

Cytotaxonomy of Iberian loaches with some remarks on the karyological evolution of both families (Pisces, Cobitidae, Homalopteridae)

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SUMMARY — The three species of cobitoids inhabiting the Iberian Peninsula were cytogenetically analysed - *Cobitis calderoni*, *C. maroccana* and *Nemacheilus barbatulus*. Metaphasic plates of specimens collected in several Portuguese and Spanish streams and, for the stoneloach, also in a central European river, were compared. All the species have a diploid value of $2n = 50$ and are characterized by a high incidence of chromosomes with terminal/subterminal centromeres, particularly the cobitids. In spite of their phenotypical distinction, the spined-loaches karyotypes are grossly similar, suggesting only the occurrence of punctual chromosome rearrangements related to the respective differentiation processes. The lack of well-apparent interpopulation chromosome variability and the existence of diploid-polyploid relationships in these Euro-Asiatic families is finally discussed according to the new model of Cyprinidae karyological evolution.

INTRODUCTION

In the Iberian Peninsula there are two genera of loaches — *Cobitis* Linnaeus, 1758 and *Nemacheilus* Bleeker, 1863 — which belong, according to SAWADA (1982), to distinct families Cobitidae and Homalopteridae¹, respectively, both included in the superfamily Cobitoidea.

The genus *Cobitis*, which has a number of species with rather limited ranges, presents a Palearctic distribution — through all East Asia and even part of Mekong Basin in South Asia, Siberia, part of West Asia, whole Europe, and Morocco. The Iberian species presently recognized are the endemic *C. calderoni* Bacescu, 1961 and the ibero-african endemic *C. maroccana* Pellegrin, 1929.

¹ This nomenclature should be replaced, according to KOTELLAT (1988a), by the senior synonym «Balitoridae» but the option here was to avoid additional confusion.

The former inhabits the septentrional catchments, while the latter, more common and widespread, is found in the central and meridional rivers and also in Morocco (see VASIL'eva *et al.* 1992).

The genus *Nemacheilus*, with almost 200 species awaiting a taxonomic redefinition (see BANARESCU 1990), ranges through Eurasia (with the exception of: most northern areas, most of Iberian, Italian and Arabian Peninsulas, western slope of Balkans, southern Greece, south of Tigris and Euphrates Basins) and has one isolated species in Lake Tana (East Africa). The type-species — *N. barbatulus* (Linnaeus, 1758) — although recently captured in a Douro tributary (GONZÁLEZ *et al.* 1989) is typically found in NE Spanish rivers (DOADRIO 1986).

The aim of this paper is to present a comparative cytotaxonomic analysis of the three loach species inhabiting Iberian waters. First results on Iberian loaches karyology were presented by ELVIRA and BUSTAMANTE (1983a, 1983b) but never published. Recently, VASIL'eva *et al.* (1992) studied the morphology of both spined loaches, and included karyological data of *C. maroccana* Portuguese specimens, which were compared with the karyotype of *C. calderoni* (only known from an Abstract presented by ELVIRA and BUSTAMANTE 1983a).

Afterwards, a more complete and general survey is now offered. It includes the analysis of specimens of both cobitid species collected in Spanish and Portuguese streams, and also the comparison of the karyotype of the Iberian stoneloach with the one found in stoneloaches captured by the first author (J.M. Madeira) in a tributary of the European River Rhine.

MATERIALS AND METHODS

Specimens were captured by electrofishing or by cast nets. The Iberian collection sites are represented in Fig. 1: *Cobitis calderoni* - Portugal (1-3, Douro Basin, N = 11), Spain (4-5, Douro B., N = 7); *C. maroccana* - Portugal (6, Vouga B.; 7, Samarra B.; 9-13, Tagus B.; 14, Sado B.; 15-16, Mira B.; 17, Arade B.; 18-20, Guadiana B., N = 56) and Spain (8, Tagus B., N = 5) and *Nemacheilus barbatulus* - Spain (21, Ebro B., N = 16). The stoneloach sample from Rhine Basin (N = 5), was directly obtained in River Rotbach, Urmasch tributary (Switzerland, 21-09-1988 - T.L. = 41—48 mm).

Standard procedures for chromosome preparation and respective classification were used as described in COLLARES-PEREIRA (1983). Silver NOR staining was performed following the technique of HOWELL and BLACK (1980). Gill and kidney tissues were analysed in Portuguese and Swiss material, but only gill tissue was used in Spanish material. The homologous chromosome pairs were assembled into the groups: M (metacentric, $r = 1.0-1.7$), S (submetacentric, $r = 1.8-3.0$) and A (subtelo-, acro- and telocentric, $r > 3.0$). For FN estimation, the first two groups were scored as bi-armed and the latter as uni-armed.

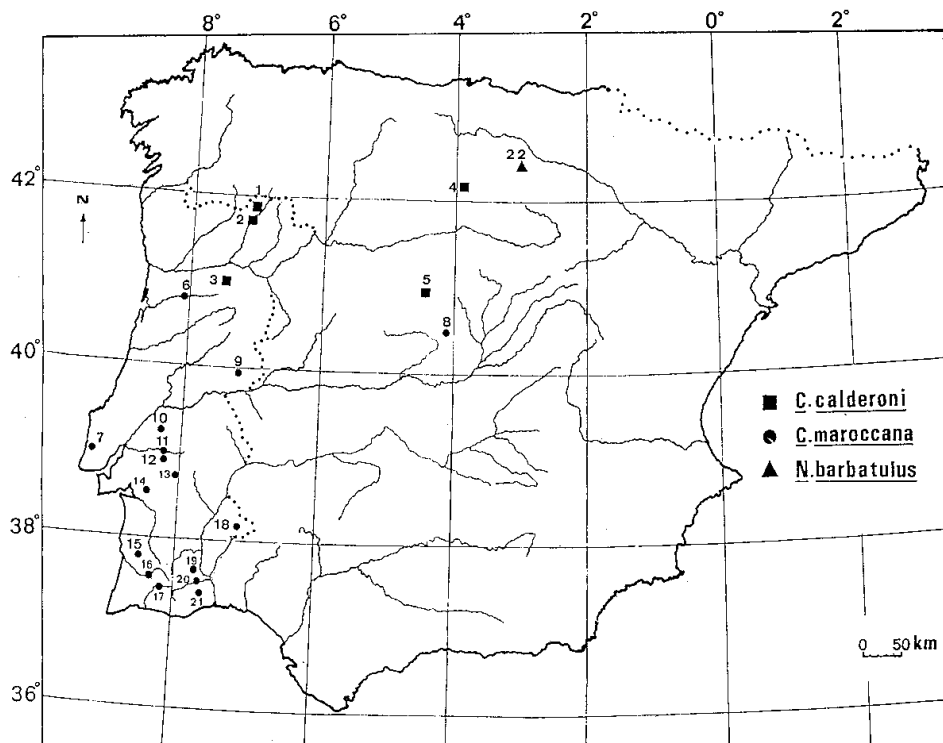


Fig. 1. — Collection sites of the Iberian material.

RESULTS

The three species analysed have 50 elements in their respective diploid sets, and both male and female karyotypes did not exhibit any apparent distinction at general morphological level (Table 1).

TABLE 1 - Karyotypic formulations and FN from modal metaphases of the Iberian loaches now analysed (S - Spain; P - Portugal; SW - Switzerland).

Species	Loc.	No. Specimens			Karyot. form.			FN
		♂	♀	?	M	S	A	
<i>Cobitis calderoni</i>	S	1	6		6	14	30	70
	P		4	7	6	14	30	
<i>C. maroccana</i>	S	2	3		6	12	32	68
	P	22	30	4	6	12	32	
<i>Nemacheilus barbatulus</i>	S	5	11		8	20	22	78
	SW			5	8	20	22	

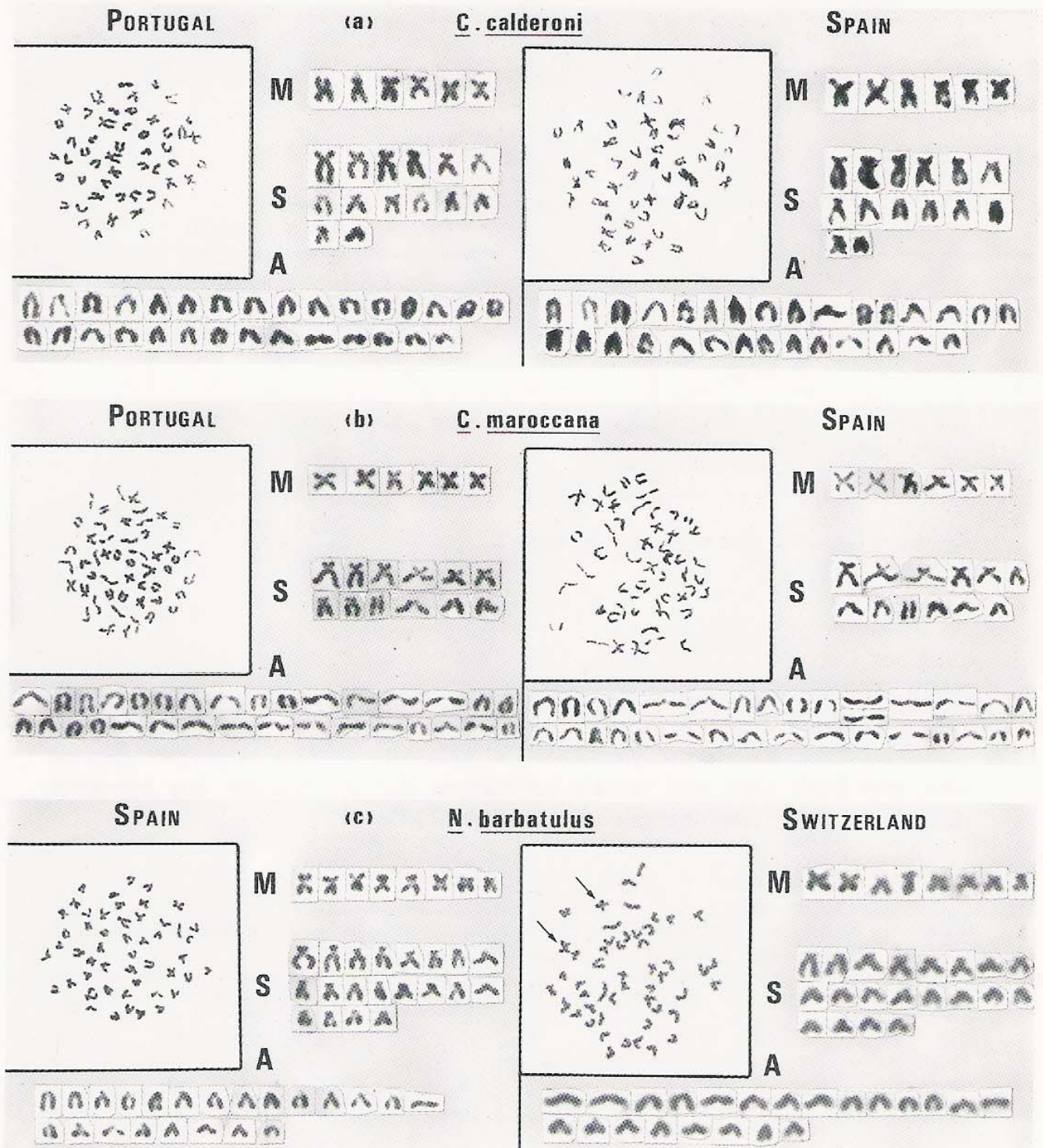


Fig. 2. — Metaphasic plates and karyotypes of specimens of (a) *C. calderoni* (Portugal: no. 1; Spain: no. 4); (b) *C. maroccana* (Portugal: no. 18; Spain: no. 8); (c) *N. barbatulus* (Spain: no. 22; Switzerland) (see Fig. 1 for collecting place reference). Arrows indicate Ag-NOR pair.

Cells not yielding modal counts were almost all negatively skewed, probably due to loss during preparation, overlap or miscounting. Also the very reduced number of hypermodal values may be explained by those artifacts or by both breakage and/or premature chromatid separation.

Cobitis calderoni. — All the specimens analysed (N = 18) exhibit identical karyotypes, with a reduced number of metacentric elements (three pairs). Besides, one of them presents an arm ratio near the M-S border, specially in Spanish material, and its classification was not unequivocal in all the photographed plates. However, the karyotypic formulations obtained in fishes collected both in Spanish and Portuguese tributaries of Douro Basin may be considered similar (6M: 14S: 30A, FN = 70) (Fig. 2a).

C. maroccana. — The karyotype description of this ibero-african species previously presented (VASIL-EVA *et al.* 1992) is confirmed by the analysis of more material from the same and distinct aquatic systems (N = 61), both in Portugal and in Spain. The karyotypic formulations obtained in material from several catchments are also identical (6M: 12S: 32A, FN = 68), without any well-apparent differences (Fig. 2b). The Ag-NORs were always detected in the biggest metacentric pair.

Nemacheilus barbatulus. — The karyotype seems to be characterized by the existence of 14 pairs of meta- and submetacentric chromosomes and by a clearly inferior number of acrocentric elements (karyotypic form. 8M: 20S: 22A, FN = 78). There is also an apparent reduction in the TCL (total complement length) when compared with the analysed cobitid species. Conspicuous differences between the iberian and the non-iberian material now studied (N = 21) were not detected. Chromosomal NORs were found to occur identically in the biggest metacentric pair (Fig. 2c).

DISCUSSION

All the species have the same diploid complement and are characterized by a high incidence of chromosomes with terminal/subterminal centromeres, particularly the cobitids. Moreover, and based on the evidence given by the conventionally Giemsa-stained chromosomes, the apparent similitude within and between the karyotypes of these *Cobitis* is really striking, specially if the clear distinction at phenotypical level is taken into account (for details see VASIL'EVA *et al.* 1992). In fact, the general chromosome pattern of the two spined-loaches, differing only in a small submetacentric pair, did not allow the detection of any more significant difference, suggesting karyological stability.

The same unexpected loss of apparent interpopulation variability was also enregistered in *N. barbatulus*. In fact, this species, which karyotype may be

distinguished from the now analysed cobitids by the presence of more meta- and submetacentric chromosomes, presents gross chromosomal identity in the specimens of both iberian and non-iberian populations. So, without using more resolution and more reliable technical procedures, the existing differences with formerly published data (SOFRADZIJA and VUKOVIC 1979; VASIL'EV 1985), referring to the presence of a higher number of acrocentric elements in stoneloaches collected both in Bosnia and USSR, respectively, should not be at present interpreted.

As for the number of chromosomes, the populational differences already described in three distinct congeneric taxa — *C. taenia*, *C. biwae* and *C. sinensis* — apparently involving hybridization and unisexuality phenomena (UENO and OJIMA, 1976; SOFRADZIJA and BERBEROVIC 1978; VASIL'EV *et al.* 1989, 1990), were not found in Iberian cobitids. Moreover, a diploid-triploid-tetraploid complex was very recently described in Cobitidae by the last team, suggesting the production of stable tetraploidy through the hybridization of two diploid bisexual species (*C. taenia* and *C. granoei*).

Therefore, the family Cobitidae contains both «diploid» and «tetraploid» species, the first group ranging from $2n = 40$ to $2n = 62$, with a modal value of $2n = 50$, the second one varying between $2n = 98-100$. The latest were until now described in three distinct genera — these referred to *Cobitis*, *Botia* and *Misgurnus* (Table 2) — and have been assumed as the product of independent

TABLE 2 - List of know diploid values of Cobitidae and Homalopteridae genera (see MAZIK and TOKTOSONOV 1984; VASIL'EV 1985; KHUDA-BUKHSH *et al.* 1986; YU *et al.* 1987, 1989).

Genera	No. species analyzed	$2n$
Cobitidae		
<i>Acanthopthalmus</i>	1	50
<i>Acanthopsis</i>	1	50
<i>Barbatula</i>	2	48
<i>Botia</i>	5	50-100
<i>Cobitis</i>	7	48-100
<i>Lefua</i>	2	50
<i>Lepidocephalichthyes</i>	1	62
<i>Leptobotia</i>	6	50
<i>Misgurnus</i>	2	48-100
<i>Parabotia</i>	3	50
<i>Paramisgurnus</i>	1	48-49
<i>Sabanejewia</i>	3	50
Homalopteridae		
<i>Hemimyzon</i>	1	50
<i>Nemacheilus</i>	9	36-50
<i>Micronemacheilus</i>	1	50
<i>Triplophysa</i>	2	48
<i>Vanmanenia</i>	1	50

polyploidization events arisen in their lineages (see VASIL'EV *et al.* 1989). So, $2n=50$ has been considered the plesiomorphic condition for the family (VASIL'EV *et al.* 1989; YU *et al.* 1989; Buth *et al.* 1991), assumption almost exclusively supported by commonality.

Regarding the Homalopteridae, all the already analysed taxa present diploid values ranging between $2n=36-50$, most commonly $2n=50$ (Fig. 3). However, SOFRADZIJA and VUKOVIC (1979) observed the existence of spermatogenetic polyploid meiosis in *N. barbatulus* from Bosnia.

Diploid-polyploid relationships have been also discussed in another cypriniform family, besides the biggest fish family - Cyprinidae. Extant «polyploids», that represent already more than $\approx 20\%$ of the ≈ 450 analysed cyprinids, after being also assumed as derivated from the most primitive «diploids» were recently divided, according to COLLARES-PEREIRA and COELHO (1989), into two distinct groups: primary polyploids, those who retained the ancestral condition (just as another Cypriniformes family — the Catostomidae) and, the remaining, as secondary polyploids. Such new model for the karyological evolution of Cyprinidae is in some way also supported by the strong correlation found between the occurrence of polyploidy and the geographic distribution of southeastern asiatic taxa, very probably the center of origin and radiation of the family.

Such remarkable fact was objectively related by ZAN *et al.* (1986) with the uplift of Qinghai-Xizang Plateau through the suddenly induced temperature decrease, and by KHUDA-BUKHSH *et al.* (1986) with the termic variation and exigent ecological conditions of the rapidly-flowing Himalayan streams where polyploids are also very abundant.

Although Homalopteridae are not known to have polyploid taxa it would be interesting to analyse in the near future the karyotypes of the several species of *Yunnanilus* Nichol, 1925, which are characterized by having both a wide distribution range and habitat preferences. In fact, KOTTELAT (1988b) described a «miniature species flock» from the chinese Yunnan Plateau nearby the area anteriorly focussed. So, if the referred to theoretical assumption of the origin of cyprinid polyploidy is valid and if these nemacheiline were already differentiated, considering their relatedness, it is expectable that they will share this apomorphic character with the cyprinids. If not, inference about an eventually most recent differentiation process from a well-established already diploidized cyprinid ancestor may be supported.

Effectively, the superfamily Cobitoidea with a total of ≈ 48 genera and ≈ 300 species (see NELSON 1984), constitute the second most important group of Cypriniformes. However their karyotypes have a striking difference in relation to Cyprinidae: the relatively high number of chromosomes with subterminal/terminal centromeres, only observed in some very few indian cyprinids (see TRIPATHI and SHARMA 1987). Such fact may favour an origin for loaches from a cyprinid ancestor already reduced to $2n=50$, and so the

occurrence in this family of only secondary polyploids probably by an indirect via, as already evidenced and cited above. Those telocentric elements could have been produced in the course of the differentiation processes by distinct kinds of structural rearrangements such as pericentric inversions and/or centric transpositions, what in part may counteract with the apparent karyological stability now observed in Iberian cobitids.

However, the great variability observed in genome sizes of loaches is somehow remarkable pointing that, as in cyprinids, considerable changes have occurred during or associated to cobitid speciation episodes. Data from MURAMOTO *et al.* (1968), HINEGARDNER and ROSEN (1972) and ZAN *et al.* (1986) report a range from 0.8 pg to 4.6 pg for cobitids, suggesting that *Misgurnus anguillicaudatus* may really represent a secondary polyploid with its two kinds of populations ($2n=50$ - 1.4 pg; $2n=100$ - 4.6 pg). Nevertheless, *Botia macracantha* ($2n=98$) has a DNA content similar to *Acanthopthalmus khulii* ($2n=50$) and very closed to the inferior limit described for the family. This may obviously suggest an origin for «polyploid» species also from a polyploid ancestor, and the subsequent occurrence of other types of structural rearrangements such as, at least initially, centric fusions.

In conclusion, to answer to this complex question, most extensive work must absolutely be carried out in loaches, regarding not only genome size determinations but also the obtention of high resolution chromosome images. Improvement of banding techniques will certainly increase the information potential of chromosomes, since the establishment of the presumed homologies will be reliable. Then, more extensive inferences on loaches phylogeny and on the real role of polyploidy in the evolution of this important Cypriniformes group, will be allowed.

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REFERENCES

- BANARESCU P., 1990. — *General distribution and dispersal of freshwater animals*. In «Zoogeography of fresh waters», Vol. 1, 511 pp., Aula-Verlag Wiesbaden.
- BUTH D.G., DOWLING T.E. and GOLD J.R., 1991. — *Molecular and cytological investigations*. In «Cyprinid Fishes. Systematics, biology and exploitation», ed. by I.J. Winfield and J.S. Nelson, pp. 83-126, Chapman & Hall, London.
- COLLARES-PEREIRA M.J., 1983. — *Cytotaxonomic studies in Iberian Cyprinids. I. Karyology of Chondrostoma lusitanicum Collares-Pereira*, 1980. *Cytologia*, 48: 753-760.
- COLLARES-PEREIRA M.J. and COELHO M.M., 1989. — *Polyploidy versus diploidy: a new model for the karyological evolution of Cyprinidae*. *Arq. Museu Boc. N.S.*, 1 (26): 375-383.
- DOADRIO I., 1986. — *Nuevas localidades de Noemacheilus barbatulus (L., 1758) (Ostariophysi, Homalopteridae) en España*. *Misc. Zool.*, 10: 391-392.

- ELVIRA B. and BUSTAMANTE A.D., 1983a. — *Karyotypes of the iberian loaches Cobitis calderoni Bacescu, 1961 and C. paludicola De Buen, 1935 (Pisces, Cobitidae)*. II Jornadas de Ictiologia Iberica, Barcelona, 23-28 May, (Abstract).
- , 1983b. — *Cariología de los cobítidos ibéricos, con la descripción del cariotipo de Noemacheilus barbatulus (L., 1758) (Pisces, Cobitidae)*. XIX Jornadas de Genética Luso-Espanhola, Coimbra, 20-22 September (Abstract).
- GONZALEZ G., DOMINGUEZ J. and PENA J.C., 1989. — *Primera cita de Noemacheilus barbatulus L., 1758 (Cypriniformes, Homalopteridae) en la Cuenca del río Duero*. Misc. Zool., 13: 201-202.
- HINEGARDNER R. and ROSEN D.E., 1972. — *Cellular DNA content and the evolution of teleostean fishes*. Am. Nat., 106: 621-644.
- HOWELL W.M. and BLACK D.A., 1980. — *Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method*. Experientia, 36: 1014-1015.
- KHUDA-BUKHSH A.R., CHANDA T. and BARAT A., 1986. — *Karyomorphology and evolution in some indian hillstream fishes with particular reference to polyploidy in some species*. Proc. 2nd. Int. Conf. Indo-Pacific Fishes pp. 886-898.
- KOTTELAT M., 1988a. — *Indian and Indochinese species of Balitora (Osteichthyes: Cypriniformes) with descriptions of two new species and comments on the family-group names Balitoridae and Homalopteridae*. Rev. Suisse Zool., 95: 487-504.
- , 1988b. — *Revision of Yunnanilus with descriptions of a miniature species flock and six new species from china (Cypriniformes: Homalopteridae)*. Environm. Biol. Fishes, 23: 65-93.
- MAZIK E.Y. and TOKTOSONOV A.T., 1984. — *The karyotypes of three species of loaches of the genus Noemacheilus Hasselt (Cypriniformes, Cobitidae)*. Tsitologiya, 26: 960-961.
- MURAMOTO J., OHNO S. and ATKIN N.B., 1968. — *On the diploid state of the Fish Order Ostariophysi*. Chromosoma, 24: 59-66.
- NELSON J.S., 1984. — *Fishes of the world*, 2nd. ed., 523 pp., John Wiley & Sons.
- SAWADA Y., 1982. — *Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes)*. Mem. Fac. Fish. Hokkaido Univ., 28: 65-223.
- SOFRADZIJA A. and BERBEROVIC Lj., 1978. — *Diploid-triploid sexual dimorphism in Cobitis taenia taenia L. (Cobitidae, Pisces)*. Genetika, 10: 389-397.
- SOFRADZIJA A. and VUKOVIC T., 1979. — *Chromosome complement of Nemacheilus barbatulus (Linnaeus, 1758), Cobitidae, Pisces*. Ichthyologia, 11: 43-49.
- TRIPATHI N.K. and SHARMA O.P., 1987. — *Cytological studies on six cyprinid fishes*. Genetica, 73: 243-246.
- UENO K. and OJIMA Y., 1976. — *Diploid-tetraploid complexes in the genus Cobitis (Cobitidae, Cyprinida)*. Proc. Jap. Acad., 52: 446-449.
- VASIL'EV V.P., 1985. — *Evoljucionnaja kariologija*. 300 pp., Nauka Publishers, Moscow.
- VASIL'EV V.P., VASIL'EVA K.D. and OSINOV A.G., 1989. — *Evolution of a diploid-triploid-tetraploid complex in fishes of the genus Cobitis (Pisces, Cobitidae)*. In: «Evolution and ecology of unisexual Vertebrates», ed. by R.M. Dawley and J.P. Bogart, pp. 153-169, New York State Museum, Albany, N.Y.
- , 1990. — *Reticulate evolution in vertebrates: the diploid-triploid-tetraploid complex in the genus Cobitis (Cobitidae)*. III. *Origin of the triploid form*. Voprosy Ichthyol., 30: 543-550.
- VASIL'EVA K.D., COLLARES-PEREIRA M.J. and MADEIRA J.M., 1992. — *Variability, divergence and taxonomy of spined loaches (Cobitidae) from Iberian Peninsula*. Voprosy Ichthyol., 32: 41-55.
- YU X., ZHOU T., LI K., LI Y. and ZHOU M., 1987. — *On the karyosystematics of cyprinid fishes and a summary of fish chromosome studies in China*. Genetica, 72: 225-236.
- , 1989. — *Chromosomes of chinese fresh-water fishes*. 179 pp., Science Press, Beijing, China.
- ZAN R., SONG Z. and LIU W., 1986. — *Studies on karyotypes and nuclear DNA contents of some cyprinoid fishes, with notes on fish polyploids in China*. Proc. 2nd. Int. Conf. Indo-Pacific Fishes, pp. 877-885.

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