



Cytotaxonomy of *Parodon nasus* and *Parodon tortuosus* (Pisces, Characiformes). A case of synonymy confirmed by cytogenetic analyses

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Abstract

Morphological and cytogenetical studies were carried out on the freshwater fish *Parodon nasus* and *Parodon tortuosus* in order to evaluate a putative synonymy. The diploid chromosome number observed in both species was $2n = 54$ (48M/SM and 6ST) with no differences between the sexes. Despite slight differences in the pattern of heterochromatin distribution and the number of cusps in symphysean teeth, the location of nucleolar organizer regions (NORs) and 5S rRNA genes (both species-specific features) were similar in both species. The remarkable similarity observed between these allopatric species supports recent taxonomical reviews indicating that *P. tortuosus* is a synonym for *P. nasus*.

Key words: cytogenetics, Parodontidae, systematics, 18S rDNA, 5S rDNA.

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Introduction

Over the last three decades, cytogenetic studies in Neotropical fishes have widely contributed to the taxonomy of Neotropical fishes. It has been pointed out that karyotypical analyses provides useful information for evolutionary and phylogenetic studies and aids in the identification of controversial species (Bertollo *et al.*, 1986), and, indeed, such techniques have been successfully applied to characterize phenotypically similar cryptic species. Cytogenetical analyses of some fish species (e.g. from the genera *Leporinus* (Galetti *et al.*, 1981a, b) and *Brycon* (Margarido and Galetti, 1996)) have agreed with their taxonomic status but cytogenetical studies of species such as *Astyanax scabripinnis* (Moreira-Filho and Bertollo, 1991), *Hoplias malabaricus* (Bertollo *et al.*, 2000), *Eigenmannia virescens* (Almeida-Toledo *et al.*, 2002) and *Hoplerhythrinus unitaeniatus* (Diniz and Bertollo, 2003) have diverged from taxonomic classifications, suggesting that these groups should be taxonomically reviewed.

The Parodontidae, a relatively small freshwater fish family within the order Characiformes, comprises fish commonly known in Brazilian Portuguese as ‘canivetes’ or

‘charutos’ (‘penknives’ or ‘cheroots’) (Pavanelli, 2003) which are widely distributed throughout nearly all South America. Three genera are reported for this family: *Parodon* Valenciennes, 1850; *Apareiodon* Eigenmann, 1916; and *Saccodon* Kner, 1964. Since 1849, 13 *Apareiodon*, 18 *Parodon* and 3 *Saccodon* species have been described for the Parodontidae, although after the detailed review of Pavanelli (2003) this number was reduced to a total of 21 species. Parodontid fish present distinct, non-overlapping, reproductive periods (Barbieri *et al.*, 1983) and a pale gray streamlined body (Nomura, 1979), the coloration being basically composed of stripes, bars and blotches providing an obliterative effect in their rocky and turbulent freshwater habitats (Sazima, 1980). The pectoral and pelvic fins of members of this family are well developed and adapted to stabilizing the fish on the rocky bottoms of the rivers and streams where these fish are usually found grazing on algae (Sazima, 1980).

Dentary structure is an important feature of the family Parodontidae and is helpful in the identification of the three genera (Garavello, 1977), small lateral teeth being present in the genus *Parodon* but absent in *Apareiodon* and *Saccodon*. In the Parodontidae the pre-maxilla is surrounded by a series of tiny teeth with rounded cusps, the number of cusps of pre-maxilla teeth being a reliable character for the diagnosis of *Parodon* and *Apareiodon* species but not for *Saccodon* species, which are characterized by extensive teeth polymorphism (Garavello, 1977).

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Cytogenetic analyses carried out on 12 species of Parodontidae (Table 1) have shown a conserved diploid number of $2n = 54$ chromosomes but differences related to karyotypical structure. A multiple sex chromosome ZZ/ZW₁W₂ type system has been reported for *Apareiodon affinis* (Moreira-Filho et al., 1980) but a ZZ/ZW sex chromosome system for *Parodon hilarii* (Moreira-Filho et al., 1993), *Parodon* sp. (Centofante et al., 2002) and *Apareiodon* sp. (Margarido et al., 2004).

Britski (1972) states that *Parodon tortuosus* Eigenmann and Norris, 1900 (type locality Tietê river, Upper Paraná basin, São Paulo state, Brazil) is the only species of this genus in the Paraná river basin, whereas *Parodon nasus* Kner, 1859 (type locality Cuiabá river, Paraguay

river basin, Mato Grosso, Brazil) is restricted to the Paraguay river basin (Britski et al., 1999). However, the taxonomical review performed by Pavanelli (2003) considered *P. tortuosus* to be a junior synonym for *P. nasus*.

The aim of the work described in the present paper was to better characterize *P. tortuosus* and *P. nasus* using both cytogenetic analysis and a comparative analysis of the number of teeth cusps in these two taxonomic units.

Material and Methods

Specimens of *P. tortuosus* were collected in the Passa-Cinco river (Upper Paraná river basin) and *P. nasus* in the Cuiabá river (Paraguay river basin). Mitotic chromosomes were obtained from 20 specimens (10 males and 10

Table 1 - An overview of karyotypical data in Parodontidae species.

Species	Locality	Sex	2n	Chromosome formulae	FN	Sex system	NORs	Ref
<i>Parodon</i>								
<i>P. tortuosus</i>	Passa-Cinco River, SP	F-M	54	48 M/SM, 6 ST	108	-	Pair n. 25(ST)	1
	Ivaí River, PR	M	54	-	108	-	-	2
	Tibagi River, PR	F-M	54	54 M/SM	-	-	-	3
	Paíol Grande stream, SP	F-M	54	48 M/SM, 6 ST	108	-	Pair n. 25 (ST)	4
<i>P. pongoensis</i>	Araguaia River, MT	F-M	54	50 M/SM, 4 ST	108	-	Pair n. 2 (SM)	5
<i>P. hilarii</i>	Porta stream, MG	F	54	53 M/SM, 1 ST	108	ZW	Pair n. 15 (SM)	6
		M	54	54 M/SM	108	ZZ	Pair n. 15 (SM)	
<i>Parodon</i> sp.	Paíol Grande stream, SP	F	54	54 M/SM	108	ZW	Pair n. 15 (SM)	7
		M	54	54 M/SM	108	ZZ	Pair n. 15 (SM)	
<i>Apareiodon</i>								
<i>A. ibitiensis</i>	Passa-Cinco River, SP	F-M	54	50 M/SM, 4 ST	108	-	Pair n. 26 (ST)	8
	Ivaí River, PR	M	54	50 M/SM, 4 ST	108	-	-	9
	Tibagi River, PR	F-M	54	52 M/SM, 2 ST	108	-	-	10
<i>Apareiodon</i> sp.	Piquiri River, PR	F	54	50 M/SM, 4ST	108	ZW	Pair n. 26 (ST)	11
		M	54	50 M/SM, 4ST	108	ZZ	Pair n. 26 (ST)	
<i>A. piracicabae</i>	Passa-Cinco/Mogi-Guaçu Rivers, SP	F-M	54	52 M/SM, 2 ST	108	-	Pair n. 27 (ST)	12
		M	55	52 M/SM, 2 ST, 1B	110	-	-	13
<i>Apareiodon</i> sp. A	Passa-Cinco River, SP	F-M	54	50 M/SM, 4 ST	108	-	Pair n. 26 (ST)	14
<i>Apareiodon</i> sp. B	Frio stream, MG	F-M	54	50 M/SM, 4 ST	108	-	Pair n. 26 (ST)	15
<i>Apareiodon</i> sp. C	Barreiro stream, MG	M	54	52 M/SM, 2 ST	108	-	-	16
<i>A. vittatus</i>	Araguaia River, MT	F-M	54	52 M/SM, 2 ST	108	-	Pair n. 27 (ST)	17
<i>A. affinis</i>	Iguaçu River, PR	F	55	51 M/SM, 4 ST	110	-	Pair n. 26 (ST)	18
	Passa-Cinco, Mogi-Guaçu and Piracicaba Rivers, SP	M	54	50 M/SM, 4 ST	108	-	Pair n. 26 (ST)	
<i>A. affinis</i>	Lower Paraná basin (Argentina)	F-M	54	a = 50 M/SM, 4 ST	107	-	Pair n. 26 (ST)	19, 20
		M	54	b = 50 M/SM, 2 ST, 2 A	106	-	Pair n. 26 (ST)	
		F-M	54	c = 50 M/SM, 3 ST, 1 A	107	-	Pair n. 26 (1ST, 1A)	
		F-M	54	d = 50 M/SM, 2ST, 2 A	106	-	-	
		F-M	54	e = 50 M/SM, 1 ST, 3 A	105	-	-	
		F-M	54	f = 50 M/SM, 2 ST, 2 A	98	-	-	
		F-M	54	g = 50 M/SM, 1 ST, 3 A	105	-	Pair n. 26 (2A)	
		F-M	54	h = 50 M/SM, 4 A	104	-	-	
		F-M	54	i = 49 M/SM, 5 A	103	-	-	
		F-M	54	j = 48 M/SM, 6 A	102	-	-	
<i>A. affinis</i>	Cuiabá River, MT	F-M	54	a = 42 M/SM, 12 ST/A	98	-	Pair n. 23	21
		F-M	54	b = 44 M/SM, 10 ST/A	102	-	-	22
<i>A. affinis</i>	Tibagi River, PR	F	55	-	-	ZW ₁ W ₂	-	23
		M	54	-	-	ZZ	-	
<i>A. affinis</i>	Paraná River, Porto Rico, PR	F	55	-	-	ZW ₁ W ₂	-	24
		M	54	-	-	ZZ	-	
<i>A. cf. affinis</i>	Rio Sapucaí, MG	F	55	-	-	ZW ₁ W ₂	-	25
		M	54	-	-	ZZ	-	

Legend: 2n (diploid number), FN (fundamental number), NOR (nucleolar organizer regions), F (female), M (male), M/SM (metacentric/submetacentric), ST/A (subtelocentric/acrocentric). Reference: 1 - Moreira-Filho et al. (1984, 1985), Jesus and Moreira-Filho (2000a); 2 - Sato et al. (1998); 3 - Schneider and Dias (2002b); 4 - Centofante et al. (2002); 5 - Jesus and Moreira-Filho (2000a); 6 - Moreira-Filho et al. (1993), Jesus and Moreira-Filho (2000a); 7 - Centofante et al. (2002); 8 - Moreira-Filho et al. (1984, 1985), Jesus and Moreira-Filho (2000a); 9 - Sato et al. (1998); 10 - Schneider and Dias (2000); 11 - Margarido et al. (2004); 12 - Moreira-Filho et al. (1984, 1985), Jesus and Moreira-Filho (2000b); 13 - Falcão et al. (1984); 14 - Jesus and Moreira-Filho (2000b); 15 - Jesus and Moreira-Filho (2000b); 16 - Jesus and Moreira-Filho (2000b); 17 - Jesus and Moreira-Filho (2000b); 18 - Moreira-Filho et al. (1980, 1984, 1985), Jesus and Moreira-Filho (1999), Jorge and Moreira-Filho (2000); 19 - Jorge and Moreira-Filho (2000); 20 - Jorge (2000); 21 - Jesus and Moreira-Filho (1999); 22 - Jesus (1996); 23 - Schneider and Dias (2002a); 24 - Endo and Júlio Jr. (2001); 25 - Leite and Maistro (2000).

females) of *P. nasus* and 22 specimens (8 males and 14 females) of *P. tortuosus* using the methodology described by Bertollo *et al.* (1978). Silver nitrate (Ag) staining was used to detect the nucleolar organizer regions (Ag-NORs) according to the method of Howell and Black (1980). Fluorescent *in situ* hybridization (FISH) with 18S rDNA probes obtained from *Prochilodus argenteus* (Hatanaka and Galetti, 2004) was performed according to the protocol of Pinkel *et al.* (1986). This same procedure was employed for FISH with a 5S rDNA probe obtained from *Leporinus elongatus* (Martins and Galetti, 1999). Differentiation of GC-rich chromosome regions was carried out by Schmid's method (Schmid, 1980) followed by staining with the GC-specific fluorochrome Chromomycin A₃, and the constitutive heterochromatin being analyzed by the C-banding technique (Sumner, 1972). The chromosome types were classified according their arm ratio, as proposed by Levan *et al.* (1964). The teeth cusps analyses were carried out on 23 specimens of *P. tortuosus* and 33 specimens of *P. nasus* according to the methodology described by Moreira-Filho and Garavello (1994).

Results

The *P. tortuosus* karyotype was $2n = 54$ (48M/SM + 6ST) for both sexes (Figure 1-a), C-banding showing evident pericentromeric marks on chromosome pairs 2, 4, 10, 11 and 19 as well as telomeric blocks on pairs 1, 6, 7, 17, 25, 26 and 27 (Figure 2-a).

This paper presents for the first time a cytogenetical analyses of *P. nasus*, the diploid number obtained by us being $2n = 54$ (48 M/SM + 6ST) for both sexes (Figure 1-b), C-banding showing conspicuous pericentromeric bands on chromosome pairs 4, 5, 16 and telomeric blocks on pairs 1, 6, 17, 25, 26 and 27 (Figure 2-b).

A single Ag-NOR pair was found in both *P. nasus* and *P. tortuosus*, located at the terminal position on the long arm of the 25th chromosome pair (Figure 1). Four GC-rich signals were observed for both species, two on the short arms and two on the long arms (coincident with the NOR sites) of chromosome pair 25 (Figure 1). A single NOR pair on the long arms of chromosome pair 25 was confirmed by FISH with the 18S rDNA probe (Figure 3 a-b). However, while 5S rDNA FISH showed regions in a single pair on *P. tortuosus* chromosomes, this probe showed regions on three *P. nasus* chromosomes (Figure 3 c-d).

The number of symphysean teeth cusps ranged from 16 to 19 in *P. tortuosus* (Figures 4-a, 5) and from 17 to 21 in *P. nasus* (Figures 4-b, 5).

Discussion

The results obtained for diploid number ($2n = 54$), karyotype formulae, NORs, GC-rich regions, C-banding and 5S rDNA location were quite similar in *P. tortuosus* and *P. nasus*. Our *P. tortuosus* data supports that previously

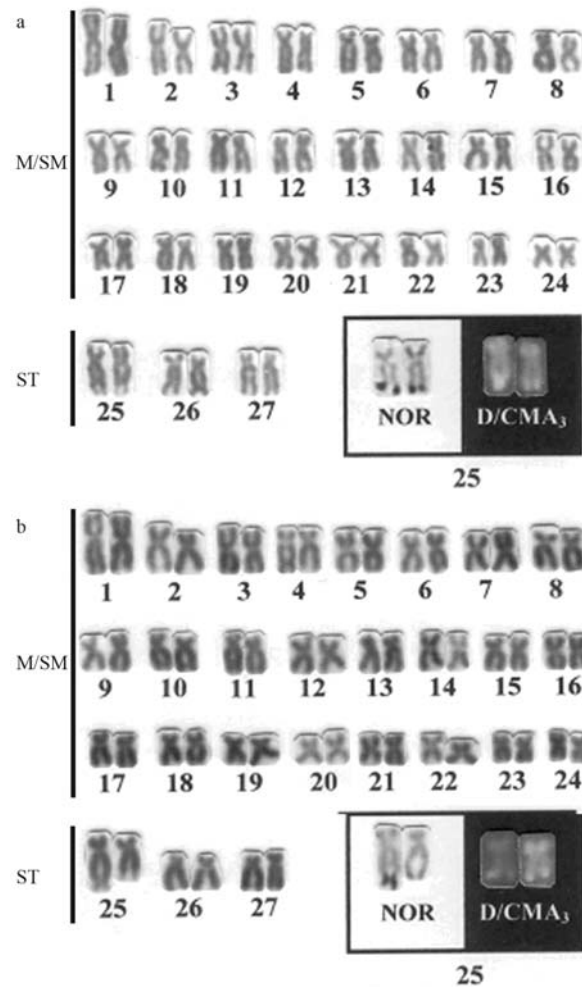


Figure 1 - Karyotypes of: a. *Parodon tortuosus* and b. *Parodon nasus* with conventional Giemsa staining. The nucleolar organizer regions after silver nitrate (Ag-NOR) and D/CMA₃ staining are showed in the boxes.

published by Moreira-Filho *et al.* (1984, 1985), Jesus and Moreira-Filho (2000a), Vicente *et al.* (2001) and Centofante *et al.* (2002). A diploid number of 54 has been reported for all Parodontidae species (Table 1), with the exception of *A. affinis* females from Upper Paraná which present a female heterogamety characterized by a multiple ZW₁W₂ sex chromosome system. While *Apareiodon* species present extensive karyotypical similarity (Moreira-Filho *et al.*, 1985), a diversification of chromosomal morphology is found within the genus *Parodon*, with a ZZ/ZW sex chromosome system distinguishing *P. hilarii* and *Parodon* sp. from the other species of this genus (Centofante *et al.*, 2002).

A single NOR-bearing chromosomal pair has been found in Parodontidae fish (Table 1). The location of the NOR seems to be more conserved within *Apareiodon*, except that *A. piracicabae* shows intra- and inter-individual polymorphism related to the occurrence of double NORs in

each chromosome of a subtelocentric pair (Moreira-Filho *et al.*, 1984).

The NORs of *P. hilarii*, *P. pongoensis* (also known as *Parodon* sp.) and *P. tortuosus* have been considered to be species-specific markers located on different chromosomes (Jesus and Moreira-Filho, 2000a), and our results show that the NORs of both *P. tortuosus* and *P. nasus* are located on the same chromosomal pair, reinforcing the idea of synonymy between these two species.

Chromomycin A₃ staining showed that the NORs of *P. tortuosus* and *P. nasus* were GC-rich, a result identical to those published for other Parodontidae species (Jesus, 1996). The distribution of constitutive heterochromatin in *Parodon* and *Apareiodon* chromosomes occurs preferentially at the centromeric and, sometimes, the telomeric regions (Jesus and Moreira-Filho, 2000b). Our C-banding results were quite similar for *P. tortuosus* and *P. nasus*, with slight differences found in only a few chromosomes.

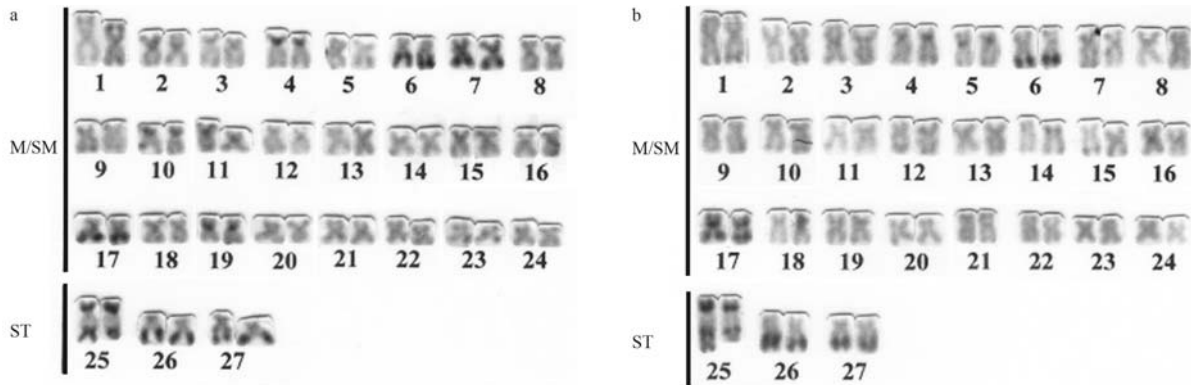


Figure 2 - C-banded karyotypes of: a. *Parodon tortuosus* and b. *Parodon nasus*.

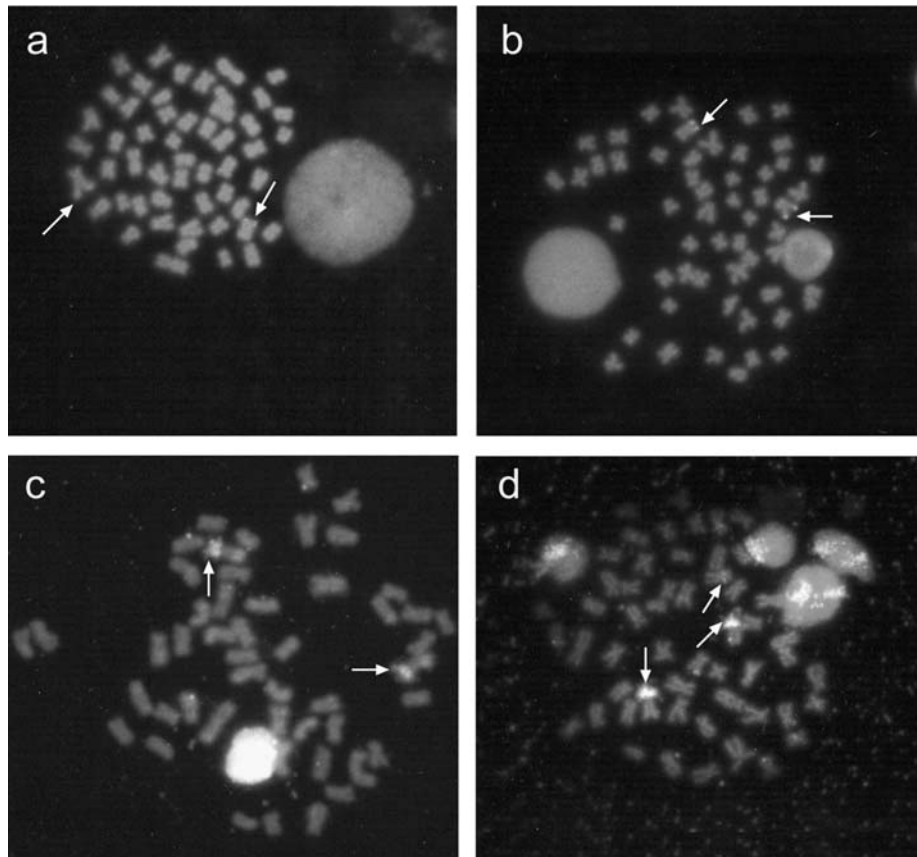


Figure 3 - Fluorescent *in situ* hybridization with the 18S rDNA probe (a. *Parodon tortuosus* and b. *Parodon nasus*) and the 5S rDNA probe (c. *Parodon tortuosus* and d. *Parodon nasus*).

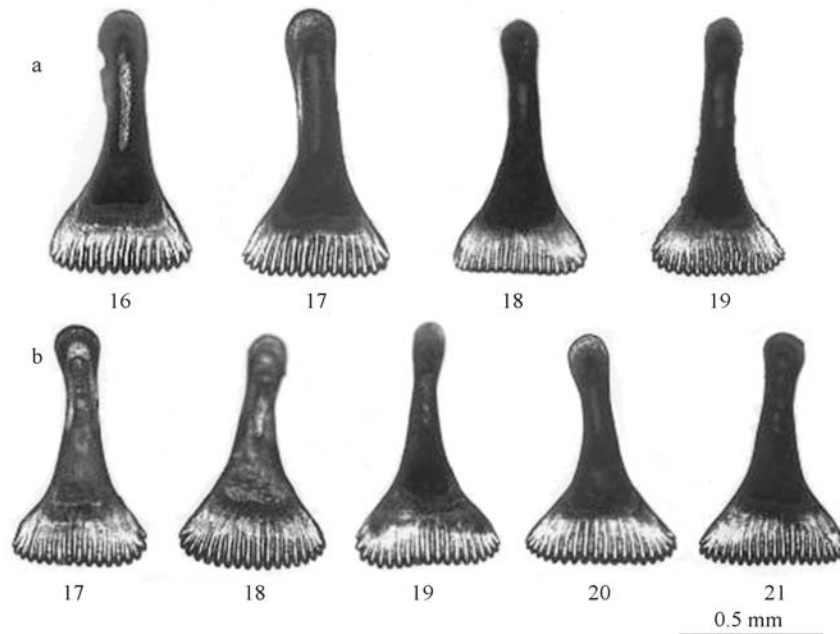


Figure 4 - Symphysean teeth of: a. *Parodon tortuosus* and b. *Parodon nasus*, with the variation of the number of cusps for each species.

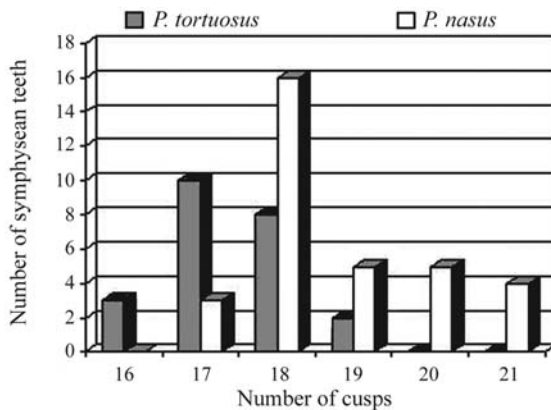


Figure 5 - Histogram showing the distribution of frequency of cusps by teeth in *P. tortuosus* (grey) and in *P. nasus* (white).

These slight differences have also been observed in *P. tortuosus* by other authors (Jesus and Moreira-Filho, 2000a; Vicente, 2001; Centofante *et al.*, 2002) and also in *P. hilarii* (Jesus, 1996; Vicente, 2001), and are probably related to technical artifacts and/or interpopulation differences. These observations lead us to conclude that in the family Parodontidae the pattern of constitutive heterochromatin cannot be considered a useful marker for species diagnosis, contrasting with the situation in the genera *Leporinus* (Galetti *et al.*, 1991a) and *Brycon* (Margarido and Galetti, 1996) where the C-banding pattern is species-specific.

Vicente *et al.* (2001) detected a major and a minor 5S rDNA cluster on distinct chromosomal pairs of *P. hilarii*, *P. pongoensis* and *P. tortuosus* providing a species-specific marker. The occurrence of 5S rRNA genes in clusters involving more than a single chromosomal pair has been reported in several fish species, including those from the genera *Schizodon* (Martins and Galetti, 2000), *Leporinus* (Martins and Galetti, 1999) and *Brycon* (Wasko and Galetti, 2001). In these species, as in *Parodon*, besides the presence of two clusters there are also differences related to the location of 18S and 5S rDNA, which seems to represent a common situation in fish (Martinez *et al.*, 1996). According to Martins and Galetti (1999), this distinctive location of ribosomal genes is required for the efficient maintenance of the conserved sequence displayed in tandem arrays.

We found that in *P. nasus* and *P. tortuosus* only the 5S rDNA major cluster was well-defined and on the same chromosome (syntenic) as the NORs, similar to the situation in *Salmo salar* described by Pendás *et al.* (1994). Since *P. tortuosus* is the only species within the genus *Parodon* to present such a feature, our results concerning the location of the 5S rDNA major clusters reinforces the similarity between *P. nasus* and *P. tortuosus*. In *P. nasus*, however, a third chromosome also contained the 5S rDNA major cluster, probably representing a homologue from a second 5S rDNA-bearing pair (a minor cluster) absent from the *P. tortuosus* specimens analyzed by us. The small size of these clusters (*i.e.* only a few copies of the 5S rRNA genes) in *P. nasus* may have been why this homologue was not detected by FISH, as it was reported for the genus *Brycon* (Wasko and Galetti, 2001).

Our morphological analyses based on the number of symphysean teeth cusps showed a variation of 16-19 for *P. tortuosus* and 17-21 for *P. nasus*. According to Jesus and Moreira-Filho (2000a) such an overlap in the number of cusps is uncommon among *Parodon* species, although a symphysean teeth analyses of some Parodontidae species has shown a similar number of cusps in distinct species such as *Apareiodon ibitiensis* and *Apareiodon* sp. which were found to have 8-11 cusps (Jesus, 1996).

Taken together, our results demonstrate a high degree of similarity between *P. tortuosus* from the Passa-Cinco river and *P. nasus* from the Cuiabá river, supporting the taxonomical review carried out by Pavanelli (2003) who states that *P. tortuosus* should be considered a synonymy for *P. nasus*.

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