DOI: http://dx.doi.org/10.1590/1519-6984.13212

Cytogenetics analyses among populations of the fish *Iheringichtys labrosus* (Kröyer, 1874) (Siluriformes, Pimelodidae). Karyotype analysis, C- banding and AgRONs distribution

Sanchez, S.[a], Swarça, AC.[b] and Fenocchio, AS.[c]*

[a]Instituto de Ictiología del Nordeste, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste – UNNE, Sargento Cabral, 2139. CP 3400, Corrientes, Argentina
[b]Departamento de Histologia, Centro de Ciências Biológicas – CCB, Universidade Estadual de Londrina – UEL, Rod. Celso Garcia Cid, Pr 445, Km 380, CP 10011, CEP 86057-970, Londrina, PR, Brazil
[c]Departamento de Genética, Universidad Nacional de Misiones – UNaM, Félix de Azara, 1552, CP 3300, Posadas, Misiones, Argentina
*e-mail: swarca@uel.br

Received: July 16, 2012 – Accepted: December 18, 2012 – Distributed: February 28, 2014
(With 3 figures)

Abstract

Karyotypic data on *Iheringichthys labrosus* from several populations of Paraná River/Argentina are presented. The diploid number was 2n=56 and the karyotype consisted of 42m/sm + 14st/a (NF= 98). The AgNORs were observed in telomeres of the long arm of a st/a chromosome pair. These sites were also positive after C-banding. Heterochromatin was observed in the telomeric position in some chromosomal pairs and can be found in one or both metacentric chromosome arms. The obtained results were compared with those reported for different populations from Brazilian rivers and the similarity among them was evident. However, discordant chromosome formulae and karyotype features between samples should be carefully taken because, in some cases, they seem to be more technical artifacts than real differences.

Keywords: Pimelodidae*, I. labrosus*, karyotype conservatism.

Análise citogenética entre populações de peixes *Iheringichtys labrosus* (Kröyer, 1874) (Siluriformes, Pimelodidae). Análise cariotípica, bandamento C e distribuição das AgRONs

Resumo

Dados cariotípicos de *Iheringichthys labrosus* de várias populações do Rio Paraná/Argentina são apresentados. O número diploide foi 2n=56 e o cariótipo constituído de 42m/sm + 14st/a (NF= 98). As AgNORs foram observadas telomericamente no braço longo de um par cromossômico st/a. Estes sítios também apresentaram bandas positivas após bandamento C. Heterocromatina foi observada em posição telomérica em alguns pares cromossômicos e podem ser encontradas em um ou ambos os braços de cromossomos metacêntricos. Os resultados obtidos foram comparados com aqueles reportados em diferentes populações de rios brasileiros e foi evidente uma similaridade entre eles. Entretanto, fórmulas cariotípicas discordantes e características cariotípicas entre exemplares deveriam ser cuidadosamente observadas porque, em alguns casos, eles parecem ser mais artefatos de técnica que diferenças reais.

Palavras-chave: Pimelodidae, I. labrosus, conservantismo cariotípico.

1. Introduction

The order Siluriformes (Fowler, 1951) comprises a large group of fish without scales, whose body is completely uncovered or covered totally or partially with lines of bony plates. This group comprises 30 families with a wide distribution around the world, predominates in freshwaters, and is very well represented in the Neotropical region (Reis et al., 2003).

Cytogenetic studies in Siluriformes show the occurrence of groups with a broad chromosome number variability, ranging from 2n=24 in *Leiobagrus marginatus* (Günther) (Amblycipitidae) (Yu et al., 1989) to 2n = 134 in *Corydoras aeneus* Hensel (Scheel et al., 1972; Henigardner and Rosen, 1972), as well as, examples of a relative stability with respect to diploid numbers, i.e.: Sorubiminae and *Pimelodus* Lacépède, 1803, that show 2n= 56 in almost all studied species (Swarça et al., 2007). LeGrande (1981) proposed a hypothetical ancestral karyotype for Siluriformes with 2n= 56±2 and high Fundamental Number (NF), which was supported by other studies in different species of the order (Oliveira and Gosztonyi, 2000).

Iheringichthys labrosus (Lütken, 1874), belongs to the Pimelodidae family and it is being widely used for sport fishing along the Paraná River channel and its main tributaries. Chromosomal studies performed in different Brazilian populations of this species showed karyotyopes composed of 2n=56 and NF usually reported being greater than 100, AgNORs present in one chromosomal pair and conspicuous C bands predominantly located in the telomeric regions (These data are summarised in Table 1). Some populations showed B chromosomes (Vissotto et al., 1999; Carvalho et al., 2004; Carvalho and Dias, 2005a). Papers on Neotropical fish cytogenetics show differences in karyotypes and formulas into the same species that are frequently attributed to "polymorphisms" however, in many cases the results could be due to technical problems and/or to the subjectivity of the researcher. This situation has been reported for species of the genus *Pimelodus*, a catfish nearly related to *Iheringichthys* by Swarça et al. (2007), and justify the need to study several populations in view of a correct karyotypic characterisation.

In the present work, *I. labrosus* from several localities along Paraná River (Argentina) were cytogenetically studied with the objective of reach an almost correct karyotypic characterisation and the results were compared with those reported for different populations from Brazilian river basins.

2. Material and Methods

Twenty five specimens of *Iheringichthys labrosus,* 19 females (f) and 6 males (m), caught in the Parana River (NE Argentina), lagoons alongside river, and small tributaries were cytogenetically analysed: the sample localities are shown in Figure 1: Posadas (Misiones Province: S 27° 21' 06"; W 55° 53' 54" -2f / 1m-), Reconquista (Santa Fé Province: S 29° 14' 11"; W 59° 34' 46" -2f / 1m), Ituzaingó (S 27° 29' 57"; W 56° 42' 42" -6 f-), Itá Ibaté (S 27° 25' 46"; W 57° 21' 40" -2f / 1m), Yahapé (S 27° 22' 15"; W 57° 39' 11" -1 m), Puerto Abra (S 27° 18' 48"; W 57° 53' 33" -3 f), and Corrientes (S 27° 27' 23"; W 58° 49' 06" -4f / 2m) (Corrientes Province). The specimens were deposited in the collection of the Laboratory of the Instituto de Ictiología del Nordeste, Universidad Nacional del Nordeste/Argentina. The chromosome preparations were obtained from cephalic kidney cells, with either of the following protocols: short-term culture cells (Fenocchio et al., 1991) or the conventional method (Foresti et al., 1993). Heterochromatin regions were detected according to Sumner (1972) and the nucleolar organiser regions (AgNORs) were analyzed after silver nitrate staining (Howell and Black, 1980). The best metaphases were photographed and amplified and measured with the help of a 0.02 mm precision caliper to obtain centromeric index in order to proceed to the construction of the karyotype. The chromosomes were classified as metacentrics (m), submetacentrics (sm), subtelocentrics (st) and acrocentrics (a) according to their morphology and arm ratios (Levan et al., 1964; Guerra, 1988). In order to determine the Fundamental Number (NF) the st/a chromosomes were considered as monoarmed.

3. Results

Satisfactory results were obtained in 18 of the 25 specimens analysed and after counting 238 metaphase plates a modal diploid number of 56 chromosomes were determined. The centromeric index was estimated measuring chromosomes from 20 metaphases. Karyotypes were composed by 42m/sm + 14st/a (NF= 98) (Figure 2). There were no sex-related chromosome heteromorphism.

The AgNORs were observed in a st/a pair staining telomeric regions on the long arms (Figure 3a), only cells with one or two active nucleoli were identified.

Table 1. Cytogenetic data of *Iheringichthys labrosus*. Paraná State (PR), São Paulo state (SP), Diploid Number (2n), number of arms (NF), location of nucleolar organising regions (AgNORs), location of constitutive heterochromatic regions (BC), number of B chromosomes (Bs); q: long arm; p: short arm; per: pericentromeric; int: interstitial; tel: telomeric, cent: centromeric.

Locality	2n	Karyotypic Formula	NF	AgNORs	BC	Bs	References
Mogi-Guaçu River (SP, Brazil)	56	40 m/sm + 16 st/a*	96			0-2	Dias and Foresti (1990)
Paraná River (PR, Brazil)	56	42 m/sm + 14 st/a	98	st, q, tel	per/ tel	-	García et al. (1990)
Tibagi River (PR, Brazil)	56	40m/sm+16st/a*	96	st, q, tel	tel	0-3	Carvalho et al. (2004), Carvalho et al. (2010)
Capivara Reservoir, Sertanópolis and Porecatu (PR, Brazil)	56	38 m/ sm+18 st/a*	94	st, q, tel	tel	0-1	Carvalho and Dias (2005a, b)
Jurumirim Reservoir (SP, Brazil)	56	40m/sm+16st/a*	96	st, q, tel	per/ int/tel	+ (1)	Vissotto et al. (1999)
Guaraúna River (PR, Brazil)	56	46m/sm+10st/a*	102	sm, p, tel	tel /cent	-	Ribeiro et al. (2008)
Paraná River (7 localities from Argentina)	56	42m/sm+14st/a*	98	st, q, tel	tel/int	-	Present paper

*NF recalculated by the authors.

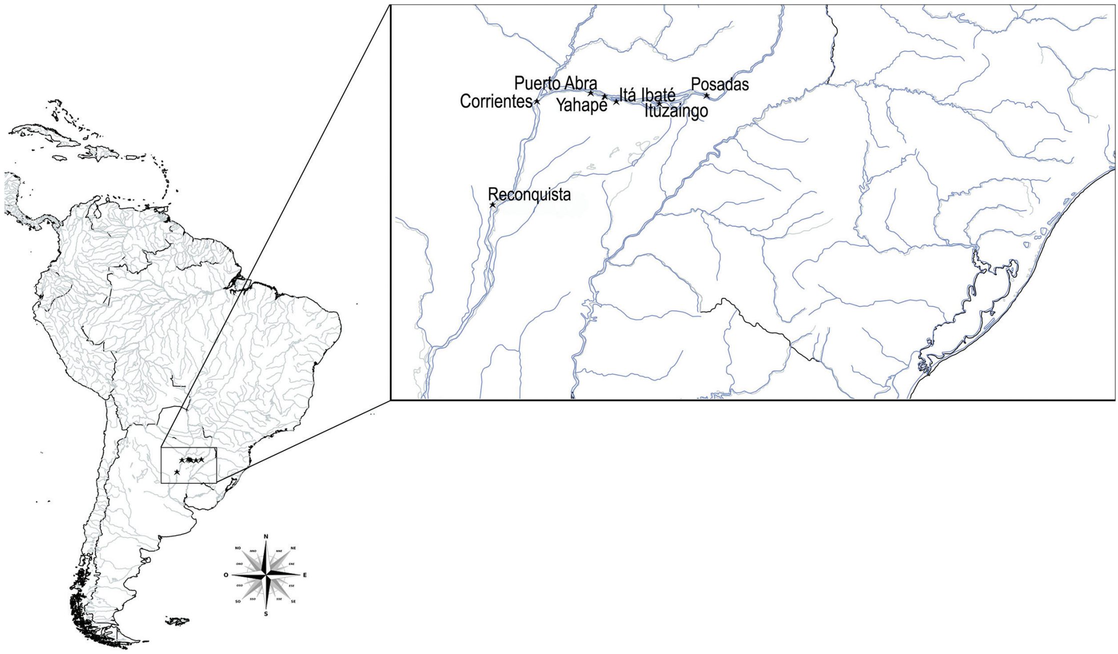

Figure 1. Map of South America, the box indicates the studied region in Argentina. Numbers correspond to the sample sites: 1- Reconquista (Santa Fé State); 2- Corrientes; 3- Puerto Abra; 4- Yahapé; 5- Ita Ibaté; 6- Ituzaingó (Corrientes State); 7- Posadas (Misiones State).

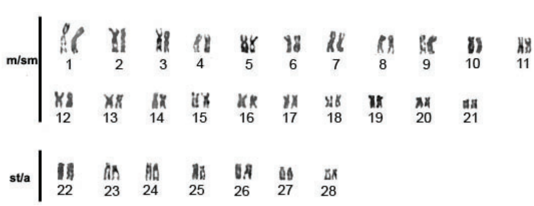

Figure 2. Karyotype of *Iheringichthys labrosus*, Giemsa staining.

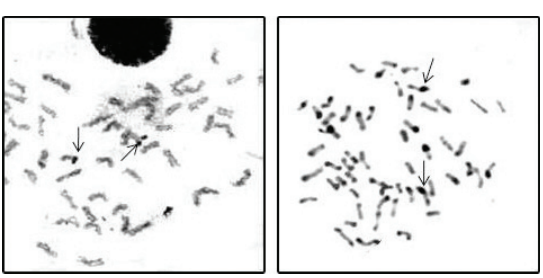

Figure 3. Metaphase plates of *Iheringichthys labrosus* stained with $AgNO_3$ (a - partial plate) and C-banding (b). Arrows indicate AgNOR-bearing chromosomes.

Heterochromatin was observed almost exclusively in the telomeric position of some chromosomal pairs and can be found on one or both chromosomal arms of several m\sm chromosomes. Heterochromatic bands stain centromeric regions and entire short arms of some st\a chromosomes (Figure 3b). The AgNORs also are C-positive.

4. Discussion

Previous cytogenetic studies in *I. labrosus* report the same features, i.e., the diploid number of 56 chromosomes, the absence of sex chromosomes, and the presence of simple AgNORs, coincident with the heterochromatic segments located in the terminal position in the long arm of a st/a pair (Vissotto et al., 1999; Carvalho et al., 2004; Carvalho and Dias, 2005a). A remarkable feature evidenced in this work is that all the seven populations studied along the Paraná river show the same chromosome number and karyotype formula (42m/sm+14st/a). This fact makes it clear that *I. labrosus* does not have a large cytogenetic variation as observed in other species of the family, as is the example of *Pimelodus maculatus* (Swarça et al., 2007).

However, differences in chromosome formula reported by other authors could be explained in some cases by the occurrence of pericentric inversions, rearrangements that would allow the emergence of new chromosome types without affecting the diploid number of the species. Even artifacts in cytogenetic preparations or not standardised techniques (highly condensed chromosomes or with a not well-defined morphology) may lead to differences in karyotypes that are often not real. In the present study, in order to compare the fundamental number (NF) of all species some of them were recalculated (Table 1, asterisk).

It could also be mentioned that some variations were detected in the C-bands, i.e., heterochromatin was observed almost exclusively in the telomeric position of some chromosome pairs and can be found on one or both chromosomal arms of most m chromosomes. There was one exception represented by a sample from the Jurumirim Reservoir (Brazil), which revealed heteropicnotic bands in several pairs of the complement (Vissotto et al., 1999). This additional variation is associated with the presence of B chromosomes, which were described only in some populations of this species (Table 1).

It is possible that the diversification of populations could be greater from a genetic, not chromosomal, point of view since the karyotypes from diverse populations of *Iheringichthys* show subtle differences. This hypothesis is supported by the results of a molecular study including several populations of three Pimelodidae species by RAPD and isoenzyme analysis. In this study, *I. labrosus* showed the highest level of genetic variability, where all populations were clearly isolated from each other, probably as a result of sedentary habits that this species presents (Almeida et al., 1999).

After analysing samples of four families of Siluriformes, LeGrande (1981) proposed that the ancestral karyotype of this Order could present 2n = 56±2 and a fundamental number higher than 80, an assertion which was supported by other authors (Swarça et al., 2007). The karyotypes found in several *I. labrosus* populations, as well as in many genera of Pimelodidae, evidence that 2n=56 predominate, suggesting some degree of conservatism, at least in diploid number and chromosome macrostructure.

The presence of AgNORs in telomeric position on the long arm of chromosome st-a appears as a cytogenetic feature shared by closely related genera as *Bergiaria* Eigenmann & Norris, 1901 (Dias and Foresti, 1993), *Iheringhichthys* Eigenmann & Norris, 1900 (Carvalho et al., 2004; Carvalho and Dias, 2005a), *Parapimelodus* and *Pimelodus* Lacépède, 1803 (Garcia and Moreira-Filho, 2005; Swarça et al., 2001). One exception is the population from the Guaraúna River (Paraná, Brazil), where the NOR is found on the short arm of a sm pair (Ribeiro et al., 2008). In previous reports, the presence of AgNORs on the short arms of st-a chromosomes observed in some species of *Pimelodus* was considered derived, arisen from rDNA loci translocations (Borin and Martins-Santos, 2002; Souza et al., 2004).

For a more accurate cytogenetic description, populations of *Iheringichthys labrosus* from Argentina were studied and compared with available data from Brazil. Summarising the data obtained from 14 populations and joining the chromosomes m/sm and st/a, four karyotypic formulas were observed (Table 1). However, variability in chromosome formulae and karyotype features among populations should be taken carefully because, in some cases, they seem to be more technical artifacts than real differences. This situation indicates the need to devote more attention, either in the classification and organisation of the karyotypes, for a more adequate and parsimonious cytogenetic characterisation reflecting accurately the species relationships.

References

ALMEIDA, FS., FUNGARO, MHP. and SODRÉ, LMK., 1999. Estudo de *Iheringichthys labrosus* e *Pimelodus* aff. *absconditus* (Pisces, Siluriformes) da bacia do Rio Tibagi (PR) através da análise de RAPD e Isoenzimas. Genetics and Molecular Biology, vol. 22, p. 174.

BORIN, LA. and MARTINS-SANTOS, IC., 2002. Cytogenetic aspects in species of the genus *Pimelodus* (Pisces, Siluriformes, Pimelodidae) of the River Paraná basin. Cytologia, vol. 67, p. 199-204. http://dx.doi.org/10.1508/cytologia.67.199

CARVALHO, RA., GIULIANO-CAETANO, L. and DIAS, AL., 2004. Cytogenetic analysis A- and B-chromosomes of *Iheringichthys labrosus* (Pisces, Pimelodidae) from the Tibagi River, Paraná, Brazil. Cytologia, vol. 69, p. 381-385. http://dx.doi.org/10.1508/cytologia.69.381

CARVALHO, RA. and DIAS, AL., 2005a. Cytogenetic characterization of B chromosomes in two populations of *Iheringichthys labrosus* (Pisces, Pimelodidae) from the Capivara Reservoir (Paraná, Brazil). Caryologia, vol. 58, p. 269-273. http://dx.doi.org/10.1080/00087114.2005.10589462

-, 2005b. Karyotypic characterization of *Iheringichthys labrosus* (Pisces, Pimelodidae): C-, G- and restriction endonuclease banding. *Genetics and Molecular Research*, vol. 4, p. 663-667. PMid:16475110.

CARVALHO, RA., LAUDICINA, A., GIULIANO-CAETANO, L., MARTINS-SANTOS, IC. and DIAS, AL., 2010. Cytogenetic analysis of the 18S, 5S rDNA and B chromosome of *Iheringichthys labrosus* (Lütken, 1874) (Siluriformes, Pimelodidae). *Brazilian Journal of Biology*, vol. 70, p. 631-636. PMid:20730351. http://dx.doi.org/10.1590/S1519-69842010000300021

DIAS, AL. and FORESTI, F., 1990. Algumas considerações sobre o cariótipo de *Iheringichthys labrosus* (Siluriformes, Pimelodidae) do Rio Mogi-Guaçu. In *Abstract do III Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais*, 1990. Botucatu. p. 32.

-, 1993. Cytogenetic studies on fishes of the family Pimelodidae (Siluroidei). *Revista Brasileira de Biologia* = Brazilian Journal of Biology, vol. 16, p. 585-600.

FENOCCHIO, AS., VÊNERE, PC., CESAR, ACG., DIAS, AL. and BERTOLLO, LAC., 1991. Short term culture from solid tissues of fishes. Caryologia, vol. 44, p. 161-166. http://dx.doi.org/10.1080/00087114.1991.10797181

FORESTI, F., OLIVEIRA, C. and ALMEIDA-TOLEDO, LF., 1993. A method for chromosome preparations from large fish specimens using "in vitro" short-term treatment with colchicines. Experientia, vol. 49, p. 810-814. http://dx.doi.org/10.1007/BF01923555

GARCÍA, RMG., SACHETE, R. and MARTINS-SANTOS, IC., 1990. Aspectos citogenéticos de *Iheringichthys labrosus* (Pisces, Pimelodidae) do Rio Paraná, região de Porto Rico, PR. In *Abstract do III Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais*, 1990. Botucatu. p. 33.

GARCIA, C. and MOREIRA-FILHO, O., 2005. Cytogenetical analyses in three fish species of the genus *Pimelodus* (Siluriformes: Pimelodidae) from Rio São Francisco: Considerations about the karyotypical evolution in the genus. Neotropical Ichthyology, vol. 3, p. 285-289. http://dx.doi.org/10.1590/S1679-62252005000200006

GUERRA, MS., 1988. *Introdução a Citogenética Geral*. Rio de Janeiro: Ed. Guanabara. 132 p.

HENIGARDNER, R. and ROSEN, DE., 1972. DNA cellular content and evolution off teleostean fishes. American Naturalist, vol. 106, p. 621-644. http://dx.doi.org/10.1086/282801

HOWELL, WM. and BLACK, DA., 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developper: a 1- step method . Experentia, vol. 36, p. 1014-1015. http://dx.doi.org/10.1007/BF01953855

LEGRANDE, WH., 1981. Chromosomal evolution in North American catfishes (Siluriformes, Ictaluriidae) with particular emphasis on the madtons, *Noturus*. Copeia, vol. 1, p. 33-52.

LEVAN, A., FREDGA, K. and SANDBERG, AA., 1964. Nomenclature for centromeric position on chromosomes. Hereditas, vol. 52, p. 201-220. http://dx.doi.org/10.1111/j.1601-5223.1964.tb01953.x

OLIVEIRA, C. and GOSZTONYI, AE., 2000. A cytogenetic study of *Diplomystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in siluriforms. Caryologia, vol. 53, p. 31-37. http://dx.doi.org/10.1080/00087114.2000.10589178

REIS, RE., KULLANDER, SO. and FERRARIS JUNIOR, CJ., 2003. *Check list of the freshwater fishes of South and Central America*. Porto Alegre: Edipucrs. 742 p.

RIBEIRO, LB., MATOSO, DA., ALMEIDA, MC., VICARI, MR., MORAES-NETO, A., SVIDNICKI, MC. and ARTONI, RF., 2008. Karyotypic variability in *Iheringichthys labrosus* (Teleostei, Pimelodidae) from the Tibagi River basin (Paraná State, Brazil). *Genetics and Molecula Research*, vol. 7, p. 718-724. PMid:18767239. http://dx.doi.org/10.4238/vol7-3gmr456

SCHEEL, JJ., SIMONSEN, V. and GYLDENHOLM, AD., 1972. The karyotypes and some eletrophoretic patterns of fourteen species of the genus *Corydoras*. *Sonderdruck aus Z f zool Systematik u Evolutionsforschung*, vol. 10, p. 144-152.

SOUZA, L., SWARÇA, AC. and DIAS, AL., 2004. Analysis of the nucleolus organizer regions in 5 species of the genus *Pimelodus* (Siluriformes, Pimelodidae) using $AgNO_3$, CMA_3, and FISH with the 18S rDNA probe. Caryologia, vol. 57, p. 145-151. http://dx.doi.org/10.1080/00087114.2004.10589384

SWARÇA, AC., GIULIANO-CAETANO, L. and DIAS, AL., 2001. Analyses of nucleolus organizer regions and heterochromatin of *Pimelodus maculatus* (Pisces, Pimelodidae). Genetica, vol. 110, p. 97-100.

SWARÇA, AC., FENOCCHIO, AS. and DIAS, AL., 2007. An update Cytogenetic Review for Species of the Families Pseudopimelodidae, Pimelodidae and Heptapteridae (Pisces, Siluriformes). Suggestion of a Cytotaxonomical Classification. Caryologia. vol 60, p. 338-348. http://dx.doi.org/10.1080/00087114.2007.10797957

SUMNER, AT., 1972. A simple technique for demonstrating centromeric heterocromatin. Exploration Cell Research, vol. 75, p. 304-306. http://dx.doi.org/10.1016/0014-4827(72)90558-7

VISSOTTO, PC., FORESTI, F. and OLIVEIRA, C., 1999. Supernumerary chromosomes in two species of the family Pimelodidae (Teleostei, Siluriformes). Chromosome Science, vol. 3, p. 9-13.

YU, X., ZHOU, T., LI, Y., LI, K. AND Zhou, T., 1989. *Chromosomes of Chinese Fresh-Water Fishes*. Beijing: Science Press. 180 p.