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Cryptic species of the genus *Pimelodella* (Siluriformes: Heptapteridae) from the Miranda River, Paraguay River basin, Pantanal of Mato Grosso do Sul, Central Brazil

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Specimens of *Pimelodella* captured in the Miranda River, Pantanal of Mato Grosso do Sul State, present morphological features that could indicate at least four species. Therefore, karyotype analysis and molecular biology provided evidence that they were only two species, one showing 2n = 46, and the other, 2n = 52 chromosomes, with only 18% genetic similarity. The morphological analysis evidenced that the dorsal filament is a male characteristic and that the upper lobe of the caudal fin was variable and might or might not be elongated in both species. With respect to morphometric characters, the formation of two groups was evident, but with a small overlap of specimens between them. Among the species with filaments on the dorsal fin observed in the Pantanal, the one with the lesser length of adipose fin base is *P. griffini*, which corresponds to that with 2n = 46 chromosomes, whereas the species *P. taenioptera* has 2n = 52 chromosomes. Thus, the accurate detection of these cryptic taxonomic units was only possible with the use of various analysis techniques. Furthermore, it is worth noting that the identification of cryptic species is important for obtaining correct estimates of fish diversity in the Pantanal.

Exemplares de *Pimelodella* capturados no rio Miranda, Pantanal do Mato Grosso do Sul, apresentavam características morfológicas que poderiam indicar, pelo menos, quatro espécies. Entretanto, com a análise cariotípica e da biologia molecular ficou evidente que se tratava de apenas duas espécies, uma apresentando 2n = 46 e a outra, 2n = 52 cromossomos, e com apenas 18% de similaridade genética. Pela análise morfológica foi observado que o filamento dorsal é uma característica de machos, e o lobo superior da nadadeira caudal se mostrou variável, podendo, ou não, ser alongado em ambas espécies. Com relação aos caracteres morfométricos, também houve a formação de dois grupos, mas com uma pequena sobreposição de exemplares entre eles. Das espécies com filamento na nadadeira dorsal apontadas para o Pantanal, a que possui menor comprimento da base da nadadeira adiposa é *P. griffini*, o que corresponde àquela com 2n = 46 cromossomos e, ao contrário, a espécie com 2n = 52 cromossomos, é *P. taenioptera*. Assim, apenas com o emprego de diversas técnicas de análise foi possível o reconhecimento seguro dessas unidades taxonômicas que se mostravam crípticas. Ressalta-se, ainda, que a identificação de espécies crípticas é importante para que estimativas da diversidade de peixes do Pantanal sejam feitas corretamente.

Key words: Cytotaxonomy, Cytogenetics, Multivariate morphometrics, RAPD.

Introduction

The Neotropical region harbors the most representative groups of freshwater fish in the world, with about 6,000 of the 13,000 existing species (Reis *et al*., 2003). This region is a vast sedimentary wetland plain called Pantanal, which has a very rich fish fauna, with approximately 260 identified species, including small and medium-sized catfish, such as Heptapteridae (Britski *et al*., 2007). Currently, this is the most diverse family within the order Siluriformes, and *Pimelodella* Eigenmann & Eigenmann, 1888 is their most specious genus, with 71 valid species (Bockmann & Guazzelli, 2003).

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Six species of *Pimelodella* have been observed in the Pantanal (Britski *et al*., 2007) and are traditionally differentiated by the presence of elongate filaments on the dorsal fin (*vs*. absence); elongate upper lobe of caudal fin (*vs*. same length as the lower); color (dark spot on dorsal fin; dark stripe along the body); and the presence of hypertrophied lateral line pores on the ventral region of the head. Specimens of *Pimelodella* captured in the Miranda River, Pantanal of Mato Grosso do Sul State, exhibited morphological features that could involve at least four species (*Pimelodella gracilis* Valenciennes, 1835, *P. megalura* Miranda-Ribera, 1918, *P. taenioptera* Miranda-Ribeiro, 1914, and *P. griffini* Eigenmann, 1917), since some specimens did or did not present an elongate filament on the dorsal fin, as well as an upper lobe of the caudal fin elongated or with the length similar to that of the lower lobe.

Thence, the objective of this study was to analyze these specimens in the light of cytogenetics, molecular biology, and morphology, to test the hypothesis that they might belong to those four species.

Material and Methods

The specimens of *Pimelodella*, subject of this study, were collected from the left bank of the Miranda River, at Base de Estudos do Pantanal (BEP), belonging to the Universidade Federal do Mato Grosso do Sul (BEP/UFMS), Municipality of Corumbá, Mato Grosso do Sul, in the region known as Passo do Lontra, located at 19°34'37"S and 57°00'42"W (Fig. 1).

The specimens were collected by trawl with a mesh of 2 mm between the adjacent knots or fished with rod and hook, and kept alive in a tank with dechlorinated tap water at a temperature of 25ºC. For the analyses, the specimens were later killed by overexposure to the anesthetic (1.0 mL of 5% benzocaine for each liter of water).

The species were identified according to Miranda-Ribeiro (1914), Eigenmann (1917) and Britski *et al*. (2007). The specimens are deposited in the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL), Paraná, Brazil (MZUEL 6455 to 6460).

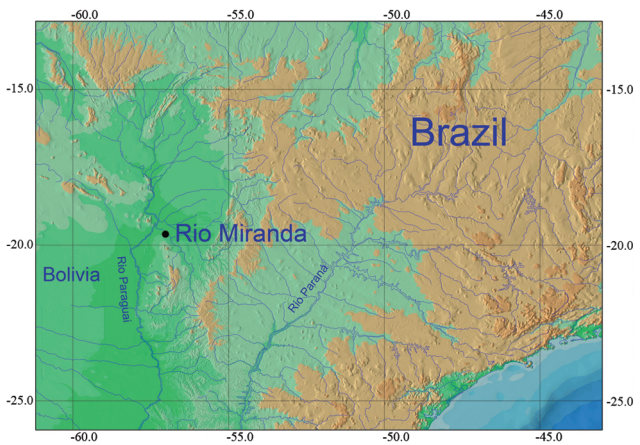

Fig. 1. Collection sites of *Pimelodella* specimens (black circle, 19°34'37"S and 57°00' 42"W) in the Miranda River basin, Passo do Lontra, Mato Grosso do Sul State, Brazil.

Mitotic chromosomes were obtained through the technique described by Bertollo *et al*. (1978) and classified according to the methodology proposed by Levan *et al*. (1964). As it is possible that different species share the same diploid number, especially if they belong to the same genus, as observed by Souza *et al*. (2004), molecular analyses were performed to verify whether those chromosome numbers actually represented only one particular species.

For the molecular analyses, samples were removed from muscle tissue and placed in 2 mL cryopreservation tubes with absolute ethanol and stored in a freezer at -20º C. The specimens were then fixed in 10% formalin and preserved in 70% alcohol. The extraction and quantification of genomic DNA were based on Almeida *et al*. (2003), and for the obtainment of genetic identity, we used the PCR-RAPD (Random Amplified Polymorphic DNA) technique. Thirty different primers (OPA, OPAC, OPC, OPM and OPX Kits from Operon Technologies Ltd.) were tested for the analysis and those presenting the best electrophoretic profile and distinct banding pattern were selected. To assess the level of reliability of the results, the coefficient of variation for the number of amplified markers (CV%) was calculated using the program dBoot v. 1.1 (Coelho, 2001). For the analysis of genetic similarity, we used the computer program NTSYS-PC (Rohlf, 2000), employing the Jaccard coefficient (J) and the UPGMA clustering method.

The body measurements were taken point to point with the aid of digital calipers with 0.01 mm accuracy, following the protocol developed by Shibatta (1998). The following measurements were taken: standard length, head length, snout length, eye diameter, pre-dorsal distance, length of dorsal fin base, length of adipose-fin base, dorsal fin spine length, caudal peduncle height, pre-ventral distance, length of anal fin base, length of pectoral fin spine, and interorbital distance. The measurements, except those for the barbel length, formed a matrix that was analyzed with the employment of multivariate statistics of the Principal Components with axis distortion, known as Shear, following the protocol of Bookstein *et al*. (1985). The statistical package utilized for this analysis is called Shear (Mcleod, 1990), and the interpretations of the axes were accomplished according to Neff & Marcus (1981). The body proportions of the measurements relative to head were calculated in relation to the lengths of the head and body, and the lengths of the head and barbel were estimated in relation to the standard length. The differences in body proportions among species were analyzed applying the t Student test using the statistical package PAST (Hammer *et al*. 2001). We also calculated the allometric coefficient for the upper lobe of the caudal fin in relation to standard length applying the least-squares method, where the angular coefficient (b) with a value higher than 1 was considered positive allometric; lower than l, negative allometric; and equal to 1, isometric. The assessment of pectoral-fin spines of the two species was made in relation to the size of the anterior

and posterior saw-toothed margins, and compared with the illustrations presented by Eigenmann (1917). One sample of each species was cleared and stained in accordance with Dingerkus & Uhler (1977) to facilitate the visualization of spines details. The presence of the elongate filament on the second dorsal-fin ray (which forms the spines) was related to the chromosomal number and sex.

Results

Cytogenetics. We analyzed 33 specimens, considering an average of 30 metaphases per individual. Of these, 15 specimens presented 2n = 46 chromosomes and 18 specimens, 2n = 52 chromosomes (Figs. 2a-b), indicating that the sample comprised two species. The species with 2n = 46 chromosomes showed 19 pairs of meta/submetacentric chromosomes and four pairs of subtelo/acrocentric chromosomes, with a secondary constriction on the second pair of meta/submetacentric chromosomes. The species with 2n = 52 chromosomes presented 21 pairs of meta/submetacentric chromosomes and five pairs of subtelo/acrocentric chromosomes, and did not present sex heteromorphism. We did not observe any supernumerary chromosomes in both species.

Molecular Biology. We selected nine primers (OPA2, OPAC 4, APAC 7, OPC 2, OPM 13, OPM 20, OPX OPX 6 and 12) that resulted in 181 loci. These proved to be sufficient for the analysis, due to the value of the coefficient of variation (CV), which stabilized around 5%, indicating a high level of reliability.

The comparative analysis of 18 specimens, 11 with 2n = 52 and 7 with 2n = 46, revealed the presence of two different RAPD profiles in all primers utilized, which had a close relationship with the two chromosomal groups. The dendrogram (Fig. 3) revealed that the similarity between the two groups was only 18%, clearly indicating the presence of two species and corroborating the cytogenetic data.

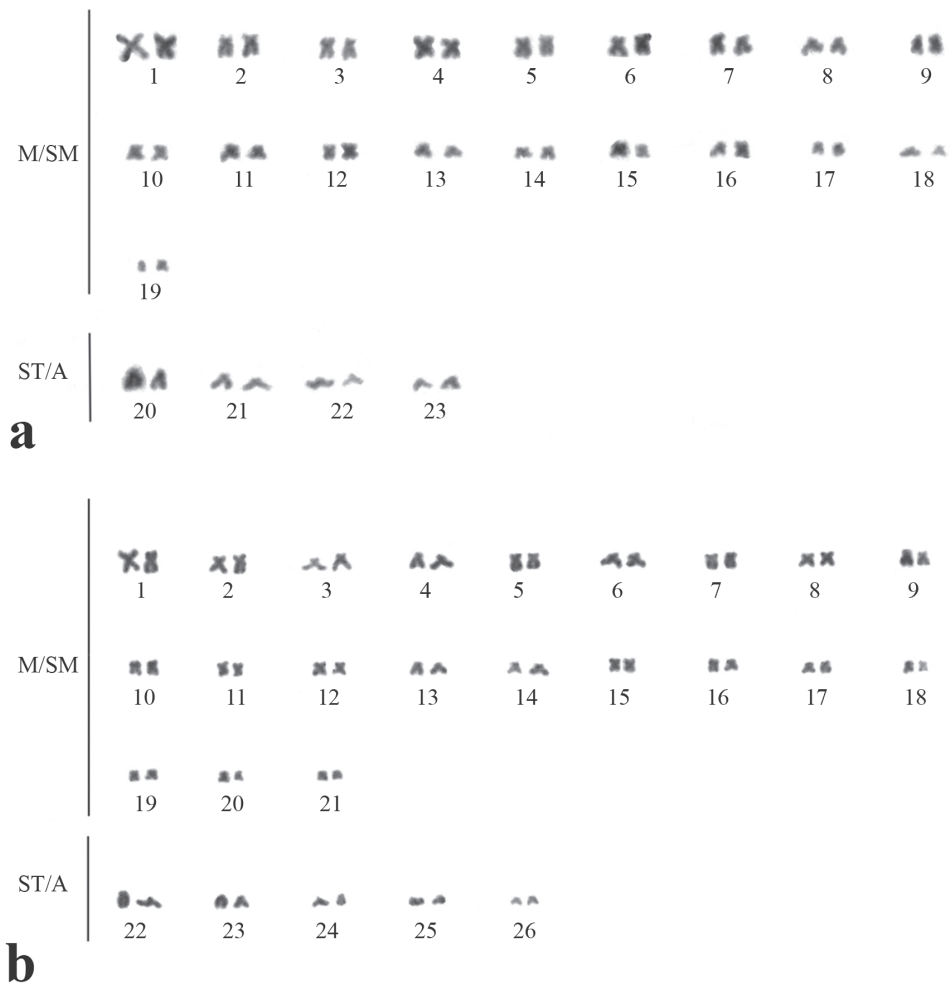

Fig. 2. Karyotypes of *Pimelodella* from the Miranda River, Passo do Lontra, Mato Grosso do Sul State. (**a**) 2n = 46, and (**b**) 2n = 52.

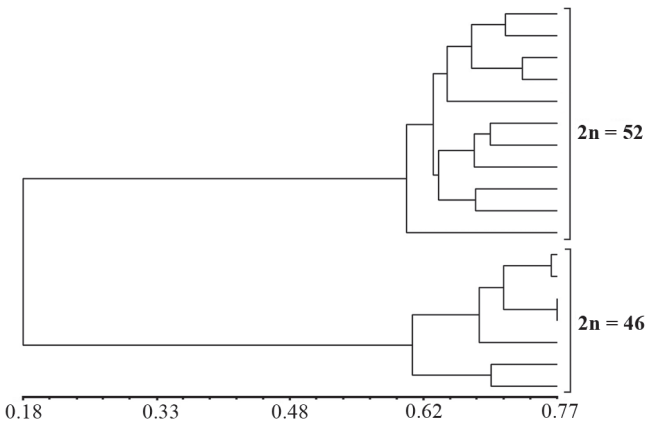

Fig. 3. Dendrogram of genetic similarity among the species of *Pimelodella* from the Miranda River based on the Jaccard coefficient and on UPGMA clustering method, showing the formation of two clusters.

Table 1. Weight of variables, eigenvalues and percentages relating to the first, second, and third eigenvectors of the Principal Components obtained from the analysis of combined samples of *Pimelodella* species.

	CP 1	CP 2	CP3
1. Standard length	0.289726	0.015837	-0.044266
2. Head length	0.258083	0.077697	-0.156947
3. Snout length	0.276907	0.069637	0.007304
4. Eye diameter	0.171324	0.217980	0.342959
5. Pre-dorsal length	0.245775	0.200042	0.188072
6. Dorsal-fin base length	0.314548	-0.316442	-0.418420
7. Adipose-fin base length	0.398125	0.026110	-0.275871
8. Dorsal-fin spine length	0.242476	0.365517	0.120618
9. Caudal peduncle depth	0.241554	-0.223113	0.034162
10. Pre-ventral length	0.283770	0.009382	-0.111584
11. Anal-fin base length	0.230008	-0.719201	0.518514
12. Pectoral-fin spine length	0.289681	0.308775	0.272182
13. Interorbital width	0.301805	-0.066202	-0.458721
Eigenvalue	0.108	0.004	0.003
Relative porcentages	90.6	3.0	2.2
Acumulated relative porcentages	90.6	93.6	95.8

Morphometry. The two species could also be differentiated by multivariate analysis of the Principal Components. The first axis, representing the size factor, retained 90.6% of the morphometric measurement variance; the second and third axes, which represent the shape, retained 3.0% and 2.2%, respectively (Table 1). The first axis revealed that most specimens with 2n = 52 chromosomes, which are distributed to the right in Fig. 4, were larger than those with 2n = 46. The differentiation of the species can be observed on the third axis, however with a small morphological overlap between the two species. The smallest specimen with 2n = 52 chromosomes was morphometrically similar to the individuals with 2n = 46 chromosomes. Moreover, the largest specimen with 2n = 46 chromosomes showed greater similarity to the group with 2n = 52. Thus, there is evidence of the need for caution when identifying specimens of these extreme sizes with the morphometric characters. Although there was a small overlap between the two species, the specimens of each remained relatively cohesive, indicating that they are really distinct.

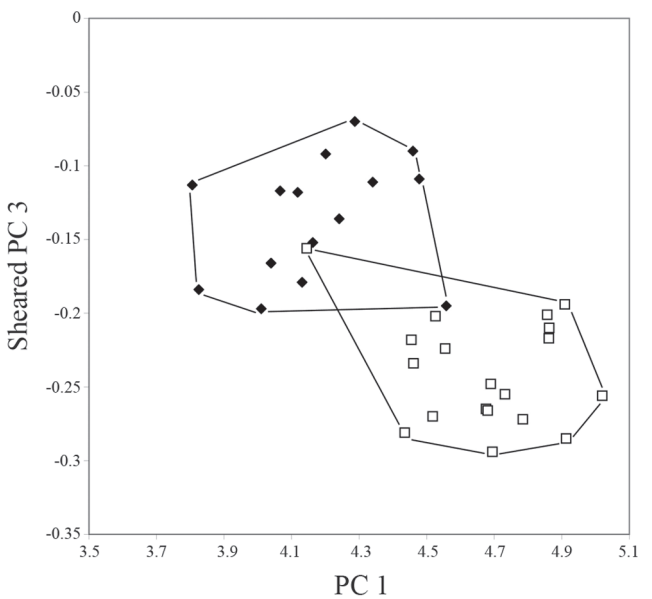

Fig. 4. Projection of the individual scores obtained through the analysis of the Principal Components of the combined samples of *Pimelodella* with 2n = 46 (diamonds) and with 2n = 52 (squares).

The variables with the highest weight for the group with 2n = 46, indicated by positive values in the third eigenvector, were: eye diameter, length of anal fin base, and length of pectoral spine. For the group with 2n = 52, the variables with higher weight were: length of dorsal fin base, length of adipose fin base, and interorbital distance, which were negative in the third eigenvector (Table 1).

The body proportion analysis (Table 2) revealed that the limits of variation of all variables overlap between the two species, but with significant differences between some means. Thus, although there is an overlap in the variation ranges, there is a shift of values in relation to the mean values, which significantly differentiate the two species from each other (according to the *t* Student test). Many variables with such differences coincide with those that were highly noticeable in the third axis of the Principal Component analysis as having the highest weights in the discrimination of the two species. In species with 2n = 46, the coinciding variable was the length of the anal fin base, and in species with 2n = 52, the length of the dorsal fin base, the length of adipose fin base, and the interorbital distance. In the analysis of means, the highest values of the pre-dorsal distance, caudal peduncle height,

Table 2. Morphometry of *Pimelodella* with 2n = 46 (13 specimens) and *Pimelodella* with 2n = 52 (19 specimens. Except barbel with 18 specimens) from the Miranda River Basin, Pantanal, MS. SD = Standard deviation; * = Means significantly different.

	2n = 46 (13 ex.)		2n = 52 (19 ex.)		*t* test (p)
	minimum-maximum	mean±SD	minimum-maximum	mean±SD	
Standard length (mm)	60.7-93.2	76.3±9.561	89.5-156.7	111.1±16.478	<0.0001*
Percentages of standard length					
Head length	21.6-24.3	23.0±0.717	20.4-24.6	22.4±0.998	0.0729
Pre-dorsal length	28.2-33.4	30.1±1.572	25.8-31.7	27.7±1.406	0.001153*
Dorsal-fin base length	14.4-19.2	16.8±1.649	15.9-19.4	18.0±0.993	0.0163*
Adipose-fin base length	31.2-39.9	35.5±2.296	36.9-44.2	41.5±1.873	<0.0001*
Dorsal-fin spine length	18.3-24.4	20.1±1.530	14.8-22.1	18.8±2.070	0.0619
Caudal peduncle depth	7.0-9.0	8.0±0.589	6.2-8.2	7.3±0.447	0.0011*
Pre-ventral length	40.0-44.9	43.5±1.442	41.6-45.2	43.3±0.886	0.6232
Anal-fin base length	10.5-16.8	12.3±1.709	8.5-11.9	10.7±0.967	0.0016*
Pectoral-fin spine length	16.9-20.3	18.6±1.049	15.4-19.8	18.0±1.16	0.1122
Maxillary barbel length	63.6-96.8	81.9±8.666	54.5-81.5	70.8±7.206	0.0031*
Percentages of head length					
Snout length	64.1-70.4	67.1±2.184	63.1-72.2	66.9±2.528	0,81396
Eye diameter	23.8-31.4	26.4±1.966	20.3-23.9	21.9±1.034	<0.0001*
Interorbital width	21.5-29.0	25.6±2.018	23.9-31.7	27.8±2.303	0.0081*

eye diameter, and length of maxillary barbel stood out in species with 2n = 46, except for the length of the pectoral-fin spine. The latter should therefore be used with caution.

Spines. The spines in the two species were very similar with regard to the saw-tooth pattern in the anterior and posterior margins of (Fig. 5) and cannot be used as a distinguishing feature between them. In both species, the anterior margin is fully serrated with wide retrorse serrations covering approximately 25 to 33% of the pointy end of the spine; heading toward the base of the spine, there are small orthogonal saw-toothed margins. The posterior margin presents larger retrorse and more robust serrations than the anterior margin with ossified saws covering 70 to 75% of the length.

Filament on the dorsal fin and caudal fin lobes. Contrary to what was initially assumed, the dorsal fin filament does not differentiate among the two species, as both possess this same feature (Fig. 6). However, it was possible to correlate their presence with sex (Table 3): males usually do present the dorsal fin filament, but females do not. The length of this filament is apparently not related to the increase in size of the specimen, since the two smaller males (SL = 107.4 mm and 108.1 mm) had the filament, while one of the largest (SL = 117.6 mm) did not bear the filament. The Pearson correlation coefficient between the length of the filament and the standard length in males of *Pimelodella* 2n = 52, corroborates this hypothesis, as it showed a very low value ($r^2 = 0.06$, N = 7).

The Pearson correlation coefficient demonstrates that the increase in standard length explains about 40% of the variation in length of the upper lobe caudal fin in *Pimelodella* 2n = 52, whereas in *Pimelodella* 2n = 46, this explanation comprised approximately 57% of that variation (Fig. 7). As occurred in the multivariate morphometric analysis, the smaller specimens of *Pimelodella* 2n = 52 tend to be confounded with the larger specimens of *Pimelodella* 2n = 46. Nevertheless, the calculation of the allometric coefficient reveals that in *Pimelodella* 2n = 52, the upper lobe of the caudal fin in males shows positive allometric growth (b = 1.11), whereas in *Pimelodella* 2n = 46, the allometric growth is negative (b = 0.61), meaning that as the standard length increases in the former species, the upper lobe of the caudal fin grows in the same proportion, while the opposite occurs with the second species.

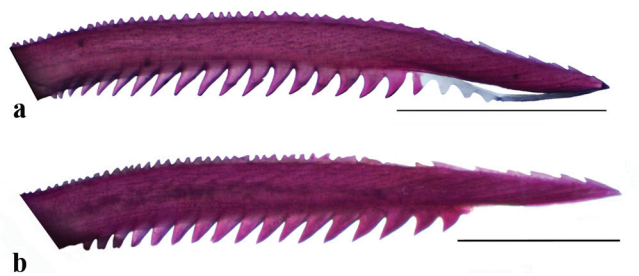

Fig. 5. Pectoral-fin spines in *Pimelodella* from the Miranda River. **a**) 2n = 46; **b**) 2n = 52. Scale bar = 5 mm.

Discussion

The hypothesis that the sample could contain four sympatric species of *Pimelodella* from the Miranda River was not confirmed by the analyses accomplished in this study. The features initially observed, such as the presence of elongated filament on the dorsal fin and the morphologic characters of the caudal fin lobes, as traditionally used, can be variable between and within species.

Hence, this study evidenced a case of morphological convergence in species with a high level of molecular and cytogenetic diversification. The existence of morphological similarities between related but different organisms is evidence

for evolution by natural selection. Thus, it is possible that environmental pressures are selecting similar body forms among different species, favoring both. Two or more species identified as one fall within the concept of cryptic species. In *Pimelodella*, some sympatric species are morphologically very similar and probably constitute a species complex, hampering their identification. According to Eigenmann (1917), "As the color and the size of the eye also vary with age and with locality, and, as all of these characters vary independently, the defining of species of *Pimelodella* becomes a delicate and difficult task." This difficulty was also observed by Martin & Bermingham (2000), while studying *Pimelodella chagresi* (Steindachner, 1876) from Central America. They utilized morphology, RFLP, and mitochondrial genes as tools to unravel the problem.

In a review on cytogenetic studies of the family Heptapteridae carried out by Swarça *et al*. (2007) it was found that the diploid number of 2n = 52 has already been observed in the genus *Pimelodella* in *P. gracilis*, from the Paraguay River, Mato Grosso do Sul State; *Pimelodella* sp., from the Paraná River, Paraná State; *P. cristata* (Müller & Troschel, 1849), from the Araguaí River, Mato Grosso State; and *P.* aff. *avanhandavae* Eigenmann, 1917, from the Tibagi River, Paraná State. However, among all above-cited species, only *P. gracilis* occurs in the Paraguay River basin (Britski *et al*., 2007), but the morphological characteristics of individuals that had 2n = 52 did not coincide with the characters used to identify *P. gracilis*, because some presented the first dorsal fin ray prolonged as a filament. According to Miranda-Ribeiro (1914) and Britski *et al*. (2007), this is a feature found in *P. taenioptera*, a name that can be applied to the species with 2n = 52 chromosomes, as they share many morphological characteristics, except for the color pattern. However, as noted by Eigenmann (1917), this character is subject to a great variation.

The diploid number of 2n = 46 is shared by several species of *Pimelodella*, among which are: *P. avanhandavae* (Vissotto *et al*., 1999), *P.* aff. *avanhandavae* (Swarça *et al*., 2003), *P. boschmai* van der Stigchel, 1964 (Garcia & Almeida-Toledo, 2010), *Pimelodella* sp. (Vasconcelos & Martins-Santos, 2004; Garcia *et al*., 2010), *P. meeki* Eigenmann, 1910 (Vidotto *et al*., 2004; Garcia & Almeida-Toledo, 2010; Borba *et al*., 2011), indicating that this number is the most commonly observed in this genus. Nevertheless, this is currently the first publication reporting the occurrence of that diploid number in a species of *Pimelodella* from the Pantanal. The other species that have the dorsal fin filament elongated is *P. griffini*, which differs from *P. taenioptera* owing to the shortest length of the adipose fin base (Britski *et al*., 2007), as observed for the species with 2n = 46. As stated by Eigenmann (1917), *P. griffini* has barbels reaching the tip of the pelvic fin or are slightly shorter, whereas in specimens with 2n = 46, the barbel is extremely elongated. Eigenmann (1917) also observed that "The length of the barbels in the same species differs with age. In the young, the barbels are relatively short. They grow disproportionately larger with the growth of the fish and then lag behind again in their increase in length. The length of the barbels of the same species not only differs with age, but sometimes also with locality".

Table 3. Frequency of *Pimelodella* individuals with or without dorsal fin filament in relation to the diploid number and gender (M = male; F = female).

	With filament		Without filament		
	M	F	M	F	Total
2n = 46	1	0	0	3	4
2n = 52	7	0	1	1	9
Total	6	0	1	4	13

In freshwater fish, several cryptic species have been detected using cytogenetics. This tool allowed the observation of intraspecific chromosomal variations, enabling the identification of species complexes, as observed in *Eigenmannia* sp. (Almeida-Toledo *et al*., 2002), *Astyanax fasciatus* (Cuvier, 1819) (Pazza *et al*., 2008) and *Hoplias malabaricus* (Bloch, 1794) (Rosa *et al*., 2009).

The differences between *P. griffini* and *P. taenioptera* were also corroborated by the molecular analysis with the formation of two distinct clusters corresponding to the karyotypes found. Individuals of these clusters also showed different degrees of intraspecific genetic similarity, evidencing the variability in each species, which was observed in the morphological characteristics.

According to Chambers *et al*. (1998), the RAPD analysis is a method that has good potential for the differentiation of closely related species. The same was ascertained by Almeida *et al*. (2003) in a comparative study of six species, which comprised four species of Siluriformes, two of them were *Pimelodella*, whereby they observed that individuals of each species remained clustered.

Morphology also proved useful in the separation of the two species. The morphometric analysis is extensively used in descriptions of species for being informative and for the ease of obtaining. The multivariate morphometric analysis was important to observe the separation of the species by morphometric characters, as well as to indicate which of these characters had the greatest weight in the separation. However, as this is an exploratory method, it was not possible to know the magnitude of change in these variables. This matter was solved with body proportion calculations.

Another character relevant to the systematics of *Pimelodella* considered by Eigenmann (1917) is the pectoral fin spine and its saws. This author presented 44 illustrations of spines in different species, where one can observe the similarity in the form of the thorns among the species analyzed in this work and *Pimelodella hasemani* Eigenmann 1917, which occurs from the Guaporé to the Madeira River basins, and also in the Amazon basin. The occurrence of this species in the Paraguay River basin has not yet been reported, but it is possible that it may also occur in that basin due to the proximity of its headwaters. However, as stated by Eigenmann (1917), *P. hasemanni* does not possess the elongated filament on the dorsal fin and the caudal fin

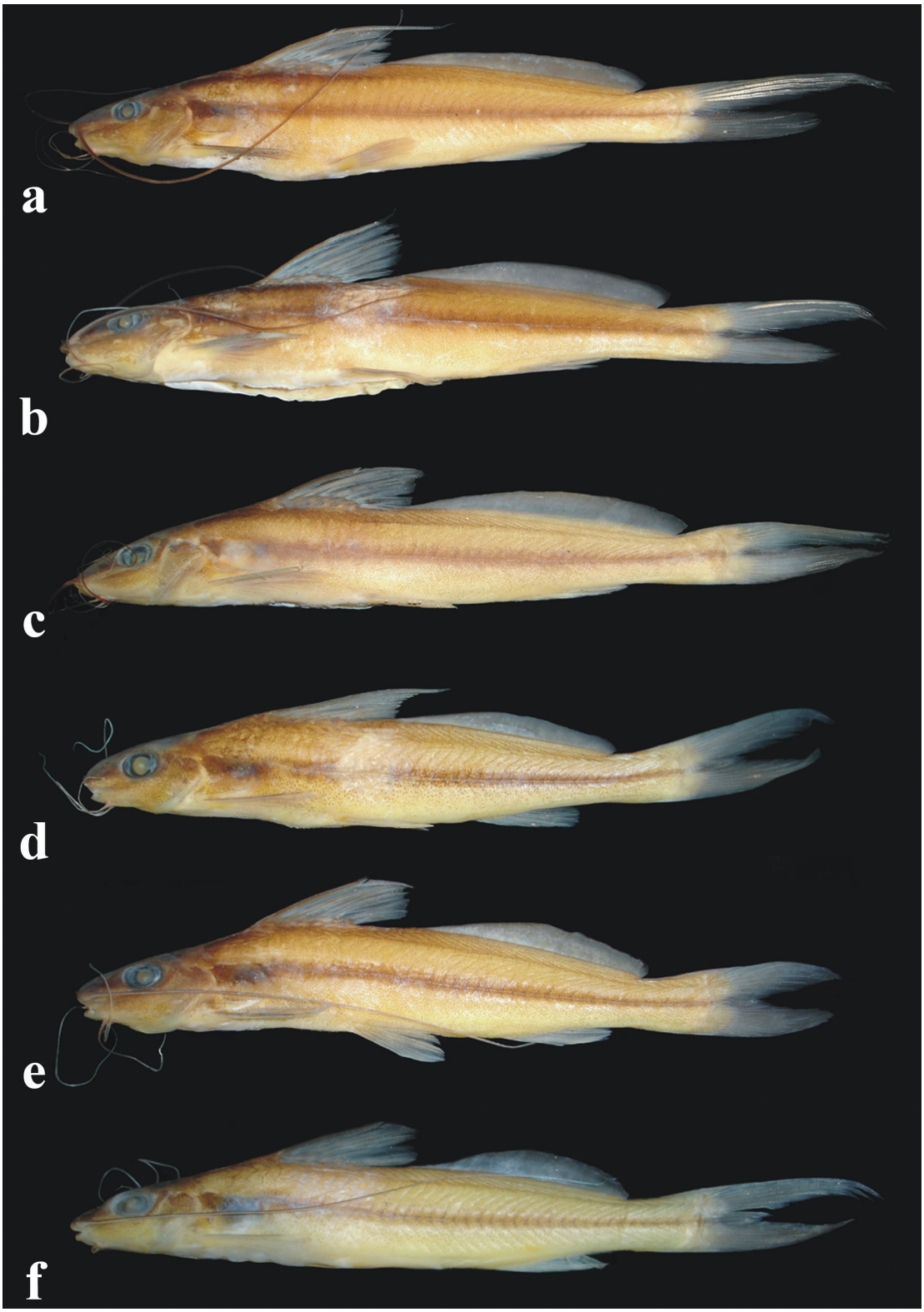

Fig. 6. **a** to **c**) *Pimelodella* 2n = 52, from the Miranda River basin, Passo do Lontra, MS: **a**) dorsal filament and upper lobe of caudal fin elongated (male, 123.6 mm SL), **b**) dorsal fin filament slightly elongated and upper lobe of caudal fin elongated (male, 106.8 mm SL), **c**) filament on dorsal fin absent and upper lobe of caudal fin not elongated (male, 117.6 mm SL). **d** to **f**) *Pimelodella* 2n = 46 from the Miranda River basin, Passo do Lontra, MS: **d**) filament on dorsal fin elongated and upper lobe of caudal fin slightly elongated (male, 82.7 mm SL), **e**) Filament on the dorsal fin slightly elongated and upper lobe of caudal fin not elongated (female, 93.2 mm SL), **f**) Filament on the dorsal fin absent and upper lobe of caudal fin elongated (female, 85.1 mm SL).

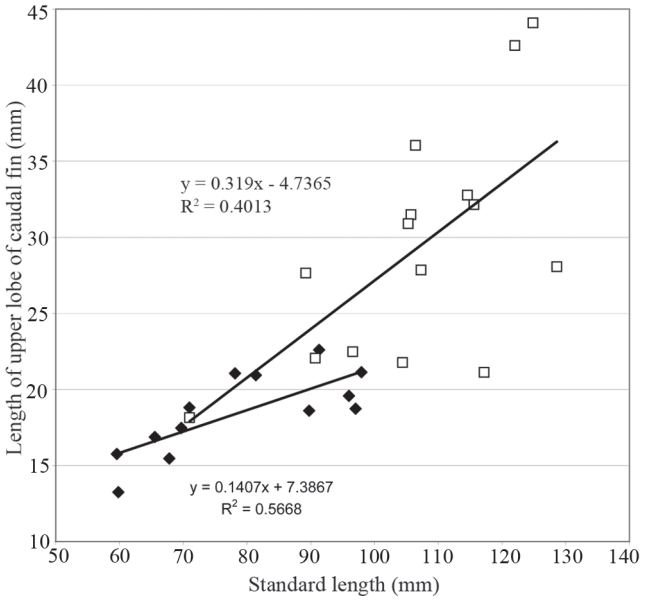

Fig. 7. Relationship between the standard length and the length of the upper lobe of the caudal fin in *Pimelodella* 2n = 46 (dark diamonds, N = 13) and 2n = 52 (light squares, N = 15).

lobes are about the same size. However, the characteristics of the thorn in this species with 2n = 46 does not permit its identification as *P. griffini*, because this species does not present strong saws on the posterior margin and the small saws on the anterior margin are slightly conspicuous. Despite the emphasis given by Eigenmann (1917) to the pectoral spine, he noted that: "The pectoral spines also vary somewhat with growth. With age the thorns increase in number by the addition of new ones toward the tip and come to occupy a larger portion of the length of the spine."

Among the other species observed in the Pantanal (Britski *et al*., 2007), *P. notomelas* Eigenmann 1917 differs from *P. griffini* and *P. taenioptera* for having a black wedge-shaped spot on the dorsal fin and for not presenting a dark lateral stripe along the lateral line. *Pimelodella mucosa* Eigenmann & Ward is also easily identified by the pore sizes of the lateral line in the lower region of the head, starting between the edges of the operculum and extending toward the mandible with a diameter approximately equal to the diameter of the pupil. *Pimelodella gracilis* and *P. megalura*, in turn, may be distinguished from *P. griffini* and *P. taenioptera* by the absence of the dorsal fin filament. However, it is possible that a considerable number of identifications of females of these two species have been incorrectly made. In this case, it is essential to identify their gender and age, since young females can cause confusion. As taxonomy is primarily based on morphological characteristics, it has often occurred that morphologically indistinguishable organisms have been identified as belonging to the same species, when, in fact, they are distinct species. *Pimelodella griffini* and *P. taenioptera* show morphological characteristics which, in principle, can cause confusion between them. However, the joint analysis of the karyotype, molecular biology and morphometry allowed distinguishing them safely.

Thus, only the employment of various analytical techniques enabled an accurate detection of these apparently cryptic taxonomic units. The non-recognition of species has several consequences that range from the underestimation of the real biodiversity of each site to the threat of extinction of rare species that can be confused with other more common species (Frankham *et al*., 2008). Also, it is worth emphasizing the importance of the identification of these sympatric, cryptic species, to ensure that estimates of fish diversity in the Pantanal are accurately made.

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