blood samples of some species from that institution.

## RESUMO

O polimorfismo da desidrogenase fosfoglicônica (PGD) foi investigado em 23 espécies de primatas do Novo Mundo, e a variação eletroforética total encontrada pode ser explicada pela ocorrência de 21 alelos autossômicos codominantes. Os alelos *PGD\*11* e *PGD\*15* foram os únicos encontrados em mais de um gênero, sendo que o primeiro agrupa *Aotus*, *Cacajao*, *Chiropotes*, *Saimiri*, *Cebuella*, *Callithrix* e *Saguinus*, e o segundo *Alouatta*, *Lagothrix*, *Callicebus* e *Cebus*. Os agrupamentos intergenéricos obtidos são comparados com as classificações taxonômicas mais recentes para os primatas do Novo Mundo.

## REFERENCES

- Lima, M.M.C., Sampaio, M.I.C., Schneider, M.P.C., Scheffrahn, W., Schneider, H. and Salzano, F.M. (1990). Chromosome and protein variation in red howler monkeys. *Rev. Bras. Genet.* 13: 789-802.
- Meireles, M.P.C., Sampaio, M.I.C., Schneider, H. and Schneider, H. (1992). Protein variation, taxonomy and differentiation in five species of marmosets (Genus *Callithrix* Erxleben, 1977). *Primates* 33: 227-238.
- Melo, A.C.A., Sampaio, M.I.C., Schneider, M.P.C. and Schneider, H. (1992). Biochemical diversity and genetic distance in two species of the genus *Saguinus*. *Primates* 33: 217-225.
- Mittermeier, R.A., Rylands, A.B. and Coimbra-Filho, A.F. (1988). Systematics: species and subspecies - an update. In: *Ecology and Behavior of Neotropical Primates*. Vol. 2 (Mittermeier, R.A., Rylands, A.B., Coimbra-Filho, A.F. and Fonseca, G.A.B., eds.). World Wildlife Fund., Washington, DC, pp. 13-75.
- Rosenberger, A.L., Setoguchi, T. and Shigehara, N. (1990). The fossil record of callitrichine primates. In: *The Platyrrhine Fossil Record* (Fleagle, J.G. and Rosenberger, A.L., eds.). Academic Press, London, pp. 209-236.
- Sampaio, M.I.C., Barroso, C.M.L., Silva, B.T.F., Seuanez, H., Matayoshi, T., Howlin, E., Nasazzi, N., Nagle, C. and

- Schneider, H.** (1991a). Genetic variability in *Cebus apella paraguayanus*. Biochemical analysis of seven loci and variation in glyoxalase-I (E.C. 4.4.1.5). *Primates* 32: 105-109.
- Sampaio, I., Schneider, M.P.C., Barroso, C.M.L., Silva, B.T.F., Schneider, H., Encarnacion, F., Montoya, M. and Salzano, F.M.** (1991b). Carbonic anhydrase II in New World monkeys. *Inter. J. Primatol.* 12: 389-402.
- Sampaio, M.I.C., Schneider, M.P.C. and Schneider, H.** (1993). Contribution of genetic distances studies to the taxonomy of *Ateles*, particularly *Ateles paniscus paniscus* and *Ateles paniscus chamek*. *Int. J. Primatol.* 14: 895-903.
- Sampaio, I., Schneider, M.P.C. and Schneider, H.** (1996). Taxonomy of the *Alouatta seniculus* group: biochemical and chromosome data. *Primates* 37: 65-73.
- Schneider, M.P.C., Sampaio, M.I.C., Schneider, H. and Salzano, F.M.** (1989). Genetic variability in a natural population of Brazilian night monkey (*Aotus infulatus*). *Int. J. Primatol.* 10: 363-374.
- Schneider, H., Sampaio, M.I.C., Schneider, M.P.C., Ayres, J.M., Barroso, C.M.L., Hamel, A., Silva, B.T.F. and Salzano, F.M.** (1991). Coat color and biochemical variation in Amazonian wild population of *Alouatta belzebul*. *Am. J. Phys. Anthropol.* 85: 85-93.
- Schneider, H., Schneider, M.P.C., Sampaio, I., Harada, M.L., Stanhope, M., Czelusniak, J. and Goodman, M.** (1993a). Molecular phylogeny of the New World monkeys (Platyrrhini, Primates). *Mol. Phy. Evol.* 2: 225-242.
- Schneider, H., Schneider, M.P.C., Sampaio, I., Montoya, M., Tapia, J., Encarnacion, F., Anselmo, N.P. and Salzano, F.M.** (1993b). Divergence between biochemical and cytogenetic differences in three species of the *Callicebus moloch* group. *Am. J. Phy. Anthropol.* 90: 345-350.
- Schneider, M.P.C., Sampaio, M.I.C., Schneider, H., Encarnación, F., Montoya, E., Pissinatti, A., Coimbra-Filho, A. and Salzano, F.M.** (1994). Comparative study of lactate dehydrogenase in fifteen genera of New World monkeys. *Rev. Bras. Genet.* 17: 321-329.
- Schneider, M.P.C., Schneider, H., Sampaio, M.I.C., Carvalho-Filho, N.M., Encarnación, F., Montoya, E. and Salzano, F.M.** (1995). Biochemical diversity and genetic distances in the pitheciinae subfamily (Primates, Platyrrhini). *Primates* 36: 129-134.
- Silva, B.T.F., Sampaio, M.I.C., Schneider, H., Schneider, M.P.C., Montoya, E., Encarnación, F., Callegari-Jacques, S.M. and Salzano, F.M.** (1993). Protein electrophoretic variability in *Saimiri* and the question of its species status. *Am. J. Primatol.* 29: 183-193.
- Swofford, D.L. and Selander, R.B.** (1981). *Biosys-1. A computer program for analysis of variation in genetics. User's Manual.* Unpublished manual. The University of Illinois at Urbana-Champaign, pp. 1-43.

(Received March 13, 1995)